Intracellular K⁺ Activity and Its Relation to Basolateral Membrane Ion Transport in *Necturus* Gallbladder Epithelium

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**Abstract** A study of the mechanisms of the effects of amphotericin B and ouabain on cell membrane and transepithelial potentials and intracellular K activity (aKi) of *Necturus* gallbladder epithelium was undertaken with conventional and K-selective intracellular microelectrode techniques. Amphotericin B produced a mucosa-negative change of transepithelial potential (Vm~) and depolarization of both apical and basolateral membranes. Rapid fall of aKi was also observed, with the consequent reduction of the K equilibrium potential (EK) across both the apical and the basolateral membrane. It was also shown that, unless the mucosal bathing medium is rapidly exchanged, K accumulates in the unstirred fluid layers near the luminal membrane generating a paracellular K diffusion potential, which contributes to the Vm~ change. Exposure to ouabain resulted in a slow decrease of aKi and slow depolarization of both cell membranes. Cell membrane potentials and aKi could be partially restored by a brief (3-4 min) mucosal substitution of K for Na. Under all experimental conditions (control, amphotericin B, and ouabain), EK at the basolateral membrane was larger than the basolateral membrane equivalent emf (Eb). Therefore, the K chemical potential difference appears to account for Eb and the magnitude of the cell membrane potentials, without the need to postulate an electrogenic Na pump. Comparison of the rate of Na transport across the tissue with the electrodiffusional K flux across the basolateral membrane indicates that maintenance of a steady-state aKi cannot be explained by a simple Na,K pump-K leak model. It is suggested that either a NaCl pump operates in parallel with the Na,K pump, or that a KCl downhill neutral extrusion mechanism exists in addition to the electrodiffusional K pathway.

**Introduction**

Gallbladder epithelium transports sodium and chloride from the lumen to the serosal side. Recent studies in *Necturus* and rabbit gallbladder have established that both the luminal (apical) and the serosal (basolateral) membranes of the transporting cells are mainly K-selective (Hénin and Cremaschi, 1975; Reuss J. GEN. PHYSIOL. © The Rockefeller University Press 0022-1295/80/07/0033/20 $1.00 33 Volume 76 July 1980 33-52
and Finn, 1975 a and b; Van Os and Slegers, 1975; Reuss, 1979). In Necturus, the luminal membrane has smaller permeabilities for Cl (Reuss and Finn, 1975 b) and Na (Reuss and Finn, 1975 b; Van Os and Slegers, 1975), whereas the basolateral membrane has a very small Cl permeability and is essentially impermeable to Na (Reuss, 1979).

Tracer uptake measurements (Frizzell et al., 1975; Cremaschi and Hénin, 1975) and ion-selective microelectrode studies (Duffey et al., 1978; Reuss and Weinman, 1979; Reuss and Grady, 1979) have revealed that entry of NaCl from the lumen into the cells is a coupled process, driven by the Na electrochemical potential difference, which results in an intracellular Cl activity higher than the one predicted from equilibrium distribution.

At the basolateral membrane, Na transport in uphill (cell potential ~−65 mV, intracellular Na activity ~22 mM; Reuss and Weinman, 1979), whereas Cl transport is downhill. However, the Cl conductance of the basolateral membrane is too small to account for Cl extrusion by electrodiffusion. Alternative mechanisms of Cl transport at this membrane have been proposed for both Necturus (Reuss, 1979; Reuss and Weinman, 1979) and rabbit gallbladder (Duffey et al., 1978).

The polyene antibiotic amphotericin B, when added to the luminal solution bathing the gallbladder, causes a mucosa-negative change of transepithelial potential (Cremaschi et al., 1971). In rabbit gallbladder, this has been interpreted to result, at least in part, from stimulation of basolateral electrogenic Na transport (Rose and Nahrwold, 1976 and 1977; Rose, 1978). A similar conclusion was drawn by Graf and Giebisch (1979) from microelectrode studies in Necturus gallbladder. This interpretation is not supported by the findings of Cremaschi et al. (1977) in rabbit and Reuss (1978) in Necturus. In these two studies it was shown that the change of transepithelial potential is accompanied by depolarization of both cell membranes and reduction of both cell membrane resistances (Reuss, 1978). Cell depolarization was thought to result mainly from a rather nonselective increase in apical membrane small cation permeability. This effect appeared to account for the transepithelial hyperpolarization without the need to postulate electrogenic Na transport.

The cardiac glycoside ouabain considerably inhibits fluid transport by Necturus gallbladder, causes a mucosa-negative change of transepithelial potential (Cremaschi et al., 1971). In rabbit gallbladder, this has been interpreted to result, at least in part, from stimulation of basolateral electrogenic Na transport (Rose and Nahrwold, 1976 and 1977; Rose, 1978). A similar conclusion was drawn by Graf and Giebisch (1979) from microelectrode studies in Necturus gallbladder. This interpretation is not supported by the findings of Cremaschi et al. (1977) in rabbit and Reuss (1978) in Necturus. In these two studies it was shown that the change of transepithelial potential is accompanied by depolarization of both cell membranes and reduction of both cell membrane resistances (Reuss, 1978). Cell depolarization was thought to result mainly from a rather nonselective increase in apical membrane small cation permeability. This effect appeared to account for the transepithelial hyperpolarization without the need to postulate electrogenic Na transport.
tial by comparing the K equilibrium potential ($E_K$) across the cell membranes with the cell membrane equivalent electromotive forces under various conditions.

Using the control data of $aK_i$, cell membrane potentials, and K conductance ($g_K$) at both cell membranes, we were able to analyze in detail the mechanism of K transport at the basolateral membrane and its relationship to NaCl transport. Our results do not agree with the notion of a simple Na,K pump–K leak model at this site.

**MATERIALS AND METHODS**

Necturi (Necturus maculosus) were obtained from Mogul-Ed Co., Oshkosh, Wis. and kept in aquaria at 4–8°C. The gallbladders were removed, washed with Ringer's solution, and mounted horizontally in a modified Ussing chamber, mucosal side up.

**Bathing Solutions**

Standard Ringer's medium (Na-Ringer) had the following composition (mM): NaCl 109.2, KCl 2.5, CaCl$_2$ 1.0, NaHCO$_3$ 2.4. The solution was equilibrated with room air and had a pH of about 8.0. Potassium Ringer's medium (K-Ringer) was prepared by total, isomolar replacement of NaCl with KCl. Ouabain was added to the serosal medium to a final concentration of $10^{-4}$ M. Amphotericin B was added to the mucosal medium to a final concentration of $5 \times 10^{-6}$ M. The mucosal and serosal solutions were replaced continuously. The experiments were performed at 24 ± 1°C.

**Electrical Measurements**

Details of the preparation and uses of conventional and K-selective microelectrodes (ME) have been published (Reuss and Finn, 1975 a and 1977; Reuss and Weinman, 1979; Reuss et al., 1979). Transepithelial ($V_{m0}$) and intracellular potentials (apical: $V_{mc}$, basolateral: $V_{cb}$) were measured with high-input impedance electrometers provided with digital panel meters, displayed on an oscilloscope (Tektronix, Inc., Beaverton, Oreg.), stored in a 1074 Signal Averager (Nicolet Instrument Corp., Madison Wis.), and plotted with a 7010-B X-Y recorder (Hewlett Packard Co., San Diego, Calif.) or a Brush 2400 three-channel pen recorder (Gould, Inc., Instruments Div., Cleveland, Ohio). The output of the K-selective ME was connected to a F223A high-input impedance dual electrometer (W-P Instruments, Inc., New Haven, Conn.). In some experiments, the signal was filtered to reduce its noise level. The output of the electrometer was displayed, stored, and recorded as described for cell membrane potentials.

The properties of the K-selective ME were not changed by amphotericin B at the concentration employed in the experiments.

The criteria for validation of the impalements were those previously described (Reuss and Weinman, 1979).

Intracellular K activities ($aK_i$) were determined according to the equation:

$$aK_i = (aK_o + kaNa_o) \exp[(V^* - V)F/nRT],$$

where $aK_o$ and $aNa_o$ are the extracellular K and Na activities, respectively, at the time of the impalement, $k$ is the selectivity coefficient of the ME (Na over K), $V^*$ is the intracellular potential measured by the K-selective ME, $V$ is the membrane potential, $n$ is a constant determined from the electrode calibration, and $F$, $R$, and $T$ have their usual meanings. To calculate $aK_i$ according to Eq. 1, both the conventional
and the K-selective ME were referred to the same bathing medium. When the tissue was exposed to Na-Ringer on both sides, the values of the apical membrane potential ($V_{mc}$) and the change in potential measured by the K-selective ME upon impalement ($V^*$ referred to mucosal solution) were employed. Of course, this is equivalent to using $V_{on}$ and $V^*$ referred to serosal solution, because the value of $V_{ms}$ is subtracted from both $V$ and $V^*$ when changing the reference from mucosal to serosal medium (see Eq. 1). Once both ME are inside cells, changes in $aK_o$ do not influence the calculation of $aK_i$, because the resulting membrane potential changes alter $V$ and $V^*$ by the same amount (see Results). Actual records of intracellular potentials of experiments in which mucosal medium $aK$ was changed are referred to the serosal bathing medium, in which the bulk solution K activity was kept constant. The calculation outlined above does not consider intracellular Na. From the selectivity of the ME and the mean $aNa_i$ value of 22 mM (Reuss and Weinman, 1979) it can be shown that the resulting error in the estimation of $aK_i$ is <1 mM under control conditions. Ouabain and amphotericin B increase $aNa_i$ (Reuss et al., 1979; Graf and Giebisch, 1979). During exposure to the polyene antibiotic, the increase of $aNa_i$ to ~75 mM (Graf and Giebisch, 1979) results in an overestimation of $aK_i$ of ~1.5 mM.

Cell membrane equivalent electromotive forces were calculated as previously described (Reuss and Finn, 1975a and b; Reuss, 1979).

**Experimental Procedure**

At least 30 min after the tissues were mounted in the chamber, when both the transepithelial potential ($V_{me}$) and the transepithelial resistance ($R_t$) were stable, impalements with conventional and K-selective ME were performed (at least six with each) in the same field, under control conditions (Na-Ringer on both sides). These values were averaged to calculate mean values of $V_{me}$, $V_{on}$, and $V^*$ for that tissue; $aK_i$ was calculated as explained above.

During experimental perturbations (Na substitutions, addition of amphotericin B to the mucosal medium, or addition of ouabain to the serosal medium), potentials and $aK_i$ were determined in one of two ways: (a) average of several impalements with conventional and ion-selective ME at given intervals, or (b) continuous records with both electrodes in cells, before, during, and after the perturbation. 

**Statistics**

Unless otherwise stated, results are expressed as means ± SE. Comparisons were made by conventional paired data analysis.

**RESULTS**

Impalement of small cells with microelectrodes can cause significant membrane damage or changes in intracellular composition and, therefore, artifactual results (Lindemann, 1975; Higgins et al., 1977; Nelson et al., 1978). For this reason, adequate criteria for the validation of impalements are a necessity in this type of study. We have described in detail the criteria employed in our previous work with Cl-, K- and Na-selective ME. Fig. 1 illustrates an impalement with a K-selective ME. Intercellular coupling and the effect of mucosal K-Ringer on membrane potentials are also shown (see also Reuss and Weinman, 1979, and Reuss and Grady, 1979).

The mean $aK_i$ values before drug addition were 86.0 and 91.6 mM in the ouabain and amphotericin B series, respectively, agreeing well with our recently obtained mean value of 87.3 mM (Reuss and Weinman, 1979). As
observed previously, valid impalements with K-selective ME always resulted in positive deflections, indicating that $aK_i$ is higher than the value predicted from equilibrium distribution.

**Effects of Ouabain on Potentials and Intracellular K Activity**

As shown in Table I, ouabain ($10^{-4}$ M, serosal solution) produced decreases in transepithelial and cell membrane potentials, which became significant at 30 min. Intracellular K activity fell progressively, from a control value of 86.0

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**Figure 1.** Impalement with K-selective ME under control conditions. Upper trace ($V_{cs}$): basolateral membrane potential. Middle trace ($V_K$): output of K-selective ME, referred to serosal bathing medium. Lower trace ($V_{cs} - V_K$): differential record. The records start with the conventional electrode in a cell ($V_{cs} = -67$ mV). At the arrow, a second cell, 120 μm distant, was impaled with the K-selective ME. The potential measured by this electrode before impalement is the transepithelial potential ($V_m$), in this case $-0.8$ mV. The upward deflection in the middle trace indicates that $aK_i$ is higher than predicted for equilibrium distribution. At the moment indicated by $P$, a current pulse ($5 \times 10^{-9}$ A, 1 s duration) was applied through the conventional ME. Note the off-scale deflections in $V_K$ and $V_{cs} - V_K$ traces. The small voltage change in the $V_K$ trace indicates that the two cells are electrically coupled. During the period indicated by the upper bar (labeled K), the mucosal medium was changed from Na-Ringer to K-Ringer. The depolarizations measured with the two ME differ by only about 1 mV (lower trace) and are completely reversible. The vertical deflections at 10-s intervals result from transepithelial current pulses. From the comparison of the deflections recorded by each ME in the cell, and the deflection across the whole tissue (not shown), the ratio of membrane resistances of the impaled cells can be calculated, after corrections for the voltage drops in the solutions. The increase in the amplitude of the deflections during mucosal exposure to K-Ringer is mainly due to the drop of apical membrane resistance (see Reuss and Finn, 1975 a and b).
mM, to 29.4 mM after 60 min of exposure to the glycoside and was significantly reduced as soon as 15 min after ouabain was added.

The difference between the measured $aK_i$ and the calculated value of $aK_i$, assuming passive distribution, decreased progressively with time of incubation in ouabain, from a control value of 62.2 mM, to a value of 21.0 mM at 60 min. In two tissues, 120 min after ouabain was added, the values of $aK_i$ were 10.3 and 5.1 mM, compared with control values of 92.6 and 94.1 mM, respectively.

The effects of a brief (3–4 min) mucosal exposure to K-Ringer on potentials and $aK_i$ were determined in tissues incubated with ouabain. As shown previously, in the absence of ouabain, exposure of the mucosal surface of the tissue to K-Ringer results in rapid depolarization of both cell membranes. Thereafter, the cell membrane potentials remain constant throughout the period of exposure to K-Ringer. Upon reexposure to Na-Ringer, the cell potentials return to their original values (Reuss et al., 1979). In the absence of ouabain, mucosal exposure to K-Ringer for periods of 1–5 min did not result in a change in $aK_i$.

When the gallbladder was exposed to ouabain for 70–100 min, the transepithelial potential fell to practically zero, the two cell membranes depolarized, and $aK_i$ was largely reduced (Table II; values before K-Ringer). At this time, the tissues were exposed to K-Ringer for 3–4 min. The results were quite different from those observed in the absence of ouabain. As shown before (Reuss et al., 1979), the immediate depolarization in K-Ringer was similar to that seen under control conditions. However, during the period of exposure to K-Ringer, $V_{mc}$ and $V_{cs}$ did not remain constant but hyperpolarized progressively. Upon the return to Na-Ringer on the mucosal side, both $V_{mc}$ and $V_{cs}$ became larger than the values measured before exposure to K-Ringer. In Table II we compare electrical potentials and $aK_i$ values immediately before exposure to K-Ringer and at the peak value of $V_{cs}$ after reexposure to Na-Ringer. The values of $V_{mc}$ and $V_{cs}$ were significantly increased after K-Ringer. This hyperpolarization was concomitant with an elevation of $aK_i$ by a factor of 3.7.

**Table I**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Transepithelial potential</th>
<th>Apical membrane potential</th>
<th>Basolateral membrane potential</th>
<th>Intracellular K activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$V_{mc}, \text{mV}$</td>
<td>$V_{me}, \text{mV}$</td>
<td>$V_{cs}, \text{mV}$</td>
<td>$aK_i, \text{mM}$</td>
</tr>
<tr>
<td>15</td>
<td>-0.8±0.2</td>
<td>-61.5±4.7</td>
<td>-62.4±4.8</td>
<td>86.0±4.9</td>
</tr>
<tr>
<td>30</td>
<td>-0.2±0.1*</td>
<td>-56.9±3.2*</td>
<td>-57.1±3.2*</td>
<td>49.9±5.3*</td>
</tr>
<tr>
<td>60</td>
<td>0.0±0.1*</td>
<td>-36.3±4.0*</td>
<td>-36.3±4.0*</td>
<td>29.4±5.0*</td>
</tr>
</tbody>
</table>

* Value significantly different from control ($P < 0.05$).

$\text{n = five experiments.}$
Effects of Amphotericin B on Potentials and Intracellular K Activity

Fig. 2 shows the result of an experiment in which a preparation was exposed to amphotericin B while a conventional and a K-selective ME was kept in the cells. Shortly after the polyene antibiotic was added to the mucosal bathing medium, the cells depolarized. After a delay of 1–2 min, $aK_i$ decreased.

**Table II**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Transepithelial potential</th>
<th>Apical membrane potential</th>
<th>Basolateral membrane potential</th>
<th>Intracellular K activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before K-Ringer</td>
<td>$V_m$, $mV$</td>
<td>$V_m$, $mV$</td>
<td>$V_m$, $mV$</td>
<td>$aK_i$, mM</td>
</tr>
<tr>
<td>0.1±0.1</td>
<td>-34.8±4.7</td>
<td>-34.7±4.6</td>
<td>15.6±3.5</td>
<td></td>
</tr>
<tr>
<td>After K-Ringer</td>
<td>-0.2±0.1</td>
<td>-63.6±4.1</td>
<td>-63.8±4.1</td>
<td>58.1±6.6</td>
</tr>
<tr>
<td>$P$</td>
<td>NS</td>
<td>&lt;0.025</td>
<td>&lt;0.025</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

$n =$ three experiments. All values were measured in the same cells in each tissue, 70–100 min after exposure to ouabain, with Na-Ringer on both sides (Before K-Ringer), and at the peak value of $V_m$ in Na-Ringer on both sides, after a brief (3–4 min) exposure of the apical surface to K-Ringer (After K-Ringer). See text.

NS, not significant.

**Figure 2.** Effect of amphotericin B on cell membrane potential and intracellular K activity. Upper trace ($V_m$): basolateral membrane potential. Middle trace ($V_K$): output of K-selective ME. Lower trace ($V_m - V_K$): differential record. Records start with conventional and K-selective ME inside cells. At the first arrow, amphotericin B was added to the mucosal medium (final concentration, $5 \times 10^{-6}$ M). Note that $V_m$ falls progressively. For about 2 min, $V_K$ changes by the same amount (no appreciable change takes place in the differential trace). After this delay, the upward deflection of $V_m - V_K$ indicates that $aK_i$ is falling. At the second arrow, the polyene antibiotic was removed from the mucosal medium. Slow reversal of the effects can be observed thereafter. The vertical deflections are the result of transepithelial current pulses.
Amphotericin B removal was followed by slow hyperpolarization of the membranes and increase in $aK_i$.

Fig. 3 summarizes the values of $aK_i$ in five preparations as a function of time after exposure to amphotericin B. The decrease in $aK_i$ was significant 2 min after the polyene antibiotic was added to the mucosal medium.

Steady-state potential and $aK_i$ values before and after amphotericin B (5-10 min) are summarized in Table III. In the presence of the antibiotic, as observed previously, the mucosa-negative transepithelial potential increased, whereas the cell membrane potentials decreased. $aK_i$ fell, but remained higher than the values predicted from equilibrium distribution across the cell membranes (apical: $2.0 \pm 0.2$ mM, basolateral: $2.5 \pm 0.3$ mM).

During exposure to amphotericin B, the effect of a mucosal substitution of K for Na on cell potentials and $aK_i$ is dramatically changed, as compared with control conditions, as shown in Fig. 4. When the cell has been depolarized by the polyene antibiotic, exposure to K-Ringer on the mucosal side results in
an increase in $V_{cs}$. This effect is the opposite of the one observed in the absence of amphotericin B (Fig. 1), where $V_{cs}$ decreases during exposure to K-Ringer. As shown in Fig. 4, the hyperpolarization of the basolateral membrane in K-Ringer is concomitant with an increase in $aK_i$.

The results of five experiments similar to the one shown in Fig. 4 are summarized in Table IV. The apical membrane undergoes a rapid cell-

![Graph showing effect of high mucosal K on cell membrane potential and intracellular K activity in the presence of amphotericin B.](image)

**Figure 4.** Effect of high mucosal K on cell membrane potential and intracellular K activity in the presence of amphotericin B. Symbols and protocol are as in Fig. 2. At the first arrow, amphotericin B was added to the mucosal medium (Na-Ringer). $V_{cs}$ began to decrease immediately, and after a delay, $aK_i$ fell also, as indicated by the upward change in the lower trace. At the second arrow, the mucosal medium was replaced with K-Ringer (plus the same concentration of amphotericin B). This resulted in a fast and large increase in $aK_i$ (to a value close to the control one), and in an increase in $V_{cs}$ (upper trace). At the third arrow, K-Ringer was replaced with Na-Ringer, and the amphotericin concentration was kept constant. At the fourth arrow, the wash with Na-Ringer plus amphotericin was repeated. $V_{cs}$ is depolarized further, and $V_{cs} - V_K$ reached a value similar to the one observed in amphotericin, before exposure to K-Ringer. See text.

Positive change in potential and, thereafter, repolarizes. The data shown in Table IV were obtained after $V_{mc}$ had reached a steady-state value. At that time, $V_{cs}$ and $V_{mc}$ had changed by essentially the same amount, whereas the $V_{mc}$ value was not different from that before K-Ringer (see Reuss, 1978). In 2–4 min, $aK_i$ rose more than sixfold.

**Effect of Amphotericin B on K Activity in the Apical Unstirred Layer**

As shown above, exposure of the apical surface of the cells to amphotericin B causes a reduction in $aK_i$, presumably because of a large K net flux from cells
to mucosal bathing medium. If the tissue is not superfused at a high rate or if
the mucosal solution volume is small and/or improperly stirred, this K flux
could result in an increase in K activity in the unstirred fluid layers near the
luminal membrane. This effect can be significant because an increase in
extracellular K activity (\(aK_o\)) can result in a transepithelial \(aK_o\) difference
and, therefore, in a mucosa-negative paracellular diffusion potential (see
Discussion).

To test this possibility, the transepithelial potential and mucosal \(aK_o\) were
measured by positioning ME (K-selective and Ringer-filled) near (~10 \(\mu\)m)
the apical cell surface. The result of one experiment is depicted in Fig. 5.
During exposure to amphotericin B, the K activity increased slowly when
superfusion was stopped and decreased rapidly during fast superfusion. These
quite different time-courses upon stopping or restarting the renewal of the
mucosal medium are what one would predict if K is leaving the cells through

| TABLE IV |
| INTRACELLULAR K ACTIVITY DURING EXPOSURE TO Na-RINGER OR K-
RINGER IN THE PRESENCE OF AMPHOTERICIN B |

<table>
<thead>
<tr>
<th>Mucosal medium</th>
<th>Transepithelial potential</th>
<th>Apical membrane potential</th>
<th>Basolateral membrane potential</th>
<th>Intracellular K activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na-Ringer</td>
<td>(V_{mns}, mV)</td>
<td>(V_{ma}, mV)</td>
<td>(V_{mb}, mV)</td>
<td>(aK_o, mM)</td>
</tr>
<tr>
<td></td>
<td>-3.7±1.0</td>
<td>-2.6±3.0</td>
<td>-6.2±3.2</td>
<td>12.0±1.6</td>
</tr>
<tr>
<td>K-Ringer</td>
<td>-27.0±5.0</td>
<td>-2.4±1.8</td>
<td>-29.4±4.6</td>
<td>79.5±3.3</td>
</tr>
<tr>
<td>(p)</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(n = five experiments\). Measurements in Na-Ringer were made after a steady-state value of \(V_{mns}\) was reached
in the presence of amphotericin B. Measurements in K-Ringer were made in the same cells, 2-4 min after
the mucosal solution substitution.

NS, not significant.

the apical membrane and accumulating in the mucosal unstirred layer. Upon
rapid superfusion, the unstirred layer thickness is decreased and, therefore,
\(aK_o\) in the fluid layers near the tissue falls. As is also shown in Fig. 5, when
superfusion was stopped for a second time, during continuous exposure to
amphotericin, \(aK_o\) increased again, but less than after the first period of no
stirring. In other experiments, this observation was made up to five times, and
in every case \(aK_o\) increased when the superfusion was stopped, suggesting that
a basolateral mechanism of K transport toward the cell interior persists during
exposure to amphotericin B. The contribution of this mechanism to the
transepithelial potential (\(V_{mns}\)) is difficult to measure during the phase of K
accumulation in the unstirred layer because this is slow and \(V_{mns}\) undergoes
spontaneous changes during exposure to amphotericin B (Reuss, 1978). Therefore,
the rapid change of \(V_{mns}\) upon superfusion was employed. Shortly after
amphotericin B (<10 min) was added, superfusion resulted in a 1.1 ± 0.2 mV
depolarization (\(n = 3\)). This value decreased with time and successive periods
of superfusion, but the depolarization was still identifiable 60 min after the
antibiotic (0.2 ± 0.03 mV) had been added. In conclusion, these experiments
show that K accumulation in the mucosal unstirred layer can contribute to the transepithelial potential change produced by amphotericin B and that, consistent with our measurements of $aK_i$, K uptake across the basolateral membrane continues in the presence of the antibiotic.

**Discussion**

The control intracellular K activities measured in these series of experiments agree well with our previous results (87.3 ± 3.3 mM; Reuss and Weinman, 1979) and, given the limitations of chemical analysis techniques, agree reasonably with determinations of intracellular K concentration in scraped epithelial cells (96 ± 7 mM; Reuss et al., 1979). In every experiment in which $aK_i$ has been measured in tissues exposed to standard NaCl Ringer's medium on both sides, its value has been higher than predicted for equilibrium distribution. This observation indicates that K is transported uphill into the cell. It seems obvious that this transport mechanism is located at the basolateral membrane (Reuss and Weinman, 1979; Reuss et al., 1979). However, the further possi-
bility of inwardly oriented apical membrane K transport cannot be excluded (Reuss and Weinman, 1979).

Transmembrane K Equilibrium Potential and Basolateral Membrane Equivalent Electromotive Force

The estimated intracellular K activity yields values of $E_K$ larger than the cell membrane potentials. The comparison between $E_K$ and cell membrane potential, however, does not have the same meaning as in nonpolar cells. In the epithelial cells of the gallbladder, the apical and basolateral membranes have different ionic permeabilities; therefore, their equivalent electromotive forces are not equal (Reuss and Finn, 1975a and b; Reuss, 1979). The existence of an intercellular high-conductance transepithelial pathway in parallel with the cell membranes (Frömter, 1972; Reuss and Finn, 1975a) creates an intraepithelial current loop. Therefore, to ascertain whether $E_K$ is large enough to account for the membrane potentials, it is necessary to compare it with the equivalent electromotive forces of the membranes. The result of the comparison between $E_B$ (equivalent emf of the basolateral membrane) and $E_K$ is shown in Fig. 6. The $aK_i$ values employed for the calculation of $E_K$ were the means of all determinations under control conditions and in the ouabain and amphotericin B experiments. It was assumed that the $aK_o$ was equal to that in the bulk serosal bathing medium (see below). $E_A$ (apical membrane equivalent emf) and $E_B$ were calculated from the values of potentials shown in

![Figure 6](https://example.com/figure6.png)
Tables I and III and the resistances recently measured under similar experimental conditions (amphotericin B: Reuss, 1978; ouabain: Reuss et al. 1979). The values of $E_a$, not shown, were always smaller than either $E_b$ or $E_K$ (see Reuss and Finn, 1975a; Reuss, 1979); $E_K$ was larger than $E_b$ under control conditions and during exposure to amphotericin B or ouabain. The significance of this observation in terms of the mechanisms of the effects of amphotericin B on transepithelial and cell membrane potentials is discussed in detail below.

Mechanisms of the Effects of Ouabain on Membrane Potentials

The results of the experiments with ouabain confirmed our previous observations (Reuss et al., 1979): the glycoside causes a slow depolarization of the cell membranes and a reduction of transepithelial potential. As shown in Table I, these changes are associated with a progressive decrease of $aK_i$. The present measurements with K-selective electrodes confirm and extend our previous estimates of intracellular K concentration before and after ouabain (Reuss et al., 1979). The reduction of $aK_i$ was small, but significant, 15 min after addition of ouabain, an effect faster than the one reported by De Long and Civan (1978) in toad urinary bladder, where $aK_i$ decreased after lag periods of 20-24 min. These authors observed a reduction of short circuit current (rate of net Na transport) after lag periods of 3-13 min, whereas the effect of ouabain on fluid transport in gallbladder was also faster than the one on short circuit current in urinary bladder: 18 percent inhibition at 5 min and 70 percent inhibition at 15 min (Reuss et al., 1979).

We have also observed that, in tissues exposed to ouabain for a period of 60 min or more, substituting K for mucosal Na resulted in immediate cell depolarization followed by hyperpolarization during the period of exposure to K-Ringer. In control tissues, the cells initially depolarize but do not hyperpolarize during exposure to K-Ringer. Upon replacement of K-Ringer with Na-Ringer, the cells of ouabain-treated preparations hyperpolarized to values of $V_{mc}$ and $V_{cs}$ greater than those measured before the exposure to K-Ringer. The present series of experiments (Table II) confirms the speculation that this phenomenon could be due to net K uptake with consequent increases in $aK_i$ and in $E_K$ across the cell membranes. Because both apical and basolateral membranes are K-selective (Reuss and Finn, 1975b; Reuss, 1979), a larger $E_K$ results in larger equivalent emf's at both borders and in cell hyperpolarization. Exposure of the tissues to K-Ringer on the mucosal side increased $aK_i$ from 15.6 to 58.1 mM. Although the latter value is lower than the one measured in the absence of ouabain, the change in $aK_i$ is large enough to account for the cell potential changes.

The observation that $aK_i$ in control tissues exposed to K-Ringer on the mucosal side for up to 5 min did not change requires discussion. Because the apical membrane is K-selective, the change in K chemical potential, by itself, should result in net K entry into the cells. However, in this experimental situation, both cell membranes depolarize (see Fig. 1 and Reuss and Finn, 1975a and b). This depolarization opposes the effect of the change in K chemical potential difference across the luminal membrane. Detailed calcu-
lations from the K electrochemical potential differences during exposure to Na-Ringer or K-Ringer on the luminal side (Reuss and Finn, 1975 a and b; Reuss and Weinman, 1979; and present data) and the K conductance at both membranes (Reuss and Finn, 1975 a and b; Reuss, 1979) show that the net result of the changes in K electrodiffusional fluxes is a net influx of 2.89 \times 10^{-6} \text{ mEq} \cdot \text{cm}^{-2} \cdot \text{min}^{-1} \text{ (area of stretched tissue). Assuming a cell height of 30 \mu m, the initial rate of change in } aK_i \text{ would be only } 0.96 \text{ mEq} \cdot \text{l}^{-1} \cdot \text{min}^{-1}, \text{ in excellent agreement with the observation that } aK_i \text{ essentially did not change over a 5-min period.}

A similar calculation is not possible for the analogous experiment with ouabain because cell membrane resistances cannot be measured at periods longer than 60 min of exposure to the glycoside (Reuss et al., 1979). An upper limit for electrodiffusional K entry can be estimated, however, from the change in K electrochemical potential difference across the apical membrane (when K-Ringer is substituted for Na-Ringer), assuming that \( g_K \) (apical), the K conductance of the membrane, is the same as during exposure to K-Ringer in the absence of ouabain. The predicted influx, assuming no net K flux through the basolateral membrane, is only half that required to account for the change in \( aK_i \) shown in Table II, which cannot be fully explained with the available data. One of several possibilities is that, during exposure to K-Ringer, apical \( g_K \) is greater in the presence than in the absence of ouabain.

In Fig. 6 we compare \( E_K \) with \( E_b \) under control conditions and at several intervals after exposure to ouabain. The data suggest that after \( \sim 15 \text{ min} \) \( E_b \) falls faster than \( E_K \). This is consistent with our recent observation (Reuss et al., 1979) of a reduction in basolateral membrane K selectivity in tissues exposed to ouabain. The ratio of cell membrane resistances (\( R_a : R_b \)) decreases in ouabain, as compared with control conditions, agreeing with our interpretation of an ouabain-induced reduction of basolateral membrane \( g_K \). After reloading the ouabain-exposed cells with K, \( R_a : R_b \) rose to control levels, suggesting that the effect of ouabain on \( g_K \) is, at least in part, secondary to the diminution of \( aK_i \), as one would expect. Our previous results that show that the effect of external K on basolateral membrane potential and equivalent emf are reduced after prolonged incubation in ouabain (Reuss et al., 1979) indicate that, in addition to the effect described above, ouabain reduces \( g_K \) by decreasing the K permeability of the membrane.

**Mechanisms of the Effects of Amphotericin B on Membrane Potentials**

The results reported here, in addition to previous observations in rabbit and Necturus gallbladder (Cremaschi et al., 1977; Reuss, 1978; Graf and Giebisch, 1979), reveal the following effects of the addition of amphotericin B to the mucosal medium: (a) large increase in apical membrane conductance, with a reduction of its K selectivity, (b) transepithelial (mucosa-negative) hyperpolarization, with cell membrane depolarization, (c) reduction in intracellular K activity, after a delay of up to 2 min, (d) moderate increase in basolateral membrane conductance, and (e) increase in K selectivity (\( P_K : P_{Na} \)) at the paracellular pathway, even though its total conductance does not change appreciably during incubation in Na-Ringer. The loss of apical membrane K
selectivity results, by itself, in a reduction of equivalent electromotive force ($E_a$) and thus in a fall of $V_m$. In addition, from the fall of $E_a$ alone, one would expect: (a) a fall of $V_m$ because the two membranes are electrically connected by the intercellular pathway, and (b) an increase in $V_{ma}$ because $(E_b - E_a)$ rises and $V_{ma}$ (if $E_a = 0$) is described by (Reuss and Finn, 1975 a and b).

\[ V_{ma} = (E_b - E_a)R_a/(R_a + R_b + R_s), \]  

where $E$ stands for equivalent emf, $R$ for resistance, and the subscripts a, b, and s denote apical membrane, basolateral membrane, and shunt pathway, respectively.

In addition to this mechanism, changes in potential during exposure to the antibiotic can occur because of changes of intracellular and extracellular K activity, with the consequent effects on: (a) $E_K$ across the cell membranes, and, therefore, $E_a$ and $E_b$, and (b) the K activity gradient across the paracellular pathway (see Fig. 5).

The recent measurements of Graf and Giebisch (1979) in *Necturus* gallbladder show that amphotericin B causes a rapid increase in intracellular Na activity ($a_{Na}$), with a time-course very similar to the $a_{K_i}$ results depicted in Figs. 2 and 4 (see their Fig. 10). The action of the antibiotic on the apical membrane results, therefore, in net Na uptake and net K loss from the cells.

In summary, the changes in cell membrane potentials and transepithelial potential produced by amphotericin B can be ascribed, in principle, to all or some of the following mechanisms: (a) rapid fall of apical membrane K selectivity (apical membrane $g_{Na}$ increases proportionally more than $g_K$); (b) somewhat slower decrease in $a_{K_i}$, which reduces the equivalent emf's of both apical and basolateral membrane; (c) stimulation of an electrogenic Na pump at the basolateral membrane; (d) in the presence of a significant mucosal unstirred layer, increase in $a_K$ in the fluid layers in contact with the apical surface of the cells. Inasmuch as the selectivity of the apical membrane is largely reduced by the antibiotic, the latter mechanism is unlikely to change $E_a$ significantly. It could, however, contribute to the transepithelial potential change because of the paracellular K diffusion potential. These effects are complicated further by the conductance changes outlined above. The increase in apical membrane conductance produced by amphotericin B is readily explained by an increase in small monovalent cation permeability (Reuss, 1978), similar to the effect of nystatin on the luminal membrane of rabbit urinary bladder (Lewis et al., 1977 and 1978). The mechanism of the increase in basolateral membrane conductance (Reuss, 1978) is unclear, particularly because the reduction in $a_{K_i}$ would be expected to decrease the conductance, since the membrane has a high K permeability. The possibility of amphotericin B penetration to the basolateral membrane is unlikely, because the membrane retains its K selectivity, as illustrated in Fig. 4 by the hyperpolarization observed during mucosal exposure to K-Ringer.

Our findings concerning the effect of amphotericin B on $E_b$ disagree with those of Graf and Giebisch (1979), who calculated an increase in $E_b$ and interpreted this result as an argument in favor of an electrogenic basolateral Na pump. Assuming that $E_a = 0$ in amphotericin, $E_b$ can be described by
(Reuss and Finn, 1975 a):

\[ E_b = V_{cs} + V_{ms} \frac{R_b}{R_s} \tag{3} \]

where \( V_{cs} \) is the basolateral membrane potential.

The main source of the discrepancy is the value of \( (R_b : R_s) \). This results from the use of both a higher \( R_b \) and a lower \( R_s \) in the calculations of Graf and Giebisch. Our cable analysis experiments indicate that \( R_b \) decreases shortly after exposure to amphotericin B (Reuss, 1978). Graf and Giebisch assumed that \( R_b \) was unchanged by amphotericin B. This, alone, accounts for about 46 mV of the difference between the two calculated values of \( E_b \). The presence of a K paracellular diffusion potential will also result in an overestimation of \( E_b \) (see Reuss and Finn, 1975 a and b). In addition, our estimation of \( E_K \) can be in error because of the assumption of an extracellular (basolateral) K activity equal to that of the bulk serosal solution. Both the high rate of K transport at the basolateral membrane and the serosa-to-mucosa net paracellular K flux can result in a fall of \( aK \) in the serosal unstirred layer, and, therefore, in larger values of \( E_K \) than those shown in Fig. 6.

In sum, our calculations suggest that, inasmuch as \( E_K \) remains higher than \( E_b \) during exposure to amphotericin B, it is not necessary to postulate a basolateral electrogenic Na pump to account for the effect of the antibiotic. Such a hypothesis can not be ruled out from our data, but it is apparent that the results of Rose and Nahrwold (1976) and Graf and Giebisch (1979) do not prove such a mechanism.

**Basolateral Membrane K Transport and Its Relation to NaCl Transport**

The results of this work show that K is transported by an uphill mechanism from the serosal bathing medium to the cells, and that the intracellular K activity decreases after exposure of the serosal surface to ouabain, suggesting that the Na pump is responsible for this uphill K transport. The demonstration of serosa to mucosa K transport during exposure of the apical surface to amphotericin B also indicates the presence of an uphill K transport mechanism at the basolateral membrane, inasmuch as \( aK_i \) remained above equilibrium distribution.

We will now turn our attention to the K fluxes across the basolateral membrane, as estimated from electrophysiological data, and to their relationship to NaCl transport. This analysis, in conjunction with previous data, suggests strongly that cation transport at the basolateral membrane cannot be explained by a simple system consisting of a Na,K pump in parallel with a K diffusional pathway.

We have shown that the Cl electrochemical potential difference across the basolateral membrane favors downhill extrusion (Reuss and Weinman, 1979; Reuss and Grady, 1979) in agreement with observations in rabbit gallbladder (Duffey et al., 1978). However, in Necturus the Cl conductance of the basolateral membrane is so low that only about 3 percent of the net transepithelial Cl flux can be ascribed to electrodiffusion across this membrane (Reuss, 1979; Reuss and Weinman, 1979). Consequently, an additional Cl transport mech-
anism, parallel to the electrodiffusional path, was proposed. This could consist of a neutral NaCl pump, downhill KCl extrusion, or a Cl-HCO₃ exchange mechanism (Reuss and Weinman, 1979). Our data on intracellular K activity, K electrochemical potential difference, and K conductance under control conditions lead us to draw a similar conclusion.

If the Na pump operates in a 1:1 or in a 3:2 Na:K exchange mode, and if all of transepithelial Na transport is through the pump, the rate of K uptake by the pump can be calculated from the rate of net Na transport (Reuss et al., 1979). The result of the calculation (in µA·cm⁻²) is 38 (neutral pump) or 25.3 (coupling ratio 3:2). Under steady-state conditions aKᵢ remains constant. Therefore, this inward K flux must be balanced by an equal outward K flux. For simplicity, let us first assume that all of this cell-to-medium flux is through the basolateral membrane. If the flux is only electrodiffusional, its magnitude can be calculated from the K electrochemical potential difference and the K conductance of the membrane (gK):

\[ I_K = \frac{(\Delta \mu_K)}{F} \cdot g_K = (E_K - V_{ea}) \cdot t_K g_b, \tag{4} \]

where the flux is expressed as current (I_K, in µA·cm⁻²), \( \Delta \mu_K/F \) is expressed in mV and gK in mS·cm⁻²; tK is the K transference number and gb is the basolateral membrane conductance (1/Rb). (E_K - V_{ea}) ranges from 17.2 to 32.3 mV (Tables I and III; and Reuss and Weinman, 1979). The maximum value will be employed for the calculation. g_b is 0.38 mS·cm⁻² (Reuss, 1979) and t_K is 0.94 (Reuss, 1979). The result is I_K = 11.7 µA·cm⁻², i.e., less than half of the value required for a 3:2 coupling ratio and less than one-third of the value required for a neutral Na pump. If the values of \( \Delta \mu_K/F \) obtained in the present series of experiments are employed, the discrepancy is even larger. If we assume that the basolateral membrane is only permeable to K, I_K increases only by 6 percent (g_K = 0.94 g_b). The value of g_b employed for the calculation is in excellent agreement with that obtained by Fromter (1972) in the same tissue. It could be argued that a coupling ratio (Na:K) of 3:1 would explain this result, but this is inconsistent with the magnitude of the cell membrane depolarization 15-30 min after ouabain, when fluid transport is largely inhibited (Table I; see also Reuss et al., 1979).

The above discussion shows that a simple pump-leak model of K transport across the basolateral membrane is not tenable unless only part of the Na flux is through the Na,K pump. If Na is transported in part by the Na pump and in part by a NaCl neutral pump, the K influx through the Na pump will be less than estimated above. For instance, if one-third of JNa⁰⁰ is through the Na,K pump, K influx would be only 12.7 (neutral pump) or 8.4 µA·cm⁻² (3:2 coupling), i.e., quite close to the predicted electrodiffusional efflux. Alternatively, it is possible that most or all of Na transport is through the Na,K pump, and, therefore, that the K influx is in the range of 25 to 38 µA·cm⁻², but that there are two pathways for K efflux: the electrodiffusional one (11.7 µA·cm⁻²) and neutral KCl extrusion. As stated before (Reuss and Weinman, 1979), both K and Cl are above electrochemical equilibrium in the cell. If a carrier mechanism for K and Cl exists at the basolateral membrane, downhill transport to the serosal solution will take place. This possibility is
appealing because it would not require metabolic energy input. A neutral NaCl transport mechanism would necessarily be uphill, because the electrochemical potential difference favoring Cl extrusion is smaller than the one opposing Na transport toward the serosal medium (Reuss and Weinman, 1979).

This argument is complicated further by the consideration of K fluxes at the luminal membrane and of NaCl and K fluxes through the paracellular pathway. Paracellular NaCl transport (solvent drag) would account for part of the discrepancy explained above. However, it appears unlikely that it explains net transepithelial Cl transport (Reuss, 1979). The K conductance of the apical membrane is smaller than that of the basolateral membrane (Reuss and Finn, 1975b; Reuss, 1979), and the diffusional K flux across the apical membrane can be predicted to be only about 30 percent of the basolateral flux. The possibility of an additional mechanism of basolateral Cl transport, by exchange with extracellular HCO₃, is suggested by the stimulatory effect of bicarbonate on fluid transport (Diamond, 1964; Martin, 1974; Heintze et al., 1979).

In conclusion, under all experimental conditions employed in these experiments, $E_K$ across the basolateral membrane was greater than $E_B$. Therefore, cell membrane potentials can be explained, in principle, by electrodiffusional mechanisms alone. In addition, a consideration of the electrodiffusional K flux through the basolateral membrane, in conjunction with the rate of NaCl transport, suggests that maintenance of a steady-state intracellular K activity requires (a) that only part of the Na transported through the membrane flow through the Na,K pump, or (b) that nonconductive K transport, i.e., a neutral KCl downhill extrusion mechanism, exists in parallel with the electrodiffusional pathway.

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