J. Membrane Biol. 49, 345–362 (1979)

# Intracellular Ionic Activities and Transmembrane Electrochemical Potential Differences in Gallbladder Epithelium

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Received 14 March 1979; revised 15 May 1979

*Summary.* Intracellular ion activities in *Necturus* gallbladder epithelium were measured with liquid ion-exchanger microelectrodes. Mean values for K, Cl and Na activities were 87, 35 and 22 mM, respectively. The intracellular activities of both K and Cl are above their respective equilibrium values, whereas the Na activity is far below. This indicates that K and Cl are transported uphill toward the cell interior, whereas Na is extruded against its electrochemical gradient. The epithelium transports NaCl from mucosa to serosa. From the data presented and the known Na and Cl conductances of the cell membranes, we conclude that neutral transport driven by the Na electrochemical potential difference can account for NaCl entry at the apical membrane. At the basolateral membrane, Na is actively transported. Because of the low Cl conductance of the membrane, only a small fraction of Cl transport can be explained by diffusion. These data suggest that Cl transport across the basolateral membrane is a coupled process which involves a neutral NaCl pump, downhill KCl transport, or a Cl-anion exchange system.

Knowledge of the intracellular activity of the transported ions is essential for an understanding of the mechanisms of transepithelial salt transport. In the present experiments on *Necturus* gallbladder, we have simultaneously measured cell membrane potentials and intracellular activities of K, Cl, and Na, using liquid ion-exchanger microelectrodes (Walker, 1971; Khuri *et al.*, 1972). From these data and from the extracellular activities it is possible to calculate single ion electrochemical potential differences across each cell membrane and to identify uphill and downhill transport mechanisms. In addition to this information, there are data available on the partial ionic conductances and permeability coefficients of the cell membranes (Reuss & Finn, 1975*a–b*; Reuss, 1979*a*) and the rates of salt and water transport across the epithelium (Reuss, Bello-Reuss & Grady, 1979). Knowledge of electrochemical potential differences, partial ion conductances of the cell membranes, and rate of salt transport should allow us to determine whether or not downhill

0022-2631/79/0049-0345 $03.60

transport mechanisms can be accounted for by diffusion. The results reported in this paper have been presented in preliminary form elsewhere (Reuss, 1979*b*).

## Materials and Methods

Mudpuppies (*Necturus maculosus*) obtained from Mogul-Ed Co., Oshkosh, Wisc., were kept in large aquaria at 4–8 °C. The gallbladders were removed and mounted as previously described (Reuss & Finn, 1975*a*, 1977) and incubated at room temperature (24 ± 1 °C) in a bathing medium of the following composition (in mmol/liter): NaCl, 109.2; KCl, 2.5; $CaCl_2$, 1.0; $NaHCO_3$, 2.4. The pH was about 8.0 after equilibration with room air. Both sides of the tissue were perfused continuously (Suzuki & Frömter, 1977).

### *Measurements of Electrical Potentials and Resistances*

Potentials and resistances were measured as previously described (Reuss & Finn, 1975*a*, 1977). Ag-AgCl pellets connected to the bathing media with 3-M KCl or Ringer-agar bridges were used to pass transepithelial current pulses. Ag-AgCl pellets or calomel electrodes, also connected to the solutions with salt bridges, were employed to measure the transepithelial potential. Conventional microelectrodes were prepared from inner-fiber glass capillaries (Frederick Haer & Co., Brunswick, Me., or W.P. Instruments, New Haven, Conn.) and filled with 3 M KCl or 4 M potassium acetate. Electrodes with tip resistance $\geqq 20$ MΩ were employed. In some instances, the microelectrodes were bevelled by the procedure described by Ogden *et al.* (1978). Impalements were performed with motorized, remote control micromanipulators (Stoelting, Chicago, Ill.), under observation, at 200–400 X, with an inverted microscope (model MS, Nikon Inc., Garden City, N.Y., or Biovert, Reichert, Austria). Intracellular (apical membrane: $V_{mc}$, basolateral membrane: $V_{cs}$) and transepithelial ($V_{ms}$) potentials were measured with high impedance ($10^{12}$ Ω) electrometers, displayed on a storage oscilloscope (Tektronix, Beaverton, Ore.) and a three-channel pen recorder (Brush 2400, Gould Inc., St. Louis, Mo.). The reference was the serosal solution.

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

### *Measurements of Intracellular Ionic Activities*

Chloride, K, and Na selective electrodes were prepared by the technique originally described by Walker (1971), with some modifications. Inner-fiber capillary glass was used to pull these electrodes with the same settings employed for the conventional ones, except when Cl-selective electrodes were prepared, in which case the tip diameter had to be somewhat larger ($R_{tip}$ with 3 M KCl ca. 10–20 MΩ). The borosilicate glass at the tip of the micropipette was rendered hydrophobic by exposure to the vapors of dimethyldichlorosilane, followed by curing at 100 °C for at least 1 hr, or alternatively by immersion in trichloromethylsilane, followed by curing at 140 °C for at least 1 hr (Spring & Kimura, 1979). The electrical connection between the resin and the electrometer was a reference electrolyte solution and a Ag-AgCl pellet, or a chlorided silver wire inserted in the resin (Orme, 1969).

The following exchangers were employed: *Cl*: Corning 477315 (Corning Glass, Corning, N.Y.) or Orion 92-17-02 (Orion Research, Cambridge, Mass.). *K*: Corning 477317, Orion 92-19-02, or 5% potassium tetrakis (*p*-chlorophenyl) borate (Specialty Organics, Inc. Irwindale, Calif.) in 3-nitro-O-xylene (Aldrich Chemical Co., Milwaukee, Wisc.). *Na*: 5% potassium tetrakis (*p*-chlorophenyl) borate in triethyl hexyl phosphate (ICN Pharmaceuticals, Inc., Plainview, N.Y.). The Na/K selectivity of the cation exchanger employed for both Na and K electrodes is determined by the solvent.

Measurements were performed with a F223A high input impedance ($10^{15}$ $\Omega$) dual electrometer (WP Instruments, New Haven, Conn.). The output was displayed and recorded as described for cell membrane potentials.

The electrodes were calibrated, at $24 \pm 1$ °C, both in pure, single-salt solutions, and in media similar in composition to the one employed in the experiments. Slopes were determined over a range that encompassed the intracellular and extracellular activities of the ion (e.g., in the case of K, from 1 to 100 mM).

During a recording session, several cells were impaled with a conventional microelectrode and other cells were impaled with an ion-selective electrode. The slope of the latter was checked frequently by removing the microelectrode from the cell and changing the composition of the mucosal medium (e.g., NaCl was replaced by KCl). If the slope changed, the previous impalements were discarded. Ionic activities in the bathing media were measured routinely.

The mean slopes of Cl, K and Na electrodes were 56.2, 58.2 and 59.1 mV, respectively. The electrical time constants were less than 0.2 sec. These values compare well with those obtained by other investigators (e.g., Walker, 1971; Spring & Kimura, 1978; Palmer & Civan, 1975; Palmer, Century & Civan, 1978). K-selective electrodes had a selectivity coefficient for Na over K ($k_{K,Na}$) of about 0.02, and a $k_{K,Ca}$ of 0.001 or less (*see also* Fujimoto & Kubota, 1976). Na-selective electrodes were only about 2.6 times more selective for Na than K. Since the intracellular K activity is much higher than the intracellular Na activity, measurements of Na activity required a large correction for intracellular K, and the result is therefore subject to a larger error. Chloride-selective electrodes exhibit sizable responses to bicarbonate (Saunders & Brown, 1977). Bicarbonate was present in the bathing solution at a concentration of 2.4 mM. Since $k_{Cl,HCO_3}$ is about 0.05, the bicarbonate contribution to the potential recorded by the Cl electrode in the bathing medium is well below 1 mV and was neglected. Intracellular bicarbonate was also neglected, assuming that its activity is not much larger than the extracellular activity. The possible interference of other intracellular anions will be discussed later.

Strict criteria were employed to validate the impalements with ion-selective electrodes. They are as follows: (1) The values of potentials recorded by the ion-selective microelectrode before and after impalement differed by at most 1 mV. (2) Upon impalement, the change in voltage was abrupt and monotonic. (3) The ratio of membrane resistances ($R_a/R_b$), measured from the voltage deflections produced by transepithelial current across the apical and basolateral membrane, was at least 1.2. (4) The intracellular record was stable, within 1 mV for at least 30 sec. (5) Cells impaled with a conventional and an ion-selective microelectrode, respectively, were shown to be electrically coupled when applying intracellular current pulses through the conventional electrode. (6) Changes in ionic composition of the mucosal bathing medium resulted in essentially equal immediate changes of the voltage output of a conventional and an ion-selective electrode placed inside cells. Criteria *1–4* were applied to all experiments. Criteria *5* and *6* were employed occassionally in most tissues (*see Results*).

Intracellular ionic activities were calculated according to the equation (Walker, 1971):

$$a_i^i + k_{ij} a_j^i = (a_i^o + k_{ij} a_j^o) \exp \frac{(V^* - V_{mc}) z_i F}{nRT} \tag{1}$$

where $a$ stands for activity, the subscripts $i$ and $j$ refer to the main and interfering ion, respectively, the superscripts $i$ and $o$ denote intracellular and extracellular activities, V* is the change in potential measured by the ion-selective microelectrode upon impalement, $V_{mc}$ is the apical membrane potential, $n$ is a constant determined from the electrode calibration, $k$ is the selectivity coefficient, and the other symbols have their usual meaning. All measurements of intracellular Na activity were corrected by determining intracellular K in the same tissues.

*Experimental Procedure*

At least 30 min after mounting the tissue in the chamber, when the transepithelial potential ($V_{ms}$) and the transepithelial resistance ($R_t$) were stable, impalements were performed with conventional and ion-selective microelectrodes (ME). At least 6 impalements with each microelectrode were performed and averaged to calculate mean values of $V_{mc}$, $V_{cs}$ and $V^*$ for that tissue. When the intracellular Na activity ($a\mathrm{Na}_i$) was measured, at least four impalements with a K-selective ME were performed before, and four after the impalements with the Na-selective ME.

*Statistics*

Unless otherwise stated, results are expressed as means $\pm$ SE.

## Results

Figure 1 shows typical records of impalements with conventional and ion-selective microelectrodes. It also illustrates the stability of the intracellular records, the measurements of cell membrane voltage deflections produced by transepithelial current, and the return to the same stable base-line after impalement. Impalements with Cl and Na-selective electrodes resulted in monotonic changes of their voltage output; a steady-state value was reached within a few seconds. Measurements with K-selective ME resulted in the same kind of trace in only about 60% of the impalements. The remaining 40% showed records such as the one illustrated in the figure, i.e., a rapid positive deflection followed by a small decay, which either approached the steady state monotonically, or undershot it slightly. These two types of records were sometimes seen in different cells of a single preparation, with the same microelectrode. The explanation is not clear to us. Similar observations have been made by Spring and Kimura in measurements of $a\mathrm{K}_i$ in *Necturus* proximal tubule cells (*personal communication*).

Figure 2 illustrates the other criteria employed to validate the impalements. First, intracellular current passage through a conventional ME resulted in voltage changes in the ion-selective ME. Second, K-for-Na substitution in the mucosal solution resulted in nearly equal changes

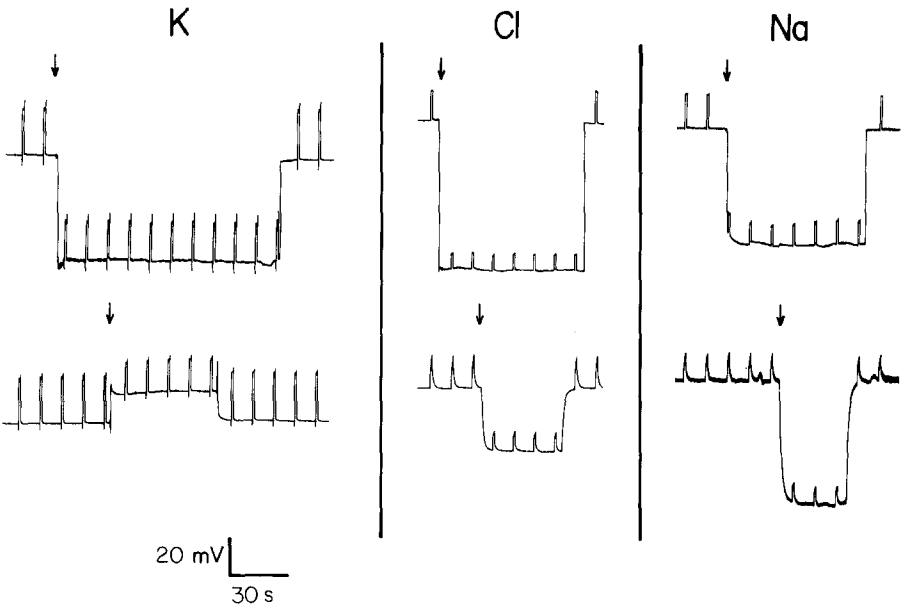


Fig. 1. Records of typical impalements, from the mucosal side, with conventional microelectrodes (upper records), and with ion-selective electrodes (lower records). Impalements are indicated by arrows. Each vertical pair was obtained from the same tissue, at about the same time. Negative potentials upon impalement are recorded downwards. The voltage deflections are the result of transepithelial current pulses. From their amplitude, the ratio of cell membrane resistances was calculated after correction for the voltage drops in the bathing media

of the potential measured with both intracellular microelectrodes. Both techniques proved to be useful criteria for impalement validation (*see Discussion*).

*Intracellular K activity:* Figure 3 (left) shows the results of all satisfactory impalements with a conventional and a K-selective ME in a typical tissue. In every impalement, in each of 16 preparations, the electrical potential recorded by the K-selective ME ($V^*$) was cell-positive, with values ranging from 5 to 35 mV. This indicates that $aK_i$ is higher than the equilibrium value. Both cell membrane potential and $V^*$ values were quite constant in a given tissue, even though sizable differences were observed from tissue to tissue (*see also* Frömter, 1972). Figure 4 shows frequency distributions of $V_{mc}$ and $V^*$ in six experiments. Both appear close to normal distributions, around means of 64 and 21 mV, respectively.

Data from 16 experiments are summarized in Table 1. The mean intracellular K activity determined from these measurements, 87.3 mM,

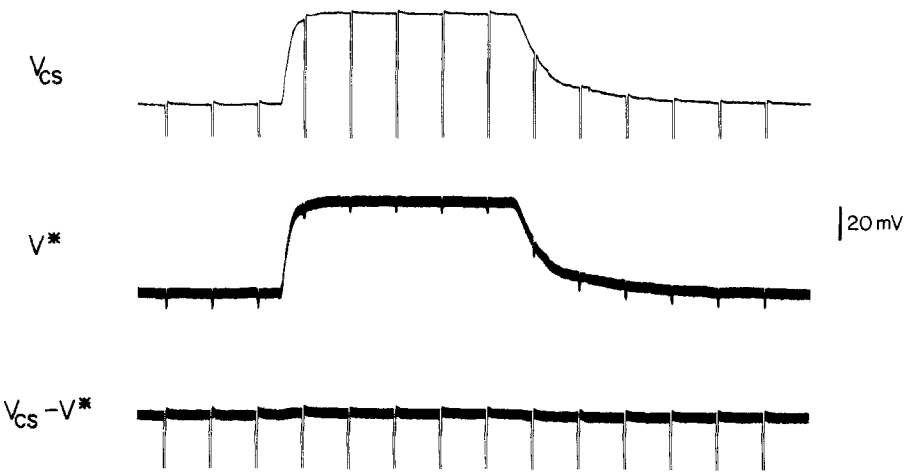


Fig. 2. Validation of impalements with a conventional microelectrode and a K-selective microelectrode. *Upper trace:* output of conventional microelectrode ($V_{cs}$); *middle trace:* output of K-selective microelectrode ($V^*$); *lower trace:* differential record ($V_{cs}$-$V^*$). Records start with microelectrodes in the cells. The interelectrode distance was about 80 μm. The voltage deflections were the result of current application ($7 \times 10^{-9}$ A, 10-sec interval) through the conventional ME (deflections off-scale in upper and lower traces). Deflections in $V^*$ record ($\Delta V_x^*$) indicate that the two cells are electrically coupled. After the third pulse, for ca. 50 sec, all NaCl in the mucosal medium was replaced with KCl. Both $V_{cs}$ and $V^*$ depolarized reversibly by ca. 52 and 51 mV, respectively. The differential trace changed by only 1 mV. $\Delta V_x^*$ decreased reversibly during exposure to high-K medium because of the fall of apical membrane resistance (*see* Reuss & Finn, 1975*a–b*)

can be compared with a mean intracellular K equilibrium activity of about 25 mM across the basolateral membrane and 24 mM across the luminal membrane. Inasmuch as the measured $aK_i$ is greater than the equilibrium activity, K is transported uphill towards the cell interior, across one or both cell membranes.

*Intracellular Cl activity:* Figure 3 (center) shows the results of all satisfactory impalements with a conventional and a Cl-selective ME in a typical experiment. In all cases, the Cl-selective ME became negative to both bathing solutions upon impalement, and $V^*$ was smaller than the cell membrane potential in that tissue. This result indicates that the Cl activity in the cell is lower than that in the bathing medium, but higher than the activity expected for equilibrium distribution. As illustrated in Fig. 5, the potentials measured with Cl-ME were distributed normally.

Data from 16 experiments are also summarized in Table 1. The mean $V_{Cl}$ was 24 mV, which corresponds to a mean intracellular Cl activity

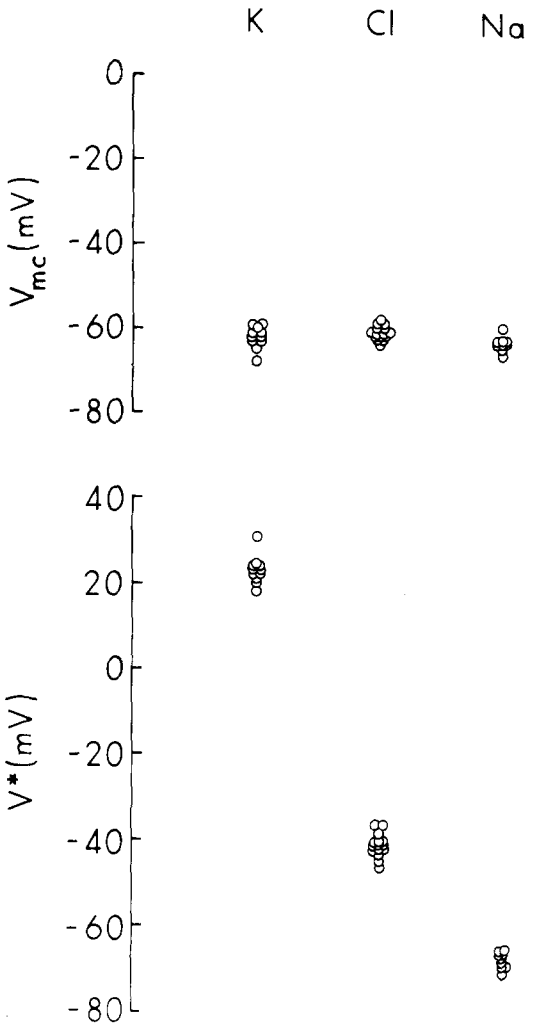


Fig. 3. Distribution of values of apical membrane potential ($V_{mc}$) and ion-selective electrode intracellular potential ($V^*$) in three representative experiments. All values for each column (K, Cl, Na) were obtained in one tissue. Note that upon impalement $V^*$ (K) is positive, $V^*$ (Cl) is negative, but less than $V_{mc}$, and $V^*$ (Na) is negative, and larger than $V_{mc}$. *See* text

of 34.7 mM. The mean calculated equilibrium activity was 8 mM. As is the case with K, intracellular Cl activity is higher than the equilibrium activity, indicating uphill transport into the cells.

*Intracellular Na activity:* The data for the Na impalements are presented in the same format as those for K and Cl, in Figs. 3 and 6, and in Table 1. As stated above, because of the low selectivity of

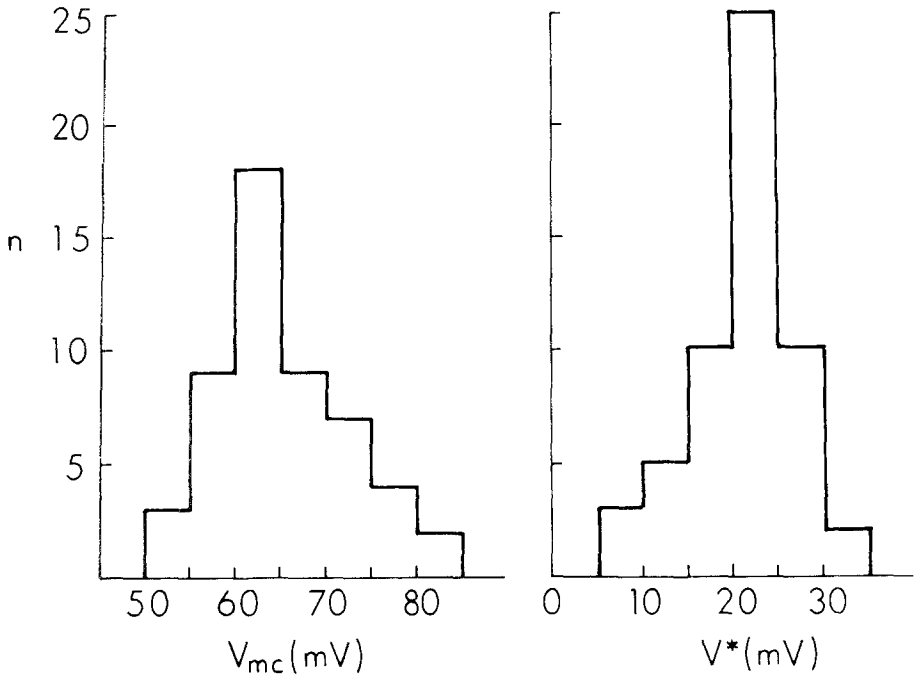


Fig. 4. Distribution of $V_{mc}$ (left) and $V^*$ (K) values (right). The results of 55 impalements in 6 tissues are shown. $V_{mc}$ values are cell negative to mucosal solution. $V^*$ values are cell positive to mucosal solution. *See* Fig. 3 and text

Table 1. Intracellular ionic activities

| Ion | $V_{ms}$ (mV) | $V_{mc}$ (mV) | $V_{cs}$ (mV) | $V^*$ (mV) | $V_i$ (mV) | $a_i$ (mM) | $E_i$ (mV) |
|---|---|---|---|---|---|---|---|
| K | $-0.8\pm0.1$ | $-64.1\pm1.4$ | $-64.9\pm1.4$ | $19.6\pm1.3$ | $83.7\pm1.3$ | $87.3\pm3.3$ | $-96.4$ |
| Cl | $-0.9\pm0.1$ | $-66.2\pm1.7$ | $-67.1\pm1.7$ | $-42.2\pm1.7$ | $24.0\pm1.7$ | $34.7\pm2.3$ | $-24.0$ |
| Na | $-0.7\pm0.2$ | $-64.1\pm1.4$ | $-64.9\pm1.5$ | $-70.0\pm0.9$ | $-5.9\pm0.7$ | $22.2\pm3.1$ | 33.4 |

$N$ (Number of tissues): 16 (K), 16 (Cl), 8 (Na). Results in each tissue were obtained from 6 to 20 impalements.

$V_{ms}$ = transepithelial potential (mucosa-serosa), $V_{mc}$: apical membrane potential (cell-mucosa), $V_{cs}$: basolateral membrane potential (cell-serosa), $V^*$: potential upon impalement with ion-selective electrode (cell-mucosa), $V_i = V^* - V_{mc}$; $a_i$: intracellular activity; $E_i$: equilibrium potential

the Na-selective resin, measurements of intracellular K activity were performed in the same tissues. This results in a large correction of the Na values and makes the absolute numbers less reliable.

In any event, the calculated intracellular Na activity (Table 1) is almost two orders of magnitude lower than the calculated equilibrium

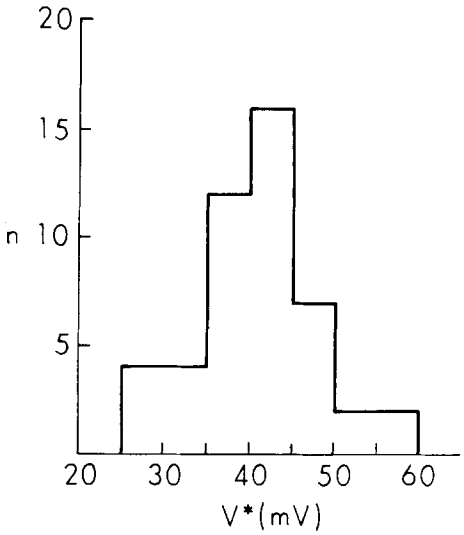


Fig. 5. Distribution of $V^*$ (Cl) values. The results of 47 impalements in 6 tissues are shown. $V^*$ values are cell negative to mucosal solution. *See* Fig. 3 and text

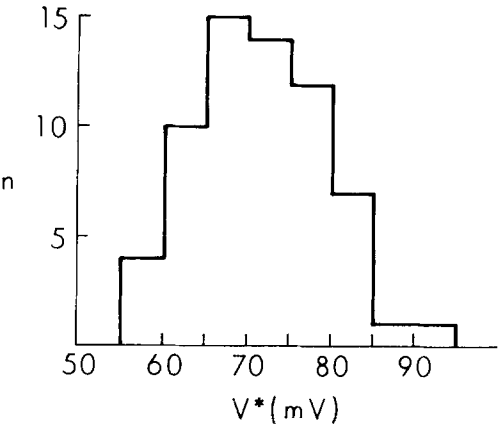


Fig. 6. Distribution of $V^*$ (Na) values. The results of 64 impalements in 8 tissues are shown. All values are cell negative to mucosal solution. *See* Fig. 3 and text

activity of about 1,100 mM, indicating that Na is extruded from the cells by an uphill (energy requiring) process.

## Discussion

The discussion of the data presented in *Results* will be restricted to the following issues: (i) Justification and validation of the techniques.

(ii) Comparison of these results with circuit analysis data published previously. (iii) Calculation of electrochemical potential differences across the two cell membranes. (iv) Mechanisms of Na and Cl transport at apical and basolateral membranes. (v) Comparison with results obtained by others in gallbladder and other leaky epithelia.

### *Justification and Validation of the Techniques*

Microelectrode studies of small cells are subject to uncertainty because of the possibility of significant cell damage (Lindemann, 1975; Higgins, Gebler & Frömter, 1977), changes in intracellular ionic composition upon impalement (DeLong & Civan, 1978; Nelson, Ehrenfeld & Lindemann, 1978), or uncontrolled changes in electrode tip potential upon penetration of the cell membrane. Since it is difficult to exclude completely the possibility of artifacts, we considered it essential to validate our measurements by a variety of criteria. A first consideration was to decide between the use of double-barrel electrodes (one barrel conventional, the other ion-selective) or single-barrel electrodes. We decided in favor of the latter, because *a priori* the possibility of damage is greater with double-barrel electrodes since, for a given barrel resistance, the tip of the double-barrel electrode is larger than that of the single-barrel electrode. In addition, impalements with conventional double-barrel microelectrodes yield, in our hands, less acceptable impalements than do single-barrel electrodes, as evidenced by lower membrane potentials and lower relative apical membrane resistance (*unpublished observations*). Finally, positioning of the two barrels in the same cell would rule out the possibility of employing the intercellular-coupling and solution-substitution criteria for validation of the impalement. Our results and those of Frömter (1972) indicate that the cell membrane potentials are acceptably constant from cell to cell in any *Necturus* gallbladder preparation, even though differences can be observed from tissue to tissue. The demonstration of unrestricted, radially symmetric coupling between the epithelial cells (Frömter, 1972; Reuss & Finn, 1975*a*) explains the constancy of the cell membrane potentials in a given tissue and, since the cell-to-cell pathway has a high electrical conductance, predicts similar ionic activities in all cells, even if their membrane properties differ. These observations justify the employment of multiple impalements to determine intracellular ion activities.

Our observations of rather constant cell membrane potentials in any

given preparation (*see* Fig. 3) do not agree with those of Zeuthen (1977) who found a rather large variation of cell membrane potential values. The reason for the discrepancy may be the employment of less strict impalement validation criteria in Zeuthen's experiments. For one thing, his observations do not appear compatible with the cable analysis experiments referred to above; this raises the possibility that cells with low potentials may have been uncoupled from the rest of the epithelium, perhaps because of impalement artifacts.

The impalement validation criteria, described in detail in *Methods*, are the strictest thus far employed in intracellular microelectrode work in epithelia. In particular, the demonstration of coupling of the two cells, and the requirement of essentially equal changes in cell potential upon ionic substitutions in the mucosal solution cannot be met with leaky impalements which result in: (i) electrical uncoupling of the impaled cells (probably because of an increase in intracellular Ca activity, *see* Loewenstein, Nakas & Socolar, 1967), and (ii) different cell membrane potential changes in two cells, upon a change in mucosal solution composition unless the two microelectrodes produce exactly the same damage upon impalement. These arguments do not rule out the possibility of some damage, more or less constant, in all impalements, which could result in artifactual values of cell potential and intracellular activity. Our main argument against this possibility is the demonstration of high ionic selectivity of the membrane of the impaled cell, as shown by the large change in cell potential produced by external K-for-Na substitutions (*see* Fig. 2, and *also* Reuss & Finn, 1975*a–b*, and Reuss, 1979*a*). The observation of no immediate change in intracellular K activity after exposure of the apical membrane to a high-K medium does not necessarily mean that $aK_i$ will remain constant at longer times. The substitution of K for Na will result in the following immediate alterations: decrease of apical membrane $E_K$, decrease of $V_{mc}$, increase of apical membrane $g$K and decrease of $V_{cs}$; eventually, the K activity in the serosal unstirred layer will rise because of transcellular and/or paracellular K flux. All of these effects will influence $aK_i$. A detailed analysis of this situation is beyond the scope of this paper.

As shown in Figs. 4–6, the potentials measured with K, Cl, and Na-selective microelectrodes were approximately normally distributed around the respective means, even though the values varied somewhat from tissue to tissue. Impalements with ion selective electrodes which did not meet the criteria described above gave consistently smaller $V^*$ values. These observations support our conclusions of: first, a homogen-

eous cell population, and second, adequate criteria for the validation of the impalements.

In addition to impalement artifacts, errors in the measurements of intracellular ionic activity could result from lack of selectivity of the electrodes. This possibility admittedly makes the measurements of Na activity, which required a large correction for intracellular K, less reliable. As shown in Table 1, the fractional standard error of the mean (SEM/$x$) was almost four times larger in the Na than in the K measurements. With the bathing solution employed in these experiments, the selectivity-dependent errors in the measurement of K are small, because of the high K/Na selectivity of the K electrode. The Cl electrodes respond to other intracellular anions, as shown by Spring and Kimura (1978) who measured an "intracellular Cl activity" of about 6 mM after prolonged perfusion of *Necturus* kidney with Cl-free Ringer, at a time at which no Cl could be detected chemically in the tissue. In our experience, however, apparent $a\mathrm{Cl}_i$ can fall to the equilibrium level when Na is removed from the mucosal bathing medium, and Cl entry is therefore reduced, suggesting that the response of the electrode to other intracellular anions is very small (*unpublished observations*).

### *Comparison of the Results with Previous Estimations of Permeability Coefficients of the Cell Membranes*

We have recently provided evidence against the possibility of a Na pump with a large electrogenic component at the basolateral membrane of this tissue (Reuss *et al.*, 1979). If in fact there are no electrogenic pumps in the system, it is possible to estimate the equivalent electromotive forces of the two cell membranes from the intra and extracellular ionic activities and the relative permeabilities of the main ions across each membrane, according to the Goldman-Hodgkin-Katz constant field equation:

$$E_m = -\frac{RT}{zF} \ln \frac{K_i + P' \mathrm{Cl}_o + P'' \mathrm{Na}_i}{\mathrm{K}_o + P' \mathrm{Cl}_i + P'' \mathrm{Na}_o} \tag{2}$$

where $E_m$ is the equivalent emf of the membrane, $P'$ and $P''$ are $P_{\mathrm{Cl}}/P_{\mathrm{K}}$ and $P_{\mathrm{Na}}/P_{\mathrm{K}}$, respectively, the subscripts $i$ and $o$ refer to intra and extracellular activities, respectively, and $R, T, z$, and $F$ have their usual meanings.

The data for apical and basolateral membrane are shown in Table 2. The values of $E_a$ and $E_b$ were calculated from Eq. (2) using the

Table 2. Cell membrane equivalent electromotive forces

| | $E_a$ (mV) | $E_b$ (mV) |
|---|---|---|
| Goldman equation[a] | 47.2 | 90.2 |
| Circuit analysis[b] | 42.4 to 51.0 | 74.9 to 87.3 |

[a] Calculated from $P_{Cl}/P_K$, $P_{Na}/P_K$ (apical membrane, Reuss & Finn, 1975*a*; basolateral membrane: Reuss, 1979*a*) and activity data in Table 1

[b] Range of values reported by Reuss (1978, 1979*a*) and Reuss *et al.* (1979).

Table 3. Electrochemical potential differences across cell membranes

| Membrane | $\Delta\mu_K/F$ (mV) | $\Delta\mu_{Cl}/F$ (mV) | $\Delta\mu_{Na}/F$ (mV) |
|---|---|---|---|
| Apical | 32.3 | −42.2 | −97.5 |
| Basolateral | 31.5 | −43.1 | −98.3 |

mean ionic activities reported above, and the permeability ratios previously published (Reuss & Finn, 1975*b*; Reuss, 1979*a*). These values are compared with those estimated by circuit analysis from measurements of potentials and resistances. The agreement of the values obtained by these two independent methods is remarkable, and provides circumstantial support for the validity of the calculation of permeability coefficients from electrophysiological data.

### *Electrochemical Potential Differences across the Membranes*

Table 3 summarizes the electrochemical potential differences ($\Delta\mu_i/F$) for Na, K, and Cl across the apical and the basolateral membrane. The values were calculated from the mean data shown in the preceding tables, according to

$$\Delta\mu_i/F = V_m - E_i \tag{3}$$

where $E_i$ is the equilibrium potential for the $i$th ion $\left(E_i = \frac{RT}{zF}\ln\frac{a_i}{a_o}\right.$, where $a_i$ and $a_o$ are the intracellular and extracellular activities$\left.\right)$, and $V_m$ is the cell membrane potential ($V_{mc}$ or $V_{cs}$).

These results indicate that under these experimental conditions Na is at a lower electrochemical potential in the cell; therefore, its transport

out of the cell is uphill. Cl and K, on the contrary, are at higher electrochemical potentials in the cell; thus, they are transported uphill toward the cell interior. Evidence to be summarized below indicates that K transport into the cell depends, at least in part, on a "classic" Na-K pump located at the basolateral membrane. Inasmuch as the apical membrane has a high K permeability (Reuss & Finn, 1975*a–b*; Van Os & Slegers, 1975), the high intracellular K activity should result in net serosa-to-mucosa transcellular K transport (basolateral uphill uptake, apical downhill diffusion). However, a large net K flux into the mucosal bathing solution could be prevented by the high K permeability of the paracellular pathway (Reuss & Finn, 1975*a–b*; Van Os & Slegers, 1975). This mechanism would explain the observation of "passive" K distribution across the rabbit gallbladder (Dietschy & Moore, 1964). Na-K ATPase has been identified at the basolateral, but not at the apical, membrane of frog gallbladder (Mills & DiBona, 1978). However, the possibility of active K uptake at the luminal membrane cannot be ruled out at present.

### *Mechanism of Na and Cl Transport at the Apical Membrane*

Transcellular NaCl transport from mucosa to serosa involves an entry step at the luminal membrane. Na entry at this site is *downhill*, driven by an electrochemical potential difference of $\sim$98 mV, whereas Cl entry is *uphill*, against an electrochemical potential difference of $\sim$42 mV. We have shown that the Na conductance of the apical membrane is very low and thus diffusion at this site accounts for only about 6% of the net transepithelial Na flux (Reuss & Finn, 1975*b*). In addition, in rabbit gallbladder Cl entry into the cells requires the presence of Na in the mucosal bathing medium (Frizzell, Dugas, & Schultz, 1975). These results are consistent with a coupled mechanism of NaCl entry, as proposed for small intestine (Nellans *et al.*, 1973) and *Necturus* proximal tubule (Spring & Kimura, 1978). The fact that the electrochemical gradient favoring Na entry is larger than the electrochemical gradient opposing Cl entry suggests that Na influx can drive Cl influx without the necessity of a metabolic energy supply (*see also* Duffey *et al.*, 1978).

### *Mechanism of Na and Cl Transport at the Basolateral Membrane*

Na transport from the cells to the serosal bathing medium is *uphill*, against an electrochemical potential difference of $\sim$98 mV. Its depen-

dence on the presence of K in the serosal medium (Diamond, 1968; Rose, 1978), the rapid inhibition of fluid transport by ouabain (Reuss *et al.*, 1979), the demonstration by Van Os and Slegers (1971) of a direct correlation between fluid transport rate and levels of Na, K activated ATPase in rabbit gallbladder, and the demonstration of the basolateral location of the Na-K ATPase in frog gallbladder (Mills & DiBona, 1978) suggest that a "classic" Na-K pump is involved in Na transport across the basolateral membrane.

Cl transport across the basolateral membrane is downhill, driven by an electrochemical potential of about 43 mV. This indicates that Cl transport can be, at least in part, diffusional. However, the Cl conductance of the basolateral membrane of this epithelium is very low (Reuss, 1979*a*). Calculations show that only about 3% of the net transepithelial Cl flux can be accounted for by diffusion across the basolateral membrane. Since paracellular Cl flux does not seem to contribute more than about 8% of the measured rate of transport, a coupled mechanism of Cl extrusion has been proposed (Reuss, 1979*a*). This could consist of a neutral NaCl pump, downhill extrusion of KCl, or a Cl-$HCO_3$ exchange mechanism.

### *Comparison with Other Results in Gallbladder and Other Leaky Epithelia*

The results of this study are in good agreement with those obtained in gallbladder by other investigators. Our values of intracellular Cl activity are close to those reported by Duffey *et al.* (1978) in rabbit gallbladder. Intracellular K and Na activities are similar to those reported by Zeuthen (1978) in *Necturus* gallbladder when the microelectrode was positioned near the basolateral membrane. Zeuthen (1977, 1978) found intracellular gradients of electrical potential and ionic activities, but our observations, with high resistance electrodes and proper support of the tissue do not show such gradients (*see also* Suzuki & Fromter, 1977, and Reuss *et al.*, 1979). The estimated Na and Cl electrochemical potential differences across the luminal membrane are also similar to those in *Necturus* proximal tubule (Spring & Kimura, 1978, and *personal communication*) and small intestine (Armstrong *et al.*, 1979). The hypothesis of a neutral mechanism of NaCl entry driven by the Na electrochemical potential difference is supported by both tracer and electrophysiological experimental results in small intestine (Nellans, *et al.*, 1973; Armstrong *et al.*,

1979), proximal tubule (Spring & Kimura, 1978, 1979) and gallbladder (Cremaschi & Hénin, 1975; Frizzell *et al.*, 1975; Reuss & Finn, 1975*b*; Van Os & Slegers, 1975).

Less information is available on the mechanism of salt transport at the basolateral membrane. Rose (1978) has raised the possibility of an electrogenic Na pump. The results of Cremaschi *et al.* (1977) with amphotericin B in rabbit gallbladder and our results with both amphotericin B (Reuss, 1978) and ouabain (Reuss *et al.*, 1979) in *Necturus* do not support this possibility. Cl extrusion at the basolateral membrane, although downhill, cannot be explained solely by simple diffusion because of the low Cl permeability of the membrane both in *Necturus* (Reuss, 1979*a*) and rabbit gallbladder (Cremaschi & Hénin, 1975).

In summary, we have shown that the steady-state intracellular ion activities in *Necturus* gallbladder epithelial cells are such that transport of Na out of the cells and transport of both K and Cl into the cells are uphill processes. Consideration of these data in conjunction with membrane conductances for all three ions suggest strongly that apical NaCl uptake is a neutral, downhill process, driven by the Na electrochemical potential difference, and that basolateral salt transport includes the operation of a Na-K pump in parallel with a nondiffusional Cl transport mechanism, i.e., NaCl pump, KCl downhill extrusion, or anion exchange mechanism.

We thank Dr. K.R. Spring for the generous gift of the cation exchanger and for advice on the use of ion-selective electrodes, and Dr. M.P. Blaustein for his comments on a preliminary version of this manuscript. We are also grateful for the assistance of T.P. Grady and the secretarial help of J. Jones. This work was supported by Grant No. AM-19580 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

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