J. Membrane Biol. 68, 215–225 (1982)

The Journal of
Membrane Biology

# Steady States and the Effects of Ouabain in the *Necturus* Gallbladder Epithelium: A Model Analysis

Hoegni J. Baerentsen, Ove Christensen, Per Grove Thomsen,* and Thomas Zeuthen

Institute of Medical Physiology A, The Panum Institute, Blegdamsvey 3c, DK-2200 Copenhagen N, Denmark

**Summary.** A simple numerical model for the *Necturus* gallbladder epithelium is presented. $K^+$, $Na^+$ and $Cl^-$ cross the mucosal and serosal membranes as well as the junctions by means of electrodiffusion; furthermore the mucosal membrane contains a neutral entry mechanism for NaCl and the serosal membrane contains an active pump for $K^+$ and $Na^+$. The values which have been used for the model are taken from the literature. The model can only attain steady states if the resistance of the serosal membrane is lower than $1000\,\Omega\,cm^2$. Values reported in the literature for the resistance of this membrane vary from about 3000 to about $100\,\Omega\,cm^2$. We shall argue, however, that the higher estimates are in error because they are derived from a model of the tissue in which each membrane and the junction are modeled by a resistor; this procedure is invalid because the resistance of the lateral intercellular space relative to the resistance of the tight junctions is neglected and consequently the resistance of the serosal membrane is overestimated by a factor of about four. Apart from predicting a realistic steady state at normal external concentrations the model can predict quantitatively several experimental results obtained from the living epithelium. We have focused on the experiments which test the permeabilities of the serosal membrane and the properties of the pump: *i)* Replacement of serosal $Cl^-$ by an impermeant ion. *ii)* Replacement of serosal $K^+$ by $Na^+$. *iii)* Inhibiting the $(Na^+, K^+)$-pump. The best correspondence between model and experiments is obtained when the pump is assumed to be electrogenic (or rheogenic) with a ratio of coupling between $Na^+$ and $K^+$ of 3:2. In this case both model and direct experiments (also presented in this paper) show an initial abrupt depolarization of 6 to 7 mV. The model also shows that it cannot be concluded from *i* and *ii* that the $Cl^-$ permeability of the serosal membrane is low. The model explains, even with high passive $Cl^-$ permeabilities, why the intracellular $Cl^-$ concentration is relatively unaffected by paracellular currents, a fact which in other epithelia has been taken as an implication of a low $Cl^-$ permeability of the serosal membranes.

**Key words** gallbladder · numerical model · effects of ouabain · steady states · effects of transmural currents

## Introduction

Ions transported by an epithelium encounter at least three barriers; the mucosal membrane, the serosal membrane and the tight junctions. As the transport can be either passive or active at these membranes, roughly 20 parameters are needed to describe the transport of $Na^+$, $K^+$ and $Cl^-$ even when a simple model is used.

Ion-selective microelectrodes have provided detailed information about the distribution of ions, especially $Na^+$, $K^+$ and $Cl^-$, in epithelial tissues. But the models by which the data have been interpreted have been too simple and have not contained the various mechanisms that are known for ion permeation. The three-resistor model, for example, in which the mucosal, the serosal and the paracellular pathway each are equated by a resistor and battery, has been useful in conjunction with the data obtained by single-barrelled electrodes. But it is too simple to predict the values that can be obtained with ion-selective microelectrodes (Zeuthen, 1981*a*, *b*). The purpose of this paper was therefore to develop a computer model which could describe the movements of $Na^+$, $K^+$ and $Cl^-$ in a leaky epithelium in steady states as well as nonsteady states. We have chosen the *Necturus* gallbladder because this epithelium is well studied and many parameters for the ion-permeations are known. We have used the model of Koefoed-Johnsen and Ussing (1958) in which each ion is allowed to permeate electrodiffusively across the membranes in such a way that the serosal membrane is predominantly $K^+$-permeable; this has recently been confirmed to apply to the choroid plexus (Zeuthen & Wright, 1981). Furthermore, $Na^+$ is extruded actively at the serosal membrane in exchange for $K^+$, and a neutral NaCl cotransport is included at the mucosal membrane. The paper shows that such a model simulates the living tissue in many of the experiments which have been performed on the epithelium so far.

A general model for epithelia has been presented (Lew et al., 1979), but the mathematical treatment

* Institute of Numerical Analysis, The Technical University of Denmark

0022-2631/82/0068-0215 $02.20

used by us is more versatile. Weinstein and Stephenson (1979) have also discussed a model of the *Necturus* gallbladder epithelium, but their aim was different from ours.

A preliminary report has been presented (Baerentsen & Zeuthen, 1981).

## List of Symbols

| | |
|---|---|
| $m, c, s$ | Index: mucosal, cellular and serosal compartment, respectively |
| $V$ | Cellular volume: $cm^3\,cm^{-2}$ |
| $Q_i$ | Amount of substance of the ion $i$: $mol\,cm^{-2}$ |
| $X^-$ | Amount of intracellular fixed anions: $mol\,cm^{-2}$ |
| $z_x$ | Average charge valency of intracellular anions |
| $C_i^a$ | Concentration of the ion $i$ in the $a$ compartment: mM |
| $z_i$ | Charge valency of the ion $i$ |
| $M_a$ | Concentration of extracellular passive ions: mM |
| $\Phi_V^{ab}$ | Volume flow through the membrane separating compartment $a$ from compartment $b$: $cm\,sec^{-1}$ |
| $\Phi_i^{ab}$ | Flow of the ion $i$ through the $i$ through the $ab$ membrane: $mol\,cm^{-2}\,sec^{-1}$, given by the Goldman equation |
| $I_i^{ab}$ | Current carried by the ion $i$ through the $ab$ membrane: $\mu A\,cm^2$ |
| $I_T$ | Total current through the epithelium: $\mu A\,cm^{-2}$ |
| $E^{ab}$ | Voltage across the $ab$ membrane with respect to the $a$ compartment: mV |
| $J_i^{ab}$ | Electrodiffusional flux of the ion $i$: $mol\,cm^{-2}\,sec^{-1}$ |
| $P_i^{ab}$ | Passive permeability of the $ab$ membrane to the ion $i$: $cm\,sec^{-1}$ |
| $F_{Na}^{cs}, F_K^{cs}$ | Ion fluxes of Na and K carried by the Na/K-pump: $pmol\,cm^{-2}\,sec^{-1}$, i.e. $F_{Na}^{cs} = F_{Na}^{max}\left[\frac{Na^c}{Na^c + K_{M,Na}}\right]^{2r}\left[\frac{K^s}{K^2 + K_{M,K}}\right]^2$ |
| $F_{Na}^{max}, F_K^{max}$ | Saturation fluxes of the Na/K pump: $pmol\,cm^{-2}\,sec^{-1}$ |
| $K_{M,Na}, K_{M,K}$ | Affinity constants with respect to intracellular Na and extracellular K: mM |
| $r$ | Coupling ratio of the Na/K pump |
| $T_{Na}^{mc}, T_{Cl}^{mc}$ | Coupled NaCl entry fluxes: $pmol\,cm^{-2}\,sec^{-1}$ assumed equal to $K_{M,NaCl}\cdot(Na^m Cl^m - Na^c Cl^c)$ |
| $K_{M,NaCl}$ | Proportionality constant: $cm^4\,mol^{-1}\,sec^{-1}$ |
| $RT/F$ | ca. 26 mV at room temperature |
| $R_{LIS}$ | Resistance of the lateral intercellular spaces ($\Omega\,cm^2$) |
| $R_{TJ}$ | Resistance of the tight junctions ($\Omega\,cm^2$) |
| $R_{ab}$ | Resistance of the membrane $ab$ ($\Omega\,cm^2$) |
| $L_p^{ab}$ | Hydraulic permeability of the membrane $ab$ ($cm\,sec^{-1}\,osm^{-1}$) |

## Materials and Methods

### *Model Description and Mathematical Methods*

The following analysis is based upon the Koefoed-Johnsen and Ussing model (1958) of the epithelial tissue. The approach is closely related to the one used by Lew, Ferreira and Mauro (1979), but it differs mathematically in that the equations are solved as a system of equations, whereas Lew et al. (1979) worked out implicit expressions for the various entities.

The tissue is thought to separate two infinte compartments, the mucosal compartment and the serosal compartment (in the following indexed $m$ and $s$). The epithelial tissue is composed of a single layer of identical cells, the internal compartment of which will be indexed $c$. The membranes separating the compartments are double-indexed according to the compartments they separate, i.e. the luminal membrane is indexed $mc$, the basolateral membrane $cs$ and the junctions $ms$.

There are two different pathways for permeation across the epithelial wall, paracellular and transcellular. The solutions in all compartments are assumed perfectly stirred and uniform. The only ions we consider are $Na^+$, $K^+$ and $Cl^-$.

In the model of the epithelial membrane six dependent variables are assumed to be sufficient to describe the state of the tissue at any time subject to a given state of the surrounding medium. The dependent variables are: *i)* $V$, the volume of the cell; *ii–iii)* $E^{mc}$ and $E^{cs}$, the potential difference across the luminal membrane with respect to the mucosal solution, and the potential difference across the basolateral membrane with respect to the intracellular solution; and *iv–vi)* $Q_{Na}$, $Q_K$ and $Q_{Cl}$, the intracellular amounts of the three ions $Na^+$, $K^+$ and $Cl^-$.

With six dependent variables it is necessary to set up six equations:

$$0 = (Q_{Na} + Q_K - Q_{Cl} - z_x X^-)/V \tag{1}$$

$$\frac{dV}{dt} = \Phi_v^{mc} - \Phi_v^{cs} \tag{2}$$

$$\frac{dQ_{Na}}{dt} = \Sigma\Phi_{Na}^{mc} - \Sigma\Phi_{Na}^{cs} \tag{3}$$

$$\frac{dQ_K}{dt} = \Sigma\Phi_K^{mc} - \Sigma\Phi_K^{cs} \tag{4}$$

$$\frac{dQ_{Cl}}{dt} = \Sigma\Phi_{Cl}^{mc} - \Sigma\Phi_{Cl}^{cs} \tag{5}$$

$$0 = E^{mc} + E^{cs} - E^{ms} \tag{6a}$$

$$0 = \Sigma_i I_i^{mc} + \Sigma_i I_i^{ms} - I_T \tag{6b}$$

where Eq. (1) is the requirement of instantaneous intracellular electroneutrality, and Eqs. (2) through (5) are the relations of mass balance, where the rate of change of the cell volume and the intracellular amounts of substance are equated to the difference of the specific flows across the luminal and the basolateral membrane. Equations (6a) and (6b) are mutually exclusive and describe the voltage-clamp condition (clamp voltage $E^{ms}$) and the current-clamp condition (clamp current $I_T$). Symbols appearing in the system of equations are: $X^-$, the nondiffusable intracellular anions with average charge valency $z_x$; $\Phi_v$, the flow of volume; $\Sigma\Phi_{Na}$, $\Sigma\Phi_K$, $\Sigma\Phi_{Cl}$, the total flow of ions, and $\Sigma I_i$ the total current carried by the ion of type $i$.

The system of equations has been solved on the computer at the Regional Computing Center at the University of Copenhagen (RECKU). The steady-state solution was obtained by using a modified version of the Gauss-Newton method for unconstrained minimization, and the transient response of the system to a predefined perturbation was computed using finite difference approximation of finite order to the time derivatives.

### *Microelectrode Measurements*

To measure the abrupt depolarization caused by the direct application of ouabain, the connective tissue was completely removed by the method described previously (Zeuthen 1982). The mesothelium was removed, and a hydrostatic pressure of 8–10 $cm^2$

**Table 1.** Parameters used to calculate steady state of model cell

| | Permeabilities ($10^{-6}$ cm sec$^{-1}$) | | | Resistances ($\Omega$ cm$^2$) | $Na^+/K^+$ pump and NaCl entry | Fixed anions |
|---|---|---|---|---|---|---|
| | Na | K | Cl | | | |
| Mucosal: | 0.1 | 1.1 | 0.7 | 3811 | $F_{Na}^{max}$: 1200 pmol cm$^{-2}$ sec$^{-1}$ $K_{M,Na}$: 7.0 mM | $X^-$: 55.0 μmol cm$^{-2}$ |
| Junction: | 10.8 | 21.2 | 2.0 | 180 | $F_K^{max}$: 800 pmol cm$^{-2}$ sec$^{-1}$ $K_{M,K}$: 0.3 mM | $z_x$: −2.0 |
| Serosal: | 0.0 | 10.0 | 5.0 | 618 | $K_{M,NaCl}$: 0.028 cm$^4$ mol$^{-1}$ μsec$^{-1}$ | |

$H_2O$ was applied from the serosal side. This lifted the epithelium from the connective tissue over areas of a diameter equivalent to 100–200 cells. Thus the connective tissue could be removed by dissection from this area without damaging the epithelium. The tips of single- or double-barrelled electrodes were positioned intracellularly under microscopic observation. When a stable membrane potential was recorded the superfusion solution was changed within 1–3 sec to one containing $10^{-3}$ M ouabain, and the subsequent abrupt depolarization was recorded (Fig. 8).

## Choice of Measurable Variables

*Ion concentrations.* $Na^c$, the electrochemically free $Na^+$ concentration in the cell is recorded in the range of 8 to 12 mM by means of liquid-ion exchanger electrodes (Reuss & Weinman, 1979; Garzia-Diaz & Armstrong, 1980; Zeuthen, 1981*c*). Glass microelectrodes give a higher estimate 25–40 mM (Zeuthen, 1978; Graf & Giebisch, 1979). We shall choose the smaller estimate because the glass electrodes via the dead space may introduce $Na^+$ into the cell, and because their tips due to their larger size may cause damage to the cell membrane. $K^c$ has been determined in a number of studies (Zeuthen, 1978; Reuss & Weinman, 1979; Garzia-Diaz & Armstrong, 1980; Reuss, Weinman & Grady, 1980). There seems to be general agreement of values in the range 110–140 mM. $Cl^c$ has been determined to be in the range of 25–60 mM (Zeuthen, 1978; Reuss & Grady, 1979; Reuss & Weiman, 1979; Garzia-Diaz & Armstrong, 1980). We shall choose the smaller values because these seem to be obtained with electrodes with the finest tips. $X^-$, the amount of intracellularly fixed negative ions of valency $z_X$ will be given by the requirement of electroneutrality. We have arbitrarily chosen $z_X = -2$.

*The intracellular potential,* $E^c$, has been found to be in the range −50 to −80 mV (for references *see above*). $E^{ms}$, the transepithelial potential difference, has been found to be of the order of 1 to 2 mV serosa positive (Frömter, 1972; Reuss, 1979; Rose & Nahrwold, 1980; Rose, 1981).

*The transepithelial transport* of isotonic saline is at normal osmolarities (about 225 mOsm) of the order 3–25 μl cm$^{-2}$ hr$^{-1}$ (Hill & Hill, 1978; Zeuthen, 1978, 1981*d*; Reuss, Bello-Reuss & Grady, 1979), which corresponds to a NaCl transport of about 400 pmol cm$^{-2}$ sec$^{-1}$.

## Choice of Derived Parameters

The assessments of permeabilities and resistance of the various pathways are ambiguous. Several authors have performed experiments with ionic substitutions in the bathing solutions and have arrived at permeability and resistance values based on an equivalent network model of the epithelia featuring only resistances and equivalent electromotive forces at each of the three cell interfaces. While this approach might be reasonable in determining the membrane properties of the luminal and the shunt pathway, the topology of the basolateral membrane indicates that the method is too simple to give a true picture of the properties of this membrane (Zeuthen, 1976, 1981*a*, *b*; Boulpaep & Sackin, 1980; *see also* Discussion).

*The Luminal Membrane.* Reuss and Finn (1975*b*) and Graf and Giebisch (1979) have deduced the sequence of permselectivity of the luminal membrane as $P_K^{mc} > P_{Cl}^{mc} > P_{Na}^{mc}$. If the relative transference number ratio $t_{Na}:t_K:t_{Cl}$ is known, it is possible to obtain the relative magnitude of the permeabilities from the relationship $P_i^{mc} = k \cdot t_i / \langle C_i \rangle$ where the index $i$ refers to the ion type, $P_i^{mc}$ is the permeability, $t_i$ is the relative transference number, $\langle C_i \rangle$ is the mean concentration in the membrane, and $k$ is a proportionality constant. The underlying condition is that no electrogenic ion transport takes place in the membrane. Reuss and Finn (1975) have obtained the relative transference number ratio $t_{Na}:t_K:t_{Cl} = 0.05:0.54:0.41$ which compares well with the ratio 0.06:0.49:0.45 estimated by Graf and Giebisch (1979). This yields a relative permeability ratio of $P_{Na}^{mc}:P_K^{mc}:P_{Cl}^{mc} = 0.06:0.62:0.32$ for the luminal membrane, assuming the mean concentrations in the membrane are $\langle Na \rangle = 70$ mM, $\langle K \rangle = 70$ mM and $\langle Cl \rangle = 102$ mM.

Values of the resistance $R_{mc}$ of the luminal membrane range from 1.0 to 4.7 kΩ cm$^2$ (Frömter, 1972; Reuss & Finn, 1977; Graf & Giebisch, 1979). By varying the total permeability and keeping the ratio between the individual permeabilities constant, it is possible to obtain a resistance of the luminal membrane comparable with reported values (*see* Table 1).

*The Shunt Pathway.* Graf and Giebisch (1979) have obtained the transference number ratio $t_{Na}:t_K:t_{Cl} = 0.80:0.05:0.15$ of the tight junctions. If we use the same approach as above and $i$, we assume that the solutions at both membrane interfaces are identical, then a relative permeability ratio $P_{Na}^{ms}:P_K^{ms}:P_{Cl}^{ms} = 0.31:0.63:0.06$ is obtained. The total resistance of the tight junctions plus the lateral intercellular spaces are reported to lie in the range 200 to 450 Ω cm$^2$ (e.g. Frömter, 1972; Reuss & Finn, 1977). Recently, Simon, Curci, Gebler and Frömter (1981) have measured directly with microelectrodes in the lateral intercellular spaces and found that the resistance of the junctions themselves is of the same order as that of the lateral spaces. We have therefore chosen the resistance of the junctions to be 180 Ω cm$^2$.

*The Basolateral Membrane.* The properties of the basolateral membrane have not been investigated directly. This is due to *a*) the inaccessibility of the membrane that faces the connective tissue and to *b*) the convoluted character of the membrane. The resistance of the basolateral membrane has been estimated from the voltage-divider ratio $\alpha$ (Frömter, 1972; Reuss & Finn, 1977; Reuss, 1979). Whether this is correct depends on the resistance and length-constant of the lateral spaces (Zeuthen, 1976, 1981*a*, *b*; *see also* Discussion). The resistance thus determined is in the range 1270 to 4000 Ω cm$^2$ (e.g., Frömter, 1972; Reuss & Finn,

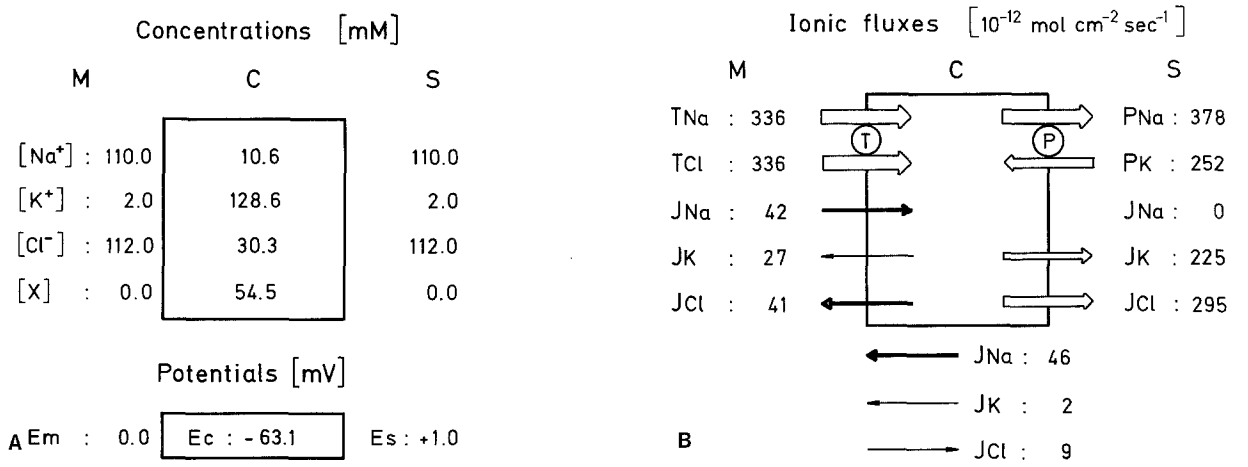


**Fig. 1.** Steady state of the model cell. *A* shows the concentrations and potentials in the mucosal, cellular and serosal compartment. *B* shows the ion fluxes across the membranes. Single arrows (*J*) indicate electrodiffusive fluxes, *T* indicate the neutrally coupled cotransport of $Na^+$ and $Cl^-$, *P* indicate the Na and K pumped by the (Na, K)-ATPase. At the lower part the junctional fluxes are shown. (Table 1 contains the parameters used to calculate the steady state.)

1975*a*, 1977)[1]. With this resistance it was not possible to obtain any steady state with our model.

We have therefore assumed that the true resistance of the serosal membrane is low enough to allow sufficient $Cl^-$ and $K^+$ ions to leave the cell by electrodiffusion when the influx is mediated by the neutral NaCl influx and the $Na^+/K^+$ pump. This seems to be the case for $K^+$ in the epithelium of the choroid plexus (Zeuthen & Wright, 1981). With a resistance of about $600\,\Omega\,cm^2$ and a ratio $P_{Cl}^{cs}/P_K^{cs}=0.5$ a steady state was possible (Table 1 and Fig. 1). It will be argued later that these choices of permeabilities and resistance do not conflict with experimental results.

### *Parameters for Active and Passive Carriers*

Relatively little is known about the values of the parameters of the pump. Zeuthen and Wright (1978, 1981) and Saito and Wright (1981) have suggested that $r=1.5$ in the choroid plexus. We shall show (p. 222) that this also applies to the *Necturus* gallbladder epithelium. We know from the work of Graf and Giebisch (1979) that the carrier is unsaturated at normal intracellular concentrations wherefore $F_{Na}^{max}$ can be assumed to be relatively high. $K_{M,Na}$ and $K_{M,K}$ are in the choroid plexus 7–20 mM and 1 mM (Saito & Wright, 1981). We shall use the parameters for the choroid plexus and an $F_{Na}^{max}$ of 1200 and an $F_K^{max}$ of $800\,pmol\,cm^{-2}\,sec^{-1}$. It turns out, however, that the steady-states are relatively insensitive to the choice of these parameters.

The contribution of the neutral Na entry flux $T_{Na}^{mc}$ (*see* list of symbols) to the total Na flux across the luminal membrane has been estimated to 85% by Graf and Giebisch (1979), to 36% by van Os and Slegers (1975) and to more than 50% by Reuss and Finn (1975*b*). Despite the quantitative differences it is agreed that the luminal electrodiffusive Na permeability is too small to permit Na entry in amounts comparable to the net $Na^+$ transport across the tissue.

We have chosen to let about 86% of the $Na^+$ influx across the mucosal membrane be carried neutrally with $Cl^-$. This means that the constant $K_{M,NaCl}$ (Lew et al., 1979) in the neutral influx (*see* list of symbols) is of the order $0.028\,cm^4\,mol^{-1}\,sec^{-1}$.

### *Parameters for $H_2O$ Transport*

The model is not primarily designed to give a realistic view of $H_2O$ transport. We have allowed the cell compartment to be in osmotic equilibrium with the serosal and mucosal compartments.

## Results

### *The Resistance of the Serosal Membrane*

The model cell could not attain steady states if the resistance of the serosal membrane was higher than typically $1000\,\Omega\,cm^2$. In that case neither the electrodiffuse effluxes of $K^+$ and $Cl^-$ were large enough to compensate for the mediated influxes; consequently the cell would swell if there was no other means for $Cl^-$ exit, i.e. a neutral cotransport. If the resistance was lower than $1000\,\Omega\,cm^2$ the influxes could be matched by electrodiffusive effluxes alone.

The exact value of limiting resistance will, of course, depend on the values assigned to the influxes of $K^+$ and $Cl^-$. In the following we shall tentatively assume that the resistance is low enough to sustain electrodiffusive effluxes and test whether such a choice is compatible with experimental facts.

A possible steady state is given in Fig. 1 and Table 1. It was constructed by making an informed

[1] Recently, Frömter et al. (1981) have presented a new set of data for the resistances of the various pathways of the *Necturus* gallbladder epithelium. The data are based on the three-resistor model and are approx. $R_{LIS}+R_{TJ}=82\,\Omega\,cm^2$, $R_{mc}=1090\,\Omega\,cm^2$, $R_{cs}=164\,\Omega\,cm^2$. As can be seen $R_{cs}$ is about ten times lower than previously estimated. The authors explain that the tissue in their study is in a better condition than in previous experimental reports. One could imagine that the lateral spaces in their study were wide, consequently $R_{LIS}/R_{TJ}\approx 0$, and the three-resistor model was valid in their hands and giving the correct low value of $R_{cs}$.

| | M | C | S |
|---|---|---|---|
| | Concentrations [mM] | | |
| $[Na^+]$ : | 110.0 | 10.7 | 110.0 |
| $[K^+]$ : | 2.0 | 131.10 | 2.0 |
| $[Cl^-]$ : | 112.0 | 22.6 | 2.0 |
| [X] : | 0.0 | 59.6 | 110.0 (Z = -1) |

| | | | |
|---|---|---|---|
| | Potentials [mV] | | |
| Em : | 0.0 | Ec : -62.2 | Es : +2.5 |

**Fig. 2.** Steady state of the model cell (parameters in Table 1) when $Cl^s$ was reduced to 2 mM by replacement with a relative impermeant ion. The replacing ion had a permeability across the junction which was 0.14 times $P_K^{ms}$

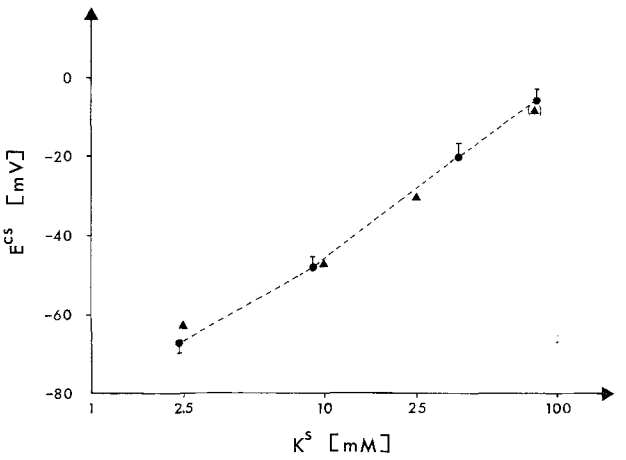


**Fig. 3.** Effect of replacement of $K^s$ by $Na^s$ on the serosal membrane potential $E^{cs}$. The filled circules are experimental results obtained by Reuss (1979); the filled triangles are points predicted by the model (Table 1, Fig. 1). The parentheses around the point at 85 mM indicates that only a quasi-steady state was obtained with the model at this concentration; however, the cellular values are constant within 5% for more than one hour after the replacement of $K^s$

guess on the derived parameters (permeabilities, carrier-characteristics, etc.), whereafter it was tested whether the model predicted the correct measurable variables (concentrations, potentials, etc.). This process was repeated until both derived and measured values were within the limits set by experiments.

*Comparisons with the Behavior of Living Cells*

Permeabilities of the basolateral membrane: Three types of experiments have been performed with microelectrodes in order to determine $P_{Cl}^{cs}$. *(i)* Serosal $Cl^-$ ions were replaced by an impermeant ion ($Cl^s$ = 2 mM) and the intracellular electrical potential was recorded (Reuss, 1979). *(ii)* $K^s$ was varied while keeping $Na^s + K^s$ constant in order to determine the ratio $P_K^{cs}/P_{Cl}^{cs}$ (Reuss, 1979). *(iii)* In the proximal tubules (Shindo & Spring, 1981) and in the gastric mucosa (Machen & Zeuthen, 1980) a current was passed transepithelially while $Cl^c$ was recorded.

*Ad i.* Figure 2 shows the effects in our model of replacing $Cl^s$ by an impermeant ion, down to $Cl^s$ = 2 mM. The depolarization of the serosal membrane $\Delta E^{cs}$ relative to the initial steady state (*compare* Table 1 and Fig. 1*A*) was 0.07 mV. In the experiments performed by Reuss (1979) $\Delta E^{cs}$ was found to be between 3 and 4 mV. Thus both the model and the experiments show that $E^{cs}$ is rather insensitive to changes in $Cl^s$. The permeability of the ion *M* which replaced $Cl^s$ was assumed to obey $P_M^{ms}/P_K^{ms} = 0.14$ across the junctions. This choice was uncritical for the effects studied.

*Ad ii.* $K^s$ was varied in our model cell and $Na^s + K^s$ were kept constant. We saw changes in $E^{cs}$ which were very close to those obtained by Reuss (1979) (Fig. 3). It should be noted that the situation obtained with the model for $K^s$ = 85 mM is a non-steady-state, the values of which, however, remain virtually constant for several hours.

*Ad iii.* Experiments on the effects of the transmural current on $Cl^c$ have not been performed in the *Necturus* gallbladder. There is, however, evidence in other epithelia, proximal tubule (Shindo & Spring, 1981) and gastric mucosa (Machen & Zeuthen, 1980) that $Cl^c$ is rather insensitive to transepithelial currents. We therefore examined the effect of transepithelial currents on $Cl^c$ in the model cell. The results are presented in Fig. 4 for transmural currents in the range of $-2000$ to $3000\,\mu A\,cm^{-2}$ from mucosa to serosa. When the resulting potential across the serosal membrane varied $\pm 10$ mV it could be seen that $Cl^c$ varied 2 mM which is equivalent to 1.7 mV in the potential measured by the electrode. If $E^{cs}$ is varied by $\pm 20$ mV, $Cl^c$ varies by 6 mM which is 5.6 mV in the potential of the electrode. This shows that the electrical potential needs to be varied more than 10 mV across the serosal membrane in order to induce measurable changes in $Cl^c$.

In the *Necturus* proximal tubular epithelium

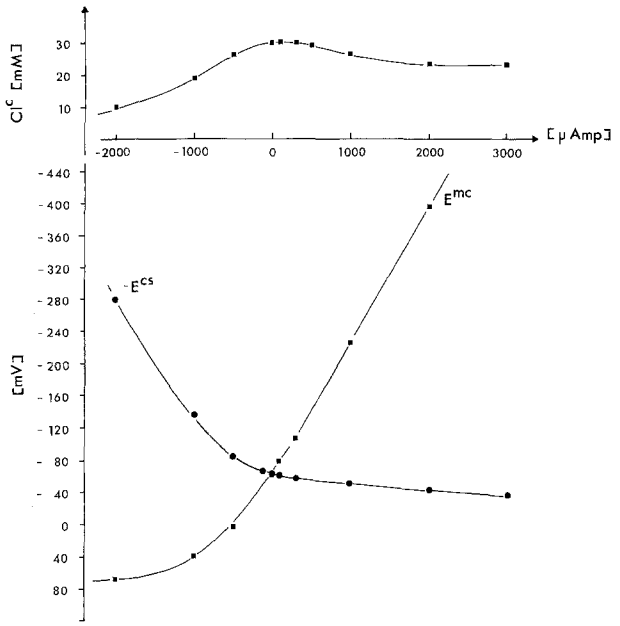


**Fig. 4.** Effects of a transmural current on the cellular concentration of $Cl^-$ and on the mucosal ($E^{mc}$) and serosal ($E^{cs}$) membrane potential. Positive current is in the direction from mucosa to serosa. Only 4% of the current is transcellular (*see* Table 1). Current is per $cm^2$

Shindo and Spring (1981) induced changes of ±20 mV across the basolateral membrane with changes of 2 to 4 mM in $Cl^c$, which is only slightly smaller than the value predicted by the model cell. Furthermore, if the convoluted nature of the lateral intercellular spaces of the proximal tubule is taken into account then the true induced variation in $E^{cs}$ may be smaller than the one estimated from the changes in potential imposed between the serosal and mucosal bath. If the values from Boulpaep and Sackin (1980) are used, then it can be seen that the true change in $E^{cs}$ may be of the order of only 10–12 mV even if the change imposed in the baths is 20 mV (*see also* Discussion p. 224).

A similar discussion applies to the work on the gastic mucosa (Machen & Zeuthen, 1980). When luminal $Cl^-$ ions were removed it was found that $Cl^c$ in the cell changed according to what could be expected from a luminal $Cl^-$ permeability of about $0.5 \times 10^{-5}$ cm sec$^{-1}$. When the luminal membrane was hyperpolarized by 15 mV (from −27 to −42 mV) $Cl^c$ only changed by a few mM.

### *The Effects of Inhibiting the $Na^+/K^+$ Pump*

Figures 5*A* and *B* show the effects on the model cell (Fig. 1*A* & *B*) of inhibiting the pump (e.g. by ouabain). It is noted that the inhibition causes an abrupt depolarization $\Delta E_{ouab} = -\Delta E^{cs} = -\Delta E^{mc}$ of

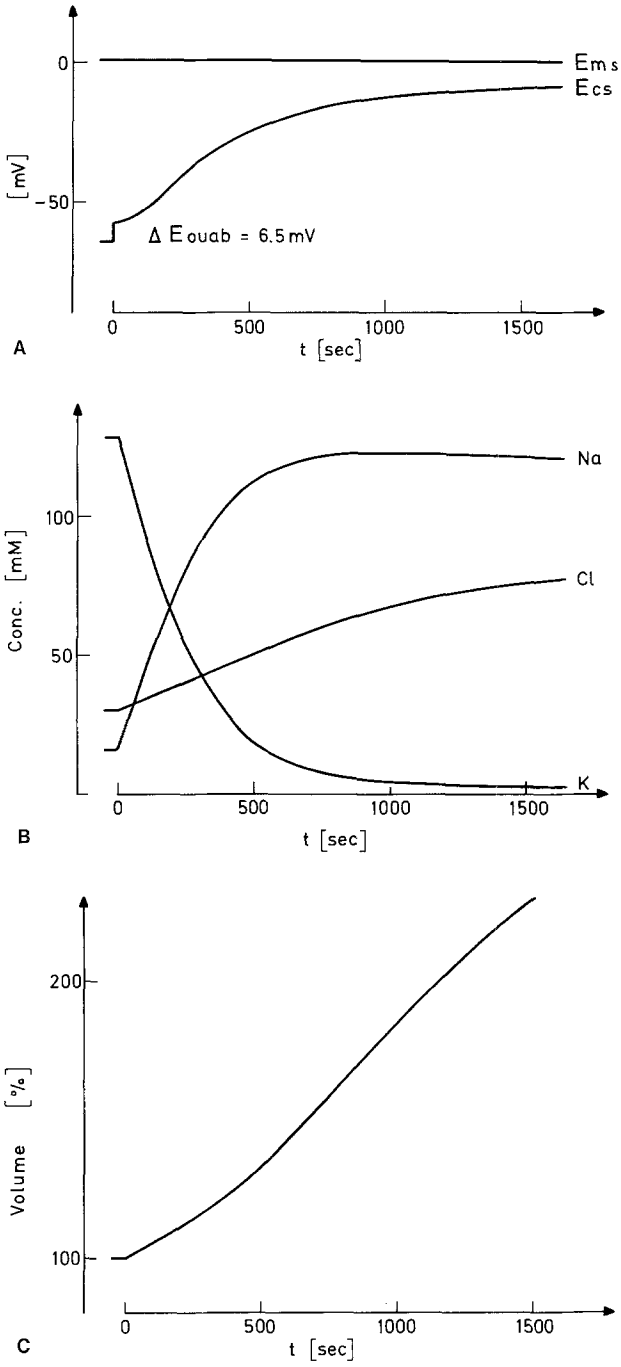


**Fig. 5.** *A* shows the effects on the electrical potentials of inhibiting the $Na^+/K^+$ pump by ouabain. The immediate effect is an abrupt depolarization $\Delta E_{ouab}$ of 6.5 mV. *B* shows the effects on the intracellular ion concentrations. *C* shows the ouabain-induced swelling

6.5 mV. If the coupling ratio $r$ was assumed to be 3, $\Delta E_{ouab}$ would have been 16.5 mV; if $r$ was 1 $\Delta E_{ouab}$ would have been 0 mV; all other parameters as in Table 1.

The only parameters apart from $r$ which influenced $\Delta E_{ouab}$ significantly were the resistance of the

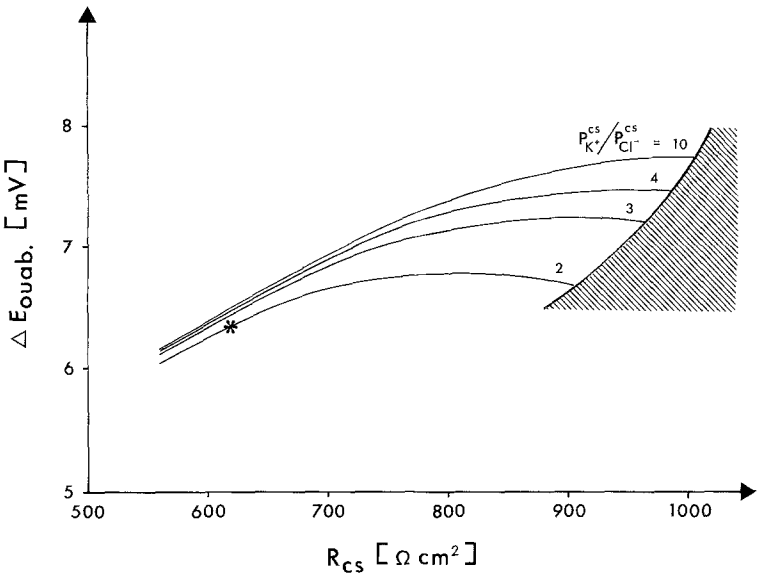


**Fig. 6.** The ouabain-dependent depolarization $\Delta E_{ouab}$ as a function of the resistance of the basolateral membrane; the ratio of the permeabilities $P^{cs}_K/P^{cs}_{Cl}$ is varied in the range 2 to 10. Other parameters are found in Table 1. The hatched area indicates values for which no steady states could be found. The asterisk indicates the steady state described in Fig. 1, Table 1

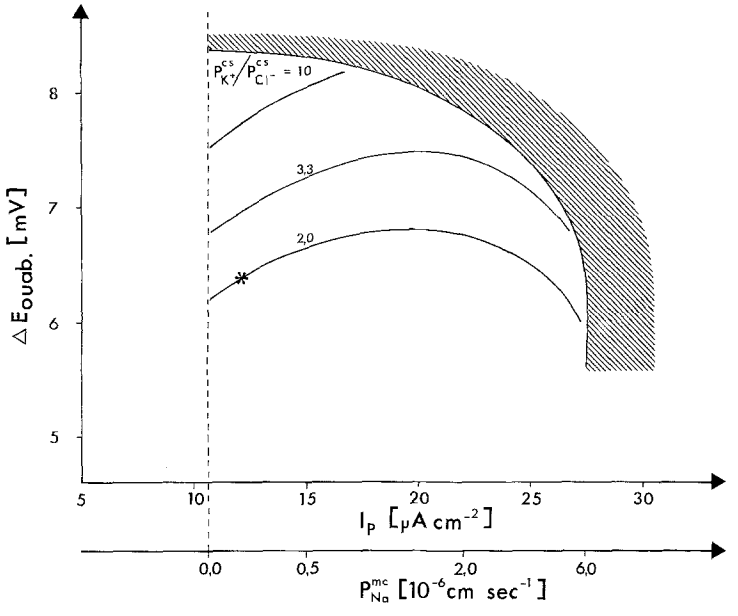


**Fig. 7.** As Fig. 6, but here the current carried by the pump $I_p$ is varied. This was done by varying the $Na^+$ permeability of the mucosal membrane ($P^{mc}_{Na}$)

basolateral membrane $R_{cs}$ and the current $I_p$ carried by the pump. Even so $\Delta E_{ouab}$ was relatively insensitive to changes in these parameters; $\Delta E_{ouab}$ only varied 2 mV, from 6 to 8 mV when the resistance and current were allowed to vary within physiological limits (Figs. 6 and 7). It was not possible to increase $I_p$ substantially by increasing the parameter $P^{max}_{Na}$ (p. 219) due to the rate-limiting step of Na entry at the luminal membrane. Consequently, $I_p$ was varied by means of varying $P^{Na}_{mc}$. Alternatively, we could have changed $K_{m,\,NaCl}$ but this would not effect the reported relationship significantly.

$\Delta E_{ouab} = 6.5$ mV is in good agreement with experimental observations. In the experiments where the connective tissue was completely removed, ouabain caused an abrupt (within 10 sec) depolarization of 6.3 mV $\pm$ 2.6 (SE, $n = 5$) (Fig. 8).

## Discussion

We have investigated a simple model of the *Necturus* gallbladder epithelium comprising electrodiffusive fluxes for $Na^+$, $K^+$ and $Cl^-$ across the cell membranes and the leaky junctions together with a $Na^+/K^+$ pump at the serosal membrane and a neutral influx of $Na^+$ and $Cl^-$ at the mucosal membrane. We have used the values for parameters, con-

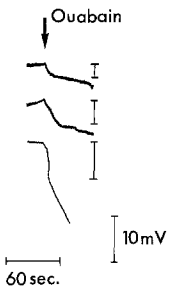


**Fig. 8.** Three recordings of the change in $E_{cs}$ induced by ouabain. The absolute value of the membrane potential was on average −60 mV. Two phases of depolarization are clearly distinguishable after the application of ouabain; an abrupt depolarization (marked by a bar) complete within 20 sec followed by a slow phase of depolarization

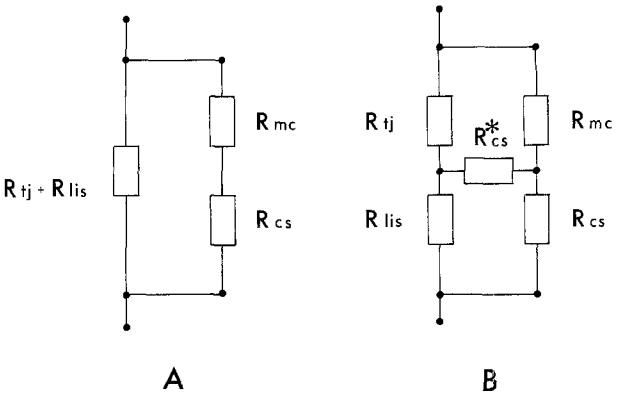


**Fig. 9.** The three-resistor model of the epithelial cell. Usually the resistance of the tight junctions $R_{tj}$ and the lateral intercellular spaces $R_{lis}$ are lumped $R_{tj}+R_{lis}$. The resistance of the mucosal membrane is called $R_{mc}$ and the resistance of the serosal membrane is called $R_{cs}$. If the ratio $R_{tj}/R_{lis}$ is of the same order as $R_{mc}/R_{cs}$, the distribution of current across the serosal membrane will be uneven and depend on the distribution of potentials in the lateral space; subsequently the route indicated by $R^*_{cs}$ cannot be ignored and the three-resistor model is invalid (*see* text)

centrations, etc., which have been found in the literature.

The first result of our analysis is that no steady state exists for our model epithelium if the resistance of the serosal membrane is of the order of $1000\,\Omega\,cm^2$ or larger; the permeabilities of the serosal membrane would be too low to allow for a sufficient efflux of $K^+$ and $Cl^-$. These high values for the resistance of the serosal membrane are obtained if the three-resistor model is applied for the epithelium. But is this model an adequate description of the tissue?

### *Validity of the Three-Resistor Model*

In the three-resistor model the mucosal and the serosal membrane and the paracellular pathway are each equated by a resistor (Fig. 9*A*). Each pathway also contains a battery but this is not interesting in the present context.

Now the paracellular pathway can be divided into a resistance of the junctions $R_{TJ}$ plus a resistance of the lateral intercellular spaces $R_{LIS}$ (Fig. 9*B*). If the ratio $R_{TJ}/R_{LIS}$ is of the same order as the ratio $R_{mc}/R_{cs}$ it can be seen from Fig. 9*B* that the three-resistor model is invalid, because a significant current will flow between the lateral spaces and the cell across the apical part of the serosal membrane ($R^*_{cs}$ in Fig. 9*B*).

A numerical example will illustrate this point. Let us assume that the junctions have a resistance of $100\,\Omega\,cm^2$ and the lateral intercellular spaces have a resistance which is $200\,\Omega\,cm^2$ giving a total resistance of $300\,\Omega\,cm^2$. Let us further assume that the mucosal membrane has a resistance which is twice that of the serosal membrane. If a transmural current produces 100 mV across the epithelium, the potential inside the paracellular pathway will be distributed as 33 mV across the tight junctions and 67 mV along the lateral intercellular spaces. The cell interior will be equipotential at 33 mV (superimposed upon the resting potential). Current will tend to pass from the apical end of the lateral spaces (which are at 67 mV) into the cell (which is at 33 mV) along $R^*_{cs}$ in Fig. 9*B*. Only towards the serosal end of the spaces does current pass from the cell interior into the lateral spaces as predicted by the three-resistor model (Fig. 9*A*). In the present example the midpoint of the lateral space has the same evoked potential (33 mV) as the potential evoked in the cell, and there is no net current across the part of the serosal membrane that lines the lateral spaces. Consequently, only the resistance of the basal part of the serosal membrane is measured. Thus if the cell was cubic with smooth walls the resistance of the serosal membrane would be overestimated by a factor of 5. Tortuosity of the lateral serosal membrane would increase this factor; tortuosity of the basal serosal membrane would decrease it.

Several estimates exist for the resistance of the lateral intercellular spaces. Frömter (1972) estimated $R_{LIS}$ to 110–180 $\Omega\,cm^2$ which was about half of the total transepithelial resistance, $381\,\Omega\,cm^2$; and Reuss and Finn (1977) found $R_{LIS} \cong 100\,\Omega\,cm^2$. This was found by opening and closing the spaces osmotically. Simon et al. (1981) measured the contribution of $R_{LIS}$ to $R_{TJ}+R_{LIS}$ directly by means of microelectrodes positioned in the lateral intercellular space and found that $R_{LIS}$ was about 50% of $R_{TJ}+R_{LIS}$. Thus it seems reasonable that $R_{TJ}/R_{LIS}$ is in the range of 3 to 1. Indirect estimates from optical data, however (Spring & Hope, 1978), indicate that $R_{LIS}$ is only 16% of the total resistance.

The ratio $R_{mc}/R_{cs}$ has been equated to the voltage divider ratio $\alpha$, defined as the ratio between the

voltage induced across the mucosal membrane $\Delta V_{mc}$, divided by the voltage induced across the serosal membrane $\Delta V_{cs}$, when a current is passed across the epithelium. Whether this equation is true will depend on the resistance and length constant of the lateral spaces (Zeuthen, 1976, 1981*a*, *b*; Boulpaep & Sackin, 1980) as discussed above. Most reports agree that $\alpha$ is in the range of 3 to 1.2. Frömter (1972), $\alpha = 1.66$; Reuss and Finn (1975*b*), $\alpha = 1.26$; van Os and Slegers (1975), $\alpha = 1.56$; Zeuthen (1978), $\alpha = 2.15$; Reuss (1979), $\alpha = 2.13$; Graf and Giebisch (1979), $\alpha = 1.49$; Suzuki and Frömter (1977), $\alpha = 3.2$. Only one report, Reuss and Finn (1977) finds a low voltage divider ratio $\alpha = 0.8$.

Existing estimates of $R_{LIS}/R_{TJ}$ are thus of the same order as estimates of $R_{mc}/R_{cs}$, in the range of 3 to 1. Consequently, the three-resistor model is invalid by the argument presented in conjunction with Fig. 9*B*.

By considering the interspaces as a cablelike structure it is possible to give a semiquantitative estimate of $R_{mc}/R_{cs}$ when $\alpha$, $R_{TJ}$, $R_{LIS}$ and $R_{mc}$ are known (Boulpaep & Sackin, 1980) (*see also* Clausen, Lewis & Diamond, 1949). If $R_{LIS}$ is 50% of the total transepithelial resistance and $R_{mc}$ is $3800\,\Omega\,cm^2$ (Frömter, 1972, $R_{mc} = 4470\,\Omega\,cm^2$; Reuss & Finn, 1975*a*, 2960–$3740\,\Omega\,cm^2$; Reuss & Finn, 1977, $3500\,\Omega\,cm^2$) and if $\alpha = 2.5$, then inspection of Fig. 5 in Boulpaep and Sackin (1980) shows that $R_{mc}/R_{cs} = 4$ which would mean $R_{cs} = 950\,cm^2$. Boulpaep and Sackin (1980) also investigated the *Necturus* gallbladder epithelium, but they only considered the data obtained by Reuss and Finn (1977) which deviate from the average findings mainly in an unusually low voltage divider ration 0.8 *vs.* normally 2 (for references *see above*).

In conclusion, with the latest estimates of the ratio of the resistance of the lateral intercellular spaces to that of the tight junction it appears that the three-resistor model is invalid. Negligence of the distributed or cablelike effects of this interspace causes the resistance of the serosal membrane to be overestimated. It should be re-emphasized that it is not the absolute value of the lateral intercellular spaces which is important, but its resistance relative to the resistance of the tight junctions[1].

### *The Steady State*

Our choice of steady state is arbitrary in that the parameters of the serosal membrane were chosen in order to allow sufficient electrodiffusive effluxes of $K^+$ and $Cl^-$. On the other hand it has been shown that the epithelia of the choroid plexus (Zeuthen & Wright, 1981) possess a sufficient $K^+$ permeability to allow for an electrodiffusive efflux, and in the gastric surface mucosa Machen and Zeuthen (1980) found that the $Cl^-$ permeability was large enough to sustain a passive $Cl^-$ efflux. Furthermore it has been shown that $Cl^c$ attains equilibrium across the serosal membrane when $Na^m$ is removed (Ellory, Ramos and Zeuthen, 1978; Reuss & Grady, 1979; Garizia-Diaz & Armstrong, 1980). This indicates that $Cl^-$ has no other means of passing the serosal membrane but electrodiffusion.

Accordingly, our model did simulate those experiments which were designed to test the properties of the serosal membrane in the *Necturus* gallbladder epithelium: If $Cl^s$ was reduced both the model and the living cells exhibited changes of the serosal membrane potential of only a few mV (Fig. 2). If $K^s$ was substituted by an impermeant ion, $Na^s$, the model predicted the depolarization observed experimentally (Fig. 3). The model also exhibited the same insensitivity in $Cl^c$ versus transepithelial current which is observed in a number of epithelia.

On the basis of the experimental results outlined above it has been argued (Reuss, 1979; Reuss & Grady, 1979; Shindo & Spring, 1981) that $P_{Cl}^{cs}$ should be insignificant relative to $P_K^{cs}$. Our analysis shows that a cell can have a relatively high $P_{Cl}^{cs}$ and still explain the measurements. The high $P_{Cl}^{cs}$ is masked by the high $P_K^{cs}$ (we use $P_K^{cs} = 2P_{Cl}^{cs}$; *see* p. 219) and by the fact that the pump is electrogenic. This becomes clear if one considers an approximated but analytical expression for the membrane potential of the serosal membrane of an epithelial cell of a leaky epithelium (Zeuthen, 1981*a*):

$$-E_{cs} = RT/F \ln \frac{(rP_K K^s + P_{Na} Na^s + P_{Cl} Cl^c)}{(rP_K K^c + P_{Na} Na^c + P_{Cl} Cl^s)}. \quad (7)$$

The permeabilities are the total permeabilities of the cells. When for example $Cl^s$ (and $Cl^m$) are reduced from 112 to 2 mM the depolarizing effects will be masked by the subsequent reduction of $Cl^c$ and by the large term $P_K K^c$ which is multiplied by the coupling ratio $r$ of the electrogenic pump.

### *Effects of Ouabain*

The immediate effect of ouabain is an abrupt depolarization of the membrane potentials. $\Delta E_{ouab}$ mainly depends on the coupling ratio $r$ of the $Na^+/K^+$ pump and to a smaller degree on the resistance of the basolateral membrane and the cur-

rent carried by the pump. These limitations and relative independences of $\Delta E_{ouab}$ on membrane resistances and pump currents signify the fact that when either of these parameters is increased, the cell potential will hyperpolarize but simultaneously there is an increase in the potential to which the cells depolarize at the application of ouabain (Zeuthen, 1981*a*).

In all leaky epithelia tested ouabain produced initially an abrupt depolarization, if it was presented instantaneously to the serosal membrane. In rat kidney proximal tubule Frömter and Gessner (1975) have observed $\Delta E_{ouab} = 10.6$ mV. In the choroid plexus Zeuthen and Wright (1978, 1981) have observed $\Delta E_{ouab} = 10.2$ mV. In *Necturus* gallbladder Rose and Nahrwold (1980) have observed a depolarization of 10.2 mV when the tissue was cooled abruptly from room temperature to 4 °C, a procedure which inhibits the pump. The last value is slightly higher than the value observed in this study (6.3 mV); this difference could be due to several factors. Even if the connective tissue is removed, ouabain may not achieve its full effect at the lateral aspects of the serosal membrane due to its foldings, and due to the convective effects of the stream of fluid. The effects may therefore differ from tissue to tissue, if the width of the lateral space varies. Also in our preparation, the connective tissue was removed from a limited area of the epithelium ranging from a diameter of 200 to 2000 μm. As the length constant for the spread of electrical signals from cell to cell is about 440 μm (Frömter, 1972), and as cells still covered by connective tissue do not immediately experience ouabain, $\Delta E_{ouab}$ may be recorded too small. On the other hand the value recorded by Rose (1981) is probably an overestimate because cooling itself introduces a depolarization via the factor $RT/F$; this could be of the order of 5 mV.

In conclusion both model and experiments show that inhibiting the pump causes an abrupt depolarization. This means that pump is electrogenic. The fact that this depolarization is between 6–10 mV strongly indicates that the coupling ratio $r$ is 1.5.[2] If $r = 1$ there would be no abrupt depolarization and if $r = 3$ there would be a depolarization of 16.5 mV (this study) or as high as 26.1 mV (Zeuthen, 1981*b*). Only by using an unphysiological set of parameters and concentrations can our model give $\Delta E_{ouab} = 6$ to 10 mV with $r = 3$.

In our model we have assumed perfect stirring of the serosal compartment as well as the lateral intercellular spaces. In the living epithelium, poisoned by ouabain the efflux of $K^+$ from the cell (Reuss *et al.*, 1979) must be influenced by unstirred layers of high $K^+$ in the lateral spaces. This makes any direct comparison of the rates of efflux difficult. It should be noted, however, that ouabain-induced $K^+$ efflux from the epithelium of choroid plexus (Zeuthen & Wright, 1981) which is unhindered by unstirred layers, resembles those predicted by the model.

It should be re-emphazised that these observations apply only to leaky epithelia where the transepithelial potential difference is small compared to the membrane potentials. In tight epithelia there is no strict relation between $r$ and $\Delta E_{ouab}$.

[2] If the low value of the resistance of the cell membrane ($\sim 164\ \Omega\ cm^2$) suggested by Frömter et al. (1981) is indeed correct then the expected depolarization should be only about 4 mV (*see* Fig. 6).

## Conclusion

A simple model of the *Necturus* gallbladder epithelium comprising a neutral entry of NaCl into the cell, an electrogenic entry of $Na^+$ and $K^+$ in a ratio of 3 to 2, and electrodiffusive fluxes of $Na^+$, $Cl^+$ and $K^+$ across both cell membranes and tight junctions, can explain most measurements performed on the tissue. There is no need, yet, to involve other transport mechanisms for $Cl^-$ (or $K^+$) across the serosal membrane.

This study was supported by the Danish Natural Sciences Research Council. Thanks are due to Mrs. U. Brat and R. Christiansen for typing various versions of the manuscript.

## References

Bærentsen, H.J., Zeuthen, T. 1981. Evidence for 3:2 coupling ratio of the Na-K pump in the serosal cell membrane of *Necturus* gallbladder epithelial cells. *J. Physiol. (London) (in press)*

Boulpaep, E.L., Sackin, H. 1980. Electrical analysis of intraepithelial barriers. *In:* Current Topics in Membranes and Transport. E.L. Boulpaep, editor. pp. 169–196. Aacademic Press, London

Clausen, C., Lewis, S.A., Diamond, J.M. 1979. Impedance analysis of a tight epithelium using a distributed resistance model. *Biophys. J.* **26**:291–317

Ellory, J.C., Ramos, M., Zeuthen, T. 1978. $Cl^-$-accumulation in plaice intestinal epithelium. *J. Physiol. (London)* **287**:12–13

Frömter, E. 1972. The route of passive ion movement through the epithelium of *Necturus* gall-bladder. *J. Membrane Biol.* **8**:259–301

Frömter, E., Gessner, K. 1975. Effect of inhibitors and diuretics on electrical potential differences in rat kidney proximal tubule. *Pfluegers Arch.* **357**:209–224

Frömter, E., Suzuki, K., Kottra, G., Kampmann, L. 1981. The paracellular shunt conductance of *Necturus* gallbladder epithelium: Comparison of measurements obtained by cable analysis with measurements obtained by a new approach based on intracellular impedance analysis. *In:* Epithelial Ion and Water Transport. A.D.C. Macknight and J.P. Leader, editors. pp. 73–83. Raven Press, New York

Garcia-Diaz, J.F., Armstrong, W. McD. 1980. The steady-state relationship between sodium and chloride transmembrane electrochemical potential differences in *Necturus* gallbladder. *J. Membrane Biol.* **55**:213–222

Graf, J., Giebisch, G. 1979. Intracellular sodium activity and sodium transport in *Necturus* gallbladder epithelium. *J. Membrane Biol.* **47**:327–355

Hill, A.E., Hill, B.S. 1978. Sucrose fluxes and junctional water flow across *Necturus* gall bladder epithelium. *Proc. R. Soc. London B.* **200**:163–174

Koefoed-Johnsen, V., Ussing, H.H. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298–308

Lew, V.L., Ferreira, H.G., Mauro, T. 1979. The behaviour of transporting epithelial cells. I. Computer analysis of a basic model. *Proc. R. Soc. London B.* **206**:53–83

Machen, T.E., Zeuthen, T. 1980. Electrophysiology of $Cl^-$ secretion in the stomach. *J. Physiol. (London)* **301**:48P

Os, C.H. van, Slegers, J.F.G. 1975. The electrical potential profile of gallbladder epithelium. *J. Membrane Biol.* **24**:341–363

Reuss, L. 1979. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder: III. Ionic permeability of the basolateral cell membrane. *J. Membrane Biol.* **47**:239–259

Reuss, L., Bello-Reuss, E., Grady, T.P. 1979. Effects of ouabain on fluid transport and electrical properties of *Necturus* gallbladder. *J. Gen. Physiol.* **73**:385–402

Reuss, L., Finn, A.L. 1975*a*. 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**:115–139

Reuss, L., Finn, A.L. 1975*b*. Electrical properties of the cellular transepithelial pathway in *Necturus* gallbladder. II. Ionic permeability of the apical cell membrane. *J. Membrane Biol.* **25**:141–161

Reuss, L., Finn, A.L. 1977. Effects of luminal hyperosmolarity on electrical pathways of *Necturus* gallbladder. *Am. J. Physiol.* **232(3)**:C99–C108

Reuss, L., Grady, T.P. 1979. Effects of external sodium and cell membrane potential on intracellular chloride activity in gallbladder epithelium. *J. Membrane Biol.* **51**:15–31

Reuss, L., Weinman, S.A. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membrane Biol.* **49**:35–362

Reuss, L., Weinman, S.A., Grady, T.P. 1980. Intracellular $K^+$ activity and its relation to basolateral membrane ion transport in *Necturus* gallbladder epithelium. *J. Gen. Physiol.* **78**:33–52

Rose, R.C. 1981. Electrolyte absorption by gallbladder: Is the pump rheogenic? *In:* Epithelial Ion and Water Transport. A.D.C. Macknight and J.P. Leader, editors. pp. 165–175. Raven Press, New York

Rose, R.C., Nahrwold, D.L. 1980. Electrolyte transport in *Necturus* gallbladder: The role of rheogenic Na transport. *Am. J. Physiol.* **238**:G358–G365

Saito, Y., Wright, E.M. 1981. Kinetics of the Na/K pump in choroid plexus. Fed. Am. Soc. Exp. Biol. **40**:363 (*abstr.*)

Shindo, T., Spring, K.R. 1981. Chloride movement across the basolateral membrane of proximal tubule cells. *J. Membrane Biol.* **58**:35–42

Simon, M., Curci, S., Gebler, B., Frömter, E. 1980. Attempts to determine the ion concentrations in the lateral spaces between cells of *Necturus* gallbladder epithelium with microelectrodes. *In:* Benzon Symposium 1980: Water Transport Across Epithelia. H.H. Ussing, N. Bindslev, N.A. Lassen, and O. Sten-Knudsen, editors. pp. 52–64. Munksgaard, Copenhagen *(In Press)*

Spring, K., Hope, A. 1978. Size and shape of the lateral intercellular spaces in a living epithelium. *Science* **200**:54–58

Suzuki, K., Frömter, E. 1977. The potential and resistance profile of *Necturus* gallbladder cell. *Pfluegers Arch.* **371**:109–117

Weinstein, A.M., Stephenson, L.L. 1979. Electrolyte transport across a simple epithelium steady-state and transient analysis. *Biophys. J.* **27**:165–186

Zeuthen, T. 1976. The vertebrate gall-bladder. The routes of ion transport. *In:* Fluid Transport in Epithelia. B.L. Gupta, R.B. Moreton, J.L. Oschman, and B.J. Wall, editors. pp. 511–537. Academic Press, London

Zeuthen, T. 1978. Intracellular gradients of ion activities in the epithelial cells of the *Necturus* gallbladder recorded with ion-selective microelectrodes. *J. Membrane Biol.* **39**:185–218

Zeuthen, T. 1981*a*. On the effects of amphotericin B and ouabain on the electrical potentials of *Necturus* gallbladder. *J. Membrane Biol.* **60**:167

Zeuthen, T. 1981*b*. Ion transport in leaky epithelia studied with ion-selective microelectrodes. *In:* The Application of Ion-Selective Microelectrodes. T. Zeuthen, editor. pp. 27–46. North Holland/Elsevier

Zeuthen, T. 1982. Relations between intravellular ion activities and extracellular osmolarity in *Necturus* gallbladder epithelium. *J. Membrane Biol.* **66**:109–121

Zeuthen, T. 1981*d*. The intracellular osmolarity during isotonic fluid transport in gallbladder. *In:* Alfred Benzon Symposium 15. H.H. Ussing, N. Bindslev, N.A. Lassen, and O. Sten-Knudsen, editors. pp. 313–325. Munksgaard, Copenhagen

Zeuthen, T., Wright, E.M. 1981. Epithelial potassium transport: Tracer and electrophysiological studies in choroid plexus. *J. Membrane Biol.* **60**:105–128

Received 19 June 1981; revised 23 October, 9 December 1981