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The Electrical Potential Profile of Gallbladder Epithelium

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Summary. In this study the relative ionic permeabilities of the cell membranes of *Necturus* gallbladder epithelium have been determined by means of simultaneous measurement of transmural and transmucosal membrane potential differences (PD) and by ionic substitution experiments with sodium, potassium and chloride ions. It is shown that the mucosal membrane is permeable to sodium and to potassium ions. The baso-lateral membrane PD is only sensitive to potassium ions. In both membranes chloride conductance is negligible or absent. The ratio of the resistances of the mucosal and baso-lateral membranes, R_M/R_S, increases upon reducing the sodium concentration in the mucosal solution. The same ratio decreases when sodium is replaced by potassium which implies a greater potassium than sodium conductance in the mucosal membrane. The relative permeability of the shunt for potassium, sodium and chloride ions is: $P_K/P_{Na}/P_{Cl} = 1.81:1.00:0.32$.

From the results obtained in this study a value for the P_K/P_{Na} ratio of the mucosal membrane could be evaluated. This ratio is 2.7. From the same data the magnitude of the electromotive forces generated across the cell membranes could be calculated. The EMF's are -15 mV across the mucosal membrane and -81 mV across the baso-lateral one. Due to the presence of the low resistance shunt the transmucosal membrane PD is -53.2 mV (cell inside negative) and the transmural PD is $+2.6$ mV (serosal side positive). The change in potential profile brought about by the low resistance shunt favors passive entry of Na ions into the cell across the mucosal membrane. Calculations show that this passive Na influx is maximally 64% of the net Na flux estimated from fluid transport measurements. The Cl-conductance of the baso-lateral membrane is too small to allow electrogenic coupling of Cl with Na transport across this membrane.

Experiments with rabbit gallbladder epithelium indicate that the membrane properties in this tissue are qualitatively similar to those of *Necturus* gallbladder epithelium.

Epithelial tissues involved in transmural Na transport have been divided into "tight" and "leaky" epithelia (Frömter & Diamond, 1972). Tight epithelia, such as amphibian skin and urinary bladder, are characterized by high transmural resistances and potential differences (PD). From ionic substitution experiments it was concluded that the transmural

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PD is the sum of two or more potential steps in the same direction (Koefoed-Johnsen & Ussing, 1958). The first step is generated across the outer or mucosal membrane, which behaves as a Na-electrode. The second or last step is generated across the inner or serosal barrier, which exhibits K-electrode behavior. The cell interior in these tissues has been shown to be electrically positive with respect to the mucosal solution (Frazier, 1962; Whittembury, 1964; Cereijido & Curran, 1965). Leaky epithelia, such as intestine, gallbladder and proximal tubules, are characterized by extracellular low resistance shunt pathways and small transmural PD's. The cell interior in these tissues is negative with respect to the mucosal solution (Boulpaep, 1971; Rose & Schultz, 1971; Frömter, 1972). Despite the detailed knowledge about the ionic selectivity of the shunt pathway there is almost no information about the ions that play a role in the electromotive forces (emf) generated across the cell boundaries in leaky epithelia. Analysis of a leaky epithelium in terms of an equivalent electrical circuit, as shown by Schultz (1972), points out that a PD measured across one of the cell membranes does not reflect *per se* the magnitude or the direction of the emf generated across that membrane. Values for all the resistive elements of the circuit are needed in order to interpret changes in transmembrane PD's induced by changes in ionic composition of the bathing solution. Since resistances of the cell membranes and of the shunt pathway have been measured in *Necturus* gallbladder epithelium (Frömter, 1972), this tissue provides a good preparation to study the ionic dependence of the emf's generated across the cell membranes of a leaky epithelium.

Materials and Methods

Tissue Preparation

Salamanders (*Necturus maculosus*) were obtained from Lemberger Co. (Oshkosh, Wisconsin) during the months of December through April. Animals were kept in tanks under running tapwater and fed with worms. Gallbladders were removed from anesthetized animals (MS 220, Sandoz). Before opening the bladder a small needle was inserted into the lumen near the duct, and most of the bile was removed by means of a syringe attached to the needle. The bladder was then opened and carefully rinsed in a large volume of Ringer's solution to remove all bile and mucous. The tissue was then incubated in aerated Ringer's solution for 30 min at room temperature and thereafter mounted as a flat sheet in a modified Ussing chamber with free access to the mucosal surface of the epithelium.

Albino rabbits of both sexes were anesthetized intravenously with Nembutal and exsanguinated by cardiac puncture. After opening the abdomen, the neck of the gallbladder was ligated and the bladder carefully removed and suspended in a gassed Ringer's solution at 38 °C. Then the bladder was cannulated and the bile removed. Care was taken to prevent overdistension of the tissue.

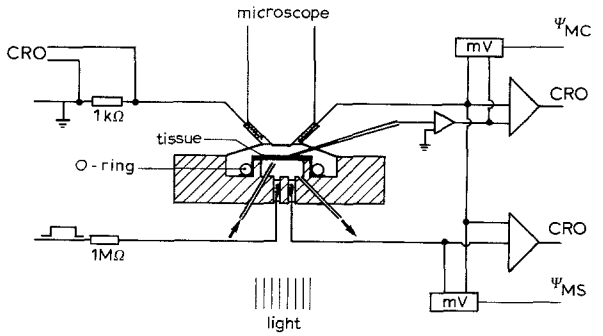

Fig. 1. Schematic view of bathing chamber and electrical equipment. The tissue, mucosal surface up, separates two compartments. The mucosal fluid is held in place by the water immersion objective. Arrangements for application of external current, measurement of ψ_{MC} and ψ_{MS} and circulation of serosal bathing solution are shown

Electrical Measurements on Necturus Gallbladder

The experimental arrangement is shown in Fig. 1 and is a modification of that used by Lindemann and Thorns (1967) in experiments on frog skin. The gallbladder is held in place by ligation around the knob and an O-ring (inner diameter 0.5 cm). This ring causes a moderate stretch which prevents folding of the epithelium. The exposed surface area is 0.38 cm^2.

Transepithelial potential differences ψ_{MS}, are measured between two Ag/AgCl wires which are connected with the mucosal and serosal solution via Ringer's agar bridges. These potentials are amplified by a differential amplifier (Tektronix 3A3) of a dual trace storage oscilloscope (Tektronix 569B) and fed parallel into an Elektrometer (Philips PM-2440).

The potential difference across the mucosal membrane ψ_{MC} is measured with a glass capillary microelectrode connected to a cathode follower (Pico-metric, IL 181) whose output is fed into the second differential amplifier of the oscilloscope and again parallel into a second electrometer. The outputs of the electrometers are connected with a two-channel pen recorder (Servogor RE 520). The tip resistance of the microelectrode is measured throughout the experiment with a repetitive calibration pulse of 1-msec duration, applied to the input of the follower stage (Lindemann & Thorns, 1967). In order to measure the transepithelial resistance, square-wave voltage pulses of 25-msec duration are passed via a 1 MΩ resistance through two other Ag/AgCl wires on either side of the epithelium. The pulses are obtained from Tektronix pulse generators (series 160). The voltage drops across the tissue ΔV_{MS} and across the mucosal membrane ΔV_{MC} are displayed and monitored on the oscilloscope screen. The current through the tissue is measured as the voltage drop across a 1 kΩ resistor. The current density is between 50 and 200 μAmps/cm^2. ΔV_{MS} is corrected for the resistance between the mucosal and serosal agarbridge and ΔV_{MC} is corrected for the resistance between the microelectrode tip and the mucosal agarbridge.

Microelectrodes were prepared from 1.5 mm O.D. Pyrex glass tubing in a horizontal puller (Narishige). The pipettes were filled by boiling in methanol under reduced pressure, placed in distilled water for 30 min, and finally placed in 3 M KCl. Electrodes were selected for a resistance between 10 and 40 MΩ and a tip potential of less then 5 mV. The microelectrode was mounted in a special lucite holder on a micromanipulator (Leitz). The tip was advanced at an angle of 15° to the epithelial surface under direct observation through a microscope with water emersion objective (Leitz). With transillumination at 500-fold magnification, the cell borders and nuclei are clearly visible. The diameter of the cells is about 20 μ. After

Table 1. Composition of Ringer's solutions

Gallbladder	Na^+	Choline	K^+	Ca^{++}	Mg^{++}
Necturus					
Normal	100	–	2.5	1	1
Na^+/Choline$^+$	x	$(100-x)$	2.5	1	1
Na^+/K^+	x	–	$(100-x)$	1	1
low Cl^-	100	–	2.5	1	1
Rabbit					
Normal	150	–	5	1	1
Na^+/Choline$^+$	15	135	5	1	1

[a] All concentrations are given in mmoles/liter.

penetration, the tip is discernible in the cytoplasm or in the nucleus of the cell. Potential measurements where the tip resistance changes after penetration have been discarded. With the tip inside a cell, the mucosal solution can be changed by gently removing the mucosal fluid with a pipette under negative pressure and applying fresh solution at the same time. The outlet of the serosal compartment has a greater diameter than the inlet and when the emerging serosal solution is collected under water, it is feasible to change the serosal solution without disturbing the epithelium. The hydrostatic pressure across the tissue was kept at zero. The compartments were unstirred and were not oxygenated. Bathing solutions were replaced every 15 min by fresh, oxygenated Ringer's solution. With this procedure cell potentials remained stable up to 9 hr after dissection. Liquid junction potentials, arising from differences in the composition of Ringer's solution in contact with the agar bridges have been measured in the following circuit:

Ag/AgCl: Normal Ringer's: mucosal: 3 M KCl: serosal: Normal Ringer's Ag/AgCl
4% agar solution 4% agar solution 4% agar

The greatest liquid junction PD observed between solutions with differences in monovalent ion concentrations is 3.1 mV. Values up to 8.5 mV are measured between Cl-Ringer's and SO_4-Ringer's solutions. All values for $\Delta\psi_{MS}$ and $\Delta\psi_{MC}$, reported under results, upon changing the ionic concentrations have been corrected for liquid junction potentials.

In this paper mean values are given with SE and number of observations in parentheses.

Electrical Measurements on Rabbit Gallbladder

Intracellular electrical potentials of rabbit gallbladder epithelium were measured as in *Necturus* gallbladder but with slight modifications. The gallbladder is now mounted in a chamber with 0.8 cm^2 surface freely exposed to the mucosal solution. The serosal solution is continuously gassed and maintained thermostatically at 38 °C. The mucosal solution is a stagnant layer of 2 mm, ungassed and not warmed. The electrical equipment is identical as described above.

Solutions

The composition of the used Ringer's solutions is given in Table 1. The pH of the solutions is 7.4 ± 0.1 and oxygenation was carried out with 100% O_2 in HCO_3^- free solutions and with 95% O_2 – 5% CO_2 in HCO_3^- containing buffers. When NaCl was exchanged for Na_2SO_4, mannitol was added to maintain a constant osmolarity. The osmolarity of the solutions was

used for *Necturus* and rabbit gallbladder[a]

Cl^-	SO_4^{--}	$H_2PO_4^-$	HCO_3^-	$TRIS^+$	HPO_4^{--}
101.5	–	0.9	–	–	2.05
109.0	–	–	–	2.5	–
109.0	–	–	–	2.5	–
4.0	48.75	0.9	–	–	2.05
132.8	–	1.2	25	–	–
144	–	1.2	13.8	–	–

measured by a freezing point depression method (Advanced Instruments Inc. Model 3A). Throughout this paper ionic concentrations are denoted as for example Na_M, which means: mucosal Na concentration.

Equivalent Electrical Circuit of Gallbladder Epithelium

The equivalent electrical circuit given in Fig. 2 is used for the analysis of changes in the cell membrane potentials upon changing the ionic concentrations. In this circuit the parameters of the mucosal or luminal membranes are E_M and R_M. E_M is the electromotive force (emf) due to ionic diffusion across this membrane. If more than one ionic species contributes to this emf, E_M takes the form of a Goldman (1943) equation. R_M is the input resistance of the luminal membrane. The parameters of the serosal cell membrane are E_S and R_S. E_S is the emf across the baso-lateral cell membrane. The possibility exists that a part of this emf is generated directly by an electrogenic Na^+ extrusion mechanism. R_S is the lumped resistance of the lateral and basal cell membrane. The shunt pathway is characterized by E_L and R_L.

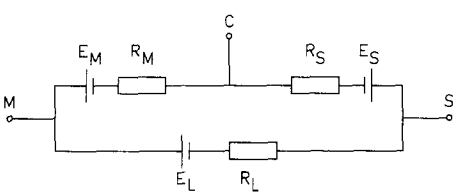

Fig. 2. An equivalent electrical circuit for gallbladder epithelium. *M*, *C*, and *S* designate the mucosal, intracellular and serosal electrodes, respectively

E_L is the emf across the shunt pathway when the ionic concentrations in the mucosal and serosal solution are not identical.

The three ionic compartments are denoted as *M*, the mucosal compartment, *C*, the intracellular compartment and *S*, the serosal compartment. The mucosal compartment is grounded via a 1 kΩ resistor (Fig. 1).

According to this circuit, the potential differences across the mucosal cell membrane, ψ_{MC}, and across the epithelium, ψ_{MS}, can be written as:

$$\psi_{MC} = [(R_S + R_L) E_M + R_M(E_S + E_L)]/(R_M + R_L + R_S) \quad (1)$$

and

$$\psi_{MS} = [R_L(E_M - E_S) + (R_M + R_S) E_L]/(R_M + R_L + R_S). \quad (2)$$

The orientation of the electromotive forces is as illustrated in Fig. 2.

Results

Mucosal Membrane Potentials in Necturus Gallbladder Epithelium

We confirm the observation of Frömter (1972), who reported that membrane potentials of *Necturus* gallbladder epithelium are unexpectedly unstable, despite the relatively large cell dimensions. Immediately after penetration of the microelectrode into the cell interior, the sudden jump in the potential of the tip is followed by irregular fluctuations of a few mV and more. In Fig. 3 some typical examples of membrane potential traces are given. As long as the amplitude of the fluctuations is greater than 10 mV, no attempts have been made to change the mucosal or serosal solution. The fluctuations always become smaller during the course of an experiment but this process takes several hours.

Criteria for acceptable impalements were: (*i*) an abrupt change in the potential with a value larger than 20 mV; (*ii*) "stable" potentials for more than 3 min, apart from spontaneous fluctuations around an average potential; (*iii*) an abrupt return to the base line after withdrawal; (*iv*) no change in tip resistance after penetration or change in tip potential after withdrawal from the cell interior. A histogram of 529 impalements on 44 specimens, which meet the four criteria, is given in Fig. 4. The mean value for ψ_{MC} is -53.2 ± 0.6 mV ($n=529$). A mean value of -59.1 ± 8.9 mV is reported by Frömter (1972). The mean value for ψ_{MS} immediately after mounting is 1.4 ± 0.4 mV ($n=49$). The experiments were performed from January until May, but membrane potentials observed in one month do not differ significantly from the mean values measured in any other month.

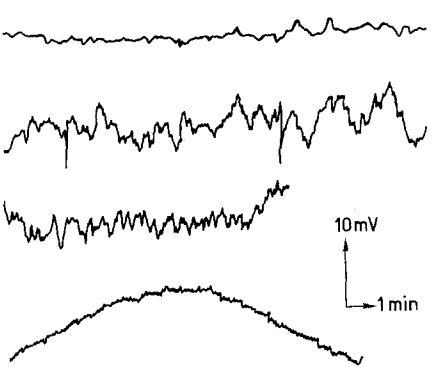

Fig. 3. Fluctuations in ψ_{MC}. No correlation between the magnitude of ψ_{MC} and the amplitude of the fluctuations is found. Fluctuation pattern varies with the bladder. The bottom trace is part of a regular synchronous potential oscillation of 13 mV amplitude and a period of 12 min (observed once in 55 specimens)

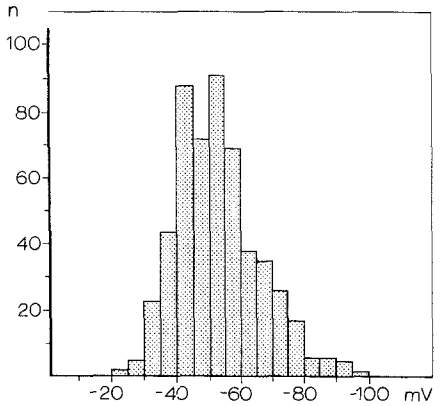

Fig. 4. A histogram of 529 membrane potentials (ψ_{MC}) measured in 44 gallbladders bathed in normal Ringer's solution

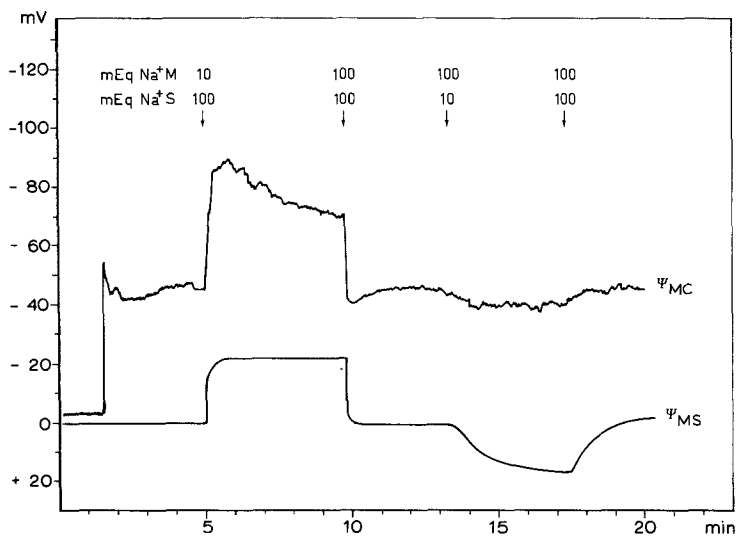

Fig. 5. Typical changes in ψ_{MC} and ψ_{MS} upon lowering the mucosal (M) or serosal (S) sodium concentration from 100 to 10 mM. Figures traced from original recording (Na exchanged against choline)

We conclude therefore that our observations on the amphibian tissue are not affected by seasonal variations. Seasonal influences have been described for *Necturus* kidney cells (Boulpaep, 1972). The large variation in ψ_{MC} (Fig. 4) is caused predominantly by variation in individual gallbladders.

From 24 gallbladders the ratio of the voltage drops across the mucosal membrane and across the tissue $\Delta V_{MC}/\Delta V_{MS}$, in response to a square current pulse, has been determined. This ratio is 0.59 ± 0.02. The voltage

divider ratio of the two cell membranes R_M/R_S is therefore 1.44 ± 0.06, which is somewhat smaller than the value (1.77) given by Frömter (1972). The mean value for the epithelial resistance is $267 \pm 37\ \Omega\ cm^2$ ($n=37$), not a significant difference from $307\ \Omega\ cm^2$, the value reported by Frömter (1972).

In order to detect contribution of an electrogenic pump to E_S, the influence of ouabain on ψ_{MC} has been studied. Addition of 10^{-4} M ouabain to the serosal solution has no fast effects. After 60 min the decrease in ψ_{MC} is smaller than 10% and after 120 min the decrease is about 30% in six gallbladders. These observations suggest that in the steady state there is no measurable electrogenic component in ψ_{MC}. The decrease in ψ_{MC} after prolonged incubation with ouabain can therefore be ascribed to dissipation of ionic gradients. The observed potentials can be considered as purely passive diffusion potentials across the various barriers in the epithelium.

Effects of Sodium Ions on the Mucosal Membrane PD and the Transepithelial PD

A sudden decrease in the Na concentration on the mucosal solution results in a hyperpolarization of ψ_{MC} and leads to the development of a transcellular PD, serosal side negative with respect to ground. A typical record of ψ_{MC} and ψ_{MS} is given in Fig. 5. The evoked transmural potential with different Na concentrations on both sides resembles that in rabbit gallbladder (Barry, Diamond & Wright, 1971). The response of the mucosal membrane potential is more complex. There is an instantaneous hyperpolarization followed mostly by a much slower depolarization. According to Eqs. (1) and (2), ψ_{MC} and ψ_{MS} are functions of three electromotive forces and the resistance of each barrier must be known in order to evaluate changes in an individual emf. Inserting $R_M = 4.47\ k\Omega\ cm^2$, $R_S = 2.88\ k\Omega\ cm^2$ and $R_L = 0.32\ k\Omega\ cm^2$, which are the values measured by Frömter (1972), in Eqs. (1) and (2) leads to

$$\psi_{MC} = 0.42\ E_M + 0.58\ E_S + 0.58\ E_L \tag{3}$$

and

$$\psi_{MS} = 0.04\ E_M - 0.04\ E_S + 0.96\ E_L. \tag{4}$$

According to Eq. (4) a change in ψ_{MS} is almost exclusively due to a change in E_L, the emf across the shunt pathway. Evaluation of the hyperpolarization of ψ_{MC} is complicated by the fact that the sign of E_L is such that this emf also leads to hyperpolarization of the mucosal membrane. E_L

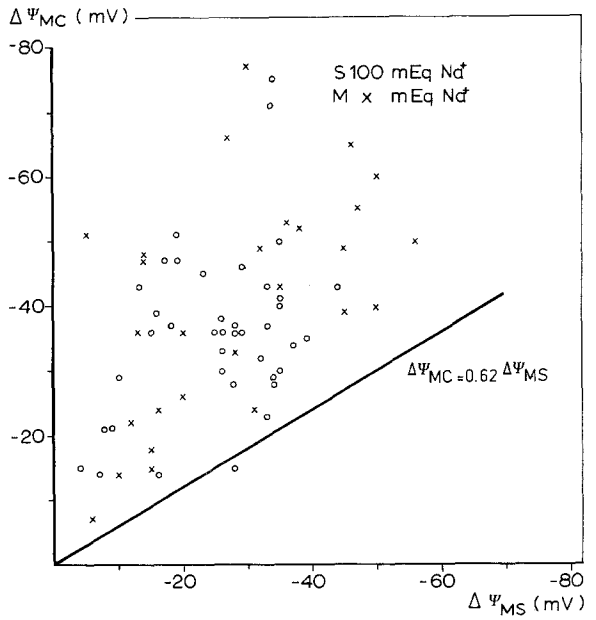

Fig. 6. Relation between $\Delta\psi_{MC}$ and $\Delta\psi_{MS}$ in response to reduction of mucosal sodium concentration. Open circles refer to a mucosal Na concentration of 10 mM, crosses to sodium concentrations between 50 and 1 mM. The solid line represents the contribution of E_L to ψ_{MC} (Eq. 5)

may be expressed as a function of E_M, E_S and ψ_{MS} on rearranging Eq. (4). Elimination of E_L from Eq. (3) leads to

$$\psi_{MC} = 0.38\, E_M + 0.62\, E_S + 0.62\, \psi_{MS}. \tag{5}$$

In Fig. 6 the instantaneous hyperpolarization, $\Delta\psi_{MC}$, upon reducing the mucosal sodium concentration, has been plotted against the change in ψ_{MS}. This figure demonstrates clearly that besides the contribution of $\Delta\psi_{MS}$ to $\Delta\psi_{MC}$ there must be a change in E_M, which implies significant Na-conductance in the luminal membrane, contributing to the emf across this membrane.

Hyperpolarization of ψ_{MC} on substituting Na in the mucosal solution has also been reported for other low resistance epithelia, e.g., proximal tubule of *Necturus* kidney (Giebisch, 1968) and rabbit ileum (Rose & Schultz, 1971). In these reports, however, the contribution of E_L to ψ_{MC} has not been evaluated.

More evidence for the presence of Na-conductance in the mucosal membrane is provided by the increase in the ratio of the mucosal and serosal membrane resistance upon reducing the mucosal Na concentration.

The ratio $\Delta V_{MC}/\Delta V_{MS}$ increases from 0.59 ± 0.02 to 0.67 ± 0.02 ($n=14$) when Na_M is reduced from 100 to 10 mmoles (Na exchanged for choline).

Amiloride has been shown to be a potent inhibitor of passive sodium movements through various cell membranes, such as frog skin (Biber, 1971), toad bladder (Bentley, 1969) and distal tubules (Duarte, Chometry & Giebisch, 1971). Interesting in this respect is our observation that 10^{-4} M amiloride in the mucosal solution fails to interfere with the sodium conductance of the luminal membrane of *Necturus* gallbladder epithelium, since no effect of this drug on ψ_{MC} nor on $\Delta\psi_{MC}$ and $\Delta\psi_{MS}$ upon reducing the mucosal sodium concentration could be observed.

Reducing the serosal sodium concentration leads to a slowly developing transepithelial PD (Fig. 5). This slow process contrasts with the fast phenomena observed on the luminal side. The large connective tissue compartment, which forms a considerable diffusion barrier, must be responsible for this phenomenon. Moreover, the perfusion rate of the serosal compartment is low, since perfusion of this small compartment must be performed without disturbing the epithelium in order to keep the electrode in the cell. The transient in ψ_{MS} on the serosal side in Fig. 5 has a half-time $t_{\frac{1}{2}} \simeq 120$ sec. This implies a total thickness of the unstirred layer of 690 μm ($t_{\frac{1}{2}} = 0.38\ \delta^2/D$, where δ = thickness of the unstirred layer and $D = 1.5 \times 10^{-5}$ cm^2/sec for NaCl) (Dainty & House, 1966). The unstirred layer may also explain the difference in magnitude of $\Delta\psi_{MS}$ upon a tenfold change in sodium concentration on the mucosal side as compared to that on the serosal side. Reducing the serosal sodium concentration has only a small effect on ψ_{MC}. Most frequently a small depolarization was observed. This can be explained by the contribution of ψ_{MS} to ψ_{MC} (Eq. 5). However, the depolarization of ψ_{MC} was usually smaller than $0.62 \times \Delta\psi_{MS}$, which suggests a small hyperpolarization. Therefore, a small sodium conductance in the serosal cell membrane cannot be excluded. This has also been reported for the peritubular membranes of proximal tubule cells of *Necturus* kidney (Anagnostopoulos, 1973).

Effect of Potassium on the Mucosal Membrane PD and the Transepithelial PD

Complete removal of Na from the mucosal solution leads to unstable cell potentials. Another complication is swelling of the epithelial cells which interferes with intracellular recordings. Moreover, the cell cytoplasm loses its translucence and becomes granular and turbid after complete replacement of Na. For this reason the sodium concentration is in most

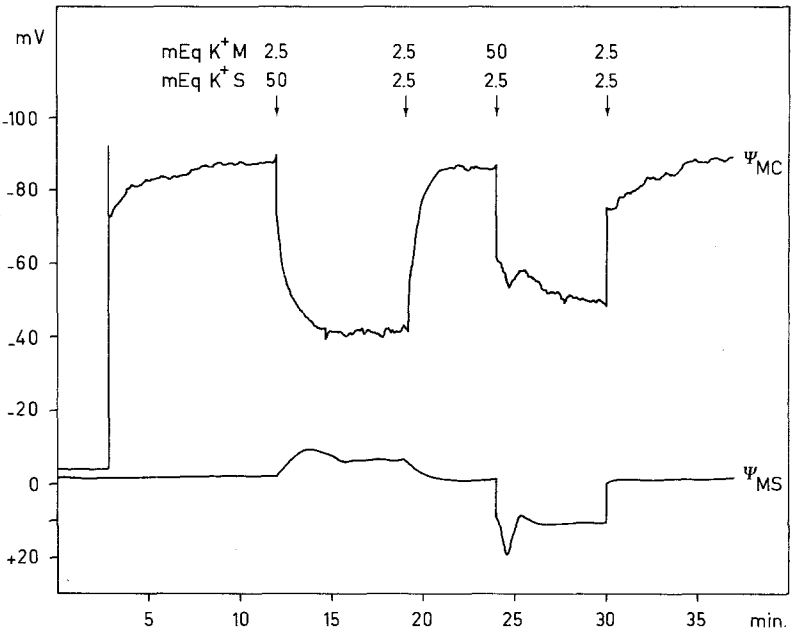

Fig. 7. Changes in ψ_{MC} and ψ_{MS} when the mucosal (M) or serosal (S) potassium is raised to 50 mM, while the sodium concentration is kept at 50 mM (choline exchanged against K). Figures traced from original recording

cases not reduced below 50 mM. In order to study potassium effects, the sodium concentration is first lowered on both sides of the epithelium by equimolar exchange for choline, then choline can be exchanged by an equimolar amount of potassium on one side only. A typical example of such an experiment is shown in Fig. 7. An increase in K concentration on the serosal side depolarizes the cell interior, which implies significant K conductance in the baso-lateral membrane. The trace of ψ_{MS} shows a transient overshoot which has also been described in gallbladders of other species (Moreno & Diamond, 1974). The transients have been ascribed to a low resistance ion discriminator in series with the discriminator determining steady-state PD's. In our experiments not all gallbladders showed such transients.

Increasing K concentrations on the mucosal side leads again to depolarization of ψ_{MC}. After the instantaneous drop in PD there is continuing depolarization. Returning to normal K concentrations ψ_{MC} returns slowly to the initial value after a fast response. Since the shunt pathway is highly permeable for K ions, a raise in K_M will lead to a significant K flux into the lateral spaces which causes depolarization of E_S

Table 2. Changes in the mucosal membrane potential upon raising K_M from 2.5 to 50 and reducing Na_M from 100 to 50 mM

Mucosal: K: 2.5 → 50 Na: 100 → 50			K: 2.5 → 50 Na: 100 → 50			
Serosal: Normal Ringer's			K: 50; Na: 50			
ψ_{MC}	$\Delta\psi_{MC}$	$\Delta\psi_{MS}$	ψ_{MC}	$\Delta\psi_{MC}$	$\Delta\psi_{MS}$	
-53.5 ± 1.8	25.3 ± 2.1	5.8 ± 0.3	-28.1 ± 1.1	12.4 ± 1.4	4.9 ± 0.3	$(n=14)$

Left: $K_S=2.5$ mM; Right: $K_S=50$ mM.

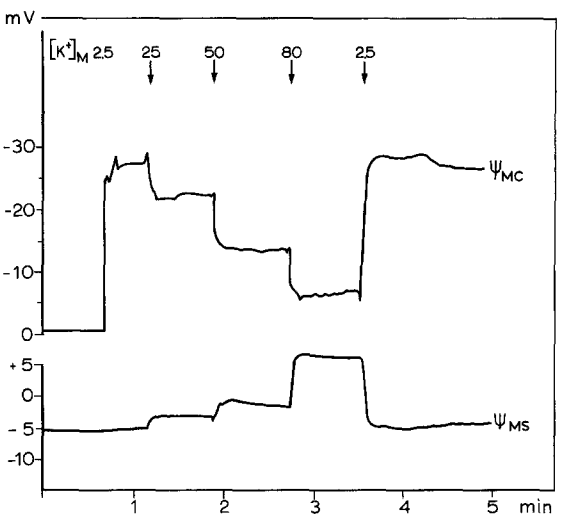

Fig. 8. Changes in ψ_{MC} and ψ_{MS} when the mucosal K concentration is raised from 2.5 to 25, 50, and 80 mM (K exchanged against Na). The serosal K concentration is kept at 80 mM

and hence of ψ_{MC}. Therefore, we have measured the response of ψ_{MS} on raising K_M in two conditions; with 2.5 mM K and with 50 mM K in the serosal solution. The results are given in Table 2. An increase in K_S from 2.5 to 50 mM reduces the response of ψ_{MC} to an increase in K_M by 50%. However, Fig. 8 shows clearly that despite a serosal K-concentration of 80 mM, the mucosal membrane PD still depolarizes on an increase in K_M from 2.5 to 25 mM. This experiment proves that the mucosal membrane is permeable for K ions. In these experiments K is exchanged equimolar for Na and therefore the ratio of K and Na permeabilities of the mucosal membrane, P_K/P_{Na}, must be greater than one.

The same conclusion can be drawn from the changes in the voltage divider ratio of the cell membranes. Increasing K_M from 2.5 to 50 reduces $\Delta V_{MC}/\Delta V_{MS}$ from 0.59 ± 0.02 to 0.44 ± 0.03 $(n=14)$.

Contribution of K ions to the emf across the mucosal membrane has been described in distal tubules of *Necturus* (Giebisch, 1961) and rat (Giebisch *et al.*, 1966), in rabbit ileum (Rose & Schultz, 1971) and in rabbit gallbladder (Henin & Cremaschi, 1975). However, in the last two studies a possible influence of lateral membrane depolarization, upon raising K_M, has not been considered.

Effect of Chloride Ions on the Mucosal Membrane PD and the Transepithelial PD

The effects of substitution of sulfate for chloride ions are small compared to the junction potentials between a chloride-Ringer's agar bridge and a sulfate-Ringer's solution (Cl-Ringer's bridge/SO_4-Ringer's solution junction PD is $+8.5$ mV with respect to Cl-Ringer's bridge/Cl-Ringer's solution).

Reducing the chloride concentration to 4 mM in the mucosal solution causes a small hyperpolarization of ψ_{MC} ($\Delta\psi_{MC} = -1.2 \pm 0.2$, $n=18$) and a small but significant transcellular PD ($\Delta\psi_{MS} = 1.9 \pm 0.4$, $n=18$). These data indicate that the chloride contribution to the shunt conductance is small, which is in agreement with reports for other gallbladders (Moreno & Diamond, 1974). The small hyperpolarization of ψ_{MC} is the opposite of what should be expected, if chloride would contribute to the mucosal membrane conductance. More likely, it is due to the fact that the sodium activity is lower in SO_4- than in Cl-Ringer's solutions (Robinson & Stokes, 1970). It is also possible that changes in Ca concentration may be responsible for the effect attributed to Cl substitution, since in SO_4-Ringer's the ionized Ca concentration is below that in Cl-Ringer's. However, in pilot experiments in which the Ca concentration has been varied between 1 and 5 mM, we have found no effects on transmural and transmucosal membrane PD's.

The changes in ψ_{MC} and ψ_{MS} upon serosal chloride substitution are -1.3 ± 0.9 and -2.9 ± 0.4, respectively ($n=9$). The change in ψ_{MC} is almost completely due to the change in ψ_{MS} (Eq. 5), which implies that chloride makes a small contribution to the serosal membrane conductance or none at all. No changes in the voltage divider ratio $\Delta V_{MC}/\Delta V_{MS}$ are observed during Cl substitution for SO_4.

Relative Permeabilities of the Shunt Pathway and the Cell Membranes

In Fig. 9 mean values of changes in ψ_{MS} are plotted against decreasing Na^+ concentrations in the mucosal solution. When the sum of Na + K

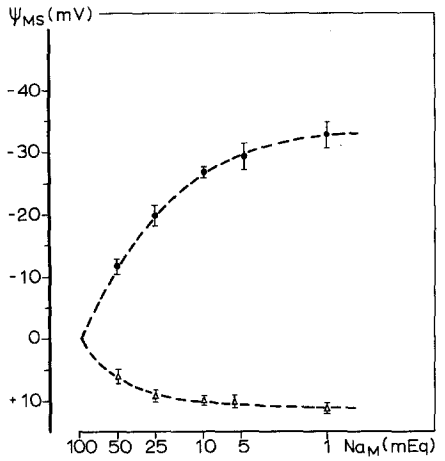

Fig. 9. Plot of ψ_{MS} against mucosal Na concentration at constant K concentration (solid circles, $n=14$) and when the sum of Na and K is kept constant (triangles, $n=7$)

is kept constant, steady-state values of ψ_{MS} are plotted. It has been shown that the constant field equation gives a reasonable fit for diffusion potentials across gallbladders (Barry *et al.*, 1971; Moreno & Diamond, 1974). According to Eq. (4) a change in ψ_{MS} is 96% of the change in E_L. Therefore we can write

$$\Delta\psi_{MS} \simeq \Delta E_L = \frac{RT}{F} \ln \frac{P_K \gamma K_M + P_{Na} \gamma Na_M + P_{Cl} \gamma Cl_S}{P_K \gamma K_S + P_{Na} \gamma Na_S + P_{Cl} \gamma Cl_M} \tag{6}$$

in which P_K, P_{Na} and P_{Cl} are relative permeabilities of the shunt, γ activity coefficients (Robinson & Stokes, 1970), and K_M, K_S are concentrations.

The data of Fig. 9 fit Eq. (6) when $P_K/P_{Na}/P_{Cl} = 1.81:1.00:0.32$. These P values are comparable to values measured in gallbladders of other species (Moreno & Diamond, 1974).

In this study we have demonstrated that the serosal membrane is almost exclusively permeable to K ions. Using the change in ψ_{MC} upon raising K_S to 50 mM (Table 2), the change in E_S can be calculated by means of Eq. (5). The calculated change in E_S is only 60% of the change expected for a pure K^+ electrode. This difference may be due firstly to a nonlinear relation of E_S with log K_S in the lower concentration region due to a small shunting effect of Cl ions, and secondly to the thick serosal diffusion layer. The K concentration in the lateral intercellular spaces is probably not the same as in the serosal solution, since K ions can diffuse through the tight junction into the mucosal solution.

Evaluation of the permeability ratio P_K/P_{Na}, of the mucosal membrane gives considerable problems. Upon reducing Na_M, the voltage divider

Table 3. Mucosal membrane potential, transmural PD and resistances of the mucosal and baso-lateral membrane and of the shunt path in condition I (normal Ringer's on both sides) and condition II (10 mM Na^+ in the mucosal solution)

Condition	mM		mV		n
	Na_M	Na_S	ψ_{MC}	ψ_{MS}	
I.	100	100	-53.2 ± 0.6	0	529
II.	10	100	-91.5 ± 4.1	-26.4 ± 1.6	43
I.	$R_{transmural}=0.27\pm0.01$ kΩ cm^2 ($n=37$)			$R_M=4.13$ kΩ cm^2	
	$R_M/(R_M+R_S)=0.59\pm0.02$ ($n=24$)			$R_S=2.87$ kΩ cm^2	
	$R_L/(R_M+R_S)=1/25$ (Frömter, 1972)			$R_L=0.28$ kΩ cm^2	
II.	$R_{transmural}=0.37\pm0.05$ kΩ cm^2 ($n=14$)			$R_M=5.83$ kΩ cm^2	
	$R_M/(R_M+R_S)=0.67\pm0.02$ ($n=14$)			$R_S=2.87$ kΩ cm^2	
	$R_S=2.87$ kΩ cm^2 (condition I)			$R_L=0.38$ kΩ cm^2	

The mean value for ψ_{MC} is the instantaneous hyperpolarization after changing Na_M from 100 to 10 mM. The resistances in condition I are calculated making use of the relation: $R_L/(R_M+R_S)=1/25$, which has been proven by Frömter (1972). In condition II, it is assumed that R_S is not changed after reducing Na_M from 100 to 10 mM.

ratio changes and upon increasing K_M, also the emf across the baso-lateral membrane depolarizes. Moreover, the cell Na and K concentrations are unknown. However, with a few assumptions it is possible to arrive at an estimate of the ratio, P_K/P_{Na}, for the mucosal membrane. An important assumption is: the sum of the extracellular Na and K activities is equal to the sum of the intracellular Na and K activities, hence $Na_C=100\,\gamma-K_C$ (γ = activity coefficient for Na and K in the extracellular fluid). Since the variation in ψ_{MC} of individual bladders is large, only mean values observed in a large number of tissues are meaningful parameters. There are two conditions which meet this criterion: condition I in which the epithelium is bathed on both sides in normal Ringer's solution and condition II in which Na_M has been reduced to 10 mM. Table 3 gives the parameters which we need to solve Eq. (1) for conditions I and II.

E_M has been shown to depend on Na and K ions and E_S only on K ions. If we use for E_L the observed transmural PD, then Eq. (1) can be written as

$$\psi_{MC}=(R_S+R_L)/R_T\times 58\log\frac{\alpha\cdot\gamma\cdot K_M+\gamma\cdot Na_M}{(\alpha-1)\,K_C+100\,\gamma}$$
$$+R_M/R_T\times 58\log\frac{\gamma\,K_M}{K_C}+R_M/R_T\cdot\psi_{MS} \qquad (7)$$

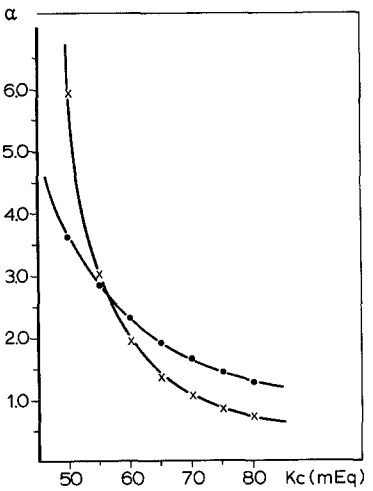

Fig. 10. Solution of Eq. (7) for conditions I and II. Cellular K activity, K_C, is plotted against α, which is the ratio P_K/P_{Na} of the mucosal membrane

Table 4. Common solution for Eq. (7) in conditions I and II when the activity coefficient γ is varied

	α	K_C	Na_C
$\gamma=1.0$	2.7	62.5	37.5
$\gamma=0.9$	2.7	56.5	33.5
$\gamma=0.8$	2.7	50.0	30.0

in which $R_T=R_M+R_S+R_L$, $\alpha=P_K/P_{Na}$, and γ is the activity coefficient in the extracellular solution.

In condition I, $E_L=0$, hence $\psi_{MS}=0$. Using the parameters given in Table 3, ψ_{MS} in Eq. (7) is only a function of α and K_C in both conditions. Fig. 10 gives a solution for Eq. (7) when K_C is varied between 50 and 80 mM ($\gamma=0.9$). A common solution for conditions I and II is obtained for $\alpha=2.7$ and $K_C=56.5$ mM.

In Table 4 the common solutions are listed for three values of γ. The ratio P_K/P_{Na} is not influenced by a change in γ. Since no values for intracellular ionic concentrations in *Necturus* gallbladder are known, we have to compare the values in Table 4 with values from *Necturus* proximal tubule cells. Several authors report Na_C between 38 and 45 mM and K_C between 90 and 108 mM (Giebisch, 1961; Whittembury, Sugino & Solomon, 1961; Khuri *et al.*, 1972). However, measurements with K-

selective electrodes in *Necturus* proximal tubule cells give K activities of 58.7 mM, considerably lower than chemically determined values (Khuri *et al.*, 1972). This K activity value for proximal tubule cells is surprisingly close to our calculated K activity for gallbladder cells (Table 4). Since our calculation leads to physiological values for the intracellular ionic concentrations the ratio $P_K/P_{Na}=2.7$ of the mucosal membrane must be close to reality.

Membrane Potentials in Rabbit Gallbladder Epithelium

More problems are met in attempting to measure membrane potentials in rabbit gallbladder epithelial cells. When the microelectrode is advanced at an angle of 45°, with observation by means of a stereomicroscope, no single stable potential can be measured. Impalement leads to a sudden jump in the potential of -40 to -60 mV, which immediately decays back to the zero line with a nearly exponential time course of about 50 sec. The same phenomenon is described by Frömter *et al.* (1971), when rat proximal tubular cells were punctured. This phenomenon seems to be due to the occurrence of leaks and is inherent to the impaling of small cells (Lassen, 1971). Advancing the electrode in a vertical position towards the tissue leads in some cases to more or less stable potentials. The same criteria for acceptable impalements as described for *Necturus* gallbladder are used, except that potentials had to be stable for 1 min instead of 3. Since small potentials tend to be less stable than the larger ones, we selected intracellular potentials larger than -50 mV.

Membrane potentials selected in this way, representing 16% of all impalements, give a mean value of -69.3 ± 1.1 mV ($n=97$). This value is 20 mV more negative than the mean value reported by Dugas and Frizzell (1974) and 10 mV more negative than reported by Henin and Cremaschi (1975). Our value is not significantly different from the membrane potentials of rat proximal tubule (Frömter *et al.*, 1971). A histogram of 97 impalements on 12 gallbladders is given in Fig. 11.

During six relatively long and stable impalements, we have demonstrated that a decrease in the mucosal sodium concentration from 150 to 15 mM (exchanged against choline) gave a hyperpolarization of ψ_{MC} ($\psi_{MC}=-21$ mV and $\psi_{MS}=-23$ mV). Exchanging mucosal Na against K leads to depolarization of ψ_{MC}, indicating that there is a K conductance in the mucosal membrane. In two gallbladders, bathed on the serosal side with 150 mM K, the largest values for ψ_{MC} observed are -20 mV, while impalements in the same bladders bathed in normal Ringer's

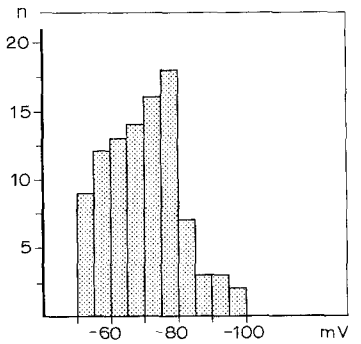

Fig. 11. A histogram of 97 mucosal membrane potentials, stable for more than 60 sec, observed in 12 rabbit gallbladders bathed in normal Ringer's solution (38 °C)

solution give values around -80 mV. Substitution of chloride by sulfate in the mucosal or serosal medium does not influence ψ_{MC}. These observations indicate that the ionic permeabilities of rabbit gallbladder epithelial cell membranes are qualitatively similar to those of *Necturus* gallbladder cell membranes.

Discussion

In this study we have shown that the mucosal membrane of gallbladder epithelium is permeable to Na and K ions, while the baso-lateral membrane is only permeable to K ions. For *Necturus* gallbladder we could evaluate the ratio P_K/P_{Na} of the mucosal membrane by using an equation for the mucosal membrane PD and those parameters which have been measured most frequently. In this way obtained values for the intracellular concentrations and the ratio P_K/P_{Na} can be used to calculate the magnitude of the emf's operating across the cell membranes of *Necturus* gallbladder.

In Fig. 12 the potential profile across gallbladder epithelium is given together with the calculated emf's, E_M and E_S. The result of a low resistance

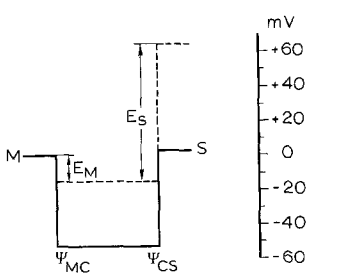

Fig. 12. Electrical potential profile of *Necturus* gallbladder epithelium. Dashed line indicates emf's operating across the cell membranes, solid line the observed PD's

shunt is that the PD across the mucosal membrane is -53.2 mV (ψ_{MC}) instead of -15 mV (E_M), while ψ_{CS} is $+55.8$ instead of $+81$ mV (E_S) and the transmural PD is $+2.6$ instead of $+66$ mV (E_M+E_S). Obviously the extracellular shunt favors passive entry of Na into the cell across the mucosal membrane since ψ_{MC} is three times more negative than E_M.

An increase in Na-influx will also lead to an increase in the rate of active Na extrusion, when the Na transport mechanism in the baso-lateral membrane is not yet saturated. In frog skin it has been shown that unidirectional uptake of Na across the outer surface of frog skin increases, when the transmural potential decreases (Biber & Sanders, 1973). The reduction in the transmural potential and the PD across the baso-lateral membrane as a consequence of the shunt, may also be favorable for active transport of Na across the gallbladder wall. In frog skin and in toad bladder epithelium the rate of net Na transport and oxygen consumption increases with decreasing transmural PD's, serosal side less positive (Nellans, 1971; Vieira, Caplan & Essig, 1972; Mandel & Curran, 1973). Moreover, Spring and Paganelli (1972) have demonstrated a close relationship between the rate of net Na transport and the direction and magnitude of the transtubular PD in *Necturus* proximal tubules. From these experiments one may postulate that a physiological consequence of the presence of an extracellular shunt is an amplification of transepithelial Na transport. Support for this postulate may be derived from experiments with substances which block the extracellular route. Recently, Moreno (1974) reported that Tri-amino-Pyrimidine is able to reduce the cation conductance of the shunt but the tissue resistance is only increased with a factor of 2.

The calculated transmural PD shown in Fig. 2 ($+2.6$ mV) is higher than our observed mean value for ψ_{MS} ($+1.4\pm0.4$ mV) but, however, identical to the value reported by Frömter (1972) ($+2.5\pm3.1$ mV). Hence in *Necturus* gallbladder, ψ_{MS} might be due to differences in E_M and E_S. For gallbladders of other species (man, monkey, goose) larger serosal positive PD's have been reported by Rose, Gelarden and Nahrwold (1973). To explain these values from differences in E_M and E_S, lower values for the ratio P_K/P_{Na} of the mucosal membrane are needed, which make E_M values less negative. On the other hand, a higher P_K/P_{Na} ratio makes E_M more negative and would explain lower transmural PD's, as reported in rabbit and guinea pig gallbladders (Rose *et al.*, 1973). An opposing emf will be generated across the shunt path by local NaCl gradients across the cation-selective tight junctions, tending to make the serosal side negative (Machen & Diamond, 1969). In addition to differences

in E_M and E_S and the presence of E_L during active transport the possibility exists that in some species electrogenic Na transport contributes to the transmural PD, since in human gallbladders the serosal positive PD is very sensitive to temperature changes (Gelarden & Rose, 1974). Recently, Bindslev, Tormey and Wright (1974) demonstrated that the transmural PD of bullfrog gallbladder is highly dependent on stirring of the mucosal solution. In view of these possible origins of transmural PD's it will be difficult to make statements about ion transport mechanisms in gallbladder epithelium which are based on the sign and magnitude of the transmural PD.

An important conclusion from our study is the fact that the ionic permeability of the cellular route in gallbladder epithelium is compatible with Ussing's model for transepithelial Na transport (Ussing, 1960). Na can enter into the cell passively across the apical membrane and can be extruded actively via a Na-K exchange pump in the baso-lateral membrane. The presence and properties of (Na-K)-activated ATPase in gallbladder epithelium have been described (Van Os & Slegers, 1971). An important question is, to what extent can passive Na influx account for the observed net salt flux. To answer this question we have measured fluid transport of two *Necturus* gallbladders in Cl-Ringer's solution. The mean value is 10.4 μl/hr cm^2 which corresponds to a net Na transport of 1.1 μEquiv/hr cm^2 assuming that fluid transport is isotonic. The passive Na influx (I_{Na}) can be calculated from, $I_{Na} = G_{Na}(\psi_{MC} - E_{Na})$, where G_{Na} is the sodium conductance of the mucosal membrane and E_{Na} the Nernst potential for the Na distribution. The upper limit for the Na-conductance of the mucosal membrane will be $1/R_M$. Taking $Na_C = 33.5$ mM (Table 4), $R_M = 4130\ \Omega\ cm^2$ (Table 3) and $\psi_{MC} = -53.2$ mV, the upper limit for passive Na influx will be 0.7 μEquiv/hr cm^2. Hence passive Na influx accounts maximally for 64% of net active Na flux.

Transcellular transport of Cl ions is difficult to explain. We have demonstrated that Cl conductance is at least very small in the apical and baso-lateral membrane. Interesting, therefore, is a recent report by Dugas and Frizzell (1974) that part of the Na flux across the mucosal membrane of rabbit gallbladder is coupled in a 1:1 ratio with Cl ions. It is possible that in our experiments a small Cl conductance of the baso-lateral membrane remains unnoticed in ion substitution experiments on the serosal side due to the large unstirred layers. A small Cl permeability has been demonstrated in peritubular membranes of proximal tubules in *Necturus* (Boulpaep, 1971; Frömter, Müller & Wick, 1971). Cl efflux across baso-lateral membrane is with a net driving force but even if we assume that all the

serosal membrane conductance is a Cl conductance, the Cl efflux will not exceed 0.4 μEquiv/hr cm^2 ($I_{Cl}=1/R_S\ (\psi_{CS}-E_{Cl})$, Cl concentration in the cell assumed to be equal to $Na_C=33.5$ mM, $R_S=2870\ \Omega\,cm^2$ and $\psi_{CS}=-55.8$ mV). Therefore the Cl-conductance of the baso-lateral membrane is far too low to allow electrogenic coupling of Cl ions with active Na transport; hence Cl transport across this membrane must be either neutrally coupled with Na or independently transported via a hitherto unknown mechanism.

In gallbladders of species with serosal positive PD's, passive Cl movement may primarily occur extracellularly. This would be similar to Cl transport in frog skin (Mandel & Curran, 1972) and in proximal tubules of rabbit (Orloff & Burg, 1971), and rat (Frömter, Rumrich & Ullrich, 1973). As a conclusion it can be said that the mode of anion transport in gallbladder epithelium is completely obscure. Elucidation of this problem needs further investigation with particular attention on the transport processes in the baso-lateral membrane.

Note Added in Proof: Since this manuscript has been submitted two abstracts on electrical measurements in *Necturus* gallbladder have been published (Reuss & Finn, 1975*a*, *b*). The results of these authors agree well with those reported above.

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