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Mechanisms of Voltage Transients During Current Clamp in *Necturus* Gallbladder

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Summary. Microelectrode techniques were employed to study the mechanisms of the transepithelial voltage transients (ΔV_{ms}) observed during transmural current clamps in the isolated *Necturus* gallbladder. The results indicate that: a) part of ΔV_{ms} is due to a transepithelial resistance change (ΔR_t), and part to a tissue emf change. b) ΔR_t is entirely caused by changes of the resistance of the paracellular pathway. At all current densities employed, the measured changes are probably due to changes in both fluid conductivity and width of the lateral intercellular spaces. At high currents, in addition to the effects on the lateral spaces, the resistance of other elements of the pathway (probably the limiting junction) drops, regardless of the direction of the current. c) The magnitude and polarity of the ΔR_t-independent transepithelial and cell membrane potential transients indicate that the largest emf change takes place at the basolateral membrane (ΔE_b), with smaller changes at the luminal membrane (ΔE_a) and the paracellular (shunt) pathway (ΔE_s). It is shown that two-thirds of the transient are caused by ΔE_s, and one-third by $\Delta(E_b - E_a)$. ΔE_s can be explained by a diffusion potential generated by a current-dependent NaCl concentration gradient across the tissue. ΔE_a and ΔE_b are caused by [K] changes, mainly at the unstirred layer in contact with the basolateral membrane.

In many salt-transporting epithelial preparations studied *in vitro* transmural dc pulses produce time-dependent voltage transients and nonlinear current-voltage relationships (Wedner & Diamond, 1969; Candia, 1970; Civan, 1970; Kidder & Rehm, 1970; Noyes & Rehm, 1971; Yonath & Civan, 1971; Helman & Miller, 1973; Spring, 1973*a*, *b*; Bindslev, Tormey, Pietras & Wright, 1974*a*; Bindslev, Tormey & Wright, 1974*b*).

Bindslev *et al.* (1974*b*) have observed that when a transepithelial constant current pulse is applied across the *in vitro* frog gallbladder, transmural voltage transients take place with three different half-times:

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of less than 1 msec (related to the electrical capacitance of the tissue), of the order of seconds (related to ion concentration changes in unstirred layers) and of the order of minutes (related to transepithelial electrical conductance changes). Neglecting the capacitive transient, when the gallbladder is exposed to standard amphibian Ringer's solution on both sides, inward (mucosa to serosa, *m* to *s*) current tends to generate a voltage transient of mucosa-negative polarity (i.e., during the pulse, the current-induced voltage *drops* with time) and a decrease of transepithelial electrical resistance (R_t). Outward (serosa to mucosa, *s* to *m*) current also produces a voltage transient of mucosa-negative polarity (i.e., during the pulse the current-induced voltage *increases* with time) but an increase of R_t. When the current density is large enough, during outward pulses R_t briefly drops and then increases. The voltage transients are conceivably caused by both the changes of tissue equivalent emf ("polarization") and total conductance. Since it has been demonstrated that the intercellular pathway accounts for more than 90% of the tissue conductance in gallbladder, Bindslev *et al.* (1974*a*) have attributed both the polarization phenomena and the resistance changes observed during current passage to changes at the paracellular (shunt) pathway.

The experiments reported here were designed to study the relative contributions of the shunt pathway and the membranes of the epithelial cells to these time dependent changes in emf and conductance in *Necturus* gallbladder. Methods previously described (Reuss & Finn, 1975*a*, *b*) allow us to calculate the electrical resistances and equivalent emf's of the cell membranes and the shunt pathway. Utilizing these techniques, it is possible to measure the contribution of each element of the circuit (cell membranes and shunt) to transepithelial polarization and conductance changes. Furthermore, the results from experiments performed with standard Ringer's solution bathing both sides of the tissue can be contrasted with those obtained after selective alterations of one or more elements of the equivalent circuit.

Part of this work has been published in abstract form (Reuss & Finn, 1976).

Materials and Methods

Necturi (*Necturus maculosus*) obtained from Mogul-Ed Co., Oshkosh, Wisconsin, were anesthetized, and the gallbladder was removed and mounted as previously described. Control determinations were started at least 30 min after mounting the tissue, if the preparation satisfied the criteria (transepithelial resistance, ion selectivity) previously described (Reuss & Finn, 1975*a*, 1977).

Bathing Solutions

Standard Ringer's solution had the following composition (mM): NaCl 109.2, KCl 2.5, $NaHCO_3$ 2.38, $CaCl_2$ 0.89, pH 7.8, gassed with room air. In particular experiments, the composition of one or both bathing solutions was changed as follows: (a) complete or partial isomolar replacement of Na with K or choline; (b) addition of sucrose to standard Ringer's, approximately to double the osmolality; (c) addition of $LaCl_3$ (1–2 mM) to Ringer's solution titrated with concentrated HCl to pH 6.8–7.0.

Electrical Measurements

Transepithelial. The transepithelial potential (V_{ms}) was measured as the PD between two Ag—AgCl electrodes (connected to the bathing solutions by Ringer's-agar bridges) with a 602 A electrometer (Keithley Instruments, Cleveland, Ohio), or with an M4A electrometer (WP Instruments, Hamden, Connecticut), and displayed on a storage oscilloscope (Tektronix Inc., Beaverton, Oregon). The serosal electrode was usually the reference.

The transepithelial resistance (R_t) was calculated from the transepithelial voltage change (ΔV_{ms}) produced by a calibrated transmural dc pulse, provided by a constant current source and applied through Ag—AgCl electrodes connected to the bathing media with Ringer's agar bridges. ΔV_{ms} was read from the pseudo steady-state value within 20 msec of the start of the current pulse. In some experiments, R_t changes were followed second to second by superimposing short dc pulses on the transepithelial current clamp (e.g., Fig. 3).

Intracellular. Microelectrodes (ME's) were prepared by pulling (two stage puller, Industrial Science Associates, Ridgewood, New Jersey) 1 mm OD, 0.6 mm ID borosilicate glass (Drummond Scientific Co., Broomall, Pennsylvania) previously threaded with fiberglass. Immediately after pulling, the micropipettes were filled with 4 M potassium acetate (with a thin needle), and beveled (Brown & Flaming, 1974) to a tip impedance of 12 to 25 MΩ. The impedance before beveling was usually 20 to 40 MΩ. Experiments in which 3 M KCl ME's were employed in the same preparations did not yield significant differences in the cell potentials.

Cellular impalements were performed always from the mucosal solution by means of mechanic micromanipulators, under visual control with a phase contrast inverted microscope (Diavert, Leitz Wetzlar, West Germany) at 200 or 320×. The criteria for appropriate impalement have been previously described (Reuss & Finn, 1975*a*). Stable cell potentials were obtained in at least 90% of the attempts.

Intracellular current pulses of 1 to 2×10^{-8} A, and up to 1 sec duration were applied from a Grass SD9 or S-48 stimulator (Grass Instruments, Quincy, Massachusetts), through the M4A electrometer. The voltage changes elicited in other cells were recorded with a second ME, as a function of the interelectrode distance.

Circuit Analysis. The equivalent circuit represented in Fig. 1*A* was analyzed by the measurements of: (a) the potentials across the luminal membrane (V_{mc}), the basolateral membrane (V_{cs}), and the whole tissue (V_{ms}), and (b) the resistances of the cell membranes (apical: R_a, basolateral: R_b) and the shunt pathway (R_s). The three resistances were calculated from: R_t, R_a/R_b, and cable analysis of the epithelial sheet (Eisenberg & Johnson, 1970; Frömter, 1972; Reuss & Finn, 1975*a*). From the values of the potentials and resistances, equivalent emf's were calculated as described before (Reuss & Finn, 1975*a, b*).

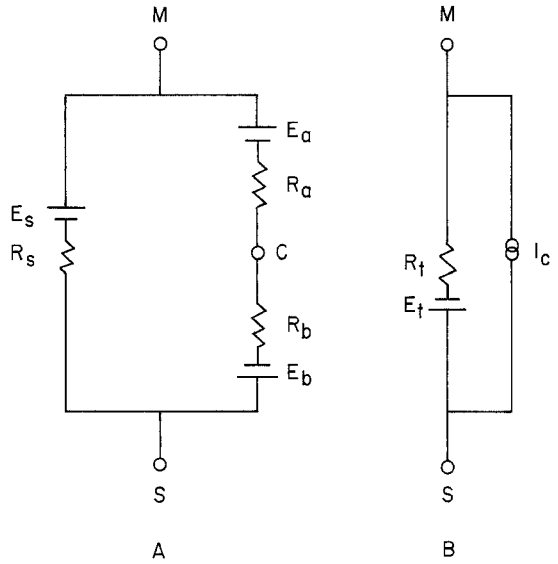

Fig. 1. Equivalent electrical circuits for *Necturus* gallbladder epithelium. M, C and S represent the mucosal solution, the cell, and the serosal solution, respectively. (*A*) Each element of the circuit is represented, according to Thevenin's theorem, by an equivalent emf (*E*'s) in series with an equivalent resistance (*R*'s). The subscripts *a*, *b* and *s* refer to the apical membrane, basolateral membrane, and shunt pathway, respectively. The polarities of E_a and E_b are based on previous data (Reuss & Finn, 1975*a*). Mean values in the experiments reported here (if E_s is assumed to be 0) are: $R_a = 4010$, $R_b = 3040$, $R_s = 370\,\Omega\,cm^2$; $E_a = 34$, $E_b = 73$ mV. V_{mc} is 55 mV, V_{cs} 57mV and V_{ms} 2 mV. (*B*) The whole epithelium is represented by a single Thevenin equivalent. $R_t^{-1} = (R_a + R_b)^{-1} + R_s^{-1}$, and E_t is a function of all emf's and resistances in the circuit. I_c is an externally applied transepithelial current *(See text)*

Estimation of the Contribution of Tissue Conductance Changes to Current-Induced Voltage Transients

To distinguish between the contribution of R_t changes and tissue emf changes to the transepithelial voltage transients during the clamps, the simplified equivalent circuit shown in Fig. 1*B* was analyzed. V_{ms} changes during a *constant* transepithelial current can result from changes of R_t (the total transepithelial resistance), E_t (the equivalent emf of the tissue), or both (see Fig. 1*B*). R_t was measured by superimposing short dc pulses on the longer lasting clamp (*see above*). Therefore, R_t changes could be followed during the clamp, their contribution to ΔV_{ms} being

$$\Delta V_{ms}^{R_t} = \Delta R_t \cdot I_c \tag{1}$$

where ΔR_t is the transepithelial resistance change during the clamp, and I_c is the clamp current density. The duration of the superimposed pulses was short enough to avoid R_t changes during the pulses themselves.

The difference between the observed ΔV_{ms} during the clamp (ΔV_{ms}^{obs}) and $\Delta V_{ms}^{R_t}$ is equal to the E_t-dependent change of V_{ms} ($\Delta V_{ms}^{E_t}$):

$$\Delta V_{ms}^{E_t} = \Delta V_{ms}^{obs} - \Delta V_{ms}^{R_t}. \tag{2}$$

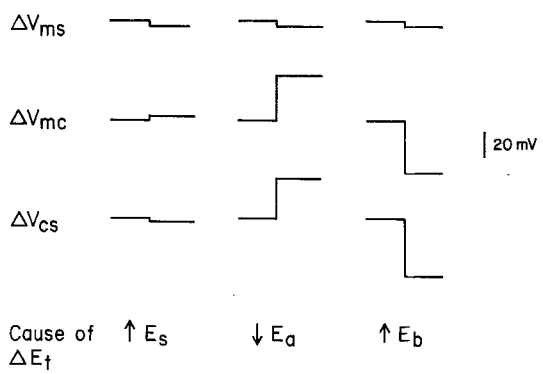

Fig. 2. Computation of magnitudes and polarities of cell membrane potential changes for a 5 mV change of V_{ms} (hyperpolarization), if ΔV_{ms} is produced by the alteration of *only one* of the emf's of the tissue. ΔV_{mc} and ΔV_{cs} were calculated from the mean control resistances (R_a, R_b and R_s) solving the equation $\Delta V_{ms}=[\Delta E_s(R_a+R_b)+\Delta(E_b-E_a)R_s]/(R_a+R_b+R_s)$, and inserting each computed ΔE into the analogous equations for ΔV_{mc} and ΔV_{cs}. Note that each emf change (lower line) produces a unique combination of polarities of ΔV's. In addition, ΔV_{mc} and ΔV_{cs} are far greater than ΔV_{ms} if E_a or E_b change. If E_s changes, ΔV_{ms} is larger than ΔV_{mc} and ΔV_{cs}

From these measurements it is possible to establish approximately what portion of ΔV_{ms}^{obs} is attributable to tissue conductance or tissue emf changes. This approach is an oversimplification, since resistance changes *per se* involve changes in V_{ms}. During the current clamp

$$V_{ms}=[(E_b-E_a)R_s+E_s(R_a+R_b)+I_c(R_a+R_b)R_s]/(R_a+R_b+R_s) \tag{3}$$

and thus if R_s is altered V_{ms} changes, independently of I_c and at constant E's. It can be shown, however, that the error involved in the use of Eqs. (1) and (2) is less than 10%.

Identification of the Site(s) of Tissue emf Change

Once the ΔE_t-dependent ΔV_{ms} value has been calculated, the site(s) of E_t change can be identified from the consideration of the circuit depicted in Fig. 1*A*. Because of the widely different resistance values, and because of the orientation of the emf's, changes in a *single emf* yield unique combinations of polarity and magnitude of the changes of V_{mc} V_{cs} and V_{ms} (*see* Fig. 2). Therefore, the magnitude and polarity of V_{ms}, V_{mc} and V_{cs} transients were determined during current clamps in either direction.

Modifications of the Composition of the Bathing Solutions

In some experiments, transepithelial and intracellular voltage transients were compared before and after modifying the composition of one or both bathing media. High serosal K was employed to test the hypothesis that the intracellular transients are caused by K concentration changes in the basolateral unstirred layer. Low Na mucosal solution (choline replacement) and hyperosmotic mucosal solution (sucrose addition) were used to increase R_s, while producing opposite effects on R_a (increase and decrease, respectively; Reuss & Finn, 1975*a*, 1977). From these experiments, the relative contributions of shunt and cell membranes to the R_t-independent voltage transient were calculated.

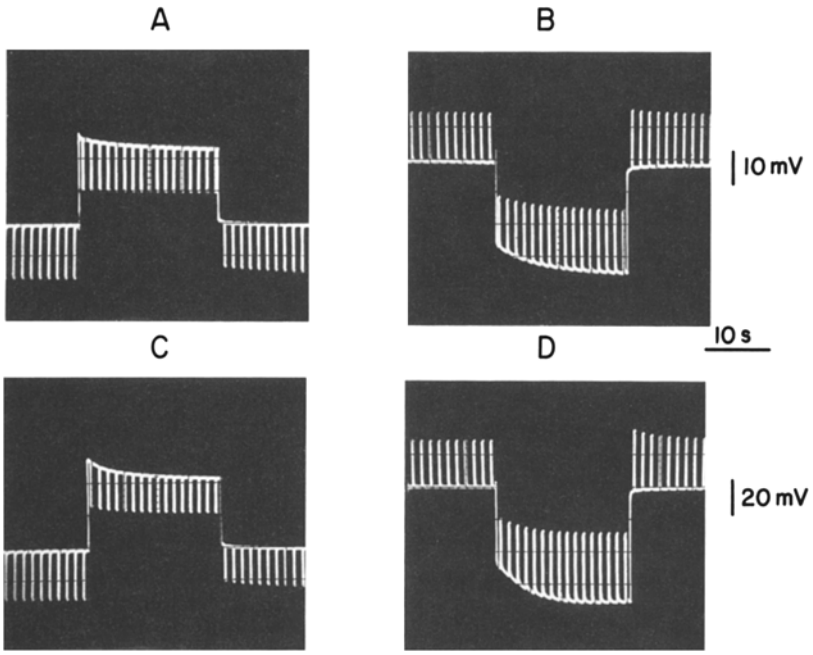

Fig. 3. Typical records of V_{ms} and R_t during transepithelial current clamps. Panels *A* and *B*, clamp current (I_{clamp}) = 50 μA/0.6 cm^2; panels *C* and *D*, I_{clamp} = 100 μA/0.6 cm^2. *A* and *C* are *m* to *s* (inward) clamps, and *B* and *D* are *s* to *m* (outward) clamps. All traces start with V_{ms} = 2.1 mV (mucosal solution negative), and R_t = 270 Ω cm^2. The short, superimposed pulses were employed to measure R_t about every second before, during, and after the clamp *(See text)*

Results

Transepithelial potentials and resistances did not differ significantly from those reported by Frömter (1972), Reuss and Finn (1975*a*, *b*, 1977), and van Os and Slegers (1975). V_{ms} ranged from 1.2 to 4.3 mV, mucosal solution negative, and R_t from 220 to 430 Ω cm^2.

Transepithelial Voltage Transients During Current Clamps

Inward (mucosa-positive, or m to s) current clamps were applied in the range of 40 to 320 μA cm^{-2}, for periods of up to 1 min. Typical records at two current densities are shown in Fig. 3. At currents smaller than ca. 100 μA cm^{-2}, the change of V_{ms} during the clamp is small, and its polarity can be either in the same direction as the initial deflection produced by the clamp (hyperpolarizing transient) or, as shown in the

Table 1. R_t changes as a function of transepithelial current density and direction

Current ($\mu A/0.6\,cm^2$)	$\Delta R_t(\Omega\,cm^2)$	
	m to *s* clamps	*s* to *m* clamps
25	-6 ± 3	$+14\pm3$
50	-16 ± 4	$+29\pm5$
100	-34 ± 7	$+51\pm9$
200	-49 ± 8	$+57\pm23$

Resistances were measured immediately before, and 30 sec after the onset of the current clamp. $n=7$ experiments. Means $\pm$ SEM.

Figure, in the opposite direction (depolarizing transient). At higher current densities, ΔV_{ms} always drops (depolarizing transient) during the clamp, roughly in proportion to the applied current. A decrease of R_t during the clamp was always observed at currents greater than $80\,\mu A\,cm^{-2}$ (Fig. 3). This change persisted immediately after turning the clamp off and reversed in a period ranging from about 20 sec to about 2 min. Very large currents required longer recovery periods or produced irreversible R_t drops. Within the reversible range, ΔR_t was roughly linear with the current density.

V_{ms}, immediately after turning the clamp off, was several mV more positive with respect to the serosal solution. This change cannot be attributed to the R_t drop, because at most V_{ms} would become 0, and not reverse, as observed in this and other experiments. Therefore, a polarization phenomenon (E_t change) has taken place during the clamp. ΔE_t is such as to tend to make the mucosal solution positive during *m* to *s* current flow.

Outward (mucosa-negative, or s to m) current clamps produce hyperpolarizing transients, i.e., ΔV_{ms} during the clamp has the same polarity as the initial current-induced voltage deflection. In the two records shown in Fig. 3 it can be seen that R_t increases during the clamp, both from the height of the short pulses and from the comparison of the "instantaneous" deflections at the start and end of the period of current passage. R_t returns slowly towards its control value after the current is off. As shown in Table 1, the change of R_t is roughly proportional (within the range of $+200$ to $-200\,\mu A/0.6\,cm^2$) to the applied current. The value of V_{ms} immediately after the end of the clamp is several mV more negative than in the pre-clamp period, indicating a mucosa-negative E_t change.

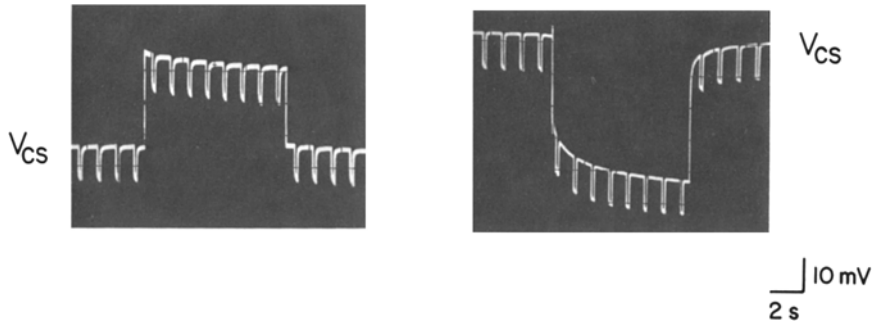

Fig. 4. Records of basolateral membrane potential changes (brief deflections) elicited by intracellular current passage before, during, and after transepithelial *m* to *s* (left) or *s* to *m* (right) current clamps. The baseline value of V_{cs} was 62 mV. $I_{clamp} = 60\,\mu A/0.6\,cm^2$. Intracellular current: 10^{-8} A. Interelectrode distance ca. 25 µm. Note that the cell potential changes elicited by intracellular current application are unchanged throughout the records

The difference between pre- and post-clamp V_{ms} values was always larger in *s* to *m* than in *m* to *s* clamps (in the same preparation, at the same current densities). As observed previously by Bindslev *et al.* (1974*b*), large *s* to *m* currents produce a short-lived decrease of transepithelial resistance, followed by a slow increase. If the current density is increased further, the increase of R_t is obliterated, and the drop becomes irreversible.

In order to get reproducible results in the experiments described above, a reasonable delay between clamps had to be allowed, because the R_t changes (and perhaps the E_t changes as well) persist sometimes for rather long periods after the clamp is off.

Mechanism of the Change of Transepithelial Resistance

The transmural resistance of the tissue lies in the epithelium itself (subepithelial contribution $\leqq 5\%$), and according to Fig. 1*A* corresponds to

$$R_t = [(R_a + R_b)\, R_s]/(R_a + R_b + R_s). \tag{4}$$

R_a is about 4, R_b 3, and R_s 0.4 kΩ cm^2; therefore R_s dominates the transepithelial resistance. Thus, the changes of R_t during the clamps have to depend almost exclusively on changes of R_s. To confirm this hypothesis, experiments were carried out to estimate cell membrane resistance changes during current clamps. Intracellular current was applied

through a microelectrode, and the membrane potential change recorded in another cell with a second microelectrode, before, during, and after transepithelial *m* to *s* and *s* to *m* clamps. A typical example of such an experiment is shown in Fig. 4. The voltage deflections produced by intracellular current remained unchanged during and after the clamp. Repeated observations in several preparations yielded the same results, regardless of the polarity of the intracellularly applied current, the distance between the current-passing and voltage-sensing electrode, or the magnitude of the transepithelial current clamp. In addition, the ratio of resistances of the cell membranes (R_a/R_b) remains essentially unchanged during transmural current passage.

Mechanism of the Change of Transepithelial Equivalent emf (Polarization)

To find out which emf or which combination of emf's of the circuit change during the current clamps, the potentials across the apical and basolateral membrane were recorded continuously before, during and after transepithelial clamps of current similar to those employed for transepithelial studies. Our reasoning, as explained in Materials and Methods, was that if only one of the three emf's (E_a, E_b or E_s) changes, the set of effects on V_{mc}, V_{cs} and V_{ms}, in terms of direction and polarity, will be specific (*see* Fig. 2). Conversely, the pattern of changes of transepithelial and cell membrane potentials should indicate which one is the quantitatively predominant change if two or more emf's vary.

The result of this experiment is illustrated in Fig. 5. The four panels show effects of *m* to *s* or *s* to *m* clamps on V_{mc} and V_{cs} transients produced by superimposed inward or outward pulses. These and several other observations consistently revealed the following features: (1) V_{mc} and V_{cs} transients are more prominent during outward (lower panels) than during inward (upper panels) transepithelial current clamps, and the effects of the pulses on the cell potentials follow the same pattern. (2) An inward (*m* to *s*) current clamp abolishes the hyperpolarizing transients produced by outward (*s* to *m*) short pulses (panel *B*). (3) Outward current clamps enhance the magnitude of V_{mc} and V_{cs} transients produced by outward short pulses (panel *D*), and induce depolarizing V_{mc} and V_{cs} transients when the short pulses are applied in the inward direction (panel *C*). (4) Appreciable V_{mc} and V_{cs} transients produced by the clamp or the short, superimposed pulses are always in the same direction (e.g.,

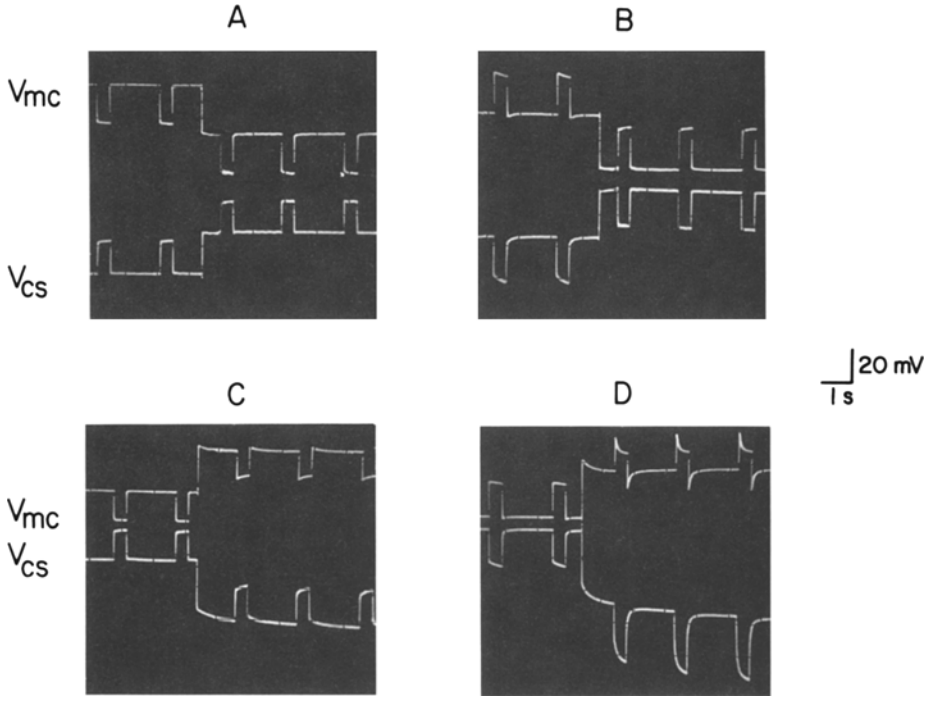

Fig. 5. Cell membrane potential transients ($\Delta V_{mc}, \Delta V_{cs}$) during transepithelial constant current clamps with superimposed short d.c. pulses. All records were obtained in the same cell of a preparation bathed bilaterally with standard Ringer's solution. Control parameters: $V_{ms} = 2.5$ mV, $V_{mc} = 56$ mV, $V_{cs} = 58.5$ mV, $R_t = 360\,\Omega\,\text{cm}^2$, $I_{\text{clamp}} = 100$, $I_{\text{pulse}} = 55\,\mu\text{A}/0.6\,\text{cm}^2$. Panels *A* and *B*: *m* to *s* clamp; panels *C* and *D*: *s* to *m* clamp. Short current pulses were applied, in addition to the clamp, inwards (*A*, *C*) or outwards (*B*, *D*)

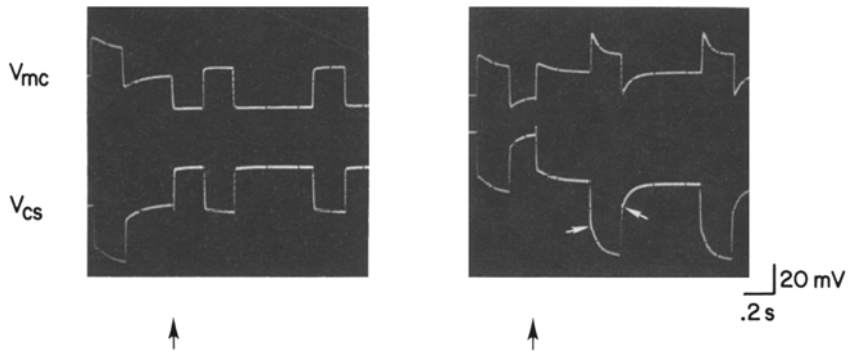

Fig. 6. V_{mc} and V_{cs} records before and during $50\,\mu\text{A}/0.6\,\text{cm}^2$ current clamps, in the *m* to *s* direction (left) or in the *s* to *m* direction (right). Start of the clamps indicated by lower arrows. Short, superimposed transepithelial pulses allow one to distinguish the start of the hyperpolarizing transient of V_{cs} and its higher value immediately after the pulse is off (first and second white arrows). In the first pulse during the *s* to *m* clamp, $\Delta V_{mc} = 13$, and $\Delta V_{cs} = 19$ mV

panels *B* and *D* before clamp, panel *D* after clamp). (5) Within the short time in which these records were taken, transepithelial resistance changes $[(\Delta V_{mc}+\Delta V_{cs})/I_c]$ are small or absent. Therefore, the direction of the V_{mc}, V_{cs} and V_{ms} transients (*see* Fig. 2) indicates that the *largest* emf change during the clamps takes place at the basolateral membrane. During outward current, V_{ms} and V_{cs} increase. Since V_{ms} also increases, and $V_{ms}=V_{cs}-V_{mc}$, $\Delta V_{cs}>\Delta V_{mc}$. When clamps applied in the opposite direction generate discernible transients, these are characterized by falls of V_{mc}, V_{cs} and V_{ms}. Again, $\Delta V_{cs}>\Delta V_{mc}$. The difference between ΔV_{mc} and ΔV_{cs} during *s* to *m* clamps is demonstrated in a faster record in Fig. 6. It should be noted that the time course of the cell potential changes is much faster than that of the change of transepithelial potential.

Mechanism of the E_b Changes During Transepithelial Current Clamps

The available information about the ionic selectivity of the basolateral membrane of gallbladder epithelial cells indicates that this membrane *behaves* as a K electrode both in *Necturus* (Reuss & Finn, 1975*b*; van Os & Slegers, 1975) and rabbit (Hénin & Cremaschi, 1975). If the membrane is in fact highly K-permselective, a likely explanation of the changes of E_b during the clamps would be K accumulation or depletion (according to the direction of the current) in the unstirred layer in contact with the basolateral membrane. To test this hypothesis, the concentration of K in the serosal solution was changed. If our interpretation is correct, we would predict that lowering $[K]_o$ should increase, and raising $[K]_o$ should decrease the voltage transients. The first experiment (low $[K]_o$) did not yield clear-cut results. Small reductions of $[K]_o$ did not alter the transients significantly, and decreases to less than 1 mM produced irreversible drops of cell potential. Increases of K concentration in the serosal solution produced the dramatic effects illustrated in Fig. 7. ΔV_{mc} and ΔV_{cs} disappear when the preparations are exposed to K-Ringer's on the serosal side, and the effect is reversible upon replacement of Na-Ringer's. The resistance changes during the clamp, although diminished, were present during exposure to serosal K-Ringer's.

Contribution of E_s and/or E_a Changes to ΔE_t During Transmural Current Clamps

Information on the contribution of ΔE_s to the voltage transients was obtained from experiments in which the magnitude of ΔE_s was modified

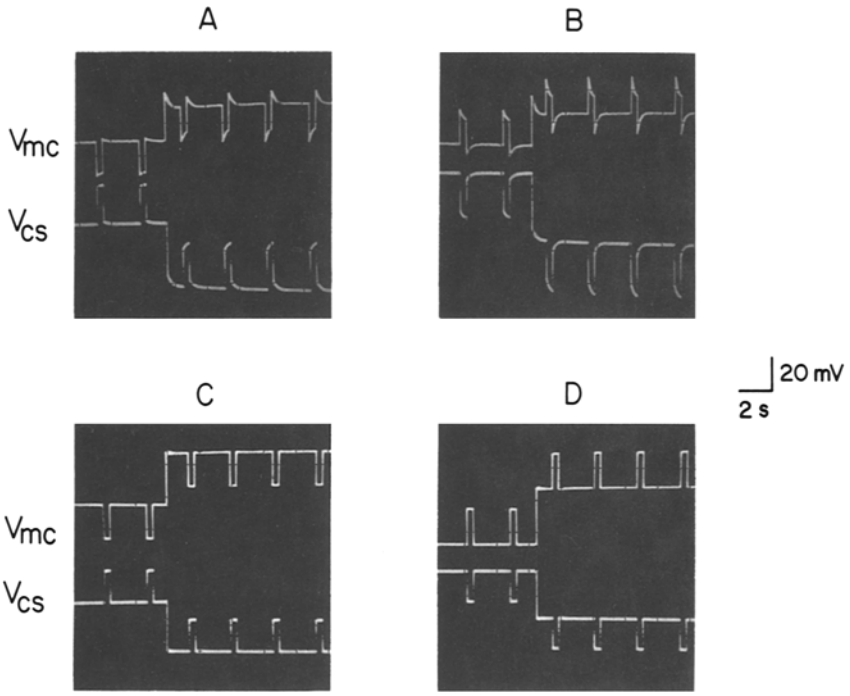

Fig. 7. Effect of high-K serosal bathing solution on V_{mc} and V_{cs} transients during *s* to *m* transepithelial current clamps. *A* and *B*: control; *C* and *D*: K-Ringer's on the serosal side. Note that the direction of the superimposed pulses is *m* to *s* in panels *A* and *C*, and *s* to *m* in panels *B* and *D*. V_{ms} was 3.0 mV (mucosa negative) under control conditions, and 2.7 mV (mucosa positive) after exposure to K-Ringer's. R_t values were 250 and 170 $\Omega\,cm^2$, respectively. V_{mc} was 61 mV in Na-Ringer's and 2 mV in K-Ringer's (cell negative). Note that in K-Ringer's the transients across both cell membranes are abolished. I_{clamp} was 100 $\mu A/0.6\,cm^2$ in Ringer's. Clamp and short pulse current densities in K-Ringer's were adjusted to yield voltage deflections similar to control

by an experimental alteration of the permselectivity of the shunt. In a control period, V_{ms}, R_t, V_{mc} and V_{cs} were measured before, during and after current clamps. Then, $LaCl_3$ was added to the mucosal bathing medium to a final concentration of 2 mM. As observed by others (Wright & Diamond, 1968; Machen, Erlij & Wooding, 1972), the transepithelial resistance rose, and P_{Na}/P_{Cl} across the shunt decreased, and usually reversed, as indicated by a reduction or reversal of a 2:1 NaCl transepithelial dilution potential. After these effects were stable, the transepithelial and cellular measurements were repeated. As expected from the reversal of P_{Na}/P_{Cl}, the resistance changes during exposure to lanthanum are opposite to these observed under control conditions; i.e., R_t rises during *m* to *s* clamps and drops during *s* to *m* clamps.

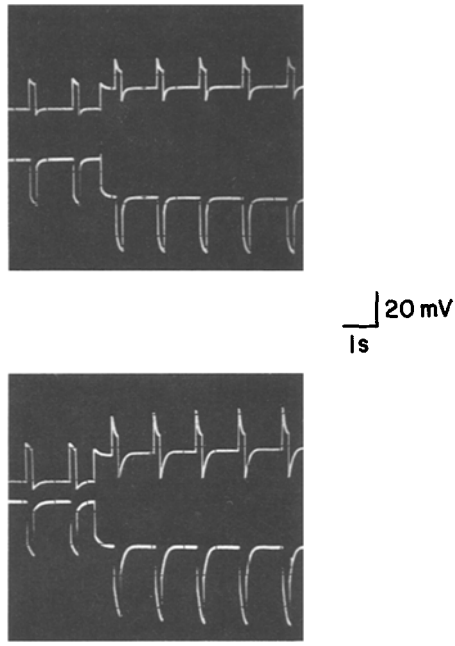

Fig. 8. Effect of La addition to the mucosal solution (final concentration 2 mM) on V_{mc} and V_{cs} transients during *s* to *m* current clamps. Upper panel: control in Ringer's. Lower panel: record 1 hr after exposure to La. R_t was 260 Ω cm^2 under control conditions and 410 Ω cm^2 in La. The upper records of each panel correspond to V_{mc}, and the lower ones to V_{cs}. I_{clamp} was 80 μA/0.6 cm^2 in control, and 50 μA/0.6 cm^2 in La. Note that, in spite of the smaller current, V_{mc} and V_{cs} transients are more prominent in La, before and during the clamp

In contrast, $\Delta V_{ms}^{E_t}$ does not reverse: 5.9 $\pm$ 0.7 mV (control), and 5.2 $\pm$ 1.2 mV (La), for 100 μA cm^{-2} outward pulses ($n=5$, no significant difference). ΔE_s would be expected to have the same polarity before and after exposure to La, because the ion will reverse both the side of current-induced NaCl accumulation and the polarity of the resulting diffusion potential. The experiment is complicated because La increases R_s. If ΔE_s were the sole cause of $\Delta V_{ms}^{E_t}$ during the clamps, in the presence of La $\Delta V_{ms}^{E_t}$ should drop, and the cell membrane transients should drop as well. The experimental observations are no change of the transepithelial transient and increases of the cellular transients (Fig. 8). These results indicate that in La the contribution of changes of E_b and/or E_a to $\Delta V_{ms}^{E_t}$ is increased. A comparison of the polarization potentials before and after La allows us to calculate the contribution of cellular and shunt emf's to $\Delta V_{ms}^{E_t}$ (*see* Discussion).

Table 2. $\Delta V_{ms}^{E_t}$ as a function of transepithelial current density and direction

Current ($\mu A/0.6\,cm^2$)	$\Delta V_{ms}^{E_t}$(mV)		P
	m to s clamps	s to m clamps	
25	-1.0 ± 0.3	1.2 ± 0.3	NS
50	-2.3 ± 0.5	2.9 ± 0.5	NS
100	-3.9 ± 0.5	5.5 ± 0.7	<0.001
200	-5.8 ± 0.8	10.0 ± 1.2	<0.001

Clamp duration = 30 sec. $n=7$ experiments. Means $\pm$SEM. Differences between $|\Delta V_{ms}^{E_t}|$, dependent on the polarity of the clamp, were analyzed by t-test for paired samples.

Table 3. Effects of Na-choline substitution or sucrose addition to the mucosal solution on $\Delta V_{ms}^{E_t}$

		+50	−50	+100	−100	+200	−200
$\Delta V_{ms}^{E_t}$ (mV)	Control	−3.0	2.5	-3.5 ± 0.9	4.3 ± 1.1	-5.5 ± 1.7	9.4 ± 2.6
	Sucrose	−6.4	6.2	-9.0 ± 0.8	11.2 ± 0.9	-12.2 ± 1.3	20.0 ± 1.6
	n	2	2	5	5	5	5
$\Delta V_{ms}^{E_t}$ (mV)	Control	−3.0	2.5	-4.8 ± 0.9	6.9 ± 1.7	-6.4 ± 1.5	9.4 ± 3.7
	Choline	0.0	4.9	$+1.2\pm0.7$	14.1 ± 3.4	0.0 ± 0.9	18.2 ± 1.2
	n	2	2	4	4	3	3

Clamp duration = 30 sec. First line indicates current density ($\mu A/0.6\,cm^2$) and polarity ($+ = m$ to s; $- = s$ to m). Means $\pm$ SEM.

Sucrose addition to the mucosal solution has been shown to increase R_s and decrease R_a. The cell potential drops and the luminal membrane undergoes a nonselective increase in monovalent ion permeability (Reuss & Finn, 1977). During exposure to a mucosal hyperosmotic medium, a larger fraction of the applied transepithelial current flows through the cells, and a smaller fraction through the shunt (because R_s is higher and (R_a+R_b) lower than control). On this basis, one would expect ΔE_s to fall and ΔE_b to rise. Again, if ΔE_s were the sole cause of $\Delta V_{ms}^{E_t}$, the value of the latter should fall in hyperosmotic medium as compared to control. Table 2 shows that, on the contrary, transepithelial polarization is enhanced. Intracellular records show, as with La, that the cellular transients are larger in the presence of sucrose in the mucosal bathing medium than in control. Not shown here, the changes in transepithelial resistance during the clamp are also increased by exposure to sucrose-containing Ringer's solution on the mucosal side.

The replacement of Na with choline in the mucosal solution would be expected to yield similar results to those observed in the presence of sucrose, because R_s increases proportionally more than (R_a+R_b), and ΔE_s is likely to diminish (inward current), since $P_{\text{choline}} \sim P_{\text{Cl}}$ across the shunt. The results of choline substitution on $\Delta V_{ms}^{E_t}$ are shown in Table 3. Note that in fact ΔV_{ms} increases for *s* to *m* pulses, as with sucrose. In addition, consistently, ΔV_{mc} and ΔV_{cs} are also increased during outward current clamps. However, ΔV_{ms}, ΔV_{mc} and ΔV_{cs} are essentially abolished during *m* to *s* current. This effect is probably related to the action of choline on apical membrane g_K (Reuss & Finn, 1975*a*).

Discussion

The results described above allow us to establish that during transepithelial current clamps the resistance changes take place at the paracellular (shunt) pathway, whereas the changes of tissue equivalent emf result from transient changes of ion concentration gradients across both the shunt pathway and the cell membranes mainly the basolateral one.

This discussion will be limited to the mechanisms of the resistance and emf changes and the possible site(s) at which these alterations take place.

Mechanism of the Current-Produced Changes of Transepithelial Resistance

From the equivalent electrical circuit shown in Fig. 1*A*, it appears obvious that a change of R_t could be caused by a change of R_s, (R_a+R_b), or both. From the control values of the resistances it can be shown that the maximum increase of R_t (over control) attributable to (R_a+R_b) is about 5%. On the other hand, decreases of the transcellular resistance could account for the measured drops of R_t during *m* to *s* pulses only if (R_a+R_b) diminishes by about one order of magnitude. Since the spread of current into the epithelium during current clamps was shown to be unchanged (Fig. 4), such large drops of (R_a+R_b) do not occur. In conclusion, the changes of transepithelial resistance during current clamps are caused by changes of the shunt resistance.

Our results are consistent with the interpretation of Bindslev *et al.* (1974*b*); i.e., two sites of current-dependent resistance changes within the shunt pathway: a) the lateral intercellular spaces, which increase (*s* to *m* current) or decrease (*m* to *s* current) R_s by changes of fluid conductivity, width, or both, and b) the junctional complexes, which tend to decrease

R_s regardless of the direction of current passage. The latter effect predominates at very large currents and can be irreversible.

Direct observations *in vitro* (Frömter, 1972), and transmission electronmicroscopy (Bindslev *et al.*, 1974*b*) substantiate the hypothesis of widening or narrowing of the lateral spaces during current passage. Our observations (phase contrast, 320×) did not yield clear-cut results. The mechanism of the changes of width of the interspaces would be the "transport number effect" (Barry & Hope, 1969*a*, *b*).

Alternatively, the resistance of the lateral spaces (R_{ls}) can change by current-induced alteration of the resistivity of the fluid they contain (ρ_{ls}). A rough calculation from our data shows that this effect can be sizable: from an estimated control R_{ls} of 100 Ω cm^2 (Reuss & Finn, 1977), and a drop of mean $[NaCl]_{ls}$ of about 30 mM during a 100 μA/0.6 cm^2 *s* to *m* pulse (*see below*), R_{ls} could rise by about 40 Ω cm^2 (Chambers, Stokes & Stokes, 1956; MacInnes, 1961). The observed ΔR_s is 51 Ω cm^2 (Table 1). Analogous calculations at other current densities indicate that a large fraction of ΔR_s could be due to changes of ρ_{ls}, during both inward and outward clamps. Even though this computation requires several difficult-to-test assumptions, it indicates that this mechanism cannot be neglected in this tissue. The situation appears to be different in frog gallbladder, where, at currents far larger than those applied in our experiments, only a small fraction of ΔR_s can be attributed to changes of ρ_{ls} (Bindslev *et al.*, 1974*b*).

Mechanisms of the Current-Produced Changes of Tissue Equivalent emf

The experimental evidence presented above can be summarized as follows: (1) A "polarization phenomenon", or change of tissue emf (ΔE_t) takes place during *m* to *s* and *s* to *m* currents. At low currents, it is roughly linear with the current density. (2) The polarity of ΔE_t is such as to tend to make the mucosal medium positive during *m* to *s* clamps, and negative during *s* to *m* clamps. (3) The E_t changes are larger during outward clamps. (4) During the transepithelial potential transients, both cell membrane potentials change in the same direction (increase during *s* to *m* clamps; decrease during *m* to *s* clamps). (5) The cell potential changes are larger than the transepithelial potential change. (6) The changes of cell membrane potential are faster than the E_t-dependent overall change of transepithelial potential.

From these results, we conclude that the largest equivalent emf change in the tissue takes place at the basolateral membrane. This does

Table 4. Computed changes of E_b and E_s during an outward current clamp for several assumed values of ΔE_a

ΔE_a (mV)	ΔE_b (mV)	ΔE_s (mV)
0	25.6	6.0
−5	29.4	5.5
−10	33.1	5.0
−15	36.9	4.5
−20	40.6	4.1
−25	44.3	3.5
−30	48.1	3.0

ΔE_b and ΔE_s were calculated assuming arbitrarily the values of ΔE_a, substituting in the circuit equations the mean values of R_a, R_b, R_s, E_a and E_b (under control conditions), and inserting the measured values of ΔV_{ms}, ΔV_{mc} and ΔV_{cs}, 30 sec after the start of an outward current clamp. ΔV's were corrected for the resistance change. Similar results, with lower values for the three Δemf's, are obtained if m to s clamps are used for the calculation.

not exclude the possibility of changes at the luminal membrane and/or the shunt, and does not imply that $\Delta V_{ms}^{E_t}$ depends predominantly on ΔE_b. In fact, from the control resistances of the three elements of the circuit it is clear that small changes of E_s can exert larger effects on ΔV_{ms} than large changes of E_b. In addition, the two time constants mentioned above suggest a fast change in cell membrane emf's, and a slower change in shunt emf. The present methods do not allow experimental determinations of ΔE_a, ΔE_b and ΔE_s during the clamps. However, estimations of the magnitude of the changes at each site can be obtained if one assumes a value for one of them (e.g., ΔE_a) and computes the other two (ΔE_b and ΔE_s) from the changes of V_{ms}, V_{mc} and V_{cs} during the clamp. The results of such a computation, for a typical measured change of V_{ms}, V_{mc} and V_{cs} 30 sec after the start of the clamps are shown in Table 4. A long clamp was preferred even though changes in R_t complicate the computation of resistance-independent ΔV's, because at very short times changes in ($E_b - E_a$) can well be over, while the E_s change has not reached its half-time. Every set of emf changes in the Table could account for the observed change of transepithelial and cellular potentials, independently of the shunt resistance changes. The solutions for $\Delta E_a > 0$ are unreasonable, because the conductance of the luminal membrane does not change during the clamps, and because its K selectivity is large. During s to m current E_a should depolarize and not hyperpolarize, since K flow from the cell to the mucosal medium will decrease the transmembrane concentration gradient. Therefore, regardless of the value of ΔE_a (0 or

negative), *both* E_b and E_s change during the clamp. From $E_b \sim 80$ mV (Reuss & Finn, 1975*b*), $[K]_{cell} = 80$ mM (Frizzell, Dugas & Schultz, 1975), and $[K]_{out} = 2.5$ mM, assuming activity coefficients equal to unity, and no change in cellular K during the clamp (*see below*), the change in $[K]_{out}$ required to yield the computed E_b changes can be calculated. For $\Delta E_b = 25.6$ mV, $[K]_{out}$ should drop to 0.91 mM, and for $\Delta E_b = 48.1$ mV, $[K]_{out}$ should fall to 0.37 mM, from a control concentration of 2.5 mM. From $(P_{Na}/P_{Cl})_{shunt} \sim 3$, it is possible to calculate the analogous $[NaCl]_{serosal}$ necessary to account for the required ΔE_s at the two extreme values of ΔE_b shown in the Table. The results of these calculations are 67 mM (if $\Delta E_s = 6$ mV), and 86 mM (if $\Delta E_s = 3.0$ mV). The [NaCl] in the bulk serosal solution is ca. 110 mM.

An additional argument for the contribution of both ΔE_s and ΔE_b to the polarization phenomenon can be put forward as follows: From the resistances of the cell membranes and the shunt pathway, their ion transference numbers, and the clamp current, it is possible to calculate the relationship between $\Delta[K]$ and $\Delta[Na]$ in the serosal solution unstirred layer. The example that follows is a computation of the *virtual* volume of serosal solution in which both [Na] and [K] would drop to 0.1 of their control values in 1 second, during an *s* to *m* current clamp at $100\,\mu A\,cm^{-2}$. i_{Na} is assumed to be exclusively through the shunt, while i_K has both cellular and shunt components. From $g_{Na} \sim 75\%\ g_t$ (shunt), $i_{Na} = 71.3 \times 10^{-5}\,\mu eq/cm^2$, while $i_K = 8 \times 10^{-5}\,\mu eq/cm^2$ (cells and shunt). Considering the Na and K concentrations in the bulk serosal solution, the current-produced fluxes are contained in $0.64 \times 10^{-5}\,cm^3$ (Na) and $3.3 \times 10^{-5}\,cm^3$ (K). Therefore, K would be depleted 5 times faster than Na to yield the same 10-fold change in concentration gradient across the corresponding membrane. Furthermore, a 10-fold rise in [K] gradient across the basolateral membrane will increase E_b by about 58 mV, while a 10-fold [NaCl] gradient across the shunt will change E_s by only 28 mV (calculated from P_{Na} and P_{Cl} across the shunt). Therefore, for a given current, $\Delta E_b > 10 \cdot \Delta E_s$. This does not mean that ΔE_b causes 90% of ΔV_{ms}, since the effects of equal changes of E_b and E_s on V_{ms} are very different, because of the relative resistances of the cell membranes and the shunt (*see above*). Taking into account that under control conditions

$$\Delta V_{ms} = 0.96\,\Delta E_s + 0.04\,\Delta(E_b - E_a) \tag{5}$$

and the calculations summarized above, it is possible to conclude that shunt polarization accounts for two-thirds, and cell membrane polarization for one-third, of the transepithelial potential change. A comparison

with the computations shown in Table 4 indicates that the ratios of emf changes could be ca. $\Delta E_b:\Delta E_a:\Delta E_s = 1:-0.5:0.1$, corresponding to the following concentration changes in the serosal unstirred layer: K from 2.5 to 0.5 mM, Na from 110 to 78 mM. The lower value of ΔE_a (when compared to ΔE_b) is consistent with the lower value of g_K/g_t at the luminal membrane, and the greater restriction to diffusion in the basolateral side of the tissue. Although this is difficult to prove directly, the geometry of the lateral intercellular spaces (in particular their small width) makes them the most likely site at which basolateral [K] changes take place.

Changes in cell ionic concentrations could have a role in these cell membrane emf changes, but a simple calculation shows that they are negligible. For instance, for a $100\,\mu A\,cm^{-2}$ *s* to *m* pulse, J_K into the cells ($g_K = g_t$, basolateral) will be $5 \times 10^{-5}\,\mu eq\,sec^{-1}$. If none of the current flowing out of the cell (to the mucosal solution) were carried by K, the increase in $[K]_{cell}$ would be maximum, and equal to $300 \times 10^{-5}/30 \times 10^{-4}\,\mu eq\,ml^{-1}\,min^{-1}$ (where the denominator is the volume of the cells per cm^2 of tissue), or, in 1 min, 1.0 mM.

In summary, these results, and the computations described above, indicate that the conductance-independent change in transepithelial potential observed during current clamps in *Necturus* gallbladder is the consequence of changes in the equivalent emf's of the three elements of the circuit, i.e., the shunt pathway and the two cell membranes. Shunt emf changes are the result of the transport number effect, and cell membrane emf changes are likely to be caused by K accumulation or depletion in the unstirred layers in contact with the cell membranes. Because of the reasons discussed above, for the same current $|\Delta E_b| > |\Delta E_a|$. An argument in favor of this is the observation that the cell membrane potential transients are abolished with high K concentrations in the serosal solution or both sides of the tissue (Fig. 7). Predictably, the transepithelial transients (bilateral K-Ringer's) *increase*: for $100\,\mu A\,cm^{-2}$, ΔV_{ms} control was 2.3 mV (*m* to *s*) and 2.9 mV (*s* to *m*), and after exposure to K, the analogous values were 3.8 and 4.8 mV, respectively (means of two experiments). This is due to the fact that $(P_K/P_{Cl}) > (P_{Na}/P_{Cl})$ across the shunt, and therefore, ΔE_s is larger in K-Ringer's for the same current. Unilateral substitutions are more difficult to analyze, since ΔE_s will be a function of the direction of current passage.

An independent evaluation of the contributions of the shunt and the cell membranes to ΔV_{ms} during the clamp can be obtained from the lanthanum experiments. As stated before, control $100\,\mu A\,cm^{-2}$ clamps (*s*

to *m*) produced a ΔV_{ms} of 5.9 ± 0.7 mV, while in lanthanum the result in the same tissues was 5.2 ± 1.2 mV ($n=5$), not significantly different from control. P_{Na}/P_{Cl} across the shunt was measured before and after La, and shown to be about 3.3 and 0.6, respectively. Therefore, in the presence of La ΔE_s will be less, because of the absolute lower selectivity of the shunt. In addition, the contribution of the same $\Delta(E_b - E_a)$ will be larger if R_s has increased. Assuming that the R_t change produced by La is entirely due to ΔR_s, the effect of $\Delta(E_b - E_a)$ on ΔV_{ms} will be 1.5 times larger in the presence of La, and the contribution of ΔE_s to ΔV_{ms} will be diminished to about half. Therefore, under control conditions ΔV_{ms} (5.9 mV) $= \Delta V_{ms}^{E_s}$ (3.6 mV) $+ \Delta V_{ms}^{(E_b - E_a)}$ (2.3 mV), and in La ΔV_{ms} (5.2 mV) $= \Delta V_{ms}^{E_s}$ (1.7 mV) $+ \Delta V_{ms}^{(E_b - E_a)}$ (3.7 mV). These results were obtained from the solution of Eq. (5) and the analogous equation in the presence of La, assuming that ΔE_s in La is reduced by 50%. Thus, V_{ms} transients in the presence of La can be accounted quantitatively by the changes of R_s, shunt selectivity, and transcellular current. This finding validates the use of Eq. (5) and is consistent with the view that under control conditions about two-thirds of ΔV_{ms} are attributable to the shunt and about one-third to the cell membranes.

Similar results to those obtained with La were observed after making the mucosal medium hyperosmotic or after replacing mucosal Na with choline.

In summary, in *Necturus* gallbladder both shunt emf changes and cell membrane emf changes contribute to the conductance-independent voltage transients produced by current passage. The magnitude of each effect depends on the selectivity and total conductance of each pathway. In very leaky tissues, shunt polarization will account for most of the transients, while in tighter epithelia the contribution of cell membrane emf changes will be sizable.

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