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The Effects of Electrical and Osmotic Gradients on Lateral Intercellular Spaces and Membrane Conductance in a Low Resistance Epithelium

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Summary. We have studied the effect of d-c currents and osmotic gradients on the conductance and ultrastructure of the frog gallbladder. Passing current across the epithelium produces three voltage transients which are due to (*i*) the membrane capacitance, (*ii*) ion polarization effects, and (*iii*) changes in membrane resistance. The direction and magnitude of these resistance changes depended on (*i*) the direction of the current pulse, (*ii*) the current density, (*iii*) the ion selectivity of the epithelium, (*iv*) the thickness of the unstirred layers, and (*v*) the presence or absence of osmotic gradients across the tissue. Osmotic gradients also change the resistance of the gallbladder. These resistance changes are accompanied by changes in cell structure. Resistance increases are associated with the collapse of the lateral intercellular spaces. We conclude that osmotic and electrical gradients increase tissue resistance by narrowing the spaces and thereby increasing their resistance. On the other hand, we believe that the drop in resistance brought about by both current and osmotic gradients is not fully accounted for by dilation of the spaces, but is related to changes within the epithelial membranes similar to those in high resistance tissues.

The electrical resistance of epithelia varies from less than 30 $\Omega\,cm^2$ to greater than 20,000 $\Omega\,cm^2$. In the case of low resistance epithelia, such as the gallbladder, intestine and renal proximal tubule, the so-called tight junctions act as a low resistance shunt so that the major pathway for ion permeation is extracellular (*see* Frömter & Diamond, 1972). Earlier we reported that the resistance and permeability of low resistance epithelia can be modified by the application of osmotic gradients, and, at least in the rabbit gallbladder, these changes can be accounted for by variations in the dimensions of the lateral intercellular spaces (Smulders, Tormey & Wright, 1972; Wright, Smulders & Tormey, 1972). In the present study we have investigated the effect of electrical currents on the resistance and ultrastructure of a 'leaky' epithelium – the frog gallbladder. We find that high current densities produce two effects, one related to the change in the

resistance of the lateral intercellular spaces and the other related to changes within the epithelial membranes. This latter effect appears similar to that observed in high resistance epithelia (Bindslev, Tormey, Pietras & Wright, 1974). Osmotic gradients also produce these resistance changes in this epithelium. A preliminary account of some of these results has already been presented (Bindslev, Wright & Tormey, 1973).

Materials and Methods

Frogs *(Rana catesbeiana)* were obtained from local suppliers and were kept in tap water at room temperature for about one week before use. Gallbladders were removed, cut open to form flat sheets and mounted between two Lucite half-chambers. The area of the exposed tissue was 11.4 mm^2 and the volume of the saline in each half-chamber was 16 ml. The saline in each compartment was vigorously agitated by means of magnetic stirring bars. Further oxygenation of the solutions was found to be unnecessary.

Transmural electrical potentials (p.d.'s) and conductances ($G=1/R$) were measured and recorded as described previously for the rabbit gallbladder (Wright, Barry & Diamond, 1971). The only modification was that a digitimer (Devices, Model 3290) was used to trigger the current stimulator via a Devices relay unit (actuate time 1 msec). The current was tapped off from a 90 V battery by means of a potentiometer. This modification allowed greater flexibility in the control of the width and frequency of the current pulse. A further modification was that the current and p.d. were recorded continuously on a multichannel pen recorder (Rikadenki, Model B 36 pen recorder). The maximum chart speed was 300 mm/min and the full scale pen response time was one-third of a second. In most experiments the resistance of the tissue was monitored by passing small current pulses (<10 $\mu A/cm^2$) for 400 msec at regular intervals. Both the amplitude and the frequency of the pulses were chosen to avoid transport number effects and resistance changes (*see* page 361). All voltages were measured to within 0.25 mV and, where appropriate, were corrected for junction potentials as described by Barry and Diamond (1970).

Experiments were carried out at room temperature (22 °C) and the saline in the Lucite chambers contained (in mM): 105.4 NaCl, 2 KCl, 1 $CaCl_2$, 1 $MgSO_4$, buffered with 2.5 Na_2HPO_4/NaH_2PO_4 to pH 7.3. In the preliminary experiments designed to characterize passive ion permeation across the frog gallbladder, the dilution potentials, bi-ionic potentials, conductance-concentration relations, and current-voltage relations were measured as described previously (Barry, Diamond & Wright, 1971; Wright *et al.*, 1971). The osmolarity of the mucosal and/or the serosal fluid was varied by the addition of sucrose (25 to 600 mM). Fluid transport was determined gravimetrically in sac preparations (*see* Diamond, 1962).

After experimental manipulation some bladders were prepared for light- and electron-microscopy. In these particular experiments the gallbladders were mounted in the chambers between plastic rings to facilitate handling of the tissue after fixation (*see* Smulders *et al.*, 1972) and the volume in each half-chamber was reduced to 3 ml in order to conserve fixative.

Fixation was initiated by adding a solution of 1 % OsO_4 (in 115 mM sodium cacodylate + 1 mM $CaCl_2$ at pH 7.3) to each half-chamber. As the fixative was delivered into the chamber the old solution was removed with an aspirator to maintain constant volume. The change of solution was completed within 5 sec. Osmotic and/or electrical gradients were maintained across the bladders during the initial fixation period whenever

appropriate. After about 10 min, the tissue was transferred to additional fixative for 30 to 60 min. It was then dehydrated in ethanol, embedded in Epon 812, sectioned and stained for both light- and electron-microscopy.

Results

Preliminary Observations

We begin by describing our experiments to characterize the physiological and anatomical properties of the frog gallbladder.

1. *The Spontaneous Transmural p.d.* In seven gallbladders the spontaneous p.d. was 3.0 ± 0.4 mV, serosa positive, upon setting up the epithelium between two identical saline solutions. This p.d. decayed slowly to 1.7 ± 0.3 mV over the following 3 hr. Both anoxia and the addition of 5×10^{-4} M ouabain to the serosal fluid eliminated the spontaneous p.d. Switching off stirring caused the p.d. to fall to near zero with a half time of 1.5 min. Resumption of stirring in the mucosal solution returned the p.d. to normal with a half time of 0.1 sec, whereas resumption of stirring in the serosal solution had no effect. The effect of stirring was not obtained when the spontaneous p.d. was eliminated with anoxia or ouabain. These results suggest that the spontaneous p.d. is related to the active transport of Na across the gallbladder epithelium and that the rate of pumping is directly or indirectly limited by diffusion in the solution adjacent to the mucosal surface of the bladder.

2. *Conductance.* The conductance of the bladder was 8.8 mmho/cm^2 upon setting up the tissue between two identical saline solutions, and the conductance was constant for at least 4 hr. The conductance of the tissue (per cm^2) was unchanged when the area of the window between the Lucite chambers was increased from 11.4 to 98 mm^2. This suggests that 'edge damage' does not contribute significantly to the conductance of this preparation. (*See also* Moreno and Diamond, 1974.)

3. *NaCl Dilution Potentials.* Dilution potentials were produced by varying the NaCl concentration in the saline bathing one side of the gallbladder; NaCl was replaced isosmotically with mannitol. The resulting diffusion p.d.'s yield a P_{Cl}/P_{Na} ratio of 0.37 ± 0.02 (9); i.e., the gallbladder is cation selective. The permeability ratio was largely independent of both the magnitude of the NaCl gradients across the tissue (*cf.* Fig. 2, Barry *et al.*, 1971) and the mean ionic strength of the mucosal and serosal solutions (*cf.* Fig. 8, Wright *et al.*, 1971). The dilution p.d.'s were usually quite stable throughout the duration of the experiment.

4. *Bi-ionic Potentials.* These were generated by replacing the Na in the solution bathing one face of the epithelium with another monovalent cation, e.g. potassium. The bi-ionic potentials did not exhibit the transient behavior seen in the rabbit gallbladder (Diamond & Harrison, 1966; Moreno & Diamond, 1974; Weidner & Wright, *unpublished observations*), but were stable with time. The magnitude of these bi-ionic p.d.'s give the permeability sequence: $NH_4(1.6) > Rb(1.5) \sim K(1.5) > Cs(1.4) > Na(1.0) > Li(0.7)$. This sequence corresponds to sequence III in Eisenman's terminology (*see* Diamond & Wright, 1969).

5. *Instantaneous Current-Voltage Relations.* The instantaneous (within 20 msec) I/V curves were linear up to ± 200 mV (*cf.* Fig. 6*a*, Wright *et al.*, 1971).

6. *Conductance-Concentration Relations.* The conductance of the frog gallbladder increased linearly with NaCl concentration from 40 to 215 mM (*cf.* Fig. 4, Wright *et al.*, 1971).

7. *Fluid Transport.* The rate of fluid transport across the frog gallbladder amounted to $26 \pm 11(6)$ μliter/cm^2 hr. Assuming that the fluid transported is isotonic the rate of fluid transport corresponds to a net sodium flux of 3 μEquiv/cm^2 hr.

8. *Streaming Potentials.* The addition of sucrose to either the serosal or mucosal fluid generated changes in p.d. across the frog gallbladder. These streaming potentials, i.e., osmotically induced potential changes, amounted to $2.4 \pm 0.2(6)$ mV when 75 mM sucrose was added to either side of the tissue. The relations between sucrose concentration and the streaming potentials were very similar to those reported previously for the rabbit gallbladder (*cf.* Fig. 3, Wright *et al.*, 1972).

9. *Unstirred Layers.* The thickness of the unstirred layers adjacent to the mucosal and serosal surfaces of the frog gallbladder was estimated from the time course of the build-up of streaming p.d.'s and NaCl dilution p.d.'s. From the streaming potentials the mucosal and serosal unstirred layers were $75 \pm 6(3)$ and $299 \pm 37(7)$ μ, respectively, and from dilution p.d.'s were $65 \pm 5(7)$ and $297 \pm 20(7)$ μ, respectively. We also noted that the mucosal unstirred layer could be reduced to less than 10 μ by jetting saline directly at the surface of the gallbladder.

10. *Structure.* The anatomy of the frog gallbladder is similar to that of the rabbit (Tormey & Diamond, 1967) but differs in some particulars: (a) When moderately stretched, the mucosal surface is devoid of any folds; i.e., it is almost perfectly planar. (b) The cells of the (simple columnar) epithelium are somewhat larger than those of the rabbit; i.e., about 50 μm high by 6.5 μm in diameter. (c) As can be seen on close examination of Fig. 8*d*, the majority of the mitochondria are limited to the apical one-third of the epithelial cells. This is in keeping with the requirements of the "standing-gradient model" (Diamond & Tormey, 1966). (d) The lateral intercellular spaces of the epithelium are more complex than those of the rabbit. In the basal one-third of the epithelium, this space becomes very wide and is filled with numerous very long and thin cytoplasmic projections which interdigitate in a complex pattern. (e) The remainder of the bladder wall averages 97 ± 23 μm thick in control bladders; this is essentially the same as in stretched rabbit gallbladders. However, distinct submucosal (lamina propria) and muscularis layers are not obvious, and the density of collagen fibers throughout the wall is considerably greater.

We conclude from these preliminary experiments that the properties of the frog gallbladder are very similar to those described previously for the rabbit gallbladder.

Effects of Current

The approach used in this study was to observe the transmural p.d. upon passing d-c current pulses across the gallbladder. The currents ranged from 5 to 1,000 μA/cm^2 and lasted from 20 msec to 20 min. On switching on the current, three distinct voltage transients may be observed. These transients, shown schematically in Fig. 1, are due to the capacity of the epithelial membranes, the build-up of polarization potentials, and changes in resistance of the tissue.

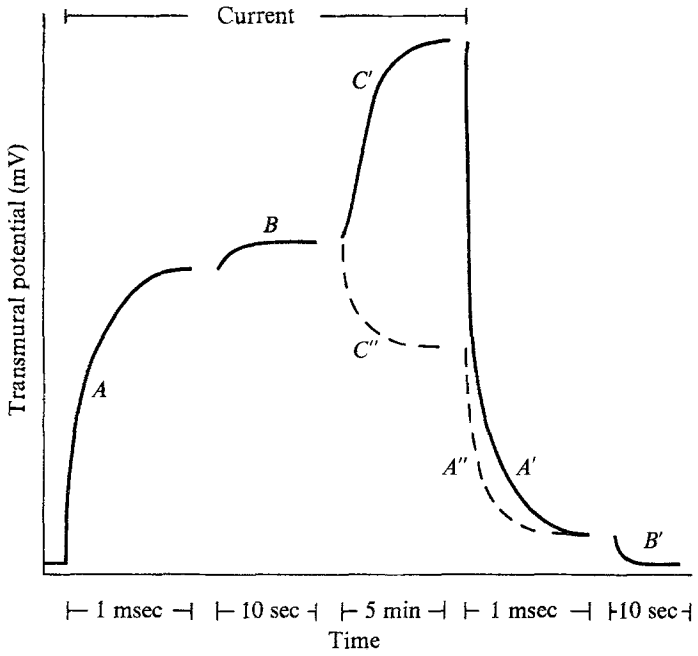


Fig. 1. The three voltage transients that are observed when direct current pulses are passed across the frog gallbladder. Transmural voltage is plotted against time. The first transient *A* (with a half time less than 0.25 msec) is related to the capacity of the membrane; the second *B* (with a half time in the range of seconds) is the polarization potential; and the third *C* (with half times in the range of minutes) is the current-induced change in membrane resistance. On switching the current off only two transients are seen: the capacitative surge and the relaxation of the polarization potentials. For additional details *see text*

1. *Membrane Capacitance.* The immediate rise in the transmural voltage after the onset of a current pulse approximated a single exponential which corresponded to a tissue capacitance of 0.5 to 0.7 $\mu F/cm^2$; i.e., the half time was about 0.1 msec.

2. *Polarization Potentials.* We observed a second transient whose time course for build-up and decay is substantially longer than the capacitive surge. For example, on passing a current of 700 $\mu A/cm^2$ from serosa to mucosa, a potential of about 5 mV developed with the half times for its build-up and decay of 15 to 30 sec and 2 to 6 sec, respectively. Passing the current in the opposite direction produced a potential of about 3.5 mV which built up and decayed with half times of 2 to 6 and 15 to 40 sec, respectively. These p.d.'s increased linearly with current density up to at least 700 $\mu A/cm^2$, and were distinct from the p.d. transients related to resistance changes which we shall describe shortly.

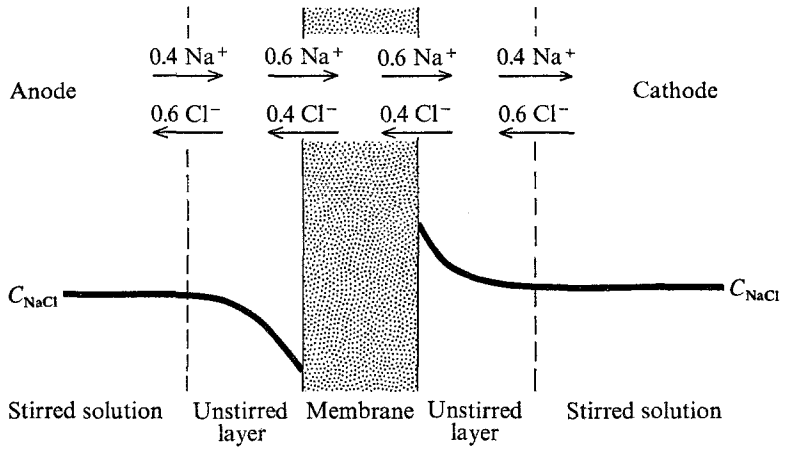


Fig. 2. A scheme showing the origin of polarization potentials in a cation-selective membrane. The membrane is shown to separate two solutions containing the same concentration of NaCl. Passing current across the membrane results in the enhancement and depletion of salt in the adjacent unstirred layers. This is due to the discontinuity of the ion transport numbers in the aqueous and membrane phases; in the aqueous solution 6/10 of the current is carried by chloride ions whereas in the cation-selective membrane most of the current is carried by sodium ions. The resulting ion concentration gradient across the membrane gives rise to both a diffusion potential and osmotic water flow. In practice the largest changes in ion concentrations occur at the serosal side of the gallbladder owing to the fact that the serosal unstirred layer is about 5× thicker than the mucosal unstirred layer. (For further discussion of this phenomenon *see text*; *see also* Barry & Hope, 1969*a*, *b*, and Wedner & Diamond, 1969)

This second p.d. transient is due to the development of polarization potentials. Passage of d-c current across ion-selective membranes leads to a build-up or depletion of salt in the unstirred layers adjacent to the membrane (*cf.* Fig. 2) due to the so-called transport number effect (Barry & Hope, 1969*a*, *b*). For a cation-selective membrane, such as the frog gallbladder, the resulting concentration gradient leads to the development of a diffusion p.d. which is superimposed on the voltage arising from the resistive properties of the tissue. These polarization potentials appear as a creep in the transmural voltage in the same direction as the initial IR step. Both the magnitude and time course of the creep depend on the thickness of the unstirred layers on each side of the membrane, the ion selectivity and the absolute permeability of the membrane.

Polarization potentials have been described previously for the rabbit gallbladder (Wedner & Diamond, 1969), the frog gastric mucosa (Noyes & Rehm, 1971), and the choroid plexus (Wright, 1972). In addition, we have observed them in the toad gallbladder, and the frog jejunum, skin, colon and arachnoid membranes.

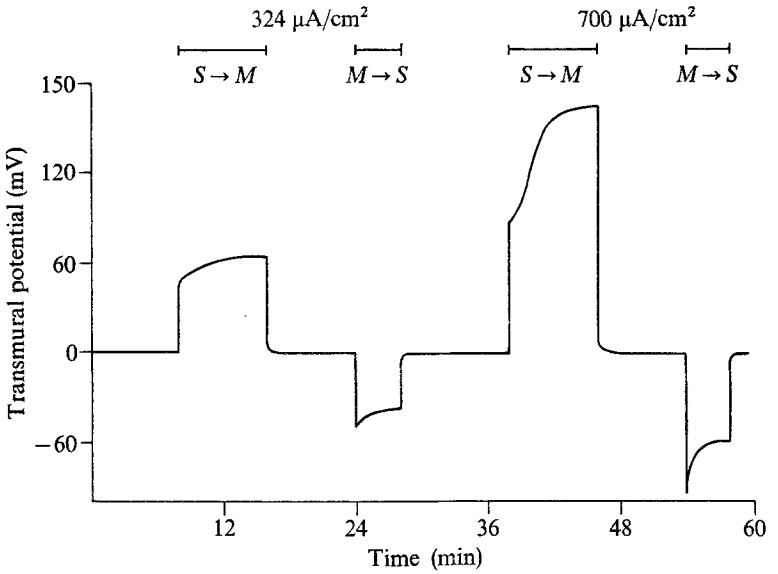


Fig. 3. The effect of current on the electrical resistance of the frog gallbladder. In this experiment d-c current pulses (324 and 700 μA/cm²) were passed across the gallbladder and the changes in transmural voltage were recorded. The voltage (in mV) is plotted against time (in minutes). As judged by the difference between the IR steps on switching the current on and off, the resistance of the gallbladder increased when currents were passed from serosa to mucosa (cathode in the mucosal fluid), and decreased when currents were passed from mucosa to serosa (cathode in the serosal fluid). *See text* for further details

3. *Resistance Changes.* On passing currents greater than 50 μA/cm² across the gallbladder the voltage creep, due to the polarization potentials, was followed by further voltage transients which we interpret as current-induced resistance changes. These voltage transients are shown in Fig. 3. In this experiment current pulses were passed across the bladder for 4 to 8 min. When the currents were passed from the serosa to the mucosa (serosa positive) there was a further increase in the p.d. after the initial capacitive and polarization transients. The half times for this third transient were 2.4 and 1.5 min for the current pulses 320 and 700 μA/cm², respectively. Comparison of the on and off voltage transients shows that this third transient was due to an increase in the resistance of the gallbladder; 40 and 106 Ω cm² for the low and high currents, respectively. These resistance increases were reversible with half times of 0.1 to 0.3 min.

Reversing the polarity of the current pulses, i.e., currents from mucosa to serosa, produced a decline in the transmural p.d. to a new steady value, and this was due to a resistance decrease. The fall in the resistance ($t\frac{1}{2} \sim$ 0.3 min) was 30 and 47 Ω cm² for currents of 320 and 700 μA/cm². These

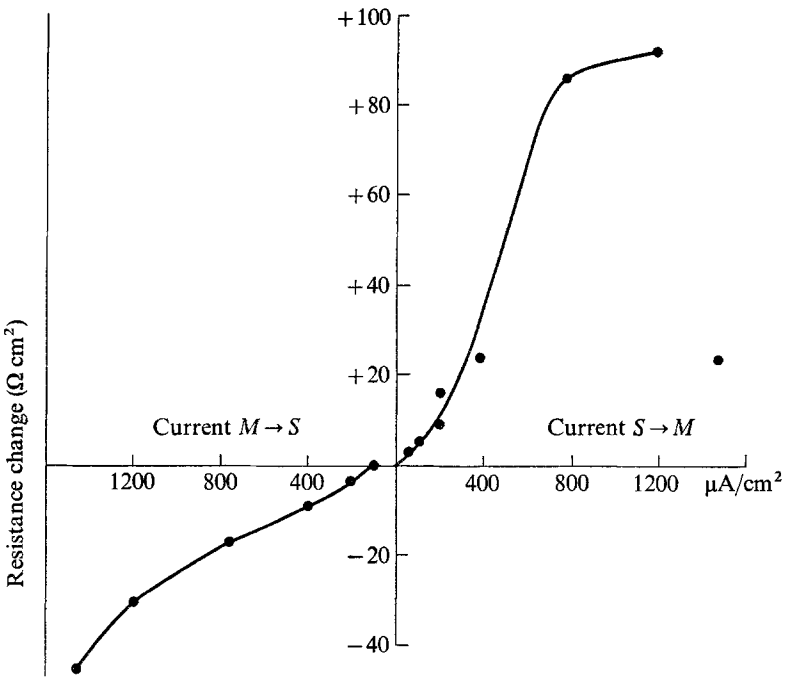


Fig. 4. The change in gallbladder resistance as a function of current density. This graph was obtained from one experiment, and the steady-state change in resistance (Ω cm^2) is plotted against the current density (μA/cm^2). The change in resistance was obtained from the difference between the IR steps on switching the current pulses on and off. The resistance change brought about by a given current pulse was allowed to relax before another current pulse was tested. Only the resistance changes brought about by the largest currents (>1200 μA/cm^2) proved to be irreversible. In this, and all other experiments, the IR steps were corrected for the potential drop in the bathing solutions

effects of current were also reversible, but in contrast to resistance increases with current of the opposite polarity, the half time for the recovery process was quite slow, i.e., 2 to 4 min.

The change in resistance as a function of current density is shown in Fig. 4. When current was passed from serosa to mucosa, the increase in resistance was roughly proportional to the current density up to 800 μA/cm^2 ($16 \pm 2(3)$ Ω cm^2/100 μA/cm^2) but at higher currents there was a saturation of the resistance increase, and at still higher currents there was even a drop in the resistance. With current in the opposite direction the decrease in the resistance was also roughly proportional to the current density up to 800 μA/cm^2, but at higher currents the drop in resistance became progressively steeper. The effects seen at high current densities, in both directions,

were associated with deterioration in the tissue as judged by the incomplete reversibility of the resistance and by a drop in the magnitude of the NaCl dilution potentials.

Factors Influencing the Current-Induced Resistance Change

1. *Ion Selectivity.* The ion selectivity of the gallbladder was varied by the addition of $LaCl_3$ to the mucosal solution (*see* Wright & Diamond, 1968). The polyvalent cation converts the gallbladder from a cation-selective membrane to an anion-selective membrane. In four experiments the P_{Cl}/P_{Na} ratio became 3.0 ± 0.3 on addition of 2 mM $LaCl_3$ to the mucosal fluid. Fig. 5 shows the effect of La^{3+} on the current-induced resistance changes in one experiment. Prior to the addition of La^{3+} we measured NaCl dilution potentials and passed 1-min current pulses (665 $\mu A/cm^2$) in both directions across the bladder. After the addition of La^{3+} we again measured the dilution potentials and passed current across the tissue. This experiment shows that when the P_{Cl}/P_{Na} ratio was changed from 0.4 to 3.2, there was a reversal in the direction of the resistance changes; i.e., whereas a current pulse increased the resistance in the absence of La (25 $\Omega\,cm^2$), the same current pulse now produced a decrease in the resistance (37 $\Omega\,cm^2$). It should also be noted that La^{3+} increased the baseline resistance of the bladder by about 25 $\Omega\,cm^2$, and that the action of La^{3+} is reversible on washing the mucosal solution with La^{3+}-free saline. La^{3+} had the same qualitative effect on three other gallbladders.

2. *Thickness of Unstirred Layers.* The effect of current was also measured in the presence and absence of stirring the solutions in contact with both faces of the epithelium. When currents were passed from mucosa to serosa, stirring had no effect on the magnitude of the current-induced resistance changes. However, the resistance changes recorded when currents were passed from serosa to the mucosa increased when we ceased stirring the external solutions; e.g., in one experiment the increase in resistance on passing a current of 334 $\mu A/cm^2$ increased from 63 to 105 $\Omega\,cm^2$ on switching off the stirring motors.

Current-Induced Resistance Changes in Other Epithelia

The increases in resistance produced by direct current have been noted previously in the rabbit gallbladder (Smulders *et al.*, 1972) and *Necturus* gallbladder (Frömter, 1972). Furthermore, Frömter noted that changes in the resistance were associated with variations in the widths of the lateral intercellular spaces. We have also observed current-induced resistance increases in the toad gallbladder (resistance 130 $\Omega\,cm^2$) and frog

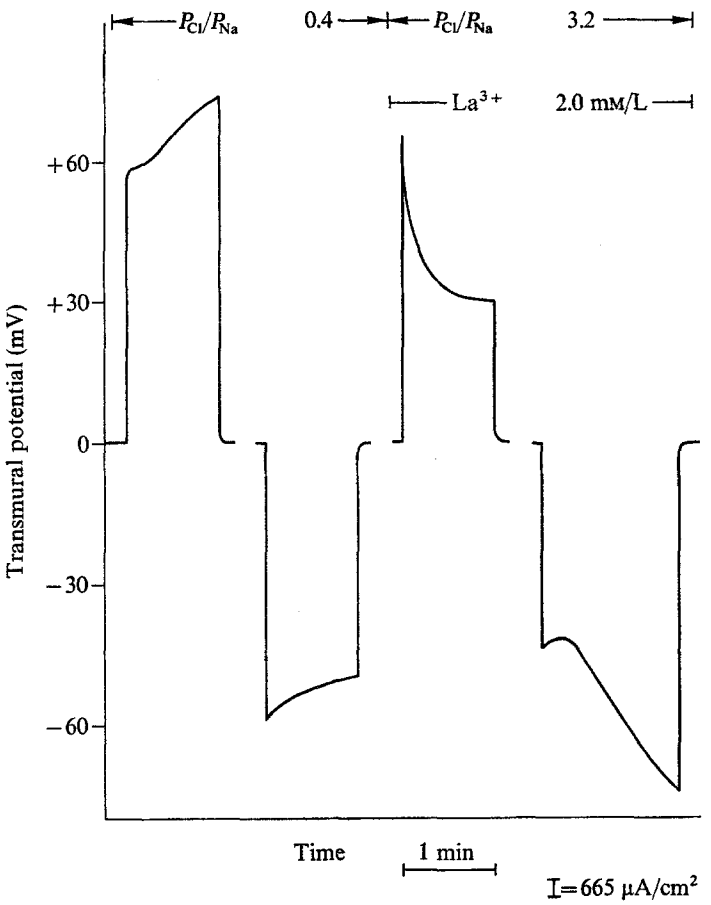


Fig. 5. Effect of La^{3+} on current-induced resistance changes. In this experiment NaCl dilution potentials and the effect of current (665 μA/cm²) was recorded before and after the addition of La^{3+} (2 mM) to the mucosal fluid. In the absence of La^{3+} (P_{Cl}/P_{Na} = 0.4), current from serosa to mucosa (cathode in the mucosal solution) *increased* the resistance, and current from mucosa to serosa (cathode in the serosal solution) decreased the resistance. La^{3+} changed the gallbladder into an anion-selective membrane (P_{Cl}/P_{Na} = 3.2) and reversed the polarity of the resistance change; i.e., placing the cathode in mucosal solution *decreased* the resistance. Not shown in the Figure is the observation that the effect of La^{3+} was reversible

jejunum (26 Ω cm²). In the choroid plexus (200 Ω cm²) we do not normally see resistance changes since $P_{Cl}/P_{Na} \sim 1$ (Wright, 1972), but the increase in resistance is observed in sulfate saline. In high resistance epithelia, such as the toad urinary bladder (3000 Ω cm²), frog urinary bladder (1250 Ω cm²), frog skin (1500 Ω cm²) and frog arachnoid membrane (2000 Ω cm²), we have been unable to detect any current-induced resistance increases. However, in these tissues we have already reported that high currents in either direction produce large reversible drops in resistance (Bindslev *et al.*, 1974).

Osmotically Induced Resistance Changes

In the rabbit gallbladder we have reported that osmotic gradients across the tissue influence the resistance (Smulders *et al.*, 1972); adding sucrose to the mucosal fluid increased it, whereas addition of sucrose to the serosal fluid had no effect. We have repeated these experiments on the frog gallbladder and these are shown in Fig. 6. Increasing the mucosal sucrose concentration increased the resistance by a maximum of 40 Ω cm^2, but addition of sucrose to the serosal fluid decreased it by a maximum of 25 Ω cm^2.

Combined Effects of Osmotic and Electrical Gradients

In two gallbladders we studied the effects of current in the presence of sucrose in the mucosal fluid. In these experiments smaller currents were required to produce a given resistance change; e.g. in the presence of 75 mM sucrose in the mucosal fluid a current of 325 μA/cm^2 increased the resistance by 115 Ω cm^2, whereas in the absence of sucrose a current of about 1000 μA/cm^2 was required to produce a similar increase in resistance. Likewise the drop in resistance produced by a current of 325 μA/cm^2 in the presence of 75 mM sucrose was about four times greater than that obtained in the absence of sucrose.

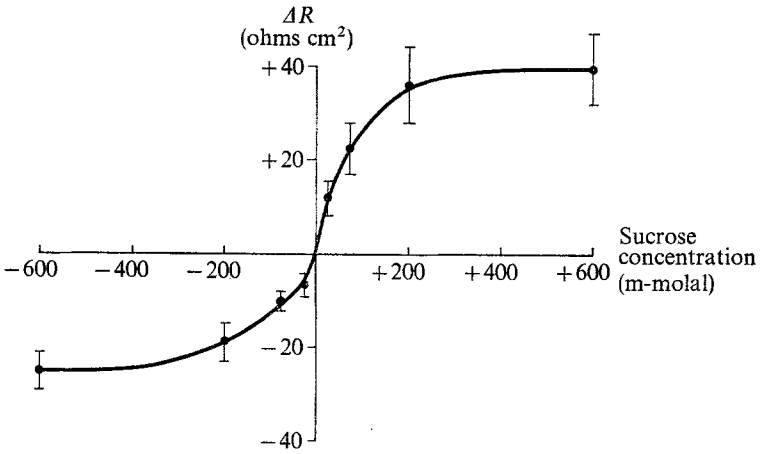


Fig. 6. The effect of osmotic gradients on the resistance of the gallbladder. In these experiments sucrose was added to the solution bathing either the mucosal or the serosal surface of the tissue. The change in resistance is plotted against the sucrose concentration (positive sucrose concentrations are used to indicate the presence of sucrose in the mucosal fluid, while negative concentrations indicate sucrose in the serosal fluid). This graph was constructed from experiments on six gallbladders and the bars represent the standard errors. Addition of sucrose to both the serosal and mucosal solutions produced no stable change in the gallbladder resistance

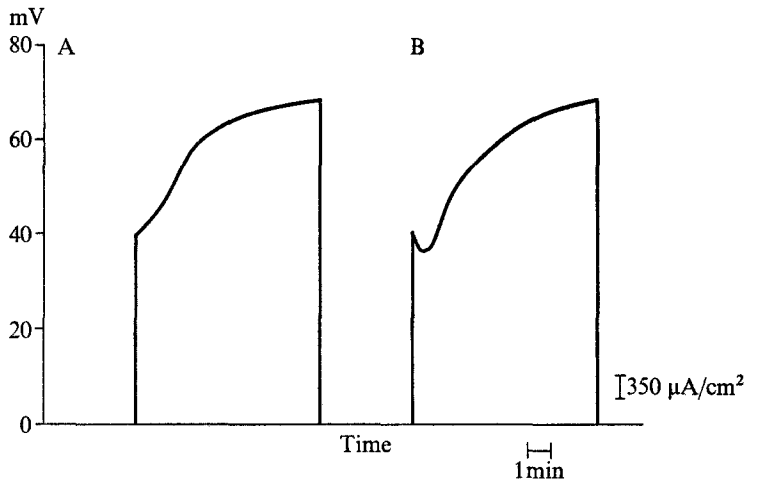


Fig. 7. Variations on the time course of the current-induced resistance increase. The usual time course of the third voltage transient is shown in part *A*. However, in this gallbladder and in others, particularly at high current densities, transients such as those shown in part *B* are frequently observed. As discussed in the text, the transient decrease in voltage (resistance) is more apparent with sucrose in the serosal fluid. Both the records in this figure were obtained by passing a current pulse (350 μA/cm²) from serosa to mucosa

The most obvious effect of sucrose in the serosal fluid concerns the time course of the resistance increase when current is passed from the serosa to the mucosa. Normally the time course of the voltage transient due to the resistance increase resembles a single exponential rise (Fig. 7*A*) but in some tissues, particularly at high current densities, there was an initial drop in the voltage before the usual increase (Fig. 7*B*). We find that addition of 5 to 15 mM sucrose to the serosal fluid makes the initial voltage drop more prominent, and eventually at 25 mM the resistance increase produced by currents up to 650 μA/cm² were abolished. In fact, in the presence of 25 mM sucrose there was an actual decrease in the resistance that corresponded to the drop usually seen with currents passed from mucosa to serosa.

These results suggest that there are two effects of current on the gallbladder. The first is a drop in the resistance which occurs when current is passed in either direction, and the second is an increase in resistance when the current is passed from serosa to mucosa. The latter is probably due to the collapse of the lateral intercellular spaces, and, at least at lower current densities, this normally outweighs the first effect.

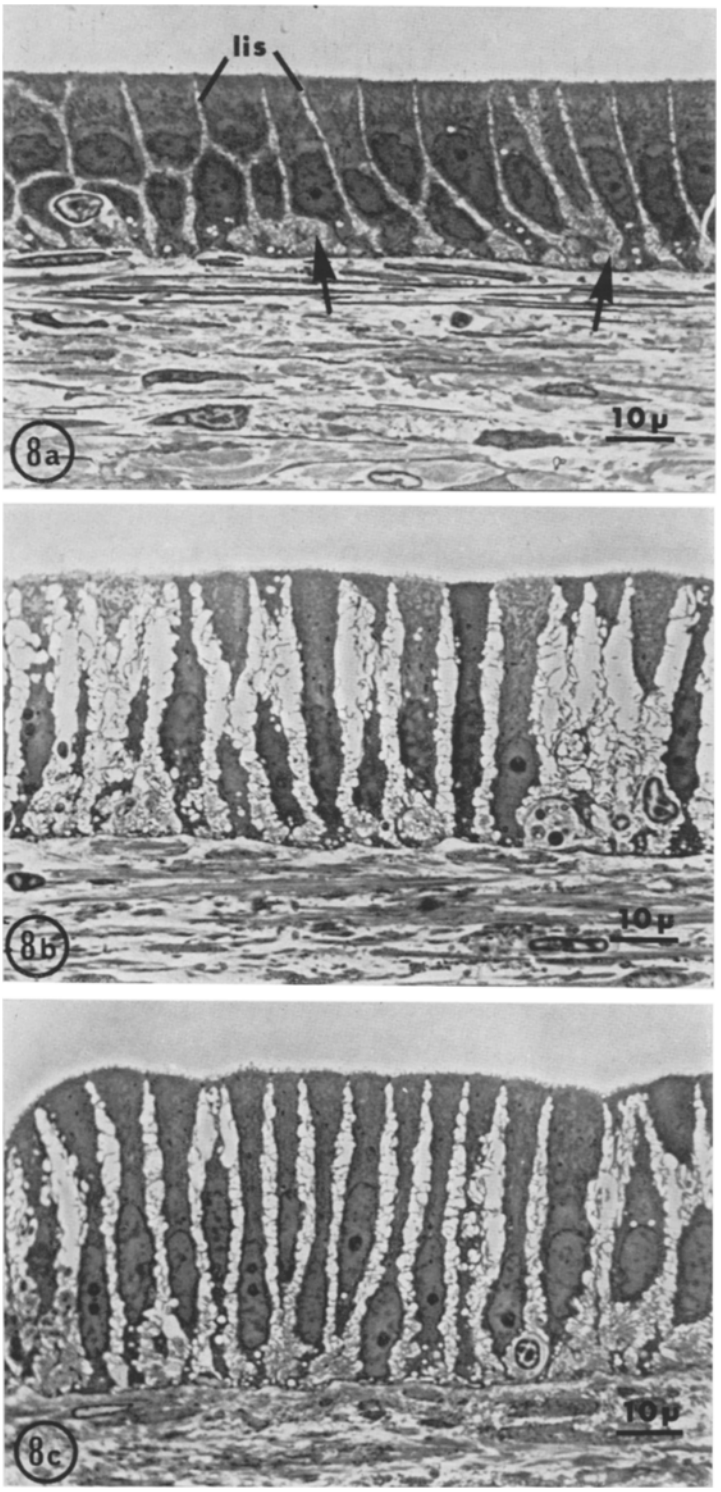
Morphological Changes

Most features of the tissue response were clearly visible by light-microscopy (as shown in Fig. 8*a–e*). In the absence of any gradients (Fig. 8*a*), the epithelium is simple columnar in form, and the individual epithelial cells are separated by clearly discernible intercellular spaces averaging less than a micron in width.

When the serosal bathing solution is made hypertonic by addition of, say, 200 mM sucrose (Fig. 8*b*), the cells become taller and narrower, and the intercellular spaces widen by nearly an order of magnitude. The rest of the bladder wall approximately doubles in thickness, and its fibrous and cellular elements become correspondingly less packed (*cf.* Fig. 7, Smulders *et al.*, 1972). Identical morphological changes occur when current lowers the tissue resistance to the same degree (Fig. 8*c*).

Opposite changes occur under conditions which raise the tissue resistance. For instance, passage of current from serosa to mucosa (Fig. 8*d*), causes

Fig. 8. Light-micrographs of frog gallbladders under various conditions. Each shows the epithelium resting on a portion of the connective tissue that makes up the rest of the bladder wall. Magnification in all cases is 900×. (*a*) *No gradient (control).* This bladder was bathed in Ringer's solution with 100 mM sucrose added to both sides. Resistance, 135 Ω cm^2. The lateral intercellular spaces (*lis*) are clearly visible between the individual epithelial cells. Note that towards the base of the epithelium these spaces (arrows) become considerably broader and more complex. (*b*) *Osmotic water flow, mucosa to serosa.* Both sides were initially bathed in Ringer's solution, and the resistance was 135 Ω cm^2. 200 mM sucrose was then added to the serosal bathing solution, and the tissue was fixed after the resistance dropped to a steady 115 Ω cm^2. The lateral intercellular spaces are dramatically broadened. The cells adjust to this by becoming taller and narrower, keeping their volumes approximately constant. (*c*) *Electrical current, mucosa to serosa.* Both sides were initially bathed in Ringer's, resistance 144 Ω cm^2. A current of 625 μA/cm^2 was then passed from mucosa to serosa, and the tissue was fixed after the resistance dropped to a steady 98 Ω cm^2. The appearance of the tissue is virtually identical to 8*b*. The experiments 8*a–c* were performed on pieces of tissue from the same bladder. (*d*) *Electrical current, serosa to mucosa.* Both sides were initially bathed in Ringer's, resistance 97 Ω cm^2. A current of 660 μA/cm^2 was then passed from serosa to mucosa, and the tissue was fixed after the resistance reached a steady 153 Ω cm^2. The lateral intercellular spaces have virtually disappeared. Also the connective tissue elements of the wall have pulled closer together, indicating that the wall has become dehydrated. The localization of mitochondria in the apical third of the epithelium is particularly evident in this preparation (circle). (*e*) *Osmotic water flow, serosa to mucosa.* Both sides were initially bathed in Ringer's solution, resistance 98 Ω cm^2. 200 mM sucrose was then added to the mucosal bathing solution, and the tissue was fixed after the resistance reached a steady 144 Ω cm^2. The lateral intercellular spaces are collapsed, and the cells are very dense indicating considerable shrinkage. The dehydration of the connective tissue is more complete than in 8*d*. Fig. 8*d* and *e* represent experiments on tissue from the same bladder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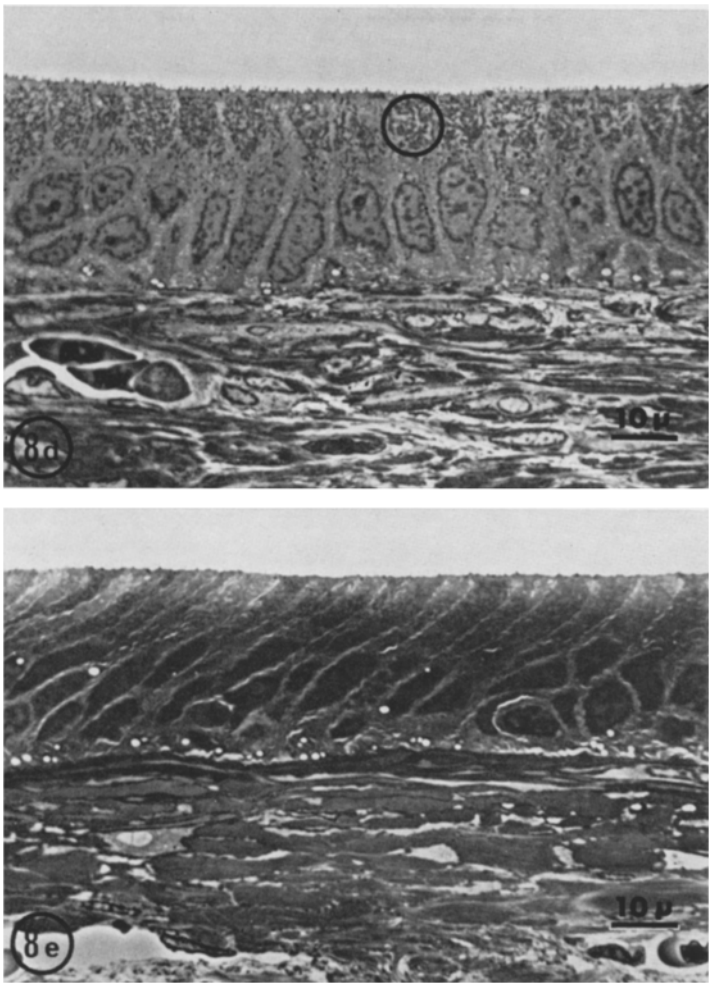
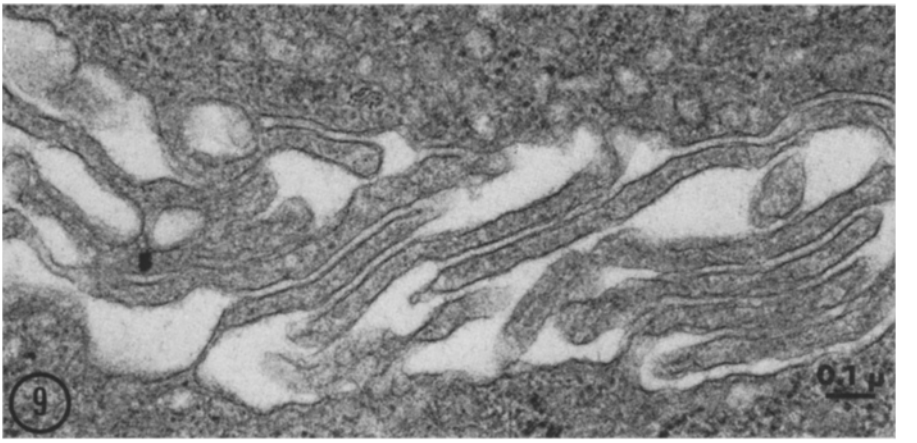


the spaces between epithelial cells to disappear below the resolution of the microscope. The remainder of the bladder wall is slightly thinner than in controls and the elements are slightly more closely spaced.

Similar changes occur when the tissue resistance is increased to the same degree by osmotic gradients (Fig. 8*e*). However, there appear to be some minor quantitative differences; the cytoplasm of the cells is somewhat denser than in any other condition studied; and the structural elements in the rest of the bladder wall appear to be particularly close-packed.

Fig. 9 is an electron-micrograph showing a portion of the lateral space between two epithelial cells in a control bladder (i.e. not subjected to any gradients). As is evident even in light-micrographs, these spaces in the more basal regions of the epithelium are considerably wider and more complex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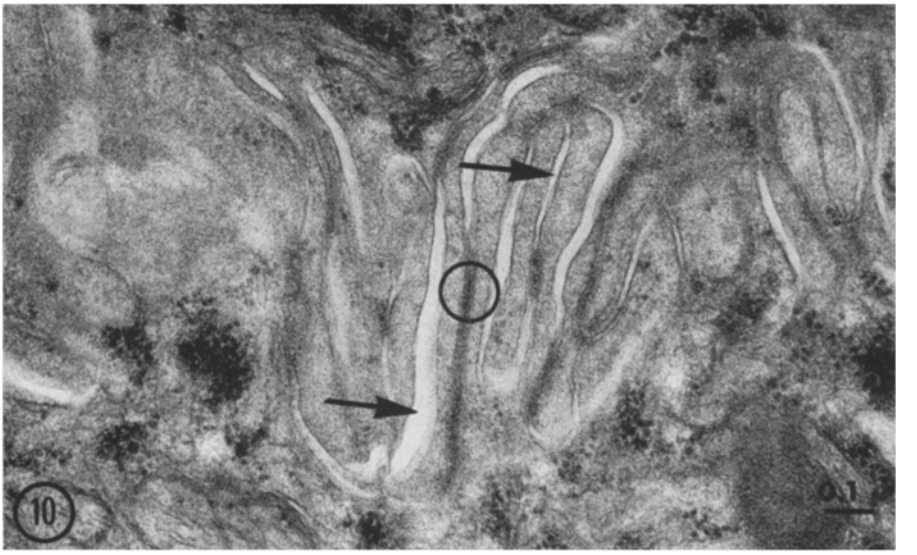
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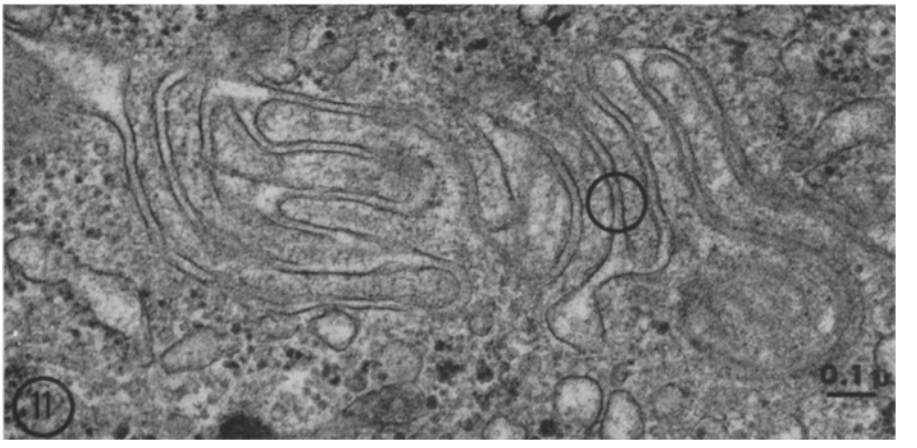
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than elsewhere. The region depicted here is representative of the more apical three-fourths of the space. It is obvious that interdigitations between adjacent cells partially close off this space, and its "open" area is therefore reduced by roughly 50%. The precise three-dimensional geometry of these interdigitations is uncertain. Almost all work on the ultrastructure of gallbladder epithelium has employed conventional transmission electron-microscopy, and the interdigitations have been interpreted as interlocking finger-like folds (e.g., Tormey & Diamond, 1967; Hayward, 1968). However, more recent scanning electron-microscope observations (of guinea pig gallbladders) suggest that they should be interpreted as "reticulated plates or microfolds" (Mueller, Jones & Long, 1972).

We can attempt to estimate the contribution of the intercellular spaces to the overall resistance of the gallbladder. For this calculation, we take the height of the average cell as 50 μm and its diameter as 6.5 μm. The lateral space in the upper three-fourths of its extent ranges between 0.4 and 0.7 μm in width. If we assume the effective open width of such spaces is 0.2 μm from base to apex the total resistance attributable to the spaces is approximately 5 $\Omega\,cm^2$. This estimate is very crude, however. In an extreme case,

Fig. 9. *No gradient (control).* This electron-micrograph shows a portion of a lateral intercellular space from the preparation shown in Fig. 8*a*. The space, which is arranged horizontally in this picture, is typical of those found apical to the more complex basal region of the epithelium. The main cell borders are separated by a distance of roughly 0.5 μm. Projecting into this space are long, slender evaginations from either cell. These folds occupy roughly half the potential volume of the space. Magnification, 66,640×

Fig. 10. *Osmotic water flow, serosa to mucosa.* Detail of a lateral intercellular space comparable to that depicted in Fig. 9 concerning arrangement and location. In this experiment the initial resistance was 71 $\Omega\,cm^2$; the tissue was fixed after addition of 600 mM sucrose to the mucosal bathing solution which increased the resistance to 102 $\Omega\,cm^2$. The interdigitating cell processes are closely pulled together, virtually obliterating the space. In many places the unit plasma membranes come into close contact (circle), but are always separated by a thin, dense layer of finely granular material. Interspersed between such areas of intimate membrane contact are pockets where the membranes are separated by apparently empty spaces ranging up to several hundred Å in width (arrows). Magnification, 66,640×

Fig. 11. *Electrical current, serosa to mucosa.* Area comparable to those depicted in Figs. 9 and 10. In this experiment the initial resistance was 56 $\Omega\,cm^2$; the tissue was fixed after passage of a current of 710 $\mu A/cm^2$ which raised the resistance to 104 $\Omega\,cm^2$. The lateral intercellular spaces are much more uniformly closed. The unit plasma membranes are separated by about 200 Å; they nowhere come as close together as in osmotic flow experiments, but neither are wide pockets found. Close inspection of areas such as that encircled suggests that most of the remaining intercellular space is occupied by partially condensed extracellular coat. Magnification, 66,640×

should the interdigitations consist of continuous *unreticulated* folds, the increased tortuosity might easily increase the effective length of the intercellular spaces, and hence their resistance, several fold.

Fig. 10 represents the "tightly closed" intercellular spaces of epithelia where the resistance has been raised by osmotic water flow from serosa to mucosa. In many places there is no obvious extracellular space remaining between the folds; the outer leaflets of the unit membranes do not appear to actually touch, as was reported for rabbit gallbladders under similar circumstances; instead they are separated by approximately 100 Å of dense, finely granular material, which we presume to be condensed extracellular coat. Interspersed with such areas are others where the extracellular space is up to several hundred Å wide; it is uncertain whether spaces are parts of a continuous, tortuous pathway or are sealed off pockets. The degree of intercellular space closure probably explains the observed increase in resistance. We have not attempted to calculate the total resistance of these spaces, as was done previously for the rabbit gallbladder, because of their greater anatomical complexity in addition to the other uncertainties that enter into such estimates.

Fig. 11 shows a "relatively closed" intercellular space typically found when the resistance has been increased by electrical current. The space between the plasma membranes is obviously wider than in the case of osmotic closure; we find no instances of close membrane approximation. There is an appreciable amount of finely granular material between the membranes, which probably is partially condensed extracellular coat. Since the resistivity of this material is unknown, we cannot calculate the contribution of these spaces to the overall tissue resistance. However, it is reasonable to presume that their closure could account for the resistance increase, especially since the passage of electrical current has reduced the normal electrolyte content of this space and thereby increased the resistivity of the remaining fluid.

Although the closure of lateral intercellular spaces provides an explanation for the increased resistance of the tissue, the converse may not be the case. If we assume that the resting resistance of the intercellular space is 5 $\Omega\,cm^2$, no degree of opening of the space could explain the observed resistance fall of 15 to 20 $\Omega\,cm^2$. Therefore, whenever currents are passed from mucosa to serosa or water flows are produced in the same direction by osmotic gradients, the explanation for the fall in resistance would lie outside the intercellular spaces.

One possible site for a resistance fall could be the underlying tissue of the bladder wall. However, examination of the fine structure of this tissue

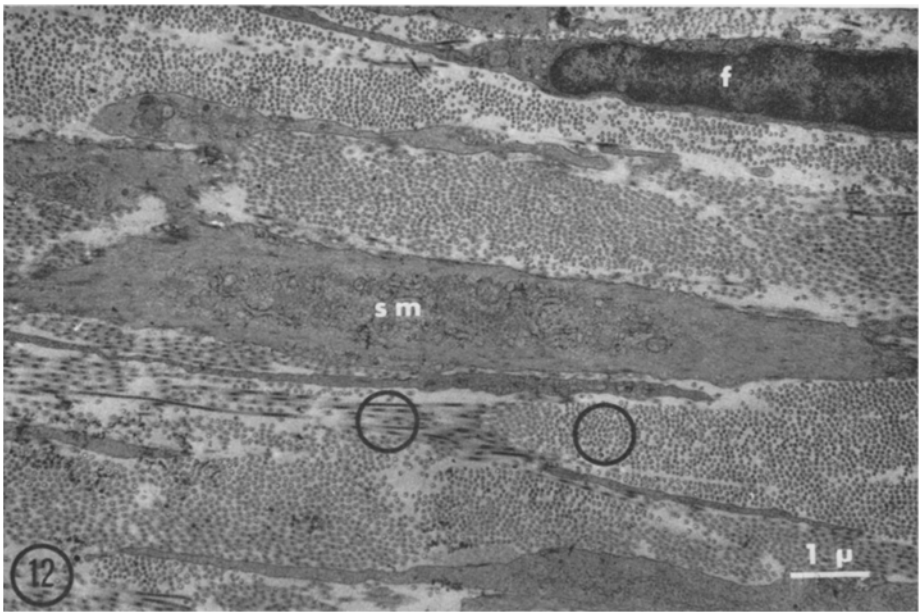

Fig. 12. Low-powered electron-micrograph of a portion of the wall of a control bladder (no gradients). The wall contains numerous collagen fibers (circles), interspersed with smooth muscle fibers (*sm*) and fibrocytes (*f*). These elements fill only approximately half the volume of the wall. Calculations show that increasing this spacing, as in mucosal to serosal water flow, could not possibly cause an appreciable drop in the overall resistance. Magnification, 10,500 ×

in control bladders (Fig. 12), shows that its elements are separated to a degree which makes their role as a significant resistance barrier unlikely [1].

Another possible site of the resistance change is the tight junctions. We have therefore carefully examined the junctions for evidence of structural change and have found none. The dimensions of the junctions are consistent from condition to condition, and we have seen no signs of internal blistering or delamination.

Discussion

Passing direct current across the frog gallbladder produces three transmural voltage transients which are shown in Fig. 1. The first is related to the capacity of the membrane (0.5 to 0.7 $\mu F/cm^2$) and lasts less than 1 msec. The second, due to polarization potentials, has the same polarity as the initial voltage response and builds up with half times ranging from 1 to

1 In three gallbladders where the epithelium was scraped off the bladder the residual resistance was $4 \pm 2\ \Omega\ cm^2$.

20 sec. The third voltage transient, which may appear as a hyperpolarization or a depolarization of the transmural p.d. shows a time course with half times from 0.5 to 4 min. It is this third transient which is of central interest in the present paper. On switching off the current pulse the voltage returns to the original level in two phases – one the capacitative decay and the other the decay of the polarization potentials. The difference between the initial voltage response on switching the current pulse on and off shows that the third slow transient is due to changes in the resistance of the tissue. The application of short current pulses of low current density after the long current pulse reveals that the resistance of the gallbladder returns to the initial value with a half time comparable to that for the onset of the resistance change during the passage of current.

In searching for the explanation of these current-induced resistance changes it should be borne in mind that the high conductance pathway for the flow of current across low resistance epithelia is through the tight junctions and the lateral intercellular spaces. Frömter (1972) has convincingly demonstrated that about 96% of the current flows across the *Necturus* gallbladder via this extracellular pathway. Thus, resistance changes in these low resistance epithelia are likely to be the result of changes in the tight junctions and/or lateral intercellular spaces. Earlier we showed that osmotically induced changes in the electrical conductance, nonelectrolyte permeability, and hydraulic conductivity of the rabbit gallbladdder could be accounted for by variations in the lateral spaces alone: i.e., changes in the dimensions of these spaces accounted qualitatively and quantitatively for the observed permeability changes.

These earlier observations of osmotic effects in the rabbit gallbladder, together with Frömter's (1972) observation that current-induced changes in the electrical resistance of the *Necturus* gallbladder were associated with visible changes in the width of the lateral spaces, led us to search for an anatomical explanation for the effects of current on the frog gallbladder.

We observed that increasing the resistance of the frog gallbladder, by either addition of sucrose to the mucosal fluid or by passing current from the serosa to the mucosa, was associated with the collapse of the lateral intercellular spaces. We feel that the collapse of these spaces can account for the observed increase in resistance. A more rigorous quantitative analysis of the anatomical changes is not possible at the present time owing to the particularly complex architecture of the spaces: e.g. the variation in the width of the spaces along the length of the cell, uncertainty about the three-dimensional structure of the highly developed cellular projections into the spaces, and the presence of surface coats on the lateral plasma membranes.

The collapse of the spaces in both the electrical and osmotic experiments is probably related to water flow across the gallbladder. As discussed previously (Smulders *et al.*, 1972), osmotic water flows from the serosa to the mucosa probably produce negative pressures within the lateral spaces, which in turn cause the spaces to collapse. The development of the negative pressures within the spaces necessitates the presence of one or more hydraulic barriers between the open end of the spaces and the serosal surface of the tissue: the location of these barriers has not yet been positively identified. In the case of current-induced resistance increases, the current flow from the serosa to the mucosa leads to the depletion of salt in the lateral spaces, due to the transport number effect (Fig. 2): this in turn leads to both polarization potentials (page 361) and osmotic water flow from the serosa to mucosa. In addition, current flow may also generate true electro-osmotic flow of water across the tissue. (Current-induced water flows across the rabbit gallbladder have been studied earlier by Wedner and Diamond, 1969, and they concluded that most, if not all, of the water flow was due to the transport number effect.) Irrespective of the relative contributions of electro-osmosis and the transport number effect, the flow of water would in turn lead to the development of negative pressures within the lateral spaces, the collapse of the spaces, and an increase in resistance.

Strong evidence to support our view about the origin of the current-induced resistance increase is: 1) both the polarization potentials and resistance increased in magnitude when we ceased stirring the external solutions. In other words, the salt gradients (and hence the osmotic gradients) across the gallbladder increased with an increase in the thickness of the unstirred layers; and 2) the direction of the resistance change was reversed by addition of La^{3+} to the mucosal solution. Lanthanum converts the gallbladder from a cation- to an anion-selective membrane, and so, for a given voltage gradient across the bladder, La^{3+} changes the polarity of the salt concentration gradients across the tight junctions. For example, when the cathode is in the mucosal solution Na^+ ions carry most of the current in the absence of La^{3+}, and so there is depletion of the salt in the lateral intercellular spaces and water flows from serosa to mucosa, but on addition of La^{3+} to the mucosal solution the bulk of the current is carried by Cl^- ions and this leads to the enhancement of salt in the lateral cellular spaces and water flows from mucosa to serosa.

The origin of the *drop* in resistance is much less certain. Although these drops are accompanied by dilation of the lateral spaces in both current and osmotic experiments, a drop in the resistance of these spaces is not likely to be the major explanation for the effect. There are two reasons for believing

this. The first is that the resistance of the intercellular spaces in the resting state is estimated to be about 5 Ω cm^2. Thus, dilation of the spaces could not account for the observed drop in the resistance. Secondly, aside from the uncertainty as to whether lateral space changes can explain the resistance decreases, we have direct evidence that current *per se* can drop the resistance of this tissue. For instance, in many experiments, particularly at higher current densities, current from serosa to mucosa often produced a transient drop in resistance before increasing it to a new steady level (*cf.* Fig. 7*B*). If low concentrations of sucrose are added to the serosal fluid this effect is emphasized, until at sufficiently high sucrose concentrations any resistance increase is abolished and the current brings about a stable drop in resistance.

These results are reminiscent of our recent experiments on the toad urinary bladder (Bindslev *et al.*, 1974) where we showed that both currents and osmotic gradients, regardless of their polarity, can cause large drops in tissue resistance. In many instances the resistance decreases were accompanied by changes ("blistering") in the tight junctions, but in certain situations no structural alterations could be found. Since in low resistance epithelia the main electrical pathway is via the tight junctions, we believe that much if not all of the resistance decreases we observe in the gallbladder can be attributed to alterations in tight junctions. Our inability to detect concomitant ultrastructural changes in these junctions is no matter of concern, since even in the toad urinary bladder large resistance changes can occur without structural alterations.

Thus the indications are that, as in the case of the toad urinary bladder, electrical and osmotic gradients may increase the intrinsic conductance of the membranes (tight junctions?) of the frog gallbladder epithelium. The difference in this low resistance epithelium is that the lateral intercellular spaces play a major role in determining the overall resistance of the tissue. Thus, when current is passed from serosa to mucosa, the resistance of the tight junctions decreases but the resistance of the intercellular spaces increases; these effects add, but in most instances, the intercellular space effect is dominant. When the current is reversed, the resistance of both junctions and spaces should decrease. The effects of osmotic gradients are probably similar to those of currents.

The final point to be considered is resistance changes brought about by changes in the average salt concentration adjacent to the epithelium. As noted in the preliminary experiments the serosal unstirred layer is about five times thicker than the mucosal unstirred layer (page 360). This means that the salt polarization is greater in the serosal unstirred layer than in the mucosal unstirred layer (*see also* Wedner & Diamond, 1969). Thus

passage of current across the gallbladder will result in changes in the average salt concentration in the solutions immediately adjacent to the epithelium. For example, passing 800 to 900 $\mu A/cm^2$ from the serosa to mucosa generates polarization p.d.'s of about 8 mV. If it is assumed that polarization of the salt occurs mainly in the serosal unstirred layer, it can be estimated from the constant field equation and the ion permeability ratios that the NaCl concentration adjacent to the serosal face of the epithelium is reduced at most by 50 to 60 mM, i.e., the average salt concentration is reduced from 110 to about 85 mM. Using this change in average salt concentration and the observation that the conductance of the tissue is directly proportional to the NaCl concentration (page 360), it is estimated that the resistance should increase by a maximum of 30 $\Omega\ cm^2$. This value should be compared with the actual increase in tissue resistance of 90 to 110 $\Omega\ cm^2$ generated by currents 800 to 1000 $\mu A/cm^2$ (Fig. 4). Thus it may be concluded that most, if not all, of the difference between the maximum increase in resistance produced by currents and by osmotic gradients may be accounted for by this change in average salt concentration. Similar calculations about the increase in concentration associated with passage of current from the mucosa to serosa also show that only a small fraction of the resistance drop can be explained in this manner. In the case of osmotic gradients the polarization potentials indicate that the changes in average salt concentration are in the opposite direction to that required to account for the resistance changes.

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