J. Membrane Biol. 1, 92–108 (1969)

Contributions of Unstirred-Layer Effects to Apparent Electrokinetic Phenomena in the Gall-Bladder

H. JAMES WEDNER and JARED M. DIAMOND

Department of Physiology, University of California Medical Center, Los Angeles, California 90024

Received 24 February 1969

Summary. Passage of electric current across rabbit gall-bladder, which is preferentially permeable to cations, causes water flow towards the negative electrode, as expected for electro-osmosis in a cation-selective membrane. Current passage also causes development of a "polarization potential difference", i.e. a transepithelial potential difference (p.d.) which transiently remains after cessation of current flow and decays back to zero with a half-time of 22 to 90 sec. The polarization p.d. is due to current-induced local changes of salt concentration in unstirred layers, mainly at the serosal face of the epithelium. These changes originate through the so-called transport-number effect. Calculation shows that much of the observed current-induced water flow represents an osmotic flow due to these local concentration changes, rather than representing true electroosmosis. By implication, a large component of streaming potentials in the gall-bladder is a boundary diffusion potential, owing to water flow producing local changes of salt concentration in unstirred layers.

Two electrokinetic phenomena arise in membranes in which frictional coupling between permeating ions and water molecules is possible: electroosmosis, a flow of water observed when an electric current is passed across a membrane; and streaming potentials (or streaming currents), the electrical potential differences (p.d.) or currents observed when water is forced to flow across a membrane under a hydrostatic or osmotic pressure difference. Observations of phenomena considered to be electroosmosis and/or streaming potentials have been reported for the gall-bladder (Diamond, 1962*c*; Pidot & Diamond, 1964; Dietschy, 1964; Diamond & Harrison, 1966) and for other biological membranes [the alga *Nitella* (Fensom & Dainty, 1963), squid nerve (Stallwothy & Fensom, 1966), intestine (Smyth & Wright, 1966; Clarkson, 1967), and frog skin (House, 1964)]. However, it has been appreciated recently that current flow across a membrane may also cause water flow due to the so-called transport-number effect (Barry & Hope, 1969*a*, *b*), i.e., osmosis caused by current-induced solute concentration changes in the unstirred layers

immediately adjacent to membranes. Similarly, water flow across a membrane may also cause p.d. due to boundary diffusion potentials, i.e., p.d. caused by flow-induced solute concentration changes in the unstirred layers. The purpose of this paper is to show that much of the current-induced water flow in rabbit gall-bladder arises from the transport-number effect rather than from true electroosmosis.

Methods

Techniques for isolating and cannulating rabbit gall-bladders, for gravimetrically measuring water flow across the gall-bladder, and for measuring electrical p.d. across the gall-bladder were in general similar to those described previously (Diamond, 1962*a*, *b*, 1964*a*; Diamond & Harrison, 1966*a*; Wright & Diamond, 1968). The gall-bladder consists of a sac lined on the inside by an epithelial cell layer in direct contact with the gall-bladder lumen and supported on the outside by connective tissue. The mucosal bathing solution is the one facing the epithelium, and the serosal bathing solution is the one facing the connective tissue. The mucosal solution is the luminal solution when the gall-bladder is in its in vivo orientation, but is the external solution after the gall-bladder has been everted. Both everted and non-everted gall-bladders were used. In all experiments, the external solution was stirred with a stream of oxygen bubbles, and the luminal solution was unstirred. All experiments were carried out at room temperature (ca. 22 °C).

P.d. were measured with a Keithley 610B electrometer and calomel half-cells, which were connected to the bathing solutions by polyethylene bridges filled with agar: saturated KCl or with agar: isotonic Na_2SO_4 for experiments in KCl or Na_2SO_4 Ringer's solutions, respectively. Electric currents were passed with similar bridges connected to AgCl electrodes and a DC voltage source and were measured by a Keithley 600A electrometer. For simultaneous passage of current and measurement of p.d., two bridges were inserted down the luminal cannula, and two others were in the external bathing solution (Fig. 1). The tip of the luminal current-passing bridge was left near the top of the cannula (ca. 4 cm from the gall-bladder wall), and the tip of the external current-passing bridge lay ca. 8 cm from the gall-bladder. The tips of the two voltage-sensing bridges were placed as close as possible (<1 mm) to the gall-bladder wall itself without actually touching it. In this experimental arrangement, the gall-bladder represents only a fraction of the total resistance between the tips of the current-passing bridges but represents almost all of the resistance between the tips of the voltage-sensing bridges. For instance, when the cell membranes of the gall-bladder were destroyed by exposure for 30 min to Ringer's solution saturated with chloroform, and the trans-gall-bladder p.d. during passage of current was measured with all bridges still in their original positions, this p.d. was found to decrease to less than 4% of the value obtained with gall-bladder cell membranes intact.

For measurements of current-induced water flows, the same arrangement with two pairs of bridges was used. At either 5- or 10-min intervals, the two luminal bridges were removed for 30 to 60 sec while the gall-bladder was being weighed to ± 1 mg on a Mettler balance. The organ was suspended from a weighing hook above the balance pan by a wire hook tied to the cannula. Since the cannula and gall-bladder wall are very light, most of the weight is the fluid in the gall-bladder sac; gain or loss of weight means flow of water across the gall-bladder wall into or out of the sac, and the slope of a graph of weight against time accurately measures the flow rate (Diamond, 1962*a*, 1964*a*). Only noneverted gall-bladders were used for these weighing experiments, since the hydrostatic pressure present during weighing causes some damage to everted preparations.

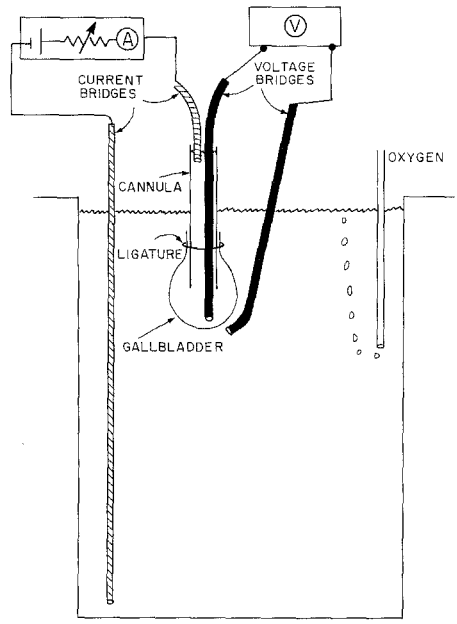

Fig. 1. Experimental arrangement for passing current across a cannulated gall-bladder with one set of agar-salt bridges (indicated by cross-hatching) while recording the potential difference across the gall-bladder with another set (indicated by solid shading). See text for details

The solution referred to in the text as KCl Ringer's solution had the composition in mM: 154 KCl, 0.25 $CaCl_2$, 2.125 K_2HPO_4, and 0.375 KH_2PO_4. The solution referred to as Na_2SO_4 Ringer's solution had the composition in mM: 118 Na_2SO_4, 3 K_2SO_4, 2.125 Na_2HPO_4, and 0.375 NaH_2PO_4. Solutions with different KCl or Na_2SO_4 concentrations were obtained by varying these concentrations without changes in other constituents; hence, isotonicity was not preserved. The pH of all solutions was 7.3.

Results

Current-Induced Water Flow

Experiments were carried out in Na_2SO_4 or KCl rather than in NaCl in order to eliminate the mucosa-to-serosa water flow associated with active NaCl transport even in the absence of applied current (Diamond, 1962*c*, 1964*b*). The first step in the procedure was to permit the gall-bladder to equilibrate for 1 hr with KCl or Na_2SO_4 Ringer's solution used symmetrically as both the luminal and external solutions. Water flow was measured in the absence of current for an additional hour and was usually found to be near zero. Flow was then measured for 30 to 45 min during passage of a constant current (interrupted during weighings). Finally, flow was measured again in the absence of current. The difference

between the flow during current and the average of the small or non-existent flows without current in the preceding and following periods was taken as the current-induced flow. Streaming potentials resulting from addition of 100 mM sucrose to the mucosal solution were measured at the beginning and end of the experiment. The average value and the standard deviation of this streaming potential (mucosa-positive) were 3.6 ± 1.0 mV for 17 gall-bladders in KCl Ringer's solution and 4.1 ± 0.3 mV for 6 gall-bladders in Na_2SO_4 Ringer's solution.

In 11 such experiments, mucosa-negative currents of 1.0 to 3.4 mA (3.4 mA in 1 experiment, 2 mA or less in the other 10) were tested in KCl Ringer's solution and were found always to cause serosa-to-mucosa water flow, at an average rate of 17 ± 9 μliters/hr, mA (average value and standard deviation). In 7 experiments, mucosa-positive currents were tested in Na_2SO_4 Ringer's solution and were found always to cause mucosa-to-serosa water flow, at an average rate of 35 ± 23 μliters/hr, mA. These values and polarities of current were selected on the basis of preliminary experiments which showed that mucosa-positive currents of this size were tolerated less well than mucosa-negative currents in KCl Ringer's solution (as indicated by deterioration of gall-bladder permselectivity) but were tolerated satisfactorily in Na_2SO_4 Ringer's solution. There was no indication of a time lag between the switching-on of current and the attainment of the steady state flow rate, but a lag of up to several minutes would not have been detected if it existed because of the time resolution of the method (a weighing every 5 or 10 min) and because of the modest flow rates.

The sign of both the current-induced flows (flow towards the negative electrode) and the flow-induced p.d. (hypertonic solution positive) is that expected for electrokinetic phenomena in a membrane in which there is greater frictional coupling between water and cations than between water and anions. Alternatively, the sign is also that expected for unstirred-layer effects (the transport-number effect and the boundary diffusion potential) in a membrane more permeable to cations than to anions (*see* Discussion). The results described in the following section show that contributions from unstirred-layer effects are present.

Current-Induced p.d.

Normally, the p.d. across a gall-bladder which separates identical bathing solutions is close to zero in the absence of an applied current, because the gall-bladder has symmetrical permeability characteristics and no electrogenic pumps (Diamond, 1962*b*; Wheeler, 1963; Dietschy,

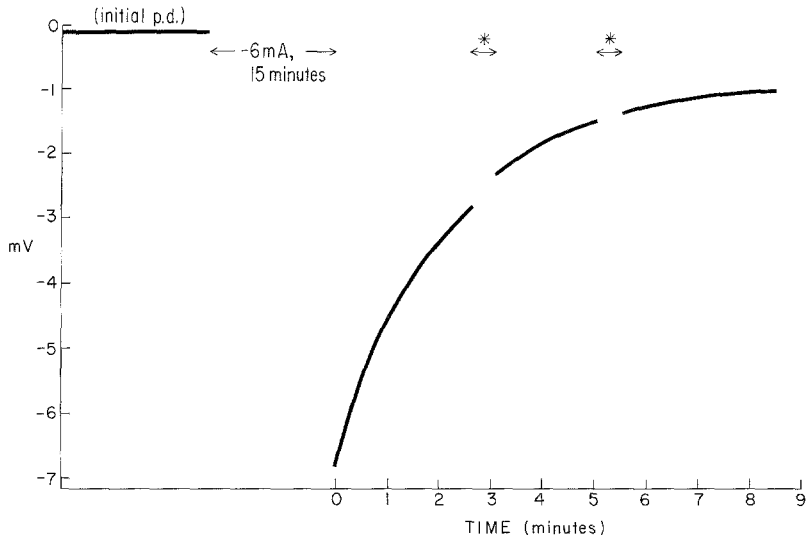

Fig. 2. The effect of renewing the luminal solution on the decay of a polarization p.d. The gall-bladder was everted so that the connective tissue faced the luminal solution and the epithelium faced the external solution. Both solutions were KCl Ringer's solution. The ordinate is the potential of the external solution with respect to the luminal solution. Initially, this was near zero, but immediately after a current of 6 mA (oriented to make the external solution negative) had been passed across the gall-bladder for 15 min, the p.d. was −6.8 mV and gradually decayed back to zero. At the two arrows marked by asterisks, the luminal solution was replaced with fresh KCl Ringer's solution. This is seen to have little or no effect on the decay of the "polarization p.d."

1964; Diamond & Harrison, 1966). An apparent exception to this rule was noticed in the course of the experiments described in the preceding sections; when relatively large currents (1 to 6 mA) had been passed for 5 to 15 min across a gall-bladder separating identical bathing solutions and the p.d. was measured immediately *after* the current had been switched off, a p.d. of up to 8.5 mV was observed (Fig. 2; table). No such p.d. was observed if no gall-bladder was present.

The principal characteristics of these "polarization p.d." were as follows: (1) The sign orientation of the polarization p.d. was the same as that of the much larger p.d. (the IR drop) present across the gall-bladder during passage of the current. (2) The polarization p.d. decayed back to zero with a half-time of 22 to 90 sec after the current had been turned off (Fig. 2). It built up with a similar half-time after the current had been turned on, as shown by briefly interrupting the current at intervals to observe the development of the polarization p.d., or as shown by observing the small gradual increase (corresponding to the amount of the polarization p.d.) in the much larger p.d. present across the gall-bladder during current

passage. In contrast, the buildup and decay of the IR drop associated with current passage were instantaneous on the time scale used (instrumental response delay, 1 sec). (3) As illustrated in Fig. 2, renewing the luminal solution (the serosal solution in this case, since the experiment of Fig. 2 was performed on an everted gall-bladder) had little or no effect on the rate of decay of the polarization p.d., which therefore is not primarily due to concentration changes in the unstirred luminal solution. (4) Polarization p.d. were an order of magnitude larger (and opposite in sign) for mucosa-negative than for mucosa-positive currents. For instance, in one gall-bladder in Na_2SO_4 Ringer's solution, a current of 1.0 mA was passed for 8 separate 10-min periods, with a mucosa-positive orientation in 4 cases and a mucosa-negative orientation in the other 4 cases. Immediately after switching-off of the current, the magnitude of the polarization p.d. averaged 3.6 ± 0.3 mV for the mucosa-negative periods and 0.16 ± 0.11 mV for the mucosa-positive periods. The reason for this asymmetry are given in the Discussion.

As explained in the Discussion section, the polarization p.d. are due to the current-induced accumulation and depletion of salt within the gall-bladder wall itself, mainly at the serosal face of the epithelial cells which are separated from the serosal bathing solution by an unstirred connective-tissue layer ca. 300 μ thick. The gall-bladder is more permeable to cations than to anions, so that transepithelial salt concentration differences result in diffusion potentials in which the dilute side goes electrically positive. The osmotic water flow resulting from these salt concentration changes would be superimposed upon true electroosmosis during current flow. To estimate the size of this effect, one needs to estimate two quantities: the local concentration change implied by a measured polarization p.d., and the osmotic flow rate caused by this local concentration change. These two relations were therefore determined empirically, as described in the next two sections.

The p.d. — Concentration Relations

An estimate of the concentration changes responsible for the polarization p.d. can be obtained by measuring p.d. during known changes in the salt concentration of the serosal solution. In electroosmosis experiments involving KCl, the applied current was mucosa-negative, and the sign of the polarization p.d. was mucosa-negative, suggesting salt depletion at the serosal face of the cells (*see* Discussion). Steady state p.d. were therefore measured as the KCl concentration of the serosal solution was lowered in steps from 154 mM (the value in the mucosal solution) to

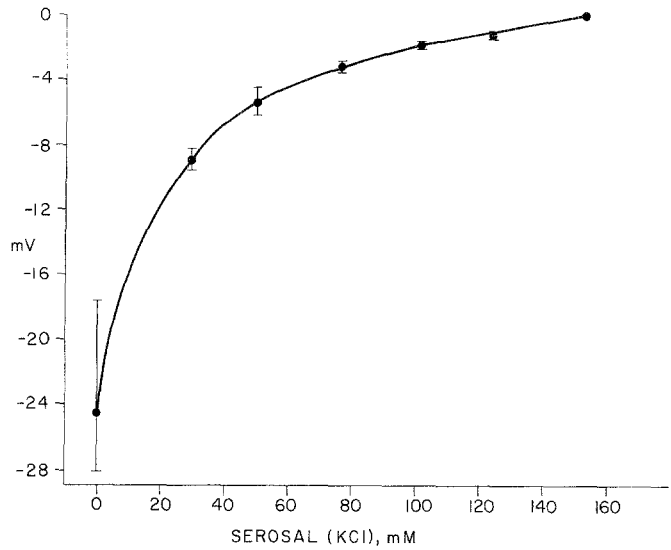

Fig. 3. KCl diffusion potentials in rabbit gall-bladder. The ordinate gives the potential of the mucosal solution with respect to the serosal solution, as a function of serosal (KCl) in mM. The mucosal solution was KCl Ringer's solution ((KCl) = 154 mM) throughout. The serosal solution differed only in having the indicated altered (KCl), and was therefore anisotonic, duplicating conditions prevailing during the existence of polarization p.d. [diffusion potentials across the gall-bladder reported in previous publications (e.g., Diamond, 1962*b*; Dietschy, 1964) have been obtained by isosmotic replacement of salt with impermeant nonelectrolyte, to minimize water flow]. Five gall-bladders were tested at each concentration: the solid circles give the average p.d.; the horizontal lines give the maximum and minimum values

0 mM. As seen in Fig. 3, which gives the averages of values from five gall-bladders, the p.d. is zero in the absence of a salt concentration gradient and becomes progressively more mucosa-negative as serosal (KCl) is reduced. In electroosmosis experiments involving Na_2SO_4, the applied current was mucosa-positive and the sign of the polarization p.d. was mucosa-positive, suggesting salt accumulation at the serosal face of the cells. P.d. were therefore measured as the Na_2SO_4 concentration of the serosal solution was raised in steps from 118 mM (the value in the mucosal solution) to 354 mM. As seen in Fig. 4 which gives the average of values from two gall-bladders, the p.d. is zero in the absence of a salt concentration gradient and becomes progressively more mucosa-positive as serosal (Na_2SO_4) is increased. The mucosa-negative p.d. of Fig. 3 are much larger than the mucosa-positive p.d. of Fig. 4, just as mucosa-negative polarization p.d. were much larger than mucosa-positive ones. This is because the direction of salt concentration changes (ΔC) was opposite in the two figures, affecting p.d. in the following two ways. Since p.d. depend

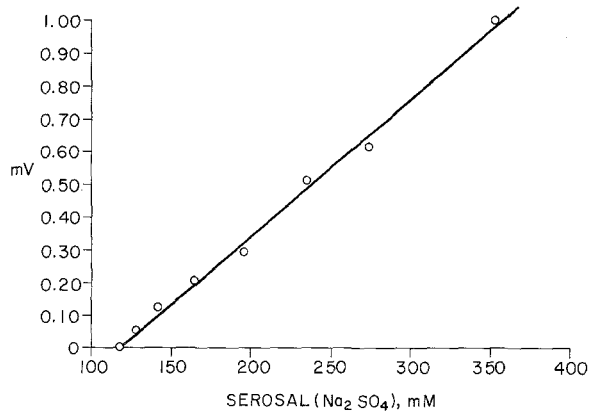

Fig. 4. Na_2SO_4 diffusion potentials in rabbit gall-bladder. The ordinate gives the potential of the mucosal solution with respect to the serosal solution. The mucosal solution was Na_2SO_4 Ringer's solution ($Na_2SO_4 = 118$ mM) throughout. The serosal solution differed only in having the indicated altered Na_2SO_4, and was therefore anisotonic (see legend to Fig. 3). Each point represents the average of values from 2 gall-bladders

upon concentration ratios rather than concentration differences between two bathing solutions, a given ΔC produces a larger concentration ratio and hence a larger p.d. if the ΔC is a decrease rather than an increase. In addition, water flow causes local salt concentration changes at the membrane oriented opposite to the imposed ΔC (*see* Discussion). These changes are proportional to absolute concentration and therefore reduce the effective salt gradient at the membrane more at high than at low salt concentrations.

The Concentration-Flow Coefficient (P_{osm})

To estimate how much of the current-induced water flow could have been due to the osmotic effect of local salt concentration changes at the serosal face of the epithelial cells, osmotic water flows were measured gravimetrically for gradients due to known changes in the salt concentration of the serosal bathing solution. In Na_2SO_4 Ringer's solution, the value of the osmotic water permeability P_{osm} obtained from increases in serosal (Na_2SO_4) of 30 to 80 mM was 0.39 μliter/hr, mosm, gall-bladder. In KCl Ringer's solution, the value obtained from decreases in serosal (KCl) of 26 to 100 mM was 0.21 μliter/hr, mosm, gall-bladder. Serosal concentration changes of these magnitudes and directions were used because they were the ones estimated as being present at the serosal face of the cells during measurements of current-induced flow, on the basis of the measured polarization p.d. and Figs. 3 and 4.

Discussion

The Origin of the Polarization p.d.

The results indicate that current flow across gall-bladder epithelium causes local concentration changes of salt mainly at the serosal face of the epithelium, in analogy to the current-induced concentration changes in unstirred layers observed in electroosmotic studies on artificial membranes (e.g., Stewart & Graydon, 1957) and in algal cells (Barry & Hope, 1969*a*, *b*). The theory of the origin of these concentration changes is well understood and is considered in the next paragraph. Specifically, applied mucosa-negative p.d. cause salt depletion, and mucosa-positive p.d. cause salt accumulation at the serosal face of the epithelium.

In the present experiments, these local concentration changes manifested themselves in three ways: (1) In the absence of ion concentration gradients and current flow, the p.d. across the gall-bladder is near zero. When a salt concentration gradient is applied, the more dilute solution goes electrically positive, indicating greater permeability to cations than anions (Diamond, 1962*b*; Diamond & Harrison, 1966). When a current is passed across the gall-bladder between nominally identical bathing solutions, oriented so as to make the mucosal solution positive, it is found that a small mucosa-positive p.d. builds up and transiently persists when the current is turned off. The orientation of this "polarization p.d." indicates that the local salt concentration at the serosal surface is now higher than that at the mucosal surface. Application of a current oriented to make the mucosal solution negative leaves a mucosa-negative polarization p.d., indicating that the local salt concentration at the serosal surface is now lower than that at the mucosal surface. (2) The decay of the polarization p.d. is unaffected by renewing the luminal solution in either an everted or a noneverted gall-bladder, indicating that the principal local concentration changes are not in the external bathing solutions but within the gall-bladder wall itself. The half-time for decay of the polarization p.d. is of the same order as half-times for diffusion from the serosal face of the cells through the connective-tissue layer to the serosal bathing solution (Diamond, 1966*b*). This suggests that the principal site of the local concentration changes is at the serosal face of the cells. (3) Salt depletion at the serosal face during mucosa-negative currents would be expected to cause serosa-to-mucosa osmotic flow, and salt accumulation during mucosa-positive currents should cause mucosa-to-serosa osmotic flow. These osmotic flows are, in each case, in the same direction as true electroosmosis but overshadow it quantitatively (calculation will be discussed later).

The origin of these local concentration changes lies in the fact that the transport number of potassium or sodium is much higher in the cell membranes of the gall-bladder than in free solution. The theory of this so-called transport-number effect has been worked out in detail and confirmed experimentally by Barry and Hope (1969*a*, *b*), who have shown that local concentration changes in adjacent unstirred layers will, in general, arise during current flow across a membrane whenever ion transport numbers in the membrane differ from those in free solution. Applying the analysis of Barry and Hope to the case of the gall-bladder in KCl solutions as an example, rabbit gall-bladder is approximately 10 times more permeable to K^+ than to Cl^- (Wright & Diamond, 1968), but K^+ and Cl^- have nearly identical transport numbers in free solution. As illustrated in Fig. 5, the passage of slightly more than two Faradays of current therefore tends to cause the local depletion of one mole of KCl in the solution at the positive surface of the epithelium and the local accumulation of one mole of KCl in the solution at the negative surface of the epithelium.

The tendency of this transport-number difference to establish a local concentration gradient of salt is balanced by three effects tending to dissipate the gradient: (1) The local accumulation or depletion of salt tends to be dissipated by diffusion into or from the well-stirred bathing solutions. The thinner the unstirred layers adjacent to the membrane, the more effective is this dissipation, and the more negligible are the local concentration changes. (2) The local gradient tends to be dissipated by back-diffusion of salt through the membrane, at a rate depending upon the membrane's permeability to salt. (3) Osmotic water flow across the membrane due to the local concentration gradient and any electroosmotic water flow coupled to ion transfer through the membrane are oriented in the direction from the low-concentration to the high-concentration side. These tend to sweep away locally accumulated salt from the high-concentration side and to sweep salt into the locally depleted boundary layer at the low-concentration side. This dissipative effect will be more important for higher linear velocities of water flow.

In the steady state, the balance between the transport-number effect and these three dissipative effects will maintain the locally raised and lowered concentrations on opposite sides of the membrane at constant levels. The gall-bladder epithelium is in direct contact with the mucosal bathing solution but is separated from the serosal bathing solution by the connective-tissue layer. Dissipation of local salt concentration changes by diffusion through boundary layers is therefore much more effective at

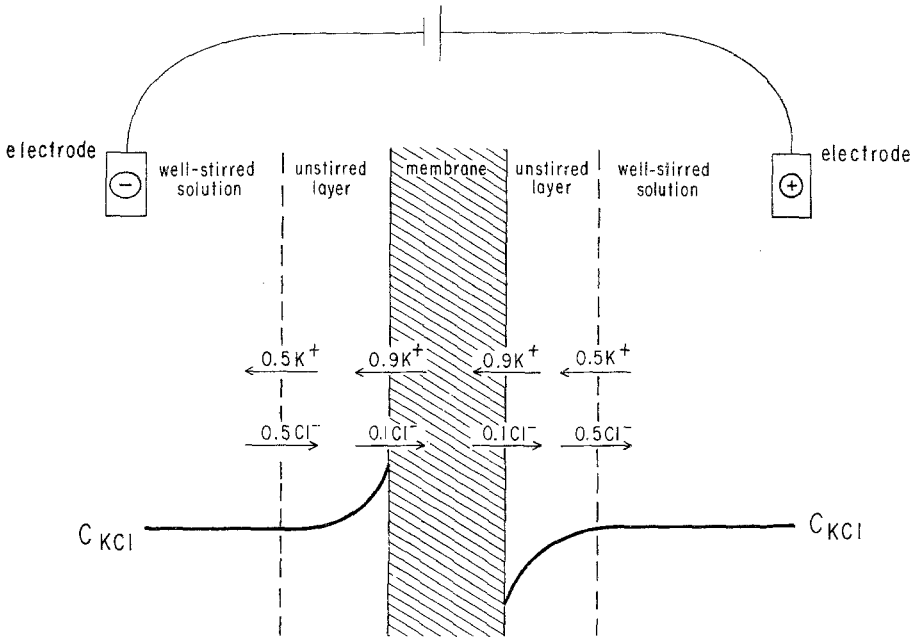

Fig. 5. Diagrammatic example of how the transport-number effect tends to establish local concentration differences across a membrane during current flow. The system consists of a membrane separated by unstirred boundary layers from well-stirred solutions into which electrodes dip for passing current. The transport numbers of K^+ and Cl^- are both nearly 0.5 in free solution but are assumed to be 0.9 and 0.1, respectively, in the membrane (these are the values for rabbit gall-bladder epithelium deduced from diffusion potential measurements; Wright & Diamond, 1968). The figure illustrates by arrows the number of moles of K^+ and Cl^- carrying current through the unstirred layers and through the membrane when one Faraday is passed. This causes the depletion of 0.4 moles KCl at the positive face of the membrane and the accumulation of 0.4 moles at the negative face. These local concentration changes tend to be dissipated by diffusion and two other effects, yielding a concentration profile in the steady state similar to that illustrated below and labeled C_{KCl}. The effect will in principle arise whenever current is passed across a membrane in which ion transport numbers differ from those in the adjacent bathing solutions, but the quantitative significance of the effect depends largely upon the thicknesses of the unstirred layers. (*See* Discussion, and Barry & Hope, 1969*a*, *b*, for further details)

the mucosal than at the serosal surface of the epithelium. At a first approximation, one may neglect changes at the mucosal boundary and consider only changes at the serosal boundary, and the observed relaxation times for the local gradients are close to those for diffusion processes in the connective tissue.

The reasons why polarization p.d. are larger for mucosa-negative than for mucosa-positive currents are analogous to those discussed in Results for the asymmetry of the p.d. – concentration relations of Figs. 3 and 4. P.d. are proportional to concentration ratios, so that depletion of a given amount of salt at the serosal face of the cells (mucosa-negative

currents) causes a larger p.d. than accumulation of the same amount of salt. The third dissipative effect, the effect of water flow, is proportional to absolute concentration and reduces the local gradient more in the accumulation than in the depletion case.

Estimation of the Osmotic Component of Current-Induced Flow

The local concentration changes produced by the transport-number effect at the serosal face of the epithelium will cause osmotic water flow during current passage, in the same direction as the expected electroosmosis. In order to discover how much of the observed current-induced flow represented true electroosmosis, we estimated the osmotic component as follows:

In 10 experiments (five in KCl, five in Na_2SO_4) where current-induced water flow was measured, the polarization p.d. was measured immediately upon switching off of the current. From Figs. 3 and 4, which give the relation between p.d. and serosal salt concentrations, the nominal serosal salt concentration corresponding to the value of the polarization p.d. was read off. The osmotic component of the current-induced water flow was then calculated from the salt concentration gradient (the difference between this calculated serosal concentration and the known mucosal concentration) and from the average P_{osm} values for KCl or Na_2SO_4 Ringer's solutions (0.21 and 0.39 μliter/hr, mosm, gall-bladder, respectively), assuming a linear relation between gradient and osmotic flow rate. In 4 of the 10 gall-bladders, both P_{osm} and current-induced water flow were measured in the same experiment, and the value of P_{osm} determined in that gall-bladder, rather than the average value for all gall-bladders, was used.

As an illustration of this calculation, in a gall-bladder in KCl Ringer's solution, a current of 1.5 mA, mucosa-negative, caused a flow of 31.9 μliters/hr. The measured polarization p.d. was 2.9 mV, mucosa-negative, corresponding in Fig. 3 to a nominal serosal concentration of 82.5 mM KCl. Since (KCl) in the mucosal solution was 154 mM, the nominal concentration gradient was 154 to 82.5 = 71.5 mM KCl. In the same gall-bladder, a gradient of 50 mM KCl (i.e., KCl = 104 mM in the serosal solution) caused a serosa-to-mucosa flow of 10.0 μliters/hr. Thus, the calculated osmotic component of the current-induced flow was (71.5)(10.0)/(50.0) = 14.3 μliters/hr.

Several approximations in the calculation require comment. First, variations in the osmotic coefficient of KCl or Na_2SO_4 with concentration were ignored, because the total variation was only 3% over the con-

centration ranges encountered (33 to 154 mM KCl, 118 to 191 mM Na_2SO_4). Secondly, the relation between concentration gradient and osmotic flow is actually somewhat nonlinear (Diamond, 1966*a*), but the values of the water flows and gradients used to obtain P_{osm} were chosen to be sufficiently close to the current-induced water flows and calculated gradients that the error introduced by the linearity approximation is minor. Finally, it should be realized that the serosal concentrations corresponding to measured polarization p.d. and read from Figs. 3 and 4 are only nominal values used to relate polarization p.d. to water flows, and are eliminated in the course of calculation. Owing primarily to the effect of the osmotic water flow induced by the serosal concentration change (the third dissipative effect listed earlier in this section), the actual concentration at the serosal face of the epithelium during either current passage or a change in the serosal bathing solution will lie between this calculated nominal value and the mucosal solution value.

The table lists polarization p.d., total current-induced flows, and calculated osmotic components of the flow for all 10 experiments in which polarization p.d. and current-induced flows were measured simultaneously. The calculated osmotic component and the total flow are of comparable orders of magnitude in all experiments. Because our method of estimating the osmotic component is an indirect one involving several approximations and assumptions, our results cannot be taken as either precluding or supporting the existence of true electroosmosis in the gall-bladder, and we feel justified in concluding only that much of the current-induced water flow originates through the transport-number effect.

In the alga *Chara australis*, Barry and Hope (1969*a*, *b*) succeeded in temporally resolving the total flow into an electroosmotic component and a local osmotic component by a method permitting much finer time resolution than that attainable in the present study; they confirmed the basis of the local osmotic component by use of an AgCl electrode near the *Chara* cell wall to measure directly the local changes in salt concentration. These experiments showed that electroosmosis does exist in *Chara* but accounts for only 40% of the total current-induced flow. Current-induced water flows have also been reported in the alga *Nitella* (Fensom & Dainty, 1963), squid axon (Stallworthy & Fensom, 1966), intestine (Clarkson, 1967), and frog skin (House, 1964), but these studies assumed the whole flow to be electroosmotic and did not consider the possibility of a component owing to the transport-number effect, the theory of which had not yet been worked out at the time these studies were performed.

Table. *Calculation of the osmotic contribution to current-induced water flow*[a]

Solution	Current (mA)	Observed flow (μliters/hr)	Polarization p.d. (mV)	Calculated osmotic component (μliters/hr)
KCl	1.6	62	6.8	48
	3.4	81	8.2	51
	1.5	22	3.5	24
	1.5	32	2.9	14
	1.0	21	1.1	20
Na_2SO_4	1.0	39	0.16	33
	1.0	46	0.20	41
	1.0	41	0.30	60
	0.5	31	0.13	25
	1.0	41	0.14	27

[a] The second column gives the applied current. The third column is the measured rate of flow across the gall-bladder caused by the current. From the polarization p.d. in the fourth column, an estimate of the local osmotic contribution to the flow is calculated as described in the Discussion section and is given in the fifth column.

Streaming Potentials

When the gall-bladder separates bathing solutions with identical ionic composition and when osmotic water flow is set up by addition of an impermeant nonelectrolyte to one bathing solution, the hyperosmotic solution goes electrically positive (Diamond, 1962*c*, 1966*a*; Dietschy, 1964; Pidot & Diamond, 1964; Diamond & Harrison, 1966). It is observed empirically that this p.d. is directly proportional to the flow rate. Detailed analyses of streaming potentials in artificial membranes (e.g., Schmid & Schwarz, 1952) have shown that the establishment of p.d. by imposed water flow across a charged membrane may involve an unstirred-layer effect as well as the true electrokinetic flow potential, just as the establishment of water flow by imposed currents may involve a local osmotic component in addition to true electroosmosis. The effect in the case of streaming potentials is that water flow across a membrane separating solutions of identical ionic composition will concentrate the solution in the unstirred boundary layer on one side of the membrane and dilute the solution in the opposite boundary layer by the factor $e^{vl/D}$, where v is the linear flow velocity, l the unstirred layer thickness, and D the solute diffusion coefficient. The resulting local concentration gradient $(Ce^{vl/D} - Ce^{-vl/D})$ causes a diffusion potential across the membrane in

the same direction as and superimposed upon the electrokinetic flow potential. In practice, when the mucosal bathing solution of the gall-bladder is hypertonic, serosa-to-mucosa osmotic water flow will locally increase the salt concentration at the serosal face of the epithelium, causing a mucosa-positive diffusion potential. Local changes at the mucosal face will be opposite in direction but quantitatively negligible because of the much thinner unstirred layer.

In the present study, the observed total streaming potential was 3.6 and 4.1 mV/100 mosm in KCl and Na_2SO_4, respectively. Of the observed current-induced water flows of 17.6 and 34.8 μliters/hr, mA in KCl and Na_2SO_4, respectively, the estimation of the local osmotic component suggests that less than one-half, and possibly much less, is likely to be true electroosmosis. If one applies the Helmholtz-Onsager equation relating electroosmosis and streaming potentials in the same membrane (Mazur & Overbeek, 1951) to one-half of the observed current-induced flows, one obtains an upper limit for the contribution that a true electrokinetic flow potential can make to the observed streaming potential: 0.6 and 1.2 mV/100 mosm in KCl and Na_2SO_4, respectively. Thus, much or most of the observed streaming potential must also be an unstirred-layer effect, the boundary diffusion potential.

While the origin of boundary diffusion potentials during water flow in the gall-bladder is qualitatively obvious, the large size of the effect is comprehensible only in the light of recent ultrastructural studies. Until recently, it was tacitly assumed that water fluxes were distributed uniformly over the surface of the gall-bladder. On this assumption it was calculated (Diamond, 1966*b*) that the maximum linear velocity of osmotic water flow for even 400 to 600 mosm gradients was about 10^{-5} cm/sec and that the maximum deviation of the factor $e^{vl/D}$ from 1.00 was 8.5%, so that boundary diffusion potentials would be negligible. However, correlated physiological and anatomical studies of the last few years have shown that gall-bladder epithelial cells are separated basally for most of their length (up to near the tight junctions at the mucosal face) by long, narrow intercellular spaces, and that these spaces provide the route for water flow linked to active solute transport (Diamond & Tormey, 1966*a*, *b*; Kaye, Wheeler, Whitlock, & Lane, 1966; Tormey & Diamond, 1967). Similar studies of ultrastructural changes during osmotic water flow caused by concentration gradients in the external bathing solutions suggest more tentatively that at least part of this flow as well goes via the lateral intercellular spaces (Tormey & Diamond, *unpublished observations*). From widths of lateral intercellular spaces measured by Tormey and

Diamond (1967), one may calculate that the spaces account for only 0.2 to 30% of the cross-sectional area of the epithelium, depending upon the experimental conditions. Thus, if most or all osmotic water flow is confined to the channels, the linear flow velocity (v) would be 3 to 500 times higher than that formerly calculated by assuming uniform flux density over the epithelium, and the factor $e^{vl/D}$ would be correspondingly larger.

Pidot and Diamond (1964) noticed that streaming potentials were associated with water flow resulting from osmotic gradients between the external bathing solutions but not with the isotonic water flow linked to active solute transport. They interpreted this to mean that the two flows went through separate channels, an interpretation that must be abandoned now that the streaming potentials have proved to be largely boundary diffusion potentials. Mucosa-to-serosa passive osmotic water flow produces a large serosa-positive boundary diffusion potential because it considerably dilutes the salt concentration of the lateral spaces. Mucosa-to-serosa solute-linked water transport produces no such boundary diffusion potential, because this transport is isotonic and is maintained by salt transport into the lateral spaces.

Contributions of boundary diffusion potentials to streaming potentials during osmotic water flow are expected to occur in other cells as well. In axons of the squid *Dosidicus gigas*, where the development of the boundary diffusion potential during osmotic flow proves to be much slower than in the gall-bladder, Vargas (1968) succeeded in temporally resolving the total streaming potential into an electrokinetic flow potential and a boundary diffusion potential, and in showing that the former is real but accounts for only 25% of the total observed p.d. The long and narrow channel responsible for the large boundary effect in the squid axon appears to be the Schwann cell slits. The p.d. which Tazawa and Nishizaki (1956) observed in association with transcellular osmosis in 5-cm-long cells of the alga *Nitella flexilis* appeared to be explicable as boundary diffusion potentials. Streaming potentials in the intestine (Smyth & Wright, 1966), which has lateral intercellular spaces similar to those of the gall-bladder, may also prove to possess a boundary diffusion potential component.

References

Barry, P. H., and A. B. Hope. 1969*a*. Electroosmosis in membranes: effects of unstirred layers and transport numbers. Part I. Theory. *Biophys. J.* **9**:700.

– – 1969*b*. Electroosmosis in membranes: effects of unstirred layers and transport numbers. Part II. Experimental. *Biophys. J.* **9**:729.

Clarkson, T. W. 1967. The transport of salt and water across isolated rat ileum. *J. Gen. Physiol.* **50**:695.

Diamond, J.M. 1962*a*. The reabsorptive function of the gall-bladder. *J. Physiol.* **161**:442.
– 1962*b*. The mechanism of solute transport by the gall-bladder. *J. Physiol.* **161**:474.
– 1962*c*. The mechanism of water transport by the gall-bladder. *J. Physiol.* **161**:503.
– 1964*a*. Transport of salt and water in rabbit and guinea pig gall bladder. *J. Gen. Physiol.* **48**:1.
– 1964*b*. The mechanism of isotonic water transport. *J. Gen. Physiol.* **48**:15.
– 1966*a*. Non-linear osmosis. *J. Physiol.* **183**:58.
– 1966*b*. A rapid method for determining voltage-concentration relations across membranes. *J. Physiol.* **183**:83.
–, and S.C. Harrison. 1966. The effect of membrane fixed charges upon diffusion potentials and streaming potentials. *J. Physiol.* **183**:37.
–, and J.M. Tormey. 1966*a*. Role of long extracellular channels in fluid transport across epithelia. *Nature, Lond.* **210**:817.
– – 1966*b*. Studies on the structural basis of water transport across epithelial membranes. *Fed. Proc.* **25**:1458.
Dietschy, J.M. 1964. Water and solute movement across the wall of the everted rabbit gall bladder. *Gastroenterology* **47**:395.
Fensom, D.S., and J. Dainty. 1963. Electroosmosis in *Nitella*. *Canad. J. Bot.* **41**:685.
House, C.R. 1964. The nature of water transport across frog skin. *Biophys. J.* **4**:401.
Kaye, G.I., H.O. Wheeler, R.T. Whitlock, and N. Lane. 1966. Fluid transport in the rabbit gallbladder. *J. Cell Biol.* **30**:237.
Mazur, P., and J.T.G. Overbeek. 1951. On electroosmosis and streaming-potentials in diaphragms. *Rec. Trav. Chim. Pays-Bas.* **70**:83.
Pidot, A.L., and J.M. Diamond. 1964. Streaming potentials in a biological membrane. *Nature, Lond.* **201**:701.
Schmid, G., and H. Schwarz. 1952. Zur Elektrochemie feinporiger Kapillarsysteme. V. Strömungspotentiale; Donnan-Behinderung des Elektrolytdurchgangs bei Strömungen. *Z. Elektrochem.* **56**:35.
Smyth, D.H., and E.M. Wright. 1966. Streaming potentials in the rat small intestine. *J. Physiol.* **182**:591.
Stallworthy, W.B., and D.S. Fensom. 1966. Electroosmosis in axons of freshly killed squid. *Canad. J. Physiol. Pharmacol.* **44**:866.
Stewart, R.J., and W.F. Graydon. 1957. Ion-exchange membranes. III. Water transfer. *J. Phys. Chem.* **61**:164.
Tazawa, M., and Y. Nishizaki. 1956. Simultaneous measurement of transcellular osmosis and the accompanying potential difference. *Jap. J. Bot.* **15**:227.
Tormey, J.M., and J.M. Diamond. 1967. The ultrastructural route of fluid transport in rabbit gall bladder. *J. Gen. Physiol.* **50**:2031.
Vargas, F.F. 1968. Water flux and electrokinetic phenomena in the squid axon. *J. Gen. Physiol.* **51(Part 2)**:123 s.
Wheeler, H.O. 1963. Transport of electrolytes and water across wall of rabbit gall-bladder. *Am. J. Physiol.* **205**:427.
Wright, E.M., and J.M. Diamond. 1968. Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions. *Biochim. Biophys. Acta* **163**:57.