

TWENTY-FIRST BOWDITCH LECTURE

The Epithelial Junction: Bridge, Gate, and Fence

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The hallmarks of epithelial physiology are two phenomena that emerge from the organization of the whole epithelium, and that cannot be explained by any one cell membrane acting alone. These phenomena are net active transport across the epithelial cell layer, and coupling between transepithelial fluxes of actively and passively transported solutes. In the 1950's physiologists tried to explain these phenomena as arising entirely from the properties of two different cell membranes in series. The junctions between the cells were tacitly assumed to be simply pieces of cement. We now know, mainly from work of the past 15 years, that the junctions are as important a key to epithelial physiology as are the cell membranes. The junctions act simultaneously as a bridge, a gate, and a fence. For each of these three junctional roles I shall recount how it was discovered by physiologists, and what are its physiological function and morphological basis. At the end I shall point out what I see as the experimental usefulness of epithelia for nonepithelial physiologists.

The Beer-can Model of Epithelia

Modern understanding of epithelial junctions began with the first detailed electron microscopic study, that of Farquhar and Palade (1963), who showed that junctions include three distinct types of morphological elements: so-called tight junctions, gap junctions, and desmosomes. This organization can best be visualized by referring to the traditional modern epithelial model, a six-pack of beer (Fig. 1). In this model each beer can corresponds to a barrel-shaped epithelial cell; the pop-top end of the can, to the so-called apical cell membrane, which faces the lumen of the epithelium-lined cavity in vivo; the walls of the can, to the lateral cell membranes; the bottom of the can, to the so-called basal cell membrane, which faces the bloodstream in vivo; the spaces between the cans, to the lateral intercellular spaces; and the plastic of the six-pack, to the tight junctions, which constitute hoops encircling the apical end of each epithelial cell and separating the luminal solution from the lateral intercellular spaces. The six-pack model fails to illustrate gap junctions and desmosomes, which are very small contact areas between lateral surfaces of adjacent cells. Whereas the absence of a desmosome analogue lets one flex the six-pack's beer cans apart at their bottoms, the function of the desmosomes is to make this impossible for the epithelium's cells. As bridges, the gap junctions regulate solute movement from inside one cell to inside another cell; as gates, the tight junctions regulate solute movement from the luminal solution directly into the lateral intercellular spaces, by-passing the cells; and, as fences, the tight junctions or associated structures presumably also regulate the movement of membrane constituents from the apical cell membrane to the lateral cell membrane.

The bridge. Of the triple junctional roles as bridge, gate, and fence, the first to be unraveled was that as bridge, in 1965. The discovery of the bridge role was a complete surprise, a by-product of experiments performed for a quite different purpose. Werner Loewenstein and his colleagues wanted to measure the electrical resistance of nuclear membranes, taking advantage of the favorable opportunity provided by the giant epithelial cells of insect salivary glands. To assess this resis-

tance, they inserted several microelectrodes into the same cell, advanced one of these electrodes into the nucleus, passed a current, and measured the resulting voltage step. As a control, they repeated the measurement with one of the voltage-sensing microelectrodes removed to a different cell, expecting to validate their procedure by observing no voltage step (because everyone then assumed the resistance between different epithelial cells to be infinite). Instead, they found to their astonishment that the voltage step was nearly as large as when both microelectrodes were in the same cell!. Evidently, there were some bridge-like structures permitting current flow (i.e., passage of ions) from cell to cell.

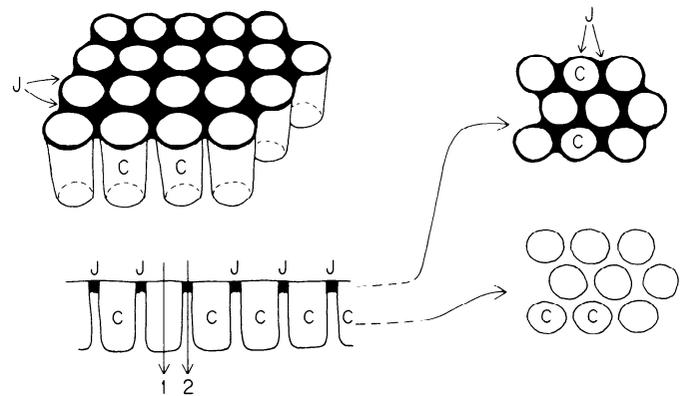


Fig. 1. Schematized conception of epithelial organization. An epithelium consists of cells (C) encircled and held together at one surface by junctions (J), and resembles a six-pack of beer extended indefinitely in two dimensions (upper left). Lower left is a section perpendicular to the epithelial sheet; upper right and lower right, sections in the plane of the sheet at the level of the junctions and lateral intercellular spaces, respectively. Alternative routes across the epithelium are via the cells or via the junctions (routes 1 and 2, lower left). The so-called apical membrane of each epithelia faces the top in the sketches of lower and upper right, while the basolateral membrane faces the sides and bottom.

Much evidence indicates that the junctional element playing this bridge role in vertebrate epithelia is the gap junction. Since the experiments on insect salivary gland in 1965, similar experiments have revealed cell-to-cell current flow under normal conditions within virtually all epithelia examined. Changes in this cell coupling during embryological differentiation and in certain cancers have attracted much interest. Yet the function of the bridges remains unknown. Structures that can transmit ions could be expected to transmit other small solutes, and this has been confirmed by dye microinjection studies. Which solutes are the ones whose exchange via the bridges is physiologically significant? Are they substances that regulate growth and differentiation? Or are they metabolites, ATP, and other high-energy compounds?

The gate. The next junctional role to be clarified was that as gate. Whereas the possible existence of bridges had not even been imagined until their accidental discovery, the question of gates did arise prior to their discovery. However, we assumed that we knew the answer to the question, and our assumption

proved wrong: we assumed the gates to be closed. That is, the tight junctions were believed to prevent transepithelial solute fluxes from by-passing the cells in all epithelia under physiological conditions. The reasons for this erroneous assumption were various: the power of suggestion of the name "tight junction" coined by 19th-century anatomists; demonstrations that junctions actually are tight to proteins and large colloidal tracers, although we now see with the wisdom of hindsight that this need not imply tightness to small ions; and the successes of Ussing and his colleagues in the 1950's in attributing transepithelial ion flow to mechanisms already demonstrated in erythrocyte, nerve, and muscle membranes. As epitheliologists short-circuited tissue after tissue in the 1950's, epithelia were found to exhibit enormous diversity in the identity of the actively transported solute(s), direction of transport, open-circuit voltage, transepithelial resistance, water permeability, and steepness of gradient built up by active transport. A book by Ussing and colleagues (1960) attempted to show that much of this epithelial diversity could in principle arise in cell membranes by different arrangements and quantitative properties of K-Na exchange pumps, K-selective membranes, and Na-selective membranes (Ussing, 1960). As late as 1969, Keynes (1969) exhaustively reviewed the facts of epithelial diversity without seeking the solution in junctional diversity.

During the 1960's, however, several observations began to hint that the junctional gates were normally open to ions in certain epithelia. Shiba (1971), Boulpaep (1971), and Sachs and colleagues (1971) pioneered in applying the technique of cable analysis to epithelia, in attempts to assess the gate question directly. The principle of these experiments was simple: measure the transepithelial resistance, use an intracellular microelectrode to measure the resistance of the apical and basolateral cell membranes, and examine whether the sum of the two cell membrane resistances in series exceeded the transepithelial resistance. A "yes" answer would imply a transepithelial shunt by-passing the cell membranes, such as an open junctional gate. However, the execution of this conceptually simple experiment was greatly complicated by the previously discovered bridge role of junctions, since current injected into one cell to determine its membrane resistances spread to adjacent cells. The bridges were no longer a fascinating surprise but a nuisance that made cable analysis necessary to calculate membrane resistances.

The first full cable analyses of epithelia were performed on renal proximal tubule and gastric mucosa, where tubular geometry or presence of multiple cell types introduced further experimental problems. To circumvent these problems, Eberhard Frömter and I turned in 1971 to *Necturus* gallbladder, a simple flat epithelial sheet with a single cell type (Frömter and Diamond, 1972; Frömter, 1972). The result of cable analysis was that the resistance of the cell membranes exceeded the transepithelial resistance by a factor of 23, meaning that a paracellular shunt accounted for 22/23 of the transepithelial ion conductance. A voltage scanning experiment, in which these shunts were detected by an extracellular microelectrode as local current sinks during transepithelial current passage, confirmed that the shunts were located at the junctions. Thus, the junctional gates of *Necturus* gallbladder, far from being tight, furnish the main pathway for passive ion permeation across the epithelium. From a survey of epithelial properties, Frömter and I concluded that the junctions are similarly "leaky" in small intestine, choroid plexus, and renal proximal tubule, but are relatively tight in frog skin, urinary

bladder, salivary and sweat duct, and probably renal distal tubule and stomach. The opposite extremes in this spectrum are rat proximal tubule and rabbit urinary bladder, with junctional resistances of $4 \Omega\text{-cm}^2$ and over $300,000 \Omega\text{-cm}^2$, respectively.

With the recognition that epithelia vary widely in junctional tightness, much of the previously observed diversity in epithelial physiology fell into place. The epithelia with low open-circuit voltages, low transepithelial resistances, and only modest solute gradients maintained by active transport proved to be the epithelia with leaky junctions, and the explanation for these properties followed immediately from the high passive permeability of the junctions of these epithelia to ions. Conversely, the epithelia with high open-circuit voltages, high electrical resistances, and steep gradients maintained by ion transport proved to be the tissues in which the junctional gates are closed.

The teleological reasons why the gates are closed in certain epithelia and open in others are only partly understood. The reason why urinary bladder, frog skin, stomach, salivary and sweat duct, and distal tubule must have "tight tight junctions" is clear: the function of these tissues is to build up steep gradients of Na^+ or H^+ , and this would be impossible if passive ion permeation down gradients were rapid. Not so clear is why gallbladder, small intestine, proximal tubule, and choroid plexus have "leaky tight junctions" and whether they would function equally well with the junctional gates closed. A possible reason is that leaky junctions are somehow involved in coupling of passive solute and water fluxes to fluxes of actively transported solute. Similarly unclear is the structural basis of differences in junctional tightness among epithelia. Is it that variation in junctional resistance is due to variation in the number of structural "strands" which the junction interposes between the lumen and lateral intercellular space? Is the variation due to different pore sizes of the junctional pathways? Or is it due to differences in charges which line the junctional wall and control ion permeability, as will be illustrated later in Fig. 3?

The fence. These gate studies showed that much of epithelial diversity arises from junctional diversity. However, epithelia also differ in their cell membranes, and this brings us to the question of the fence. The existence of a fence has been implicit in many discussions of epithelial physiology since 1950, but this problem has rarely been considered explicitly. Numerous studies have shown that the opposite-facing membranes of epithelial cells, the apical and basolateral membranes, have quite different properties. What is the fence that lets a cell maintain differences between two membranes that join each other and that presumably possess fluidity? Let us consider three examples of these differences, in order to appreciate how sharp is the dividing line at the putative fence?

1. The apical and basolateral membranes of epithelia differ biochemically. A well-known example is that the membrane-bound enzyme sucrase of the small intestine is confined to the apical membrane. Conversely, $\text{Na}^+\text{-K}^+$ -activated ATP-ase is confined to the basolateral membrane in the intestine and kidney. In intestine the membranes differ in their phospholipid-to-cholesterol ratio.

2. In several leaky epithelia, such as small intestine and proximal tubule, the apical but not the basolateral membrane has a mechanism that serves the passive, coupled entry of Na^+ and nonelectrolytes, such as sugars and amino acids. The apical membrane of small intestine and gallbladder has a similar

mechanism for passive coupled entry of Na^+ and Cl^- . In contrast, the basolateral membrane of small intestine and gallbladder has an active Na^+ pump. The basolateral membrane of these epithelia may also have a Cl^- carrier: net lumen-to-blood Cl^- fluxes across this membrane are apparently down the electrochemical gradient, but the partial conductance of Cl^- is probably much too low to account for the flux without a carrier. Thus, the apical and basolateral membranes of these leaky epithelia differ markedly in their ion transport mechanisms.

3. Just as in leaky epithelia, the two membranes of tight epithelia also differ in their ion transport mechanisms. Until recently, quantitative assessment of these differences was much more difficult than in leaky epithelia, because the high resistance of tight epithelia meant that ion conductance at the damaged tissue edge could dominate measured properties. In the last few years Higgins, Cesaro, Gebler, and Frömter (1975), and Simon Lewis and I (1976), independently devised a method for virtually complete elimination of edge damage. With this new method Lewis and I found junctional resistance of rabbit urinary bladder to exceed $300,000 \Omega \cdot \text{cm}^2$. Here is a tissue in which the tight junctions really are like cement, as classical anatomists assumed. The transcellular resistance of rabbit urinary bladder was shown by the new sealing methods to be up to $80,000 \Omega \cdot \text{cm}^2$, and nearly as high resistances were obtained in frog skin and *Necturus* urinary bladder. However, these cellular resistances were found to vary greatly with physiological state. Active Na^+ transport is associated with an electrical conductance, such that epithelial resistance of rabbit urinary bladder decreases from $80,000$ to $2,000 \Omega \cdot \text{cm}^2$ with increasing short-circuit current. These conductance changes arise entirely in the apical membrane, which possess a Na^+ entry mechanism that is probably passive and is blocked by calcium and by the drug amiloride. (Amiloride acts as a diuretic because it blocks cellular Na^+ uptake, hence Na^+ reabsorption, in distal parts of the nephron). Whether this mechanism is a carrier or pore remains unknown. A similar Na^+ entry step is probably present in the apical membranes of most Na^+ -absorbing tight epithelia. In contrast, the basolateral membranes of these epithelia have an active Na^+ pump, requiring HCO_3^- and inhibited by ouabain.

Let us now return to the question of the fence. Both in leaky and in tight epithelia, the apical membrane differs greatly from the basolateral membrane, as must be true for there to be net transport across the epithelium. Yet membranes are at least partly fluid. How is it possible that, within a few hundred Å, as one rounds the corner of the cell from the apical membrane to the basolateral membrane, one encounters such a change in membrane properties? Where is the fence that separates the amiloride-sensitive entry mechanism from the Na^+ pumps in tight epithelia, or that separates the Na^+ cotransport carriers from the pumps in leaky epithelia? Presumably the fence is at the tight junctions, which constitute hoops completely encircling each cell at this corner. Yet we have no direct evidence at all about the structural basis of the fence. Are the tight junctional structures that constitute the extracellular gate anchored to a hoop of membrane structures that constitute the fence? Is the fence part of a scaffolding that is distributed through the whole cell membrane, and that maintains the cell's shape? Cell asymmetry at the fence also raises questions of membrane assembly. Presumably there is on-going loss or destruction, balanced by synthesis, of the molecular

constituents of pumps, entry mechanisms, and cotransport carriers in the steady state. As the cell synthesizes new molecules of these mechanisms, how do these molecules get steered to the correct side of the cell?

Despite these membrane differences across the fence, the two membranes must still transport the same solutes in series at the same flux rates, and must somehow know what each other is doing. This problem may be illuminated by the discovery, in rabbit urinary bladder, of feedback from the basolateral pump on the apical conductance mechanism. Decreases in pump rate, such as those produced by ouabain or removal of HCO_3^- , decrease apical conductance; increases in pump rate increase apical conductance (Lewis, Eaton and Diamond, 1976). Existence of such feedback is obviously important to cell volume regulation, since a decrease in pump rate without a compensating decrease in Na^+ entry would cause the cell to swell and burst. The feedback problem in the asymmetrical cells of epithelia is therefore relevant also to any symmetrical cell, where the pump and entry mechanism are not spatially separate. The mechanism of this feedback in epithelia remains unknown. One candidate is changes in intracellular Na^+ concentration. If so, however, the mechanism is somehow the opposite of the simple obvious effect of "high ion concentration \rightarrow high conductance," as high intracellular Na^+ concentration would arise from a lowered pump rate, which proves to be associated with low conductance.

The existence of feedback is relevant to the unresolved question of aldosterone's site of action. As is well known, the hormone aldosterone stimulates Na^+ transport in many tight epithelia (e.g., frog skin, rabbit urinary bladder, amphibian urinary bladder, distal tubule, colon, salivary duct, sweat duct). This effect could result either from synthesis of new apical entry molecules, leading to increased cell (Na^+) and hence to increased basolateral pumping; or else from synthesis of new basolateral pump molecules (or of increased energy supply to the pump), leading to lowered cell (Na^+) and hence to increased apical entry. Discriminating between these two theories is difficult, because the presence of feedback means that aldosterone will increase both apical conductance and basolateral pumping, whichever mechanism is the primary target of aldosterone's action.

Although aldosterone acts on many tight epithelia, there is no leaky epithelium in which it has been shown to stimulate transport. This difference is not due to the aldosterone receptor being present but with its transport effects somehow shunted out in leaky epithelia: Nicolette Farman (personal communication) found that specific receptors for aldosterone can be detected biochemically in tight epithelia but not in gallbladder, a leaky epithelium. Thus, tight and leaky epithelia do not just consist of the same cells shunted by junctions of differing leakiness, but also differ systematically in their cell membranes. Two further differences between the cell membranes of tight and leaky epithelia are that only tight epithelia have the amiloride-sensitive Na^+ entry mechanism in their apical membrane, while only leaky epithelia have Na^+ cotransport mechanisms in their apical membrane. It is unclear why, teleologically, the distributions of these three epithelial mechanisms (aldosterone receptors, amiloride receptors, amiloride-sensitive Na^+ entry, Na^+ cotransport) should be so closely correlated with junctional tightness or leakiness.

Emergent Properties

Let us now consider how the roles of junctions as bridge, gate, and fence transform the emergent properties of the whole epithelium. Black-box analysis of epithelial permeability and coupling mechanisms can yield completely misleading conclusions about mechanisms at the membrane level, if one does not consider effects of epithelial organization. I shall illustrate this point by recounting misinterpretations which I proposed for two epithelial phenomena in the 1960's and which I must now dismiss as youthful follies: "electrokinetic phenomena" and osmotic water permeability.

Folly number 1: "electrokinetic phenomena." In 1962 I observed that osmotic water flow across gallbladders separating solutions of identical ionic composition give rise to electrical potential differences directly proportional to the flow rate, and hence formally identical to the flow-related potentials named streaming potentials and often observed in artificial membranes (Diamond, 1962). Other workers subsequently described similar phenomena in small intestine and choroid plexus. The electrokinetic phenomenon that is the opposite of streaming potentials and is also often observed in artificial membranes is termed electroosmosis. In 1969 James Wedner and I observed in gallbladders a phenomenon formally identical to electroosmosis: transepithelial passage of electric current gave rise to transepithelial water flow (Wedner and Diamond, 1969). While the epithelial observations do behave phenomenologically as electrokinetic effects, I drew in 1962 the incorrect inference that the "streaming potentials" also arose mechanistically as an electrokinetic effect at the membrane level--i.e., that they were due to frictional interactions between ions and water traversing the same membrane channels. We were spared this error when we observed "electroosmosis" in 1969, for we simultaneously made the observation that refuted this interpretation. When we applied a transepithelial current to obtain water flow, the voltage we recorded consisted not only of the expected IR step in phase with the current, but also of a voltage that developed more slowly and outlasted the current by many minutes. This slow voltage change showed that ion concentration gradients were being built up within the epithelium.

It turned out that both "streaming potentials" and "electroosmosis" in gallbladder are actually unstirred-layer effects within the epithelium. "Streaming potentials" arise because osmotic volume flow sweeps solute into or out of the lateral intercellular spaces, changing the ion concentrations there, and the local ion concentration gradient across the junctions sets up a diffusion potential. "Electroosmosis" arises because current flow changes ion concentrations within the epithelium through the transport-number effect (Barry and Hope, 1969a, 1969b), and the local osmotic gradient across the epithelium sets up osmotic flow. Both of these coupling phenomena arise from the whole organization of the epithelium and its internal unstirred layers, not from true electrokinetic interactions at the membrane level. No examples of true electrokinetic phenomena have been observed in any epithelium. If they do exist at all, they are below the limit of detection. The same unstirred-layer effects also contribute importantly to apparent electrokinetic phenomena in some artificial membranes.

Folly number 2: measurements of osmotic water permeability. Knowledge of the osmotic water permeability (abbreviated P_{osm}) of epithelia is of interest in several connections, such as in assessing mechanisms of solute-linked water trans-

port. In the 1960's most epitheliologists, myself included, adopted a direct approach: impose an external osmotic gradient, measure volume flow in the steady state, and calculate P_{osm} as the ratio of volume flow to imposed gradient. For leaky epithelia such as intestine, gallbladder, choroid plexus, and proximal tubule, this method yields P_{osm} values ranging from 6×10^{-5} to 8×10^{-3} cm/sec, osmolar.

However, the reinterpretation of "streaming potentials" discussed in the previous section shows that imposition of external osmotic gradients leads rapidly to solute polarization within the epithelium. From the magnitude of the "streaming potential," reinterpreted as a diffusion potential, one calculates that the effective osmotic gradient to drive water flow is reduced at least an order of magnitude below its nominal value, because the internal gradient due to solute polarization is opposite to and nearly as large as the imposed gradient. The conclusion is that measured P_{osm} values underestimate true values at least 10-fold. In elegant experiments on the alga *Chara australis*, Barry and Hope (1969b) used a fast and sensitive method to measure water flow as a function of time, while they used a chloride electrode near the membrane to monitor solute concentration changes simultaneously. Readers eager to savor the full horror of the solute polarization effect, and the errors it produces in P_{osm} measurements, will find this paper by Barry and Hope satisfying (especially their Fig. 16).

Only slightly less horrifying (because volume flows but not concentration changes were measured as a function of time) is the direct demonstration by Wright, Smulders, and Tormey (1972) of how solute polarization distorts P_{osm} measurements in gallbladder. Measuring volume flow (J_V) with a time resolution of 5 min, these authors found that J_V is at least 10 times higher at a time 5 min after imposition of an external osmotic gradient than in the steady state 30 min later. The curve of J_V versus time is so steep in its early phase that the initial J_V value must have been at least 10 times higher again than the 5-min value. Thus, if one cannot measure J_V before internal solute polarization has decreased the effective osmotic gradient, the calculated P_{osm} will be an underestimate by a huge factor. Yet the solute polarization (as gauged by the time course of "streaming potentials") has a half-time of a few seconds in gallbladder, and probably a fraction of a second in perfused renal tubule. No method for measuring J_V in epithelia remotely approaches this time resolution. Even in renal tubule a measurement of J_V takes about 30 sec. The unfortunate conclusion is that available P_{osm} determinations for leaky epithelia are literally worthless, except to indicate lower limits. We have no idea how much higher than the highest measured value of 8×10^{-3} cm/sec, osmolar the true values actually are. Here too, as with apparent electrokinetic phenomena, the organization of the whole epithelium puts a quite different interpretation on measured black-box properties.

Solute-linked water transport. The two previous sections illustrate how epithelial organization can be a nuisance to physiologists. However, this organization is essential to one of the main functions of epithelia, the coupling of water transport to active solute transport.

Virtually all epithelia generate active transepithelial fluxes of solute. These solute fluxes are accompanied by and coupled to passive water flow. In some but not all epithelia, the ratio of water flow to solute flux is such that the transported fluid is isotonic to the animal's plasma. (The correlation is that leaky epithelia, like gallbladder, small intestine, and proximal tubule, and "medium-tight" epithelia, like stomach, colon, and

ADH-treated collecting duct, transport isotonic fluids. Tight epithelia, like urinary bladder, frog skin, and salivary and sweat duct, transport hypertonic fluids.) In four of these isotonic transporting epithelia (gallbladder, small intestine, pancreas, and Malpighian tubule) the further experiment has been performed of varying the bathing solution osmolarity, with the result that transported osmolarity is equal to bathing osmolarity over a 10-fold range. The only reasonable conclusion is that water transport involves complete osmotic equilibration of actively transported solute somewhere within the epithelium. That is, active solute transport into or out of some local space within the epithelium makes this space hypotonic or hypertonic, and water flows across the epithelium down the resulting gradient between the space and the external solution. (The one recent dissenting view, Hill's electroosmotic theory, will be discussed on p. 15). In proximal tubule and perhaps small intestine there is an added complication: besides actively transported solute, much additional solute passively diffuses or is dragged across leaky junctions into the primary transported fluid, and this carries more water by codiffusion (Fordtran, 1975; Boulpaep, 1975; Andreoli, Schafer, and Patlak, 1977).

Is P_{osm} high enough in leaky epithelia to account for complete osmotic equilibration of transported solute? If the local space where equilibration took place were well-stirred, the transported osmolarity O_s would be given by the expression

$$O_s = \frac{C}{2} + \sqrt{\frac{C^2}{2} + \frac{M_o}{RTP_{osm}}} \quad (\text{eq. 1})$$

where

C = bathing solution osmolarity

M_o = active solute transport rate

R = gas constant

T = absolute temperature (Diamond, 1964).

Insertion of the P_{osm} values measured 10 years ago into eq. 1 predicted hypertonic absorbates: the P_{osm} values were apparently too low for complete equilibration.

However, combined physiological and anatomical studies showed that active solute transport took place into (or out of) the long and narrow lateral intercellular spaces, which are unlikely to constitute a well-stirred compartment (Tormey and Diamond, 1967) (Fig. 2). Direct evidence for a role of lateral spaces in osmotic equilibrium was provided by Wall, Oschman, and Schmidt-Nielsen (1970), who were able to withdraw fluid samples by micropipet from lateral spaces of transporting insect rectum and thereby demonstrated that the spaces were hypertonic. Given lack of stirring, the length of lateral spaces makes existence of standing osmotic gradients along the spaces likely. William Bossert and I derived the relevant differential equations and calculated resulting O_s values by computer (Diamond and Bossert, 1967, 1968). Subsequently, Segel (1970) obtained a limiting analytical expression for O_s :

$$O_s = C(1 - K_2)$$

where

$$K_2 \equiv \lambda [\sinh(k/\lambda)] / k \cosh k$$

$$k^2 \equiv 2 C P_{osm} L^2 / rD$$

(eq. 2)

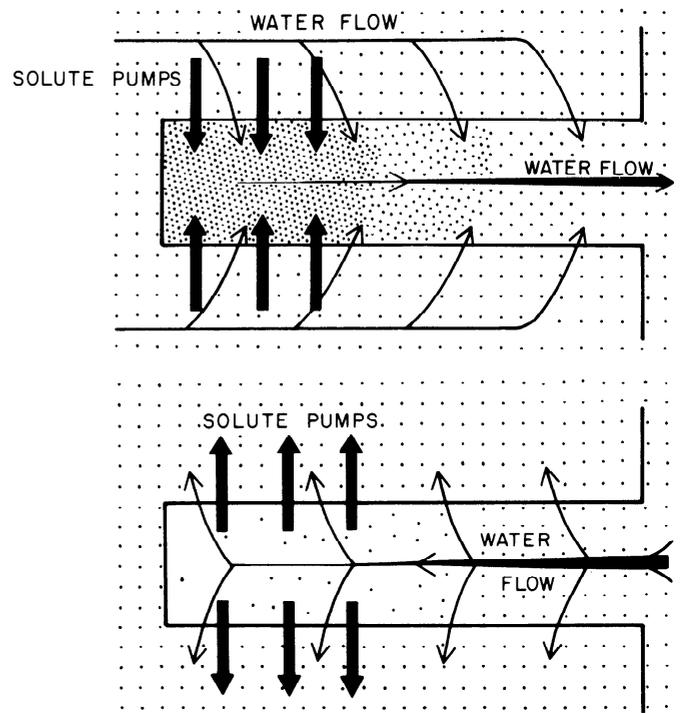


Fig. 2. Comparison of "forward" and "backward" operation of a standing-gradient flow system which consists of a long narrow channel closed at one end (e.g., a basal infolding, lateral intercellular space, etc.). The density of dots indicates the solute concentration. Forward operation (top): solute is actively transported into the channel across its walls, making the channel fluid hypertonic. As solute diffuses down its concentration gradient toward the open mouth, more and more water enters the channel across its walls due to the osmotic gradient. In the steady state a standing osmotic gradient will be maintained in the channel by active solute transport, with the osmolarity decreasing progressively from the closed end to the open end; and a fluid of fixed osmolarity (isotonic or hypertonic, depending upon the values of such parameters as radius, length, and water permeability) will constantly emerge from the mouth. Backward operation (bottom): solute is actively transported out of the channel across its walls, making the channel fluid hypotonic. As solute diffuses down its concentration gradient toward the closed end, more and more water leaves the channel across its walls owing to the osmotic gradient. In the steady state a standing osmotic gradient will be maintained in the channel by active solute transport, with the osmolarity decreasing progressively from the open end to the closed end; and a fluid of fixed osmolarity (isotonic or hypertonic, depending upon the parameters of the system) will constantly enter the channel mouth and be secreted across its walls. Solute pumps are depicted only at the bottom of the channels for illustrative purposes but may have different distributions along the channel. (From Diamond and Bossert (1968) with permission of Rockefeller University Press.)

In this expression L is the channel length, r channel radius, D solute diffusion coefficient, and λ the fraction of the channel length (measured from the blind end) over which active solute transport occurs.

Evaluation of eq. 2 requires knowledge of lateral space dimensions L and r , for which approximate ranges of values could be obtained from ultrastructural studies of epithelia by the mid-1960's. However, most of these studies either dispensed with statistical sampling and just selected "typical" electron micrographs for publication, or else relied on light microscopic techniques and were thus unable to resolve the narrower spaces. All of these studies dispensed with stereological analysis, which is essential for deducing measurements of three-dimensional structures from two-dimensional sections

(Weibel, 1973). Recently the first stereological analysis of lateral spaces in a transported epithelium has been carried out by Blom and Helander (1977), for rabbit gallbladder.

If one inserts into eq. 2 the L^2/r for rabbit gallbladder measured by Blom and Helander, together with the P_{osm} determinations of Wright, Smulders, and Tormey, one finds that osmotic equilibration along standing gradients would suffice, within a large margin of safety, to yield a virtually isotonic transported fluid (Diamond, 1977). If the equilibration space is too short for standing osmotic gradients to be significant, as may be true for proximal tubule, eq. 1 rather than eq. 2 applies. However, insertion of recent P_{osm} values for proximal tubule and gallbladder into eq. 1 still yields a nearly isotonic absorbate.

These calculations bring both good news and bad news to epitheliologists. The good news is that we are now close to a quantitative explanation of isotonic water transport in terms of measured channel dimensions and water permeability of epithelia. The bad news is that upwards revision of epithelial P_{osm} values since 1969 means downwards revision of the osmotic gradients expected to be present between lateral spaces and the external solution. The task of measuring these gradients in epithelia other than insect rectum has never seemed easy, because methods for rapid freezing, solute immobilization, and solute estimation in frozen sections must be developed. The smaller magnitude of the gradients than previously anticipated will make this task harder yet.

At this point mention must be made of two recent papers by A.E. Hill (1975a, 1975b). Many epitheliologists have been puzzled by these papers, in which Hill purported to show that "it seems virtually impossible that the intracellular and lateral spaces of fluid-transporting epithelia are in fact local osmotic coupling spaces" (Hill, 1975a, p. 99). Hill reached this conclusion by inserting what he claimed were typical P_{osm} and L^2/r values for epithelia into eq. 2. He went on to develop an electroosmotic theory of solute-linked water transport.

The explanation of this puzzle is as follows (Diamond, 1977). The P_{osm} value that Hill used, 10^{-5} cm/sec, osmolar, is nearly 1 – 3 orders of magnitude below the measured values for all leaky epithelia. It must be even further below the true values that take account of solute polarization. Hill justified the value of 10^{-5} cm/sec, osmolar on the grounds that it is representative of most cell membranes. In fact, it is below measured P_{osm} values of most cells (see for instance the tabulation by House, 1974, p. 165), and for cells other than erythrocytes these measured values are usually gross underestimates because of unstirred-layer-effects. Hill's L^2/r values were obtained from 12 publications, of which none employed stereological analysis, five only showed "typical" electron micrographs without measurements or statistical sampling, two relied on light microscopy, and one provided no morphological information at all. The L^2/r value Hill quoted for gallbladder proves to be what Tormey and I reported as the lowest extreme value rather than the average value. The resulting L^2/r values Hill used are far below that which Blom and Helander obtained by stereological analysis. Not surprisingly, Hill concluded that epithelial P_{osm} and L^2/r values are too low to account for water transport osmotically. His electroosmotic theory ignores the evidence indicating electroosmosis to be undetectably small in epithelia, and requires electroosmotic coupling efficiencies up to 550 water molecules per ion, one or two orders of magnitude above measured values for artificial membranes.

Uses of Epithelia to Physiologists

I shall conclude by considering a bridge between epithelial physiology and the rest of cell physiology. A prevalent attitude among physiologists in the 1960's went as follows: "If you want to understand basic things about cell membranes, study single cells, like nerve, muscle, and erythrocyte. Avoid epithelia because their organization is too complex."

Now that epithelial bridges, gates, and fences are better understood, physiologists can profit from some real advantages of epithelial sheets over single cells. These advantages include: durability (e.g., gallbladder withstands a pH range from 2.5 to 10.5, and osmolarities from about 60 to 600 mOsm); ready access to both sides of the preparation without need for perfusion; domination of electrical properties of leaky epithelia by a single channel, the junction; lack of the voltage dependence of ion permeabilities that makes nerve interesting but that is a nuisance for studies of permeability properties other than voltage dependence; drug control of ion permeability over one or two orders of magnitude, through amiloride; and pump-leak feedback between two separate membranes, rather than in the same membrane.

To illustrate these advantages, Figs. 3 – 7 present a brief smorgasbord of what epithelia (e.g., gallbladder) can contribute to basic permeability problems:

Fig. 3 illustrates the role of membrane charge in cation-anion discrimination. Cation conductance is constant above pH 5, then plummets with decreasing pH and is negligible below pH 3, while anion conductance changes in the reverse direction and at a lower pH. Evidently, cation conductance is controlled by membrane acidic groups with apparent pK_a near 4.5, and anion conductance is controlled by basic groups with pK_a below 3 (Wright and Diamond, 1968; Moreno and Diamond, 1974).

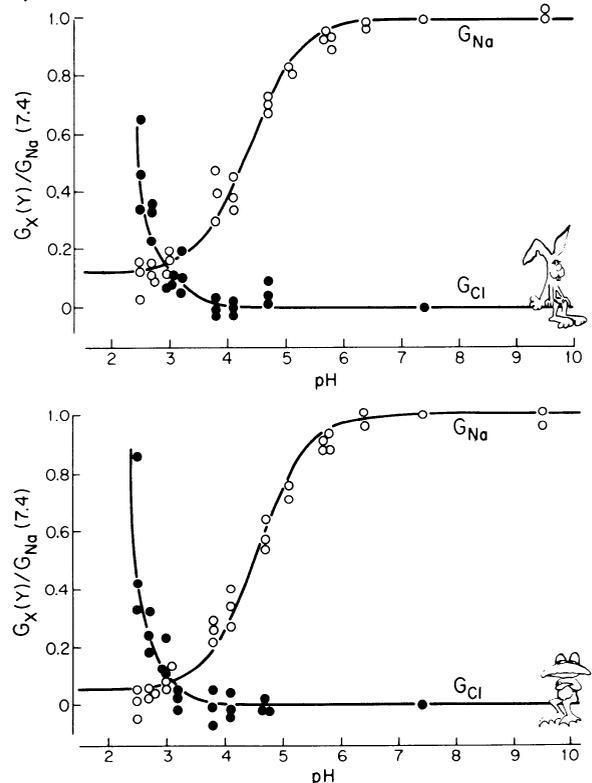


Fig. 3. pH dependence of sodium conductance (G_{Na}) and chloride conductance (G_{Cl}) in rabbit (above) and bullfrog (below) gallbladders. Ordinate is G_{Na} of G_{Cl} at the indicated pH, divided by G_{Na} at pH 7.4. After Moreno and Diamond (1974).

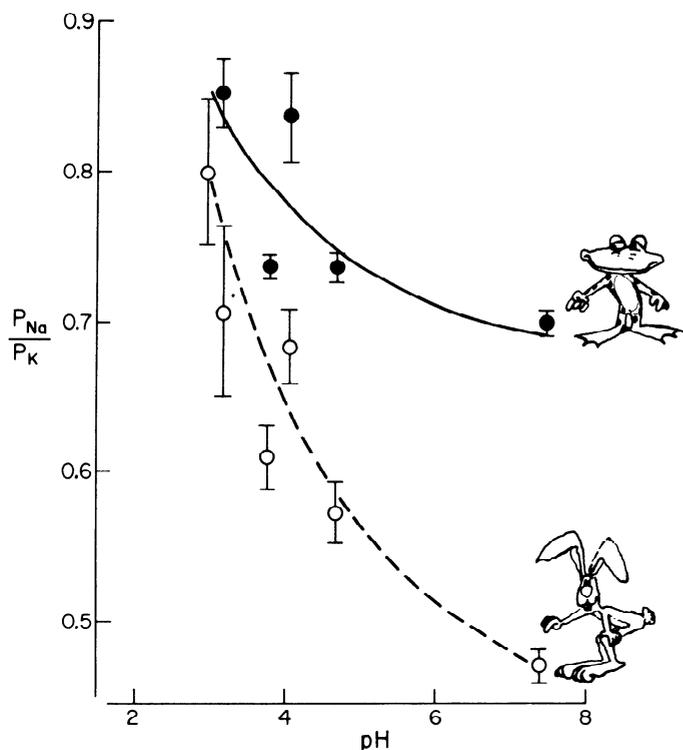


Fig. 4. pH dependence of the sodium-to-potassium permeability ratio in bullfrog (solid points and curve) and rabbit (open points and dashed curve) gallbladders. After Moreno and Diamond (1974).

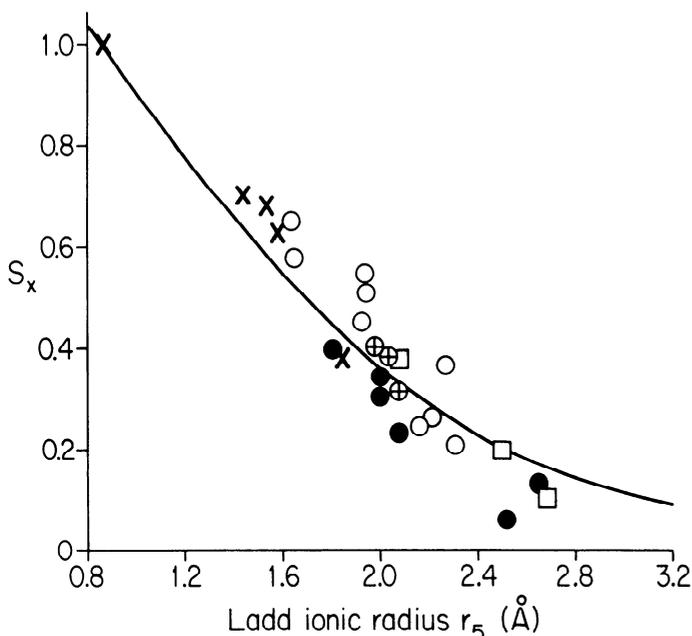


Fig. 5. Sieving of permeating cations by gallbladder tight junctions. Abscissa, the cation's ionic radius; ordinate, the cation's relative permeability coefficient in rabbit gallbladder divided by its relative permeability coefficient in bullfrog gallbladder, interpolated to the same site field strength in both species. Symbol X, inorganic cations; other symbols, organic cations. The solid curve is based on the Renkin equation with the best-fit values of effective pore radii (rabbit 5 Å, bullfrog 8 Å), (From Moreno and Diamond, 1975.)

Fig. 4 illustrates that electrostatic forces and membrane charge also play a role in cation-cation discrimination, as predicted by Eisenman (1962). The permeability ratio P_{Na}/P_K shifts with pH, as does P_{Cl}/P_{Na} (Moreno and Diamond, 1974).

From Fig. 5 one can conclude that cation-cation discrimination depends not only on electrostatic forces but also on steric effects. Ratios, between rabbit and bullfrog gallbladder, of relative permeability coefficients for numerous inorganic and organic cations vary with cation radius, once species differences in electrostatic forces have been removed. The variation suggests an effective channel radius around 5 Å for rabbit gallbladder, and around 8 Å for bullfrog gallbladder (Moreno and Diamond, 1975).

Fig. 6 depicts relative permeability coefficients of organic cations, corrected for the above-mentioned sieving. Permeability increases with the number of protons that the cation has available for hydrogen bonding, up to four protons. Evidently the membrane's cation binding sites are proton acceptors, presumably oxygen functions, that can form up to four strong hydrogen bonds with permeating cations (Moreno and Diamond, 1975).

In Fig. 7 we see that nonelectrolyte reflection coefficients (σ 's) decrease with increasing oil/water partition coefficient, and that in some but not all membranes σ 's are lower for small polar solutes than for other solutes with similar partition coefficients. This suggests that most nonelectrolytes cross membranes by dissolving in the lipid region, and that pores may also be present in some but not all membranes (Wright and Diamond, 1969; Hingson and Diamond, 1972).

All five experiments illustrated in Figs. 3 – 7 yield similar results if performed on nerve, muscle, or erythrocyte. However, the single-cell results are less extensive, harder to obtain, and cover a narrower pH range than the epithelial results.

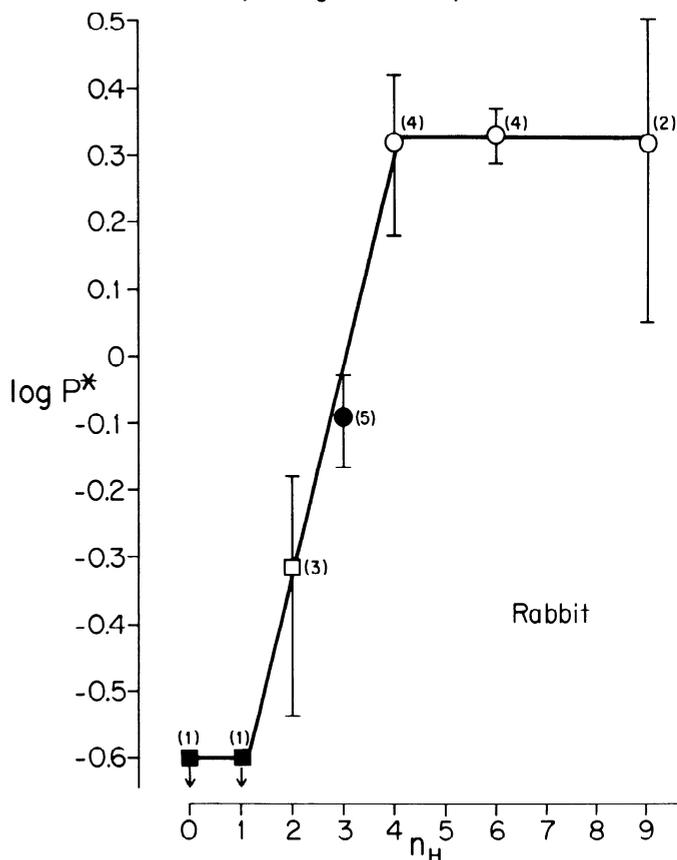


Fig. 6. Ordinate, relative permeability coefficients of organic cations in rabbit gallbladder, corrected for sieving on the basis of Fig. 5. Abscissa, number of protons on the cation capable of donation for hydrogen bond formation. Bars are standard deviations, numbers are the number of cations tested with the given n_H value. (From Moreno and Diamond, 1975.)

GALLBLADDER

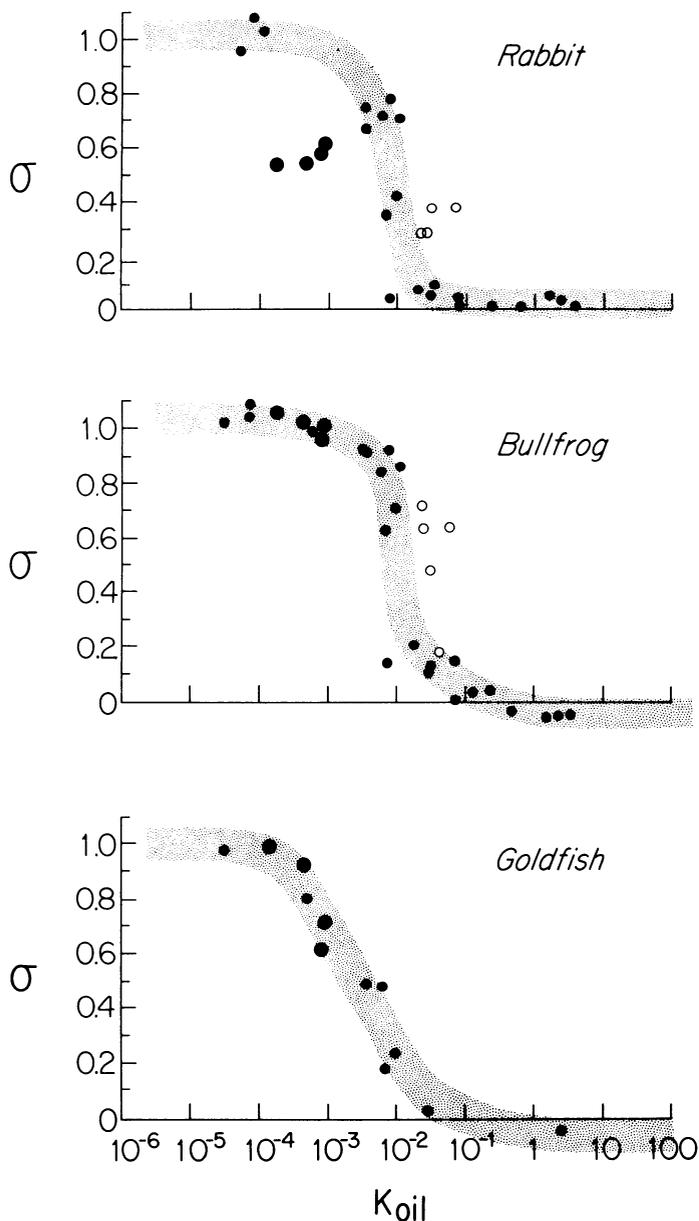


Fig. 7. Nonelectrolyte reflection coefficients (σ) in gallbladders of three species, as a function of the nonelectrolytes' partition coefficients between olive oil and water. Open circles refer to branched solutes. (After Hingson and Diamond, 1972.)

The moral of this smorgasbord is as follows. Perhaps you are not interested in the specific problems of epithelial organization; perhaps bridges, gates, and fences are all a nuisance to you; perhaps you wish to study basic questions common to all cell membranes, such as extraction of channels and carriers, or the origin of ion or nonelectrolyte selectivity. If so, consider seriously the advantages of epithelia!

With pleasure I acknowledge my debt to the colleagues who shared with me the excitement of studying epithelial organization: Peter Barry, William Bossert, Chris Clausen, Eberhard Frömter, Dickson Hingson, Simon Lewis, Terry Machen, Julio Moreno, John Tormey, James Wedner, and Ernest Wright.

This work was supported in part by grant GM14772 from the National Institute of General Medical Sciences and in part by grant AM17328 from the National Institute of Arthritis, Metabolism, and Digestive Diseases to CURE (the Center for Ulcer Research and Education).

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