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*Topical Review*

# Salt-Water Coupling in Leaky Epithelia

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**Summary.** The theory of quasi-isotonic transport by cellular osmosis (the standing-gradient theory) has been challenged on the grounds that the osmotic permeabilities of the mucosal and interspace membranes are too low; if they were as high as the theory requires then the osmotic permeability of the whole epithelium would be 2–3 orders of magnitude higher than observed. This objection has basically been accepted for it is now claimed that these enormous permeabilities do exist, but are masked by unstirred-layer effects; I show that this is incorrect because unstirred-layer corrections are small and that the situation has not changed since 1975.

The view that the route of fluid transport is junctional is replacing the cellular theory, and trans-junctional water flows seem to account for major fractions of the flow in various epithelia. I argue on grounds of general theory that these are unlikely to be osmotic flows because the junctional pores cannot satisfy both the osmotic and diffusive properties required of them, but the basic osmotic theory is also rather vague here.

Non-osmotic theories, if junctional flow is accepted, must be either electro-kinetic or peristaltic.

## Introduction

The present position with regard to salt-water coupling in epithelia must seem to the outsider a rather treacherous jungle at the moment, where if he is not careful he is likely to be severely bitten by an unstirred layer. During the last decade a "mechanics" of epithelia has arisen in relation to applied osmotic gradients, streaming potentials, diffusion potentials, and their transients, the mastery of which has seemed to many an impossible task, and the elasticity of which apparently makes it possible to explain everything. However, the prospect is not so hazy, and I shall argue that there is a much simpler structure to the subject than would at first appear and a clear set of alternatives from which to choose.

In the early seventies there were three dogmas of epithelial research: (1) all actively transported salt is pumped across cells; (2) intercellular junctions are basically seals; and (3) active fluid transfer is due to salt-water coupling by osmosis across cell membranes. The first of these is still held to quite firmly; the second has disappeared, giving rise to the nomenclature of "tight" and "leaky" epithelia (in fact, there is a continuous spectrum of leakiness) whilst the third is under extensive modification by all parties. As cellular osmosis is still held by most people to be the fundamental mechanism underlying salt-water coupling it must inevitably dominate the discussion, but I personally consider it a theory facing the gravest problems. This is not helped by the fact that much of the basic osmotic theory is still unclear in some of its most important aspects; *obscurum per obscurius.*

## Transcellular Osmosis

In 1975 I published a critical review of the standing-gradient theory (Diamond & Bossert, 1967), and in the same year there appeared the paper on osmotic fluid production in *Necturus* proximal tubule of Sackin and Boulpaep (Hill, 1975*a*; Sackin & Boulpaep, 1975). These two papers made essentially the same point, namely, that unless the osmotic permeability of the basolateral membranes of leaky epithelia in general, and proximal tubule in particular, were at least two orders of magnitude higher than the currently accepted value of $10^{-5}$ cm·sec$^{-1}$ osmolar$^{-1}$ then standing-gradient osmosis would contribute little to fluid production, given the observed geometry of the lateral intercellular spaces. If the theory is to encompass the fact that the osmolarity of the mucosal bathing solution can be substantially lowered whilst

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preserving isotonic fluid transport, then an osmotic permeability of $10^{-2}$ is needed to put the theory on a safe footing.[1] It is perhaps worth stressing a point that is often forgotten: many systems show behavior exceptionally close to true isotonicity under some conditions, within 1%, and the slope of the regression line relating the osmolarities of the bath and transported fluid can even be slightly negative (Diamond, 1964); the secretion may therefore often be *truly* isotonic, in which case no osmotic theory is tenable. Osmotic theory predicts an approximation to isotonic behavior in the very region where dependence on high $P_{os}$-values is most critical. Similar conclusions to the above were subsequently drawn for corneal endothelium (Lim & Fischbarg, 1976), and the general case for osmotically-inactive leaky junctions was treated by King-Hele and Paulson (1977).

Although the theory has been vigorously defended (Diamond, 1977; 1978), it is apparent that the analysis described above has finally been accepted. It is now claimed that enormous osmotic permeabilities really exist, permeabilities far greater than those measured in any cells to date, but that any direct observation of them is masked by unstirred layers (Diamond, 1979); unstirred layers, the argument goes, would seem to render dubious the results of any experiment that could be done to disprove the theory.

## The Great Unstirred-Layer Debate

The contention that the osmotic permeabilities of membranes are underestimated is based upon the "sweeping" effect, in which the convection distorts the initially flat concentration profiles adjacent to the membrane. When an osmotic gradient of a probe substance (usually sucrose) is applied across the epithelium, it causes osmotic volume flow which sweeps up the solute on one side, and sweeps it away on the other, resulting in the concentration profiles shown in Fig. 1. The actual osmotic difference at the membrane is lower and $P_{os}$ is underestimated. The effect occurs at the mucosal and basal membranes which are normal to the flow and along the interspaces which are parallel. As the greater part of the basolateral membrane lines the spaces in most epithelia, the sweeping effect here is of particular interest.

Whilst the effect undoubtedly occurs, its precise magnitude has never been computed by its most ardent advocates. What is truly suprising is the claim that it could mask a $P_{os}$-value of $10^{-2}$. As values of $10^{-4}$ to $10^{-5}$ are actually observed, the sweeping

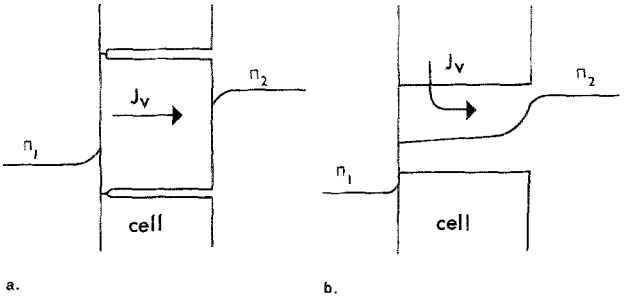


**Fig. 1.** The sweeping effect across a planar epithelial surface (*a*), which reduces the applied gradient, and along an interspace (*b*) due to longitudinal flow. The flow disperses sharply at the mouth as the channel area at the end is about 1/10 that of the epithelium

effect would have to reduce the applied gradient by at least 99%! Wright, Smulders and Tormey (1972) originally described a large transient in the establishment of osmotic flow which was interpreted by them, and by Diamond, as the establishment of an unstirred layer, although it was apparent from the start that its time constant was not only unpredictably large (Pedley & Fischbarg, 1978), but much longer than that of the establishment of "streaming potentials", which were attributed to the same cause.

This transient has recently been shown to be an artifact. When the volume flow is followed directly by volume transducers rather than by weighing a tissue sac, the steady-state flow is set up in 5 sec not 45 min as observed earlier; the transient was almost certainly due to changes in the size of the connective tissue layers which register gravimetrically (van Os, Wiedner & Wright, 1979). We can therefore ignore the small transients and deal with the steady-state flows.

A recent one-dimensional analysis of the problem at quite a rigorous level has shown that, where unstirred layers at the plane surfaces are concerned, a careful choice of parameters for the connective tissue layer ($D$, the salt diffusion coefficient; $\alpha$, the available diffusion cross-section; $\delta$, the layer thickness) leads to a sweeping effect error of 15% at the most (Pedley & Fischbarg, 1980). This treatment takes into account the sweeping effect on both the osmotic probe and the salt of the bathing solution.

What can now be said about convection in the spaces? The solution to this problem has not appeared to my knowledge, but in Table 1 I have calculated the concentration profile and hence the osmotic flow rate in channels spanning the interspace dimensions. The solution is merely that of the one-dimensional convection-diffusion equation with stationary solute gradient ($J_s=0$):

$$v_x \cdot c_x - D\frac{dc}{dx_x} = 0.$$

[1] The osmotic permeability, $P_{os}$, will always be given in units of cm · sec$^{-1}$ osmolar$^{-1}$.

**Table 1.** The sweeping effect in the lateral intercellular spaces

| Interspace width, $w/\mu m$ | $P_{os}/$ (cm·sec$^{-1}$ osM$^{-1}$) | $10^{-5}$ | $10^{-4.5}$ | $10^{-4}$ | $10^{-3.5}$ | $10^{-3}$ | $10^{-2.5}$ | $10^{-2}$ |
|---|---|---|---|---|---|---|---|---|
| 1.2 | | 0.991 | 0.964 | 0.895 | 0.740 | 0.509 | 0.301 | 0.170 |
| 0.4 | | 0.964 | 0.896 | 0.749 | 0.520 | 0.309 | 0.175 | 0.098 |
| 0.12 | $P_{obs}/P_{real}$ | 0.893 | 0.741 | 0.510 | 0.301 | 0.170 | 0.096 | 0.053 |
| 0.04 | | 0.750 | 0.522 | 0.314 | 0.174 | 0.102 | 0.057 | 0.032 |
| 0.02 | | 0.616 | 0.385 | 0.219 | 0.123 | 0.069 | 0.039 | 0.022 |

The ratio of the observed osmotic flow across an unstirred interspace to the maximum flow (well-stirred) in the steady-state, with the two baths clamped at an osmotic pressure difference (Fig. 1*b*). $P_{obs}/P_{real}$, the extent to which the $P_{os}$ value of the lateral membranes is masked by the lack of stirring, is shown as a function of $P_{os}$ and interspace width, $w$. The channel length $L$ is 25 μm. As in experiments, a 50–100 mosM gradient of sucrose $\Delta c$ was applied across the interspace already bathed in Ringer. In the integration the basal medium osmolarity $C_o$ was 0.3 osM and the diffusion coefficient $10^{-5}$ cm$^2$ sec$^{-1}$ (lower than the mean solute $D$), so that the sweeping effect is overestimated, and calculated for both the salt and the probe.

The channel is surrounded by a medium of concentration $C_o$, and the boundary conditions are:

$x=0, \quad v=0$ (closed end)

$x=L, \quad c=C_o+\Delta c$ (applied probe)

where the concentration and velocity at any point are related by

$$c_x=\frac{w}{2P_{os}}\cdot\frac{dv}{dx_x}+C_o$$

$w$ being the width of the interspace. We can see from Table 1 that there is, as predicted, a marked sweeping effect at very high $P_{os}$ values or very narrow channel widths. These results can now be used to re-examine the data of Smulders, Tormey and Wright (1972) and Wright et al. (1972) on osmotic flows in rabbit gallbladder. When the flow is serosal-to-mucosal, the spaces are virtually shut ($w \to 0$, interspace highly convoluted), and when mucosal-to-serosal they are widely open ($w > 0.4$ μm). In the former case we can regard the spaces as nonfunctional, but in the latter they are osmotically very efficient ($P_{obs}/P_{real}=0.9$–$0.96$), and the sweeping effect is quite small. Part of the interspace membrane is occluded, and from its area and the different osmotic permeabilities in the two directions it is possible to calculate its permeability; this comes out to be about $4\times10^{-5}$, and reference to Table 1 shows that this is consistent with the value of $P_{obs}/P_{real}$ chosen and near that of the epithelium.

Recently van Os et al. (1979) have obtained $P_{os}=1.6\times10^{-4}$ for this epithelium, compared to a former value of $9.2\times10^{5}$ (van Os et al., 1976). From a single $P_{os}$ value for the whole epithelium it is impossible to calculate the permeability of the interspace membranes as in the case above where a structural change occurs; the interspace membrane is 20× the area of the mucosal and so this latter dominates the overall epithelial permeability unless its permeability is very high, in which case the reverse is true. The permeability of the interspace membrane is given by $P_{os}$ of the epithelium multiplied by the sweeping factor ($P_{obs}/P_{real}$) divided by the membrane area, which for an interspace width of 0.3 μm leads to a value between 2.5 and $7.7\times10^{-6}$.[2]

The constraint on the permeability of the mucosal membrane is simple: when the epithelium is secreting fluid hypertonic by no more than 2% in Ringer (0.3 osM) at a rate of 100 μl · cm$^{-2}$ hr$^{-1}$, the water activity difference between the cell and the bath cannot exceed 0.006 osmolar (*sic*) if the transported fluid is to be produced by osmosis, i.e., down a gradient of water activity from cell to secretion, in which case the mucosal permeability must be at least $4.6\times10^{-3}$. This precludes the masking of the basolateral permeability by the mucosal membrane if it is also to function in the standing-gradient scheme.

To sum up, it seems that application of a straightforward theory of unstirred layers to the results of osmotic transient experiments does not support the qualitative arguments that they must be able to mask

[2] van Os et al. calculate that opposed to the applied osmotic gradient of 50 mosM there is a salt gradient of 40 mosM set up by the sweeping effect, and that the effective gradient is therefore only 10 mosM. $P_{os}$ is thus raised to $9\times10^{-4}$. This post-operative nursing is based upon the assumption that all of the apparent streaming potential is not only due to dilution potentials, but that it can also be translated simply into concentration differences between the bathing solutions. The authority for this is claimed from Wedner and Diamond (1969), but these authors only *implied* that a sizeable fraction of current-induced volume flow was not electro-osmosis. The complexities of this situation, and the uncertainties which surround the basic assumptions, will be discussed more fully elsewhere.

very large $P_{os}$ values, particularly those of the interspace membranes. The actual values would seem to span the range $10^{-5}$ to $10^{-4}$ which is incompatible with cellular osmosis in most epithelia. Nor has the geometry of interspaces been revised to any extent. In gallbladder the previously determined values for interspace length and width ($L=30$ μm, $w=1.5$ μm) have been revised to $L=18$ to 28 μm, $w=0.24$ to 0.93 μm by stereological analysis of electron micrographs, values which give rise to the same requirements for high osmotic permeabilities when used as the basis for calculation and which show that semiquantitative analyses of micrographs give very reasonable estimates of the dimensions (Blom & Helander, 1977).

## Paracellular Osmosis

If water has difficulty equilibrating over cells then it must cross the junctions in response to hypertonicity in the interspaces; so runs the argument, directly or by implication. It certainly seems to be true that a large fraction of the transported fluid crosses the junctions (Berry & Boulpaep, 1975; Hill & Hill, 1978; Andreoli et al., 1979; Whittembury et al., 1980), but whether this is osmotic flow is unknown. During osmotic flow experiments an upper value of the interspace membrane permeability can be obtained by assuming that the junctions do not participate ($L_p=0$, or $\sigma=0$), but the reverse assumption is never true for the cell membranes are always a complicating factor in any attempt to extricate a $P_{os}$ value for the junctions. The trans- and paracellular routes are not parallel epithelial structures but distributed. However, it is important to examine theoretically whether junctional osmosis is a viable theory for two reasons: (i) junctions are leaky and thus $\sigma$ there may be substantially smaller than 1; (ii) their area is between $10^{-3}$ and $10^{-4}$ that of the interspace membranes. These omens would not seem unduly propitious.

In Fig. 2 I have plotted the osmotic permeability $P_{os}$ against the solute permeability $P_s$ for junctional "membrane" filled with the maximum number of pores per unit area, of sizes from 2 up to 60 Å. This latter may be taken as the upper limit of junction width so that larger pores than this cannot be accommodated. The curve is based upon a Renkin formulation of both the reflection coefficient and the diffusion coefficient, but other treatments give similar results. Plotted nearby are $P_{os}-P_s$ pair values for four selected epithelia: rabbit gallbladder and corneal endothelium, and *Necturus* gallbladder and proximal tubule. The pair values are chosen in response to these two questions: (i) Assuming a transjunctional osmotic gradient of 10 mosM (5 mM NaCl), what $P_{os}$-value of the junctional substance per unit area is needed to explain the volume flows? (ii) What value of $P_s$ results in the backflux out of the junction equalling the total transepithelial flux of Na?

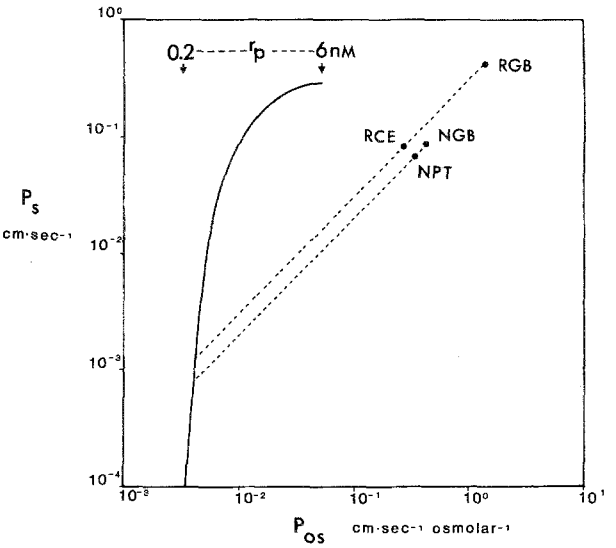


**Fig. 2.** The $P_{os}-P_s$ plane for cylindrical pores in a junction. The curve represents the locus of points representing pore radii from 0.2 to 6 nm covering the whole junctional cross-section (91%). The calculation is based on a Renkin formulation using solute radii (Na, Cl)=0.2 nm, water radius=0.15 nm, salt diffusion coefficient=$1.5\times10^{-5}$ cm/sec, and junction depth 0.4 μm. The four points represent minimum $P_{os}$- and maximums $P_s$-values for the four epithelia given below with the parameters: $L$ – junctional extent in the plane normal to flow; $J_v$ – fluid transport rate; and $C_o$ – medium osmolarity. The junction width is taken to be 5 nm maximum with a 10 mosM hypertonicity in the interspaces (*see* text).

| | | $L$ (cm cm$^{-2}$ epi.) | $J_v$ (μl·hr$^{-1}$ cm$^{-2}$ epi.) | $C_o$ (osM) |
|---|---|---|---|---|
| RGB | (rabbit gallbladder) | 4000 | 100 | 0.2 |
| RCE | (rabbit corneal) endothelium) | 1000 | 5 | 0.2 |
| NPT | (*Necturus* proximal tubule) | 800 | 5 | 0.3 |
| NGB | (*Necturus* gallbladder) | 1300 | 10 | 0.3 |

It is apparent that the points do not lie on the curve, nor to its left, in which condition they would be enveloped. In other words, the osmotic permeability of the junctions cannot really be reached by a porous system, and if it could, its salt permeability would be so large as to ensure massive salt leakage from the junction into the mucosal bath. Any point on the curve can slide along a 45° slope line because it is possible to vary $n/l$ (pore number per unit area/pore length) which is common to both $P_{os}$ and $P_s$; any $P_{os}-P_s$ pair can do likewise because it is in fact independent of the interspace hypertonicity –

a 10-fold increase in this, for example, requires a 10-fold lower value of both $P_{os}$ and $P_s$. The two datasets can be made to coincide on such a line only by shortening the junction length about 100-fold (to 4 nm) or raising the interspace concentration by the same factor (to ~1 osM), neither alternative being really possible. Thus we see that pure junctional osmosis is in rather the same state as cellular osmosis, i.e., it is rather stretched to accommodate the facts. Many workers have used experimentally determined values of $\sigma$ or $L_p$ to predict acceptable osmotic flow rates, but these are always derived coefficients in the sense that osmosis cannot be studied in isolation over the junction.

The possibility of making a judicious mix of the cellular and paracellular systems has been considered and shows signs of becoming very popular. It has been shown, however, that where two sequential osmotic equilibrations are concerned the overall result is due to the product of their efficiencies, not their sum; to achieve the required efficiency of 0.98–0.99 (a transported fluid 1–2% hypertonic) the separate efficiencies of junctional and cellular osmosis would each have to be 0.9, i.e., each would have to work very well by itself (Hill, 1977).

## Nonosmotic Theories – Cellular

There have so far been two proposals for nonosmotic transfer of water through cells. The first involves cell organelles, which may be fixed structures whose function is unclear, or moving vesicles (pinocytosis) (Frederiksen & Leyssac, 1969). Pinocytotic removal of solution from the mucosal bath not only involves a very specific rejection of other solutes which are not transported, but necessitates the turnover of enormous amounts of membrane material to accommodate the flows, due to the high surface-to-volume ratio of small vesicles. In the absence of any compelling evidence in its favor, it remains merely a possibility.

A second mechanism advanced to explain the flow is membrane (Schmid type) electro-osmosis (Hill, 1975*b*). The restrictions to this idea come from the upper limits which could be expected on coupling efficiency. The fact that quasi-isotonic flow can occur in some systems at 1/10 the normal Ringer concentration means that the ion: water coupling ratio has to reach 1:4000, but the number of water molecules in a pore (if there are any) would be about 200–300, and these could only be flushed out by one ion at least.

## Nonosmotic Theories – Paracellular

Paracellular fluid flow of this type can again occur by only two basic mechanisms: (i) by friction with moving salt, or (ii) momentum transfer from a moving component of the junctional architecture.

The first is some sort of electro-kinetic effect, of which electro-osmosis would be one manifestation, described by a Helmholtz-Smoluchowski model in which the dimensions of the pore traversing the junction is large compared to the ionic radii, and the pore core is set in motion by movement of a double layer adjacent to the wall (Overbeek, 1952). This type of electro-osmosis is an ever-present component of fluid movement in porous media and has been the subject of much study for over a century. Calculations based upon this system show that moderate zeta-potentials on the junction lining would create the conditions under which the observed flows could be driven by a transjunctional potential of one or two millivolts; in fact, it is difficult to see how some measure of electro-osmosis can fail to occur unless the junctions are strictly neutral. Such a process is not easy to investigate because as the reflection coefficient of the junctions may be low, transepithelial osmotic gradients would not evoke streaming potentials of any great size, and these would be complicated by dilution potentials due to the flow. I am currently exploring this idea, but everything is hampered by ignorance of the potential gradient in the interspaces.

The second mechanism involves either peristalsis or longitudinal propagation of a surface membrane constituent, or yet a combination of the two. In the literature of phloem transfer (which bears a striking resemblance to this one) there is even a model of propagated action potentials along tubes leading to electro-kinetic pulsatile flow.

## Future Studies

It seems that a future growing point will be the measurement of junctional flows both of water and of solutes. The work cited above on entrainment of solutes in paracellular flow, from which the fraction of water crossing the junctions during normal secretion can be assessed, will no doubt be extended to include not only other nonelectrolytes, but ions like sodium which enter cells. Sodium is of especial interest here, because if there is entrainment then it will be evident that a fraction of the salt transport must be paracellular. If this proves to be so, then we shall have to revise our first dogma that all "active" flows are cellular and include convective fields. Whether this can be achieved within the scheme of purely osmotic models is very doubtful as the volume flow has to be set up by osmosis due to the salt itself – a kind of "bootstrap" effect; osmotic theory is proving inadequate to the task of analyzing this sort

of situation and other related problems in sufficient detail to resolve questions which arise from the physiology of epithelia (Hill, 1979), and necessity may give birth to new developments of a purely theoretical nature in the basic theory of osmosis.

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