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Topical Review

Osmotic Water Flow in Leaky Epithelia

Jared M. Diamond

Physiology Department, UCLA Medical Center, Los Angeles, California 90024

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Summary. I review three currently unsolved and controversial problems in understanding solute-linked water transport in epithelia.

1. Values of osmotic water permeability (P_{osm}) calculated from steady-state osmotic flow in response to a gradient of a probe molecule tend to be underestimates, because of three unstirred-layer (USL) effects. These are: dissipation of the probe's gradient by diffusion in USL's; reduction of the probe's gradient, due to the sweeping-away effect of water flow generated by the probe itself; and solute polarization (creation of an opposing gradient of an initially symmetrically distributed solute by the sweeping-away effect). These errors increase with probe permeability, USL thickness, P_{osm}, and concentration ratio of symmetrically distributed solute to probe, and vary inversely as the fractional area available for water flow (e.g., lateral intercellular space width). The form of an osmotic transient, and the possibility of extracting a true P_{osm} value from the transient, depend on the relative values of three time constants: those for solute diffusion in USL's, for solute polarization by water flow in USL's and for measuring water flow. Sweeping-away effects cause major underestimates (by one or more orders of magnitude) in epithelial P_{osm} determinations, as shown by apparent streaming potentials during osmotic flow and by transiently reversed flows after removal of the probe. True P_{osm} values for leaky epithelia probably exceed 10^{-3} or 10^{-2} cm/sec·osm. The necessary conditions for resolving osmotic transients are set out.

2. I illustrate the difficulties in deciding what fraction of transepithelial water flow is via the cells, and what fraction via the junctions. There is no existing method for answering this question.

3. Controversies about the validity, or need for modification, of the standing-gradient theory are discussed.

Progress in this field requires new methods: to resolve osmotic transients; to separate transcellular and transjunctional water flows; and to measure solute concentrations in lateral intercellular spaces directly.

Solute-linked water transport in epithelia has become a subject of renewed interest and controversy in recent years (Segel, 1970; Weinbaum & Goldgraben, 1972; Schafer, Patlak & Andreoli, 1975; Huss & Marsh,

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1975; Hill, 1975a, 1975*b*, 1975*c*; Sacking & Boulpaep, 1975; Lim & Fischbarg, 1976; Diamond, 1977, 1978; Boulpaep, 1978; Gupta, Hall & Naftalin, 1978; Hill & Hill, 1978*a*, 1978*b*; Spring & Hope, 1978, 1979; Gupta & Hall, 1979; Andreoli & Schafer, 1979; Di Bona & Mills, 1979; Huss & Stephenson, 1979; Welling & Welling, 1979). The persistence of controversy in an area of science suggests an absence of overwhelmingly compelling evidence that would let one discriminate among competing interpretations. This seems to me accurately to describe the present state of research into solute-linked water transport. I perceive three major problems in this area as remaining not only unsolved, but unsolvable by existing methods: the magnitude of the errors that unstirred-layer (USL) effects introduce into determinations of osmotic water permeability (P_{osm}) in epithelia; the relative importance of the transcellular and transjunctional routes of water flow; and the validity of the standing-gradient theory.

This review aims to point out why these three questions remain unsolved, and to refute claims that they are already answerable. My hope is that epitheliologists will thereby be motivated to seek the new techniques that could answer these questions.

Background

In the 1950's and 1960's it became clear that water transport in epithelia is a passive consequence of active solute transport and that the coupling mechanism involves local osmotic equilibration of transported solute within the epithelium (Curran & Solomon, 1957; Curran & McIntosh, 1962; Diamond, 1964; Tormey & Diamond, 1967).[1] The discovery that the route of water transport in gallbladder is via the long and narrow lateral intercellular spaces (abbreviated LIS) made the existence of standing osmotic gradients seem inevitable (Diamond & Bossert, 1967, 1968) – *if* water flow were transcellular, as then assumed. (Standing gradients would also help explain how water flow can be generated although the final transported fluid is isotonic within experimental error.)

[1] One of the few recent dissents to this conclusion is the view of Hill. As a result of choosing unrealistic values for P_{osm} and channel dimensions in epithelia, Hill (1975*a*) concluded that local osmotic effects could not explain epithelial water transport, and he instead proposed electroosmosis as the explanation (Hill, 1975*b*). The errors in his analysis have been discussed elsewhere (Diamond, 1977, p. 15; 1978; pp. 260 and 272). It is unclear from Hill's recent papers (Hill & Hill, 1978*a*, 1978*b*) whether he has abandoned his electroosmotic theory.

However, Frömter and I (1972) subsequently showed by a quantitative cable analysis that the transjunctional route accounted for 97% of passive ion permeation in *Necturus* gallbladder. When we went on to classify other epithelia as tight or leaky to ions, we noted that the leaky epithelia tended to have higher measured values of P_{osm} than the tight epithelia. This led us to speculate that junctions may contribute significantly to P_{osm} and to solute-linked water flow in leaky epithelia.

This speculation still awaits a clear test, because of two difficulties: (i) Whereas cable analysis can resolve the transcellular and transjunctional routes for passive ion permeation, no comparable method exists to resolve the route of water. (ii) In the 1960's membrane physiologists reluctantly began to recognize the major artifacts caused by neglect of unstirred-layer effects (e.g., the transport-number effect, "creep", current-induced resistance changes, and underestimate of P_{osm} and diffusional permeabilities P_D). Epithelia are especially subject to such artifacts because of their often thick USL's and the geometry of their permeation pathways. The problem of eliminating these artifacts from P_{osm} determinations in leaky epithelia is still far from solution.

Let us now consider these dilemmas further.

Unstirred Layer Effects on P_{osm}

Theory

It was formerly believed that USL effects could cause serious underestimates of P_D's but not of P_{osm}'s (Dainty, 1963). Eventually it became recognized that P_{osm} may also be seriously underestimated, due to a USL effect termed the sweeping-away effect. Detailed experimental (Wright, Smulders & Tormey, 1972; Andreoli, Schafer & Troutman, 1978; Schafer *et al.*, 1978; van Os, Wiedner & Wright, 1979) and theoretical (Schafer, Patlak & Andreoli, 1974) studies, and briefer treatments (Diamond, 1977, 1978) are available for epithelia and even more detailed ones for single cells (Barry & Hope, 1969). Nevertheless, epitheliologists often still dismiss or misunderstand this effect or attempt to convince themselves of its unimportance. Let us therefore try again to explain this problem as clearly as possible (Fig. 1):

Consider an epithelium initially separating two identical solutions in which the main solute will generally be NaCl. At zero time ($t=0$) an osmotically active solute is added to one solution at a bulk concentra-

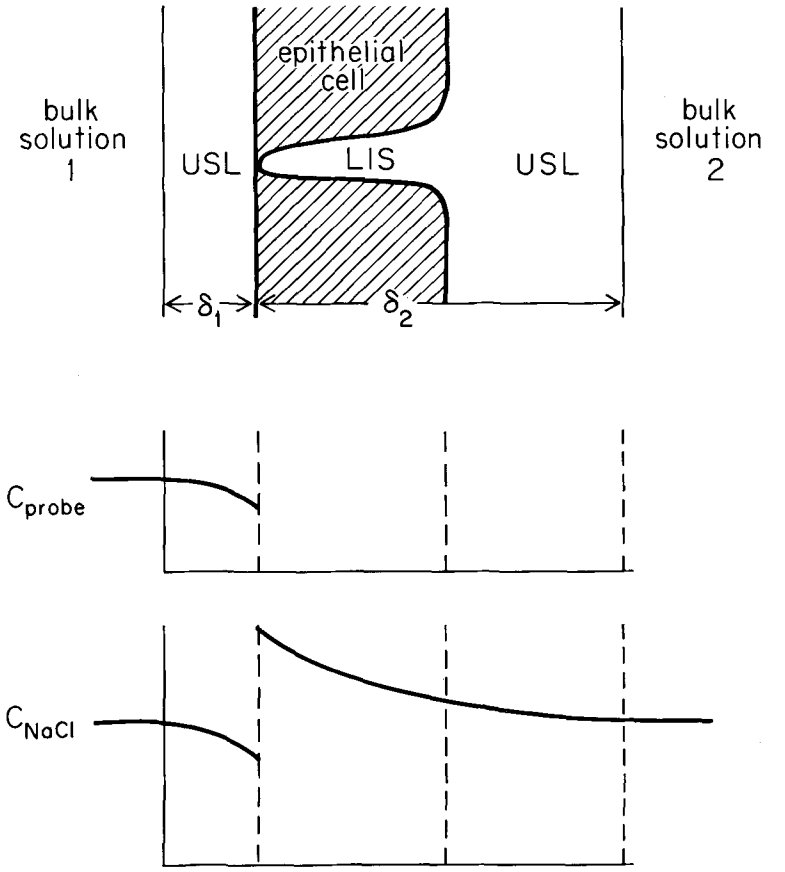

Fig. 1. *Top:* Diagram of an epithelium, with lateral intercellular space (LIS), unstirred layer (USL) of thickness δ_1 at the apical surface, and USL of thickness δ_2 (including the LIS, serosal connective tissue, and adjacent unstirred layer of bathing solution) at the opposite surface. *Middle:* Concentration profile of an osmotic probe solute added to the left bathing solution. *Bottom:* Simultaneous concentration profile of NaCl, present in both bathing solutions at equal concentrations. The sweeping-away effect from osmotic water flow (in direction solution 2 towards solution 1) lowers C_{probe} and C_{NaCl} in the left USL adjacent to the epithelium, and raises C_{NaCl} adjacent to the epithelium in the LIS and right USL. In this figure the probe is assumed to be impermeant, but this will not necessarily be true in actual cases of interest. The NaCl profile will be reversed if the osmotic probe is instead added to the right bathing solution

tion C_1, and P_{osm} is naively calculated as the steady-state water flow J_v divided by σC_1 (or by $\sigma(C_1 - C_2)$ if the solute is also present in the other solution at a bulk concentration C_2). In these expressions, σ is the solute's reflection coefficient. This steady-state calculation in general underestimates P_{osm}. Whether the true value of P_{osm} can even

be extracted from analysis of the whole osmotic transient (e.g., from a peak J_v before the steady state) depends on the relative values of the three time constants: those for solute diffusion in USL's, for solute polarization by water flow in USL's, and for measuring water flow.

If the membrane is impermeable to the osmotic probe solute and J_v is very low, the half-time τ_D for the solute to attain concentration equilibrium adjacent to the membrane is $0.38\ \delta_1^2/D_1$, where δ_1 is the USL thickness on the solution-1 side of the membrane and D_1 is the solute's diffusion coefficient in that USL. For a solute of the molecular weight of sucrose, τ_D is ~ 10 sec for a USL thickness ~ 100 μm (e.g., mucosal surface of gallbladder), several minutes or more for $\delta_1 \sim$ several hundred μm (e.g., serosal surface of gallbladder, mucosal surface of intestine), and $\leqq 0.1$ sec for $\delta_1 \leqq 10$ μm (e.g., renal tubule). (If the probe solute is permeant, part of the solute gradient will be dissipated in the USL's, and the effective osmotic gradient in the steady state (again assuming J_v very low) is not $\sigma(C_1 - C_2)$ but only,

$$\sigma(C_1 - C_2) \Big/ \left[1 + P\left(\frac{\delta_1}{D_1} + \frac{\delta_2}{D_2}\right)\right],$$

where P is the solute's permeability coefficient. Ignoring this correction causes an underestimate of P_{osm}.)

If J_v is not negligible, water flow perpendicular to the membrane causes solute to accumulate at one membrane-solution interface and to be depleted at the other. This is the sweeping-away effect. For an impermeant solute the factor by which the concentration at the membrane differs from the bulk concentration is $e^{\pm v\delta/D}$, where v is the linear velocity of water flow (i.e., J_v (cc/cm^2 · sec) divided by f, the fractional membrane area actually available for flow). Let us explicitly note six points about the sweeping-away effect:

1. Even for an impermeant osmotic probe, P_{osm} will be underestimated, because the effective gradient is reduced from the nominal $(C_1 - C_2)$ to an actual $(C_1 e^{-v_1\delta_1/D_1} - C_2 e^{v_2\delta_2/D_2})$.

2. The sweeping-away effect skews the concentration not only of the osmotic probe but also of any other solute present in the solution. In particular, if both bathing solutions contain NaCl at the same bulk concentration C_{NaCl}, water flow due to the probe's gradient will establish a gradient $C_{\text{NaCl}}\ (e^{-v_1\delta_1/D_1} - e^{v_2\delta_2/D_2})$ across the membrane: i.e., will locally polarize the salt concentration. This gradient is opposite to the probe's gradient, hence further reduces the total effective osmotic gradient and

causes a further underestimate of P_{osm}. Since the bathing salt concentration usually exceeds the concentration of the osmotic probe, salt polarization (i.e., establishment of such a local salt gradient by the sweeping-away effect) causes an even larger error than sweeping-away of the probe itself. To take a simple example, if NaCl is present in both solutions at the usual mammalian concentration of 150 mM and mannitol is added to solution 1 at 50 mM and if $\delta_2 \ll \delta_1$ and the difference between $D_{mannitol}$ and D_{NaCl} is neglected, then an osmotic flow rate sufficient to reduce $C_{mannitol}$ at the solution-1 interface of the membrane to 45 mM would reduce C_{NaCl} there to 135 mM. The effective osmotic gradient (neglecting σ's and osmotic coefficients) would not be 50 nor 45 mM but $45-2 \cdot(150-135)=15$ mM: i.e., only 30% of the nominal gradient. If δ_2 were not very low, C_{NaCl} would be correspondingly raised at the solution-2 interface, and the error would be worse.

This salt polarization is what creates the local salt gradient and boundary diffusion potential (formerly interpreted as a streaming potential) during osmotic water flow in many epithelia (Wedner & Diamond, 1969). In rabbit gallbladder a potential difference of 5 mV is observed in 150 mM NaCl solutions during osmotic flow created by a 100 mM sucrose gradient. From this voltage and the measured P_{Cl}/P_{Na} ratio, one calculates that the local salt gradient established by the sweeping-away effect is 44 mM NaCl or 80 mosM: i.e., that the nominal osmotic gradient is reduced 80% by the resulting NaCl gradient alone, even without considering the further reduction due to the sweeping-away effect on sucrose itself. For details of such calculations, *see* van Os *et al.* (1979), especially their Fig. 7 and their Discussion section entitled "Osmosis". Similar voltages related to osmotic flow in small intestine (Smyth & Wright, 1966), choroid plexus (Wright & Prather, 1970), and renal proximal tubule (Frömter & Lüer, 1969) imply large sweeping-away effects on salt in these other leaky epithelia as well.

3. The magnitude of the underestimate in P_{osm} calculated from steady-state J_v's increases with P_{osm} itself (since v is proportional to P_{osm}). Hence, the leakier the epithelium (to water), the harder it is to measure P_{osm} meaningfully.

4. The magnitude of this underestimate similarly increases with δ, the unstirred-layer thickness.

5. This underestimate increases with $1/f$ (where f is the fractional area available for water flow), because v increases with $1/f$ for a given J_v. This is a major reason why sweeping-away effects are likely to be so much more serious in epithelia than in flat membranes, such as the giant algal cells where the effects were first analyzed: epithelial water

flow is channeled through narrow spaces like the LIS, which account for only a fraction of the epithelium's cross-sectional area. Failure to consider this fact is the reason why I incorrectly dismissed the importance of the sweeping-away effect in earlier studies (e.g., Diamond, 1966, corrected in Wedner & Diamond, 1969), and is part of the reason why the USL calculations used by Fischbarg, Warshavsky, and Lim (1977) and Pedley and Fischbarg (1978) to justify dismissing the importance of the sweeping-away effect are wrong.[2]

6. The half-time τ_{sweep} for establishing solute gradients by the sweeping-away effect increases with the volume of the space (e.g., with δ and f) in which polarization is occurring, and with $1/P_{osm}$. The relatively small LIS of epithelia will tend to make τ_{sweep} brief, especially in leaky epithelia with high P_{osm}. In gallbladder τ_{sweep} in the LIS cannot exceed a few seconds and may be much less.[3]

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[2] The theoretical treatment of osmotic transients by Pedley and Fischbarg (1978) calculated that the sweeping-away effect is likely to cause only negligible to moderate concentration changes in biological membranes, not exceeding an order of magnitude. Fischbarg *et al.* (1977) similarly calculated a correction of only 25% in corneal endothelium. These two calculations are grossly in error, in part because the authors tacitly assumed J_v to be distributed homogenously over the epithelium, whereas it is funneled through the lateral spaces (i.e., they equated v with J_v rather than with J_v/f, which is 3–500 times higher). The treatment of Pedley and Fischbarg is further in error for three additional reasons: (i) It considers sweeping-away only of the osmotic probe itself, whereas sweeping-away of bathing-solution salt will generally be a larger effect (*see* point 2 in the text). (ii) By restricting consideration to impermeant probes, Pedley and Fischbarg also neglect the underestimate of P_{osm} due to the finite permeability of most probes: i.e., the correction term $1+P\left(\frac{\delta_1}{D_1}+\frac{\delta_2}{D_2}\right)$.
(iii) Pedley and Fischbarg treat a membrane with a USL only on one side, whereas there may be significant USL's on both sides. These errors in v and δ have a large effect on the calculated concentration change, as it is given by $e^{\mp v\delta/D}$. Measurements of "streaming potentials" in epithelia exposed to osmotic gradients, and of reversed osmotic flow immediately after a bulk osmotic gradient is removed, confirm order-of-magnitude changes in the gradient due to salt polarization (Machen & Diamond, 1969, p. 211; Wedner & Diamond, 1969, pp. 106–107; Wright *et al.*, 1972; Fig. 1, reproduced as Fig. 3 of the present paper).

[3] When an osmotic gradient is established across gallbladder by addition of sucrose to the mucosal solution, an electric potential difference ("streaming potential") builds up with a half-time of 2–10 sec, depending on stirring conditions and consequent unstirred layer thickness in the mucosal solution. This potential arises from the local NaCl gradient (e.g., locally raised C_{NaCl} in the LIS) generated by the sweeping-away effect. After correction for the differing D's of sucrose and NaCl, these half-times are the same as the half-times for build-up of a diffusion potential following a change in C_{NaCl} in the mucosal solution. Hence salt polarization in the LIS must be rapid compared so salt or sucrose diffusion through the mucosal unstirred layer 50–100 μm thick: i.e., $\tau_{sweep} \ll \tau_D$. In addition, there is a slower polarization of salt in the underlying connective tissue on a time scale of many minutes (Wright *et al.*, 1972).

Having considered τ_D and τ_{sweep}, consider finally τ_{meas}, the time constant for measuring J_v. Most J_v and P_{osm} determinations in epithelia have been based on weighing sacs or determining chamber volumes at intervals for 5 or 10 min. Intervals of 30 sec have been used in some studies of renal tubule (e.g., Dellasega & Grantham, 1973; Schafer *et al.*, 1974). Recently reported methods use intervals of several seconds in chambers (Fischbarg *et al.*, 1977; van Os & Wiedner, 1977; van Os *et al.*, 1979).

Thus, values of P_{osm} based on *steady-state* J_v in response to an osmotic gradient tend to be underestimates for three reasons: dissipation of the probe's gradient by diffusion in unstirred layers; reduction of the probe's gradient, due to the sweeping-away effect of water flow generated by the probe itself; and creation of an opposing gradient of an initially symmetrically distributed solute (e.g., NaCl), again due to the sweeping-away effect. These errors increase with five factors: the probe's permeability; δ_1 or δ_2; v, hence with $1/f$; P_{osm}; and the ratio of the symmetrically distributed solute's concentration to that of the probe.

Given these errors in steady-state methods, could one instead determine the true P_{osm} by monitoring transient changes in J_v following imposition of an osmotic gradient? For example, if J_v rose rapidly to a maximum before declining to a steady-state value, perhaps the maximum J_v might yield a good estimate of P_{osm}? Whether this hope can be realized depends on the relative magnitudes of τ_D, τ_{sweep}, and τ_{meas}, as illustrated in Fig. 2 for four possible cases:

$\tau_{sweep} \gg \tau_D \gg \tau_{meas}$ (Fig. 2*a*). If polarization is slow compared to diffusion and if J_v can be measured very rapidly, a broad maximum in J_v with time can be detected and does yield an approximately correct value of P_{osm}. This situation was realized by Barry and Hope (1969) for osmotic transients in the alga *Chara australis*, where it was also possible to use a AgCl electrode near the membrane to demonstrate a local salt gradient created by the sweeping-away effect.

$\tau_D \gg \tau_{sweep} \gg \tau_{meas}$ (Fig. 2*b*). One can still observe that there is an osmotic transient, in which early J_v values are below steady-state ones because of diffusional delays. However, the build-up of polarization, hence the early maximum in J_v and the possibility of extracting a realistic P_{osm}, are completely lost in the slower diffusional delay: J_v rises monotonically to a steady-state value yielding a P_{osm} value far below the true value. Examples are P_{osm} determinations in epithelia with a thick serosal USL, good measuring time resolution, and the osmotic probe added to the serosal solution.

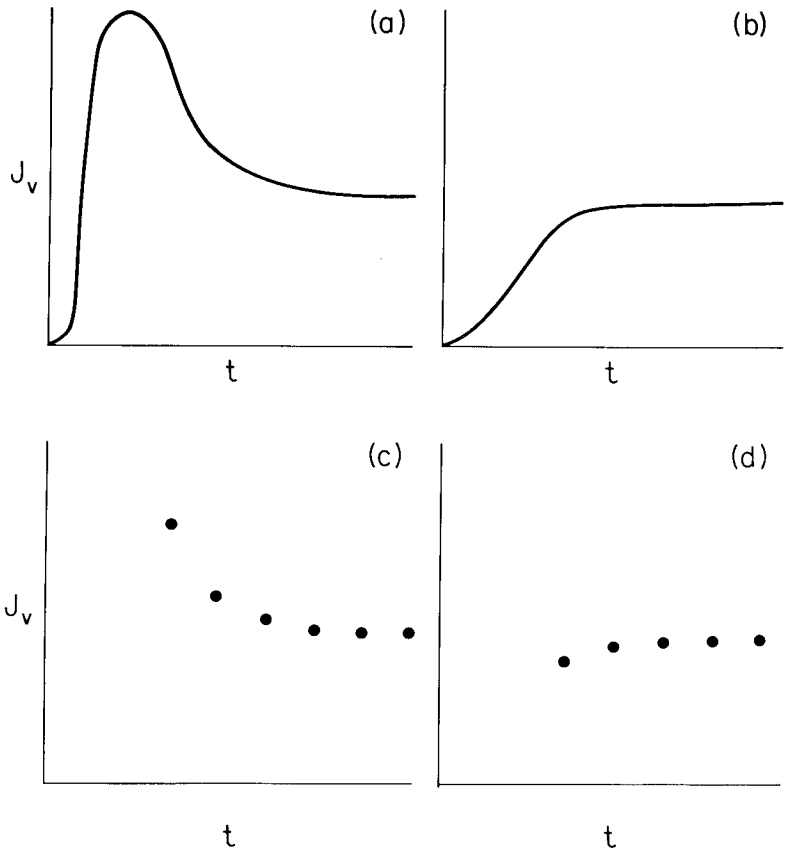

Fig. 2. Expected forms of osmotic transients across a membrane under four conditions. Left (*a* and *c*): Diffusional delays short compared to solute polarization effects; right (*b* and *d*): vice versa. Above (*a* and *b*): J_v measurements very rapid compared to diffusional delays and solute polarization effects. Below (*c* and *d*): Membrane as in *a* and *b*, respectively, but J_v measurements slow, so that only a few measurements can be taken over the time span of *a* and *b*

$\tau_{meas} \geqq \tau_{sweep} \geqq \tau_D$ (Fig. 2*c*). Compared to the case of Fig. 2a, the case assumes a similar membrane but much poorer time resolution for measuring J_v. The first part of the transient, including the peak, is lost, but enough of the transient may be recorded to make clear at least the qualitative existence of a peak. This case corresponds to the experiments of Wright *et al.* (1972), Hays (1972), and B. Hughes and T. Machen (*personal communication*) on osmotic flow across several epithelia with hypertonic mucosal solutions (*see* below for details).

$\tau_{meas} \gg \tau_D > \tau_{sweep}$ (Fig. 2*d*). This corresponds to the situation in most epithelial P_{osm} determinations to date. The possibility of detecting either diffusional delays or the development of the sweeping-away effect is lost. J_v measurements are relatively so slow that the first measured value is already close to the steady-state value.

In short, accurate measurement of P_{osm} requires a membrane in which the sweeping-away effect is either negligible or else slow compared to diffusional delays, and in which J_v measurement are rapid compared to both.

For a quantitative treatment of osmotic transients with special relevance to epithelia, *see* Schafer *et al.* (1974). In their Fig. 4, the upper solid line and both dashed lines correspond to the cases of my Fig. 2*a* and *b*, respectively.

Experiments in Epithelia

The most detailed study of osmotic transients in epithelia is by Wright *et al.* (1972) for rabbit gallbladder mounted as an everted sac. These authors determined J_v by weighing at intervals of 5 min. When an osmotic probe was added to the mucosal solution, the J_v curve of Fig. 3 (corresponding to the case of Fig. 2*c*) was obtained. J_v fell steeply from the first measured value at 5 min (yielding $P_{osm} \sim 6 \times 10^{-4}$ cm/sec·osmol) to a steady-state value after ca. 30 min (yielding $P_{osm} \sim 6 \times 10^{-5}$ cm/sec·osmol). Removal of probe from the mucosal solution transiently yielded a reversed J_v (Fig. 3), because of the local NaCl gradient opposite to the former probe gradient and set up by the sweeping-away effect. The initially measured reversed J_v on removal of the osmotic probe was nearly as large as the initially measured forwards J_v on application of the probe. This implies that the effective osmotic gradient during application of the probe had eventually declined to only a small fraction of the nominal bulk gradient (because the local opposing gradient became nearly as large as the bulk gradient). Similarly, Barry and Hope (1969, Fig. 16) demonstrated in *Chara*, by reversed flow measurements and by direct measurements of local concentrations, that most of the bulk osmotic gradient became effectively cancelled by the local opposing gradient set up in unstirred layers.

When Wright *et al.* (1972) added a probe to the serosal solution, a curve as in Fig. 2*b* or *d* was obtained. The difference in the transient arises because the serosal USL is thicker than the mucosal USL (i.e., τ_D is greater for addition of probe to serosal solution). The steady-state

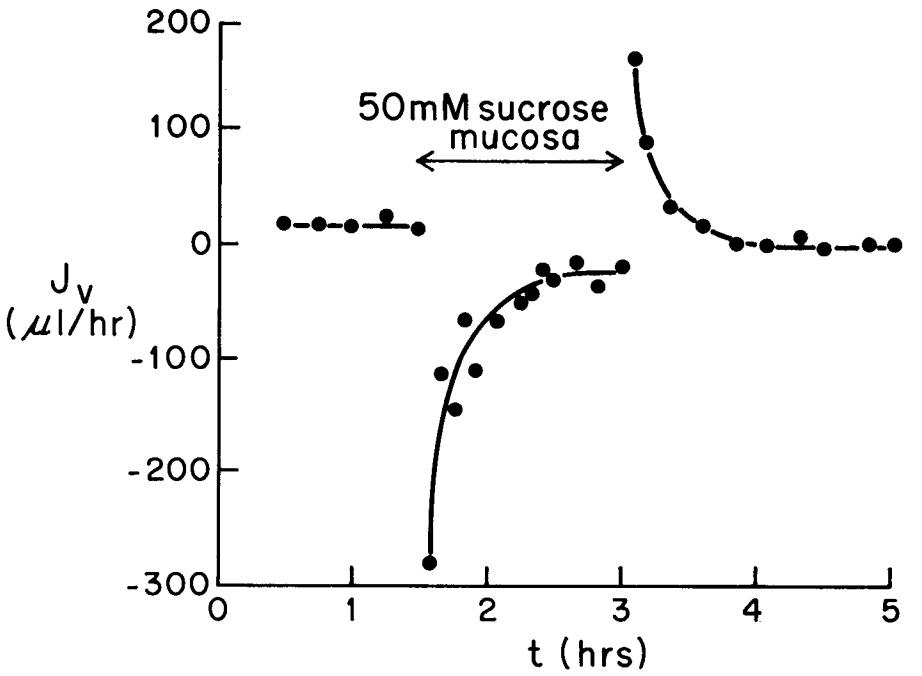

Fig. 3. Osmotic transients in everted sac of rabbit gallbladder, after Fig. 1 of Wright *et al.* (1972). J_v (ordinate) is plotted against time (abscissa). Initially and finally, both bathing solutions were symmetrical NaCl Ringer's solutions. Between the arrows, the mucosal solution was made hypertonic by addition of 50 mM sucrose. A positive J_v value means water flow from mucosa to serosa. Note the transiently large mucosa-to-serosa flow *after* removal of the sucrose gradient, due to the local salt gradient that had been built up within the epithelium by the sweeping-away effect during sucrose-induced serosa-to-mucosa flow

P_{osm} is the same for addition of probe to either solution. Wright *et al.* (1972, p. 210; *see also* van Os *et al.*, 1979) point out that much of the sweeping-away effect seen in these experiments was probably accounted for by a slow polarization, and swelling and shrinking, with a half-time of many minutes in the thick serosal connective tissue, rather than by rapid polarization with a half-time of a few seconds in the LIS.

B. Hughes and T. Machen (*personal communication*) similarly measured osmotic transients in fish intestine by weighings at 1-min intervals and obtained a curve like Fig. 2*c*, J_v declining several-fold in the first 15 min. Hays (1972) reported similar findings for ADH-treated toad urinary bladder.

Schafer *et al.* (1974) searched for osmotic transients in isolated cortical collecting tubules and found that their first J_v measurement at 30 sec

was already equal to the steady-state value at 20 min (case of Fig. 2*d*). As Schafer *et al.* note, the absence of osmotic transients in their experiments is probably because τ_D and $\tau_{sweep} \ll \tau_{meas}$: their tubule preparation lacked serosal connective tissue, and the tubule lumen radius was only ~ 10 μm, hence $\tau_D \sim 100$ msec compared to a measuring delay of 30 sec. Subsequently, Schafer and colleagues measured osmotic flow in rabbit proximal tubule as a function of perfusion rate and extrapolated the results to infinite perfusion rate to obtain $P_{osm} \sim 7\text{–}10 \times 10^{-3}$ cm/sec·osmol for proximal tubule (Andreoli *et al.*, 1978; Schafer *et al.*, 1978; Andreoli & Schafer, 1979). This procedure is surely successful at eliminating underestimates of P_{osm} due to gradual osmotic equilibration of the test solution. The procedure does not, however, address itself to possible underestimates arising from a sweeping-away effect. The existence of such effects is likely, as $\tau_{sweep} \ll \tau_{meas}$. Hence $7\text{–}10 \times 10^{-3}$ cm/sec·osmol is a lower bound on P_{osm} for proximal tubule.

What is the true value of P_{osm} in rabbit gallbladder? Since the measurements of Wright *et al.* (1972) in Fig. 3 missed the peak of the transient, all that can be said for sure is that the true P_{osm} is much higher than the 5-min value of 6×10^{-4} cm/sec·osmol. The peak probably comes at a time around 10 sec, τ_D for the mucosal USL. As a glance at Fig. 3 makes obvious, the fall in J_v from 5 to 10 min is so steep that a value an order of magnitude higher at 10 sec is likely. A more recent experimental study of rabbit gallbladder, employing a flat epithelial sheet (not a sac, as used by Wright *et al.*, 1972) and a method of measuring J_v with a time resolution of several seconds, yielded $P_{osm} \sim 1 \times 10^{-3}$ cm/sec·osmol after using streaming potentials in an attempt to estimate polarization effects (van Os *et al.*, 1979). In this study as in all earlier gallbladder studies, the time course of streaming potentials was still limited by mixing times in the mucosal solution – i.e., $\tau_{sweep} < \tau_{meas}$ still.[4] Schafer *et al.* (1974) substituted estimated gallbladder parameters into their mathematical model of osmotic transients and concluded that the true P_{osm} might be ~ 30 times the steady-state value of Wright *et al.*; i.e., $\sim 2 \times 10^{-2}$ cm/sec·osmol.

For leaky epithelia other than rabbit gallbladder, P_{osm} values calcu-

[4] In gallbladder (van Os *et al.*, 1979) as in other epithelia (summary in House, 1974), L_p values measured by hydrostatic pressure gradients greatly exceed those measured by osmotic pressure gradients. Much evidence indicates that this discrepancy arises from a small number of large pores, in which flow can be generated by hydrostatic gradients but not by osmotic gradients (because solute reflection coefficients are near zero in the large pores).

lated from steady-state J_v's range from 1×10^{-4} to 1×10^{-2} cm/sec·osmol. In the absence of osmotic transient measurements, there is no way to guess how much higher the true P_{osm} value is than these measured values. The low measured P_{osm} values are for epithelia with thick USL's and slow τ_{meas} (e.g., intestine, choroid plexus, gallbladder), the high values for epithelia with thin USL's and rapid τ_{meas} (proximal tubule, cortical collecting duct). Evidently, apparent differences among leaky epithelia in measured P_{osm} reflect measuring artifacts rather than real differences.

Over the last 15 years there has been technical progress in measuring epithelial P_{osm} values: improved stirring and hence reduction of USL thickness, more rapid methods of volumetric measurement, and appreciation of osmotic transients and sweeping-away effects. This progress has been reflected in ever higher estimates of P_{osm}. For example, estimates of P_{osm} in rabbit gallbladder have increased from 4.8×10^{-5} cm/sec·osmol in 1964 (Diamond, 1964: 5-min time resolution, noneverted sacs, no appreciation of osmotic transients), to 6×10^{-4} cm/sec·osmol in 1972 (Wright *et al.*, 1972: 5-min time resolution, discovery of osmotic transients made possible by use of everted sacs), to 1×10^{-3} cm/sec·osmol in 1979 (van Os *et al.*, 1979: time resolution of several seconds, use of streaming potentials to estimate sweeping-away effects). Some authors nevertheless continue to accept P_{osm} values 15 years out of date, to believe that USL effects do not cause significant underestimates of P_{osm}, and to seek explanations of osmotic transients in phenomena other than the sweeping-away effect. At the risk of being repetitious, it is therefore worth restating the two most obvious, qualitative indications that osmotic flow in leaky epithelia is accompanied by large sweeping-away effects causing underestimate of P_{osm}. These indications are that:

1. Osmotic flow in leaky epithelia with P_{Na}/P_{Cl} far from 1 is accompanied by apparent streaming potentials, caused by local accumulation of NaCl on the side of the epithelium opposite to the osmotic probe, or by local dilution on the same side as the probe. Calculation of the NaCl gradients responsible for the streaming potential shows that they are comparable in magnitude to the probe gradient itself. That is, most of the nominal probe gradient becomes effectively cancelled by the sweeping-away effects.

2. Immediately after removal of the osmotic probe from the bulk bathing solution, fast-time resolution volumetric methods reveal a transient reversed volume flow comparable in magnitude to the initial forwards flow (Barry & Hope, 1969; Wright *et al.*, 1972; Fig. 3 of the

present paper). This reversed flow is another expression of local NaCl accumulation due to the sweeping-away effect, and again implies effective cancellation of most of the probe gradient.

In short, the true values of P_{osm} for leaky epithelia probably exceed 10^{-3} or 10^{-2} cm/sec·osmol, and will remain unknown until two conditions are met: (i) USL's must be thin enough that $\tau_D < \tau_{sweep}$. (ii) J_v measurements must be rapid enough that $\tau_{meas} < \tau_D$. If τ_{sweep} for polarization effects in the LIS is in fact $\leqq$ a few sec, as suggested by footnote 3, then USL thickness must be $\leqq 30$ μm, and time resolution for J_v measurements must be ~ 1 sec. A solution to this technical problem is not presently available. Convincing evidence that these two problems have been solved would consist of recording an osmotic transient similar in form to Fig. 2*a*, so that P_{osm} could be calculated with confidence from the peak J_v.

Until these problems are solved, it is pointless to calculate transported osmolarities for particular epithelia by inserting estimated parameter values into standing-gradient equations and then to consider such calculations a test of the standing-gradient theory, as Sackin and Boulpaep (1975), Lim and Fischbarg (1976), and Hill and Hill (1978*b*) have recently done, when the value of a key parameter is uncertain within orders of magnitude. These tests raise additional questions stemming from their numerous other unknown parameter values. For example, the model of Sackin and Boulpaep (1975) involves about 47 parameters, many of whose values are unknown or guessed. The original standing-gradient model (Diamond & Bossert, 1967) involved five parameters, of which the values of four (all except P_{osm}) are known.

Transcellular *vs.* Transjunctional Route of Water Flow

A second major unsolved problem in solute-linked water transport concerns what fraction of transepithelial water flow is via the cells, and what fraction via the junctions. No direct or reliable method to approach this question exists at present. Some authors cite indirect evidence to support a predominantly transcellular route for leaky epithelia (e.g., Wright *et al.*, 1972; van Os & Slegers, 1973; Moreno, 1975; van Os *et al.*, 1979). Other authors cite indirect evidence to support a predominantly transjunctional route (e.g., Sackin & Boulpaep, 1975; Fischbarg *et al.*, 1977; Hill & Hill, 1978*a* and *b*). Let us consider some examples to help us appreciate the problems involved in this indirect reasoning.

Fischbarg *et al.* (1977) measured P_{osm} in rabbit corneal endothelium.

They state that measured P_{osm} is either high (115 μm/sec) or low (26 μm/sec), depending on the experimental conditions. They argue that the high and low values represent the transjunctional and transcellular routes, respectively, but this interpretation is merely a speculation. In addition, Fischbarg *et al.* (1977) present no evidence that the distribution of P_{osm} measurements is actually bimodal; the differing experimental conditions are not stated; a glance at the enormous scatter in their Fig. 1 indicates unresolved technical problems in their method of measuring J_v; and corneal endothelium differs from typical epithelia in having junctions permeable to horseradish peroxidase.

Whittembury (1967), Boulpaep and Sackin (1977), and Boulpaep (1978) review experimental studies of P_{osm} in renal tubule by Whittembury *et al.* (1959), Whittembury, Sugino, and Solomon (1960), and Grandchamp and Boupaep (1974). These experimental studies report P_{osm} measurements, either for the whole epithelium (P^c_{osm}) or for the cells alone (P^c_{osm}). Since the reported P^c_{osm} values exceed the reported P^c_{osm} values, Whittembury (1967), Boulpaep and Sackin (1977), and Boulpaep (1978) infer that transjunctional water flow is significant. However, these P_{osm} determinations exemplify the USL problems already discussed. J_v determinations were at intervals of 10–30 min in the study by Whittembury *et al.* (1959), 3–4 min for Whittembury *et al.* (1960), and an unstated interval for Grandchamp and Boupaep (1974). In the absence of knowledge of diffusional and polarization delays or of resolution of osmotic transients in these renal tubules, we can have no idea how much the effective osmotic gradient at the membrane had altered by the time of the first J_v determination, and what the real P^c_{osm} and P^c_{osm} values were.

Hill and Hill (1978*a*, 1978*b*) studied transport by *Necturus* gallbladder in very dilute bathing solutions (down to ~ 1 mosM). They found that the transported fluid remained isotonic to the bathing solutions over this osmolarity range, and that sucrose concentration in the absorbate equalled that in the bathing solution for 50 mosM bathing solutions. Through various indirect arguments Hill and Hill interpreted these results to mean that most of the water and sucrose flux is transjunctional rather than transcellular. Apart from the weakness of their indirect arguments, the meaning of their experimental results is highly suspect because gallbladders become leaky at low osmolarities. For example, at bathing osmolarities above 60 mosM, phenol red and sucrose are impermeant in rabbit gallbladder and do not enter the absorbate, but they become so permeant at lower osmolarities that their absorbate concentration

becomes equal to their bathing concentration (Diamond, 1964). As Hill and Hill studied sucrose transport only at 50 mosM and not at normal osmolarities, the same pattern could apply to *Necturus* gallbladder. A further problem overlooked by Hill and Hill is that gallbladders become very leaky at low calcium concentrations. Hill and Hill do not state the composition of their 1 mosM (!!) bathing solution, but their text (p. 152) suggests that it might have been simply a dilution of their 205-mosM bathing solution, which would mean $(Ca^{++}) = 2/205 =$ only 0.01 mM. Whatever the location of the leak that develops in gallbladders at these very low osmolarities and calcium concentrations, the experimental results obtained under these conditions are unlikely to apply to gallbladder under normal conditions and with intact permeability barriers.

These several studies just discussed exemplify our present dilemma: present indirect methods for resolving P_{osm} of cells and junctions yield conflicting and unconvincing results. A further problem is that the relative magnitude of J_v by the two routes depends not only on their relative L_p's but also on their relative σ's of actively transported solute. For instance, in leaky epithelia that transport NaCl, measured σ_{NaCl} values for the whole epithelium are often well below 1.0, presumably because of low transjunctional rather than low transcellular σ. For equal L_p's, then, the transcellular route would contribute more to solute-linked water transport.

Existence of Standing Gradients

Numerous authors (*see* first sentence of this review for references) have recently debated the validity, or need for modification, of the standing-gradient theory of solute-linked water transport which Bossert and I proposed in 1967 (Diamond & Bossert, 1967, 1968). This debate testifies to the fact that, in the 12 years since the standing-gradient theory was proposed, it has not yet been possible to devise a definitive test of the theory. The difficulties are technical: we still await a method for measuring solute gradients over a distance of ~ 10 μm, in spaces $\leqq 1$ μm wide, after having frozen the epithelium very rapidly and prevented solute translocation during both the freezing procedure and the analytical procedure.

What about the more modest goal of testing whether the LIS are hypertonic in forwards epithelia (Diamond & Bossert, 1967) and hypotonic in backwards epithelia (Diamond & Bossert, 1968), as predicted by the standing-gradient theory? The few tests made have been confirma-

tory. LIS hypertonicity has been estimated at ~ 20 mosM in rabbit gallbladder by an electrical method (Machen & Diamond, 1969), at ~ 130 mosM in insect rectum by micropuncture (Wall, Oschman & Schmidt-Nielsen, 1970), at ~ 50–100 mosM in rabbit ileum by electron microprobe (Gupta & Hall, 1979), and at ~ 60–80 mosM in *Calliphora* salivory glands by electron microprobe (Gupta & Hall, 1979: canalicular hypertonicity). A test will be especially difficult in renal proximal tubule, where P_{osm} is so high ($\sim 10^{-2}$ cm/sec·osmol) that LIS hypertonicity adequate to support water transport, if it does exist, would be only a few mosM (Sackin & Bouppaep, 1975; Andreoli & Schafer, 1979).

The only attempt to measure actual standing gradients – i.e., local concentration differences within the LIS – comes from the electron microprobe studies of Gupta, Hall, and colleagues (summarized by Gupta & Hall, 1979). These authors do report concentration differences along the LIS, and offer tentative interpretations. The measurements are technically demanding, innovative, and subject to many possible artifacts. Until the methods of Gupta, Hall, and colleagues can somehow be validated independently – e.g., by establishing a known concentration gradient across a suitable artificial membrane and examining how closely the microprobe results duplicate the known gradient – it will not be possible to assess the results reported for epithelia.

Of the criticisms and modifications raised in recent theoretical analysis of the standing-gradient theory, I shall mention here four. First, several authors have modified the model to allow for the possibility of water flow across junctions as well as across cell membranes (e.g., Huss & Marsh, 1975; Sackin & Boulpaep, 1975; Schafer *et al.*, 1975). When the theory was first proposed in 1967, it was widely believed that epithelial junctions were impermeable to both ions and water. It is now known that the junctions of leaky epithelia are permeable to ions, and there are ground to suspect that they also have some permeability to water. As discussed in the section "Transcellular *vs.* Transjunctional Route of Water Flow", we have no idea whether junctional water permeability accounts for 5 or 95% of P_{osm} in leaky epithelia. For a given value of total (i.e., transcellular plus transjunctional) epithelial water permeability, the transported fluid will be most nearly isotonic if there are standing gradients, sequential equilibration along a channel, and solely transcellular water flow, and furthest from isotonicity if all water flow is transjunctional.

A second question involves the closeness of approach to osmotic equilibrium. The fundamental assumption of the standing-gradient theory

is that the transported fluid asymptotically approaches equilibrium as it passes down the length of a long and narrow channel. As with all asymptotic processes, one strictly speaking never reaches equilibrium. The question is, instead, how closely is equilibrium approached? – in our case, how anisotonic does the final transported fluid remain, and is this residual anisotonicity measureable experimentally? This basic point about asymptotic processes has been misunderstood by Boupaep and Sackin (1977) who state (their p. 120; *also* Sackin & Boulpaep, 1975; Boulpaep, 1978, p. 300): "Even if different boundary conditions are used in Diamond and Bossert's theory, it is impossible to achieve exact isotonic reabsorption with this class of models." Of course! Sackin and Boulpaep (1975) nevertheless consider it necessary to document this point at length by model calculations, to compute, for example, that one of Bossert's and my graphs corresponded to a transported fluid hypertonic by 0.2% rather than exactly isotonic, and to propose an unstirred layer at the open end of the channel in order to eliminate this residual hypertonicity and the inevitably not-so-perfectly-flat solute profile at the open end. In fact, the emergent osmolarity from a standing-gradient channel may approach isotonicity to any arbitrary degree depending on channel parameters. Numerous parameter sets believed physiologically reasonable yield fluids isotonic within a few percent. This is within the experimental error for measuring osmolarities of epithelial absorbates or secretions, even without considering uncertainties in reflection coefficients and hence in effective tonicity.

Third, Boupaep and Sackin believe it to be an important assumption of the standing-gradient model that "active transport should be confined to a small region at the apical end of the interspace" (Boulpaep, 1978, p. 302; *see also* Sackin & Bouplpaep, 1975; p. 672). Since evidence on this point is nonsupportive or equivocal, Boupaep and Sackin construe this a criticism of the model. In fact, this is not an assumption of the model. Rather, Bossert and I showed that, for a given set of parameters, the emergent fluid is more nearly isotonic, the more that active transport is confined to the apical end. If active transport is spread uniformly over the whole channel length, the magnitude of the residual anisotonicity, and the question whether it is experimentally detectable, depend on values of the other parameters such as P_{osm}.

Finally, there remains uncertainty about actual values of some model parameters in epithelia. In particular, Hill (1975*a*) claimed that actual epithelial values of P_{osm} and channel dimensions were incompatible with generation of effectively isotonic transported fluids by a standing-gradient

mechanism. As discussed elsewhere (Diamond, 1977, 1978), Hill's assumed values were in error. At this stage, the validity of the standing-gradient model is unlikely to be settled by further model calculations and debate over parameter values, but only by experimental measurements of osmolarities in epithelial channels.

Conclusion

My present understanding of solute-linked fluid transport in epithelia is as follows. Although the evidence for an osmotic mechanism remains compelling, we don't know the relative importance of the transjunctional and transcellular routes of water flow, nor can we specify more than a lower bound on P_{osm}, nor do we know the forms of the osmotic gradients in the LIS (if they exist), nor do we presently have methods capable of solving these three problems. It seems futile to debate these questions further until someone thinks of new techniques to solve them.

The purpose of belaboring these negative conclusions is that scientists are justifiably reluctant to devote effort to the search for new methods as long as they can believe existing methods to be adequate. Hence we need to appreciate clearly the inadequacies of existing techniques that have stalled understanding of epithelial fluid transport. Specifically, new methods are required:

(1) to measure J_v in epithelia with sufficiently high time resolution and thin unstirred layers that the whole course of an osmotic transient can be observed, and a P_{osm} value uncontaminated by the sweeping-away effect extracted;

(2) to resolve directly the water flows by the transcellular and transjunctional route.

(3) to make direct measurements of solute concentrations in lateral intercellular spaces.

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