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ORIGINAL ARTICLES

Fluid Transport: A Guide for the Perplexed

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Abstract Readers of the physiological literature may be excused if they feel that fluid transport has become a complex and confusing field that is difficult to understand and to assess. The major theories of fluid-transporting epithelia are examined here with respect to their ability to explain quasi-isotonic fluid transport and its modulation by salt transport, osmotic permeability and basal tonicity. The basics of each theory are set out concisely, and their pros and cons are made explicit. Finally, a comparison is made in table form indicating their overall performance in relation to the problems of this difficult but important field.

Keywords Fluid transport · Epithelial model · Osmotic coupling · Electro-osmosis · Cotransporter · Osmosensor

Introduction

The transport of fluid by epithelia is one of the fundamental processes in physiology, as important as the mechanism of nerve conduction or muscular contraction. Despite a voluminous literature, however, there is no clear idea of how it occurs. Instead, the problem has largely been shelved, with most textbook writers content to append an arrow (*water* =>) to diagrams of transporting epithelial cells, assuming that the reader knows that somehow "water follows salt"–and thus bypassing the issue. The nonspecialist may be excused for thinking that there is no really interesting problem there at all and wondering why it was not solved long ago.

There has been a long history of grappling with this central problem. Some treatments have been obscure and complicated, involving computer modeling that few have chosen to follow. Others have been much simpler but little more than schemes which need fleshing out before they can be accepted as doing justice to the richness of the phenomenon. Yet others have been enmeshed in the characteristics of special areas, ruled by the approaches and dogmas of their respective guilds (e.g., those of renal, salivary or intestinal workers).

I have chosen to examine here five theories which are considered modern contenders for providing an explanation and which should satisfy some basic constitutional requirements. They should potentially be general; just as nerves and muscles have basic mechanisms that carry across the whole animal kingdom, so do epithelial fluid transporters. They must also be dependent upon ion transport by the cells (Na pumping), and fluid transport must stop when this is inhibited. To this we may add a first and a second amendment. First, the theories should predict quasi-isotonic flows; i.e., the fluid is transferred with a tonicity very close to that of the source bath. Second, and by no means the least important, the system should be *self-regulating*; i.e., it should run quasi-isotonically without having to be reset (tweaked) every time there is a modest change in the salt pumping or bath tonicity: the system, as engineers say, must be robust to key parameter variation.

The five approaches examined here comprise two cellular models in which water crosses the cell membranes–(1) the osmotic coupling theory and its two add-ons, (2) the

This paper is dedicated to Professor Werner Loewenstein, founder of the Journal of Membrane Biology and its Editor-in-Chief until 2006. A hard act to follow but one to be emulated.

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cotransporter theory and (3) the Na-recirculation theory–and two largely paracellular models–(4) the electro-osmotic theory and (5) the osmosensor-feedback theory. These are presented briefly, with their strengths and weaknesses (pros and cons) made quite explicit, for those readers who may want an overall view without going into too much detail. In addition, I have included a "Notes" section where more analytical and technical points have been stressed for readers (and authors) more familiar with the field, together with brief historical notes that put the theories into context where they are spread over several papers.

The Osmotic Coupling Theory

The osmotic coupling theory is the prevalent current theory. In this model salt is pumped across the epithelial cell into a space, thus creating an osmotic difference. Water is driven across the cell membranes by osmosis into this space, and the solution emerges to form the fluid (Curran & MacIntosh, 1962). The first amendment (isotonicity) is satisfied if the bounding osmotic permeabilities, P_{os}, are high enough to allow for virtually complete equilibration, in which case the fluid is quasi-isotonic. The second amendment (robustness) is also obeyed because, in this condition, the tonicity becomes insensitive to changes in pumping rates and is always quasi-isotonic. Changes in the tonicity of the source bath also have little effect on the quasi-isotonic flow because osmotic equilibration is dominant.

It became clear that the best candidate for this local space would be the interspace, and this gave rise to the "standing gradient osmotic theory," or SGOT (Diamond & Bossert, 1967). In this, interspace osmotic coupling can give rise to quasi-isotonic flow if the dimensions are right (Fig. 1). This dependence on geometry is because the coupling space is not stirred, so concentration gradients play a controlling part in fluid secretion.

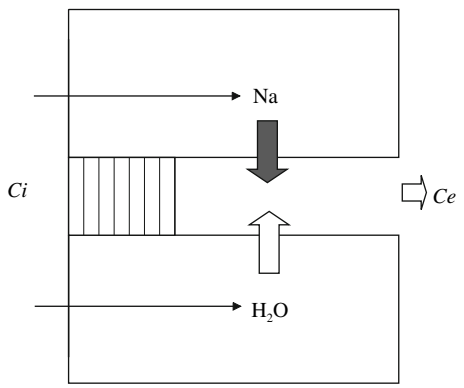

Fig. 1 Salt is pumped into the basolateral spaces, and water follows by osmosis generating an emergent osmolarity *Ce*

The modern form of this theory is as follows: If the osmotic permeability of the bounding membranes is high enough, then SGOT need not be considered in detail and all transport will be quasi-isotonic (see note 1). This is the current simplified version, precipitated by the discovery of aquaporins (AQPs), which have the potential to raise osmotic permeabilities to high values.

Pros & Cons

The theory is very appealing due to the fact that osmosis is widely considered to be a fundamental process that is intuitively understood to take place at all membranes where concentration differences of salt occur. Moreover, the essential elements of the theory are all present to the eye when perusing an electron micrograph of an epithelial cell. The pumps (active and passive ion transporters) and AQPs are present in the cell and can be localized at membranes which are water-permeable. Indeed, it is difficult to avoid some form of osmotic flow in an epithelial system unless osmotic pressure differences are virtually absent. In addition, the theory can instantly be applied to any configuration of epithelia, whether "forward-facing" or "backward-facing," if the membranes are considered water-permeable enough (see note 2).

The theory has some serious problems that have accumulated since its inception.

1. If a calculation is made of the osmolarity of the transported fluid for any coupling space, it is rarely, if ever, quasi-isotonic and usually quite hypertonic. This calculation requires knowledge of the dimensions of the space, the principal ones being its length and width, which can be obtained from electron micrographs, with an estimate of the osmotic permeability of the bounding membranes. The deciding factor is always the latter (see note 3). This argument can be turned around by calculating the osmotic permeability that would be required to achieve fluid tonicity no more than 1% hypertonic, an acceptable figure (remember that perfectly isotonic fluid is unattainable because it would require an infinite osmotic permeability). The calculated value, for many systems where the theory would be expected to apply, is almost always much greater than an experimental value or one reasonably estimated.
2. AQPs are now considered essential to the theory as they raise the osmotic membrane permeability from its basal lipid value of 10^{-4}–10^{-3} cm/s to about 10^{-2} cm/s for very permeable cells. Recently, strains of mice have been developed with knockouts for most of the major animal AQPs and it has been possible to assess

the effects of these on fluid transport. The results are not encouraging for the osmotic theory (Hill, Shachar-Hill & Shachar-Hill, 2004). Of nine epithelial systems concerned with fluid transfer, only three showed a real decrease in transport rate to 50–40% after knockouts of their AQPs; and in these, there was an unexplained fall in salt transport to 60–55%, which in itself is enough to explain most of the observed fall in fluid transport. However, in almost all cases, the osmotic permeability was decreased, in some cases by 90%. It is therefore not possible to argue that water is flowing through other pathways in the membrane because a decrease in osmotic permeability represents *all* pathways available to osmotic equilibration.

Conclusion

The osmotic coupling theory is quintessentially a geometric theory, and there has been a long history of dissatisfaction with it on the grounds that calculations do not predict quasi-isotonic flow from the parameters (Hill, 1975; King-Hele, 1979; Lim & Fischbarg, 1976; Sackin & Boulpaep, 1975; Tripathi & Boulpaep, 1989). With the advent of AQPs, all these calculations carried little weight because they could be ignored. If there is enough AQP in the membrane, the argument goes, this guarantees enough permeability to make the osmotic coupling of water to salt transport inevitable. (The calculations showed, of course, that there was not enough AQP in the membranes to bring about this state of affairs. Whoever put the AQPs there apparently did not put enough of them to allow osmotic coupling to work effectively, or at least beyond dispute.) The knockouts had either little effect on fluid transport or a partial effect that, in addition, involved a substantial fall in salt flow (Hill et al., 2004). Ironically, AQPs were all set to be the saviors of the osmotic coupling theory, but they have proved to be its major problem.

The Cotransporter Model

This is basically the osmotic coupling model of the preceding section but with a novel addition of "water pumps" at the membranes. One was always brought up to believe that water was never pumped (uphill) because it would not be worth it; the water permeability of the cell membrane would always be a massive leak pathway in parallel. However, in epithelia where water is moving *down* an osmotic gradient at too slow a rate, this argument does not apply. The theoretical and experimental basis of water cotransport has been laid out in some detail over the past several years (Loo, Wright & Zeuthen, 2002; Loo et al., 1996; Zeuthen & MacAulay, 2002; Zeuthen & Stein, 1994).

The pumps are cotransporters for ions and metabolites (especially the Na-glucose transporter or SGLT1), which also transport water as part of their turnover cycle in the epithelial membrane in a stoichiometric manner. These transporters drive ions and metabolites in the direction of net transport of salt and water. Therefore, any water trapped in the cycle and transported is an addition to any osmotic flow (*see* Fig. 2). The situation has been summed up by the leading worker in this field (Zeuthen, 2002): "Cotransporters working as molecular water pumps could be important building blocks in epithelial models…and…would alleviate the problems inherent in the traditional models based on osmosis alone."

Pros & Cons

Water transport is of great interest per se as those cotransporters transferring ions (such as SGLT1) show coupling between charge flow and water movement which is not electro-osmotic in origin. This model is also attractive in that the cotransporter water flow is geared directly to the ion flow without the need for dealing with osmotic coupling spaces.

It should be noted at the outset that there are objections to the experimental demonstration of water pumping (Charron, Blanchard & Lapointe, 2006)–on the grounds that the cotransporter water flows may be due to local osmosis created by the transport of solute across the cotransporter (see note 4). It is not clear what fraction of the water "pumping" is actually rather than apparently stoichiometric. This is an important point, of course, because if the water shifts through the transporter-membrane system are indeed osmotic, then we are simply back to the basic osmotic coupling theory again. In addition, driving water through the cotransporter with a current (Na^+ ions) would be a form of apparent electro-osmotic flow.

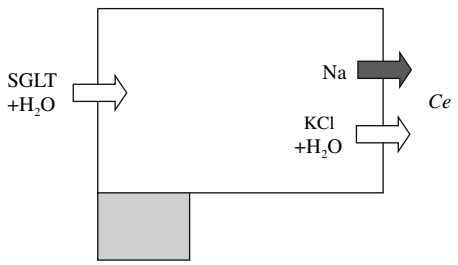

Fig. 2 Na is pumped out of the cell, and the Na-glucose transporter (SGLT1) and KCl transporter acting in response to the Na electrochemical gradient carry water across the membranes as part of their transport cycles

Besides glucose-dependent fluid transport, the other key cotransporters of central interest are the neutral Na-K-2Cl transporter (NKCC) and the KCl transporter, both of which play a major role in transcellular salt transport. Water pumping by the KCl transporter has been inferred from microelectrode studies (Zeuthen, 1994), but the Na-K-2Cl transporter has never been shown to do this. Neither has been studied in an oocyte system, which yields much more precise and quantitative data. Of those that have been assayed in this way, none, with the possible exception of the Na-glutamate transporter (EAAT1), is near isotonic because the ratio of water to solute transported is much lower than the ratio in vertebrate salines; i.e., they are hypertonic transporters by about x2 saline osmolarity (Zeuthen & MacAulay, 2002).

A problem with the role of cotransporter theory is that it has never been properly modeled in a system where the contributions of water pumping, osmosis and the ion fluxes can be investigated in more detail. It has been claimed that in small intestine and kidney the cotransporter water transport is about 33% (Zeuthen et al., 2001), but this approximate figure is based upon linear additions of contributions to water flux assessed from oocyte studies. Water fluxes do not add in a linear manner (see note 5). A subsidiary problem is that a substantial fraction of Cl ions in proximal tubule, intestine and other forward-facing epithelia must be going through the leaky junctions in response to the secretory potential, rather than using a cellular transporter. Consequently, it is very difficult to judge what contribution cotransporters could make to isotonic transfer in an epithelium.

Finally, the problem of the inadequacy of the osmotic permeability in straight osmotic coupling theory remains with this theory too, especially the role of AQP knockouts considered above (see note 6). Because water pumps are only a partial solution to isotonic flow, they cannot of themselves provide a reduced but quasi-isotonic flow when osmotic equilibration is inadequate because their contribution is hypertonic.

Conclusion

Water pumping by membrane proteins, if the uncertainty as to its magnitude can be cleared up, is interesting as an aspect of protein biophysics. However, it cannot per se solve the overall problem of isotonic water transport because the coupling ratio of the water molecules to solute particles transferred is far too low. It is difficult to assess its potential importance without a model in which isotonic fluid transport can be explored and its limits predicted, but this has not been achieved to date. If cotransporter water flow were quasi-isotonic, the first amendment (isotonicity) might be satisfied but it would be unclear as to the second (robustness). It is the osmotic coupling present along with cotransport that must guarantee these.

The Na-Recirculation Model

Like the cotransporter model, this is an addition to osmotic coupling in which Na^+ ions partially recirculate through the cell (*see* Fig. 3). In the cotransporter model, water is added to the osmotic flow; here the problem of isotonicity is solved by clawing some of the salt back again as it leaves the epithelial interspace. The amount that is recycled is just enough to reduce what would be a hypertonic solution to an isotonic one.

The model was first advanced qualitatively for fluid transport by the glands in frog skin (Ussing, Lind & Larsen, 1996), but later a quantitative assessment of the recirculation (expressed as a fraction of the Na^+ ions reentering the cells) was made for toad small intestine (Nedergaard, Larsen & Ussing, 1999). It came to about 70%. The equations used, which are crucial to the argument, stem from theoretical work on membrane fluxes (Sten-Knudsen & Ussing, 1981) subsequently applied in a modified form to epithelia (Eskesen, Lim & Ussing, 1985; Lim & Ussing, 1982). The recirculation number for toad intestine was later incorporated into a large computer model of the epithelium in which quasi-isotonic flow was generated (Larsen, Sorensen & Sorensen, 2000, 2002). Certainly, this is a complex thread to follow.

Pros & Cons

The solution to the problem looks elegant and based upon a great deal of theory and experiment over many years. The basis of osmotic coupling is retained, and to it is added a

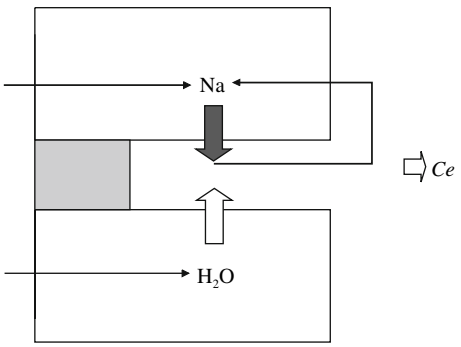

Fig. 3 Pumped Na and osmotic water are transported into the basolateral system as in Figure 1, but a fraction of the Na is recirculated across the "basal membrane," resulting in a reduction in emergent fluid tonicity, C_e

flux loop, which uses nothing but the Na-K pump and transporters that exist already in cell transport models. It does not therefore depend upon any new mechanisms for water transport, just a balancing of Na flux paths in what is a complex system of serial and parallel elements.

Unfortunately, the whole theory is founded on an assumption which is apparently incorrect. If this assumption, and the calculations which flow from it, is removed, there is no recirculation possible. This can be seen most clearly in the first intestinal study to analyze apparent recirculation (Nedergaard et al., 1999), and it suffices to concentrate on this. It is assumed that there is an Na-K-2Cl transporter on *both* sides of the cell in the normal absorptive state (mucosal to serosal) of the enterocyte. This has not been demonstrated in an epithelial cell, but if it is assumed, then recirculation follows as a matter of course (see note 7).

Furthermore, it is assumed that there are two distinct parallel pathways for Na transport, cellular and paracellular, governed by a pair of equations; but if there is no Na entry from serosa to cell via a transporter, then there are not two independent pathways as described by the equations as set out. The result of this formulation is that the measured flux ratios are processed by equations which are inevitably going to yield a value for a recirculatory component, whether it exists or not (see note 8).

In a later study, this was extended to a computed compartment model of toad intestine (Larsen et al., 2000) and later a full electrogenic model (Larsen et al., 2002), both incorporating recirculation based upon a basal Na permeability that allows Na to enter the cell from the serosa (see note 9). The model has been extended to the proximal tubule, in which recirculation is considered to play a small part but is nevertheless required for proper quasi-isotonic flow. However, in this tissue there is neither a known mechanism for Na entry at the basolateral membrane, on which recirculation must rely, nor any data from flux experiments which could be used to independently assess Na recirculation.

Conclusion

The Na-recirculation model is based upon an analysis of fluxes in intestine in which both basal Na transporters and the applicability of the flux equations involved can be called into question. It is unclear whether it satisfies either the first amendment (isotonicity) or the second (robustness) because the extent of recirculation would have to be readjusted to maintain the fluid close to a quasi-isotonic value, unless, of course, the osmotic equilibration is very high indeed in the first place. If this is true, then the recirculation is redundant. For this reason perhaps, of all the models this one is exceptionally difficult to grasp conceptually, and this difficulty is not diminished by the complexity of the computer modeling. The model is also open to the same objections with respect to AQP knockout studies as is the osmotic coupling theory, of which it is a variant.

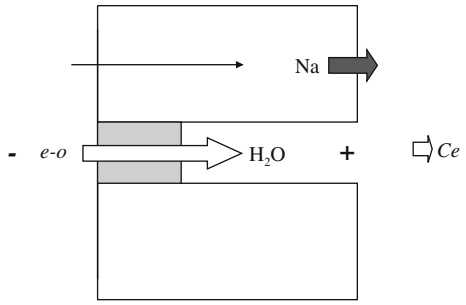

Fig. 4 Na is pumped across the cell, and the secretory potential draws ions of opposite charge through the tight junctions with electro-osmotic transfer of water in isotonic proportions

The Electro-Osmotic Theory

In its ideal form, the theory (e–o) would require flows as shown in Figure 4. Ions are pumped across the cell, generating a transepithelial potential. This draws the requisite counter-ion(s) through a selective paracellular route, setting up water flow by e-o in the tight junction. The extent of water to ion coupling then determines the tonicity of the emergent fluid.

E-o has been around for a long time, but it has never been worked on experimentally and theoretically until very recently. I shall discuss recent papers on fluid transport across mammalian corneal endothelium where a model is presented in which the e-o takes place across the tight junctions (see note 10). The theory can be considered to come in three parts: (1) the demonstration that volume flows respond to changing electrical polarization of the epithelium, (2) an electro-hydrodynamic model of e-o in the tight junction itself and (3) a model of the epithelium with ion and water fluxes, showing how it can offer a better explanation of responses to changing protocols than the osmotic coupling theory.

1. From polarization experiments on corneal endothelium (Sanchez et al., 2002) a value for the e-o coupling ratio was derived. This is the ratio of volume to current passing the junction, both of which are flows and which need not a priori be in any particular ratio. The value derived and used in subsequent analysis is 2.37 $\mu m \cdot cm^2 \mu A^{-1} hr^{-1}$ (original units). This translates to 6.35 liters of fluid per mole of univalent ions or transfer of a 0.157 M solution. Bearing in mind that mammalian Ringer is 0.150 M, this is a good omen.

2. Using a detailed theoretical model of e-o in a charged structured matrix representing a tight junction (Rubashkin et al., 2006) with certain properties (dimensions and charge density being the dominant ones, together with an ion concentration equal to that of normal saline), it is possible to approach the coupling value discussed above (see part 1).
3. Using a program that models the transporters and pumps in the endothelial cell (Fischbarg & Diecke, 2005), together with the coupling ratio from part 1, it can be shown that under different experimental conditions the volume flows are much better predicted by an e-o system than by osmotically generated (isotonic) water flows.

Pros & Cons

The theory has the merit that it departs from dependence on osmotic permeability, which has dogged the two preceding models. It also gives a role to the tight junctions other than acting as passive seals, which can be approached by electrophysiology–the junctions have been notoriously difficult to get at experimentally. Furthermore, there is experimental evidence that in certain epithelia that have been studied with extracellular probes, there is a convective component of water flow in the paracellular system, and junctional e-o might provide an explanation for that.

There is, however, a problem with the theory which, when used to model some specific experimental protocols (Fischbarg & Diecke, 2005), yields results that appear strange and require further examination. (1) After removal of HCO_3^- from the saline, the model generates a substantial flow of fluid that is grossly hypotonic, being 10% that of saline. (2) After the action of the inhibitor 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDS), the predicted fluid flow is again substantial but the net salt flux has reversed–salt and water are therefore going in opposite directions. Not only does this seem unlikely, but these flows must create hypertonicity in the stroma adjacent to the cells which has osmotic implications that are not addressed (see note 11).

A further important point requires mention. The junctional osmotic coupling coefficient, as can be seen from the theoretical study (Rubashkin et al., 2006), is very dependent upon the ion concentrations in the fluid traversing the interspace, which is obviously dominated by the adjacent fluid in the baths, particularly the source bath. This cannot be regarded as constant and equal to that of the standard saline during the different experiments analyzed with the model. Again, the problem is not addressed, but it is crucial to the theory. In fact, the theory presented indicates that the e-o coefficient must be a variable parameter and concentration-dependent (see note 12), although it is the value of this which guarantees satisfaction of the first amendment (isotonicity).

Finally, we should ask whether the theory could satisfy the second amendment (robustness). If the basal tonicity is varied, will the fluid flow remain quasi-isotonic? Most systems respond to osmotic changes induced with an impermeant solute (e.g., sucrose) by altering the fluid flow rate but preserving quasi-isotonicity. This is achieved by there being a higher salt concentration in the transported fluid, which finds a ready explanation in the osmotic coupling theory (and others, *see below*). This has not been explored in the e-o theory, and it is difficult to see how it could behave in this way: changing the osmotic pressure while leaving the ion concentrations untouched should leave e-o fluid production rates unaffected.

Conclusion

The demonstration that the passage of current causes fluid flows cannot be dismissed and is probably a widespread though variable phenomenon in epithelia. That a contribution to this is "concentration polarization," the building up of salt gradients which exert an osmotic effect on water flow and which has been a standard response for decades, is an ever-present possibility; but the quick onset of the effect and its decay point to another explanation for which e-o is a possibility.

The e-o theory is a potential solution to the problem of isotonic fluid generation, but it is very difficult to understand how it could work when all the demands upon the mechanism are considered, such as changes in bath tonicity or composition. At the heart of this is the variability of the e-o mechanism residing in the junctions, which is not robust to concentration changes.

The model advanced for the theory has excluded all osmotic parameters, although it is clear than the e-o effects must be embedded in a system with highly water-permeable membranes dominated by AQPs. In particular, where hypotonic flows are generated by the model, the exclusion of concomitant osmotic flows makes the results difficult to accept. To add an e-o mechanism to the epithelial system is interesting, but this cannot be done while excluding other elements of the system known to play an important role. If an e-o theory is postulated to replace osmotic equilibration, it must work *in conjunction* with the known permeabilities.

The Osmosensor-Feedback Theory

This is a radical departure from the theories described above and is based upon two novel mechanisms. The first is

the function of an osmosensor molecule in the membrane. The second is a mechanism for junctional fluid transfer (JFT), located in the junction but controlled by elements in the adjacent cell membrane. The rate of this is controlled by the osmosensor. The net result is that the emergent fluid from the epithelium is effectively *osmo-clamped* close to that of the source bath (Hill & Shachar-Hill, 2006). It is based upon salt pumping across the epithelium but with the osmosensor controlling the tonicity of the transported fluid by effectively mixing cellular and paracellular flows, which may be regarded individually as hyper- and hypotonic fluids. Cellular fluid flow is treated as osmotic and therefore hypertonic in origin, while paracellular flow is the forced convection of a solution through junctional channels which must discriminate against salt more than water and, thus, must be hypotonic.

The emergent fluid bathes the cell distal to the junctions, and if this becomes hypertonic, it creates a hypertonic cytoplasm (by osmotic withdrawal) which activates the osmosensor; the output is amplified (via a cell-signaling mechanism) and drives up the paracellular fluid flow, which is hypotonic. In turn, this drives down the tonicity of the emergent fluid until a balance point is reached. If the osmosensor has a high enough gain (*A*), then the fluid becomes quasi-isotonic. The system is therefore built on a simple feedback loop which homes down on the quasi-isotonic state (Fig. 5).

Osmosensing is a generally recognized phenomenon, but although there are possible candidates, no molecule has yet fitted the role completely. It has been suggested that AQPs, aside from their general property of increasing the osmotic permeability of membranes, act in this way in many cells (Hill et al., 2004). The role of AQP5 in controlling fluid transport in the salivary gland has been established by the use of a genetic mutant (Murakami et al., 2006; Murdiastuti et al., 2006) whilst the control of transport in the salivary paracellular pathway (Kawedia et al., 2007) and the osmosensing of cell swelling in the salivary gland (Liu et al., 2006) have been demonstrated to be AQP5-dependent. It looks a promising candidate in this system.

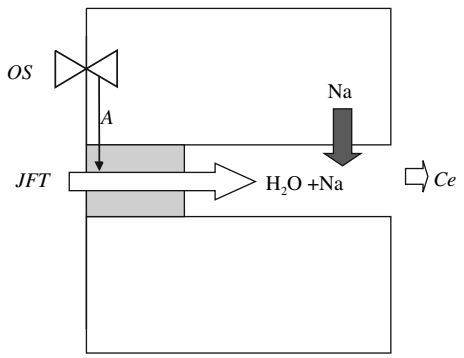

Fig. 5 Na is pumped across the cell, creating cellular hypertonicity. An osmosensor (*OS*) compares this with the source bath and sends an amplified signal (gain *A*) to a junctional fluid transfer mechanism (*JFT*), which drives down the emergent fluid to near quasi-isotonicity

Paracellular flow of fluid has been demonstrated in several epithelia using a technique of assessing fluid flow by measuring convection of probe molecules that do not pass through cells (or only slowly by comparison to the paracellular path), and it has been shown to be present in all epithelia on which the technique have been used. In the salivary gland, for example (Murakami et al., 2001), the fraction of water flow estimated to use the paracellular route via the JFT mechanism is close to 1.0; and this holds for the other epithelia tested (Shachar-Hill & Hill, 2002) (see note 13).

Pros & Cons

The theory is an attempt to depart from the present impasse and develop a theory of feedback control of fluid transport which is not based on osmotic equilibration but uses the paracellular JFT in conjunction with the cellular one. The system has been modeled in both forward-facing systems, such as gallbladder (Hill & Shachar-Hill, 2006), and backward-facing epithelia, such as salivary gland in particular (Murakami et al., 2006). It satisfies the first amendment (isotonicity) and the second (robustness). In the first, the fluid is quasi-isotonic independently of the size of the osmotic permeabilities. In the second, it is very robust to changes in the pumping rate or the tonicity of the source bath, generating a quasi-isotonic fluid as the normal steady state by clamping.

It posits a role for AQPs not only as osmosensors but also as controllers of the signal presented to the osmosensor (there are often two AQPs in epithelia, expressed at each membrane, both playing a role), and in this connection it should be noted that the model incorporates the high water permeabilities observed in experiments and does not ignore them. Although present, they contribute little to water flow directly because the osmo-clamp abolishes the gradient for water flow across the cell. Because it is the osmo-clamp which takes care of the final fluid isotonicity, the theory is insensitive to whether water transport by co-transporters is present or not. Finally, the model reacts to electrical current flow by isotonically clamping any concentration polarization that is created, therefore providing an explanation for apparent e-o effects.

There are problems associated with osmosensing. Although a model has been advanced for this based upon possible changes in the tetrameric structure of AQPs induced by solvent tension in the monomers (Hill et al., 2004), it is still speculative, and the nature of the possible interaction

with cell components is unclear as yet, although there are developments here with respect to the interaction of AQP and cell components that are not simple mediation of osmotic flows across the membranes (Kawedia et al., 2007). However, the most important problem arises with AQP knockouts. If the theory requires AQPs to play the role of controlling a JFT system, then it is as sensitive to knockouts as is the osmotic coupling theory. This only applies to those epithelia in which knockouts show no effects at all on isotonic transfer (see note 14). Furthermore, there are epithelial systems that have not been shown to have AQPs, such as small intestine and gallbladder, both carrying out quasi-isotonic fluid flow. The only real answer to this is that in these systems another molecule acts as osmosensor, such as the cystic fibrosis transmembrane conductance regulator (CFTR), in which case the theory does not always require it to be AQP. At present this is unclear.

Much more difficult to accept is the mechanism of JFT which moves salt and water in substantial amounts. In the salivary gland (Murakami et al., 2006), the data presented require that 80% of the salt flow is paracellular. If addition of the Na-pump inhibitor ouabain or, in salivary glands, the cessation of Na transport by removal of the secretagogue can stop fluid transport, would this means that the Na transfer is cellular and not substantially paracellular? The modeling demonstrates that when Na transfer by the cell is turned down, the feedback loop closes the JFT to maintain the fluid quasi-isotonic and that this compensation takes place all the way to zero Na fluxes. Some form of micro-peristalsis has been advanced for the JFT mechanism (Hill & Shachar-Hill, 1993; Shachar-Hill & Hill, 2002). However, this has yet to be properly modeled with data taken from studies on the junctional complex (see note 15), and these are extremely difficult to obtain, particularly if there are possible moving elements present, for which there is no evidence to date.

Conclusion

This theory is very new and has elements that make it surprising and possibly difficult to accept within the current outlook (which defaults to osmotic coupling). Of the two main requirements, osmosensing and JFT, the first is purely theoretical and the second, although based on experimental results that are hard to explain by any other mechanism, requires the epithelial "leaky" tight junction to be a dynamic organelle with a structure that has little experimental basis as yet. However, it predicts quasi-isotonic flow by using most of the other existing elements thought to be functional in epithelial systems in a new context. Although it is based experimentally on the interpretation of data from transport biophysics, molecular studies at the cell level will be needed to confirm many of the proposed elements and connections.

Summary

Enough has been written here about the pros and cons of the various theories. Rather than sum up by choosing a "best fit" theory, this decision may be left to the reader. To this end I have drawn up Table 1 to show how well the theories cope with the various demands made upon them and how they might appear to the unbiased physiological eye.

Notes

1. Detailed arguments why the junctions cannot represent a serious route for isotonic fluid transfer have been set out (Shachar-Hill & Hill, 2002). The equation for SGOT (Diamond & Bossert, 1967) is somewhat

Table 1 The theories and the demands placed upon them

Theory	Osmotic coupling	Cotransporter	Na recirculation	Electro-osmotic	Osmosensor feedback
Date of origin	1967 (Diamond & Bossert, 1967)	1994 (Zeuthen & Stein, 1994)	1996 (Ussing et al., 1996)	2002 (Sanchez et al., 2002)	2006 (Hill & Shachar-Hill, 2006)
Overall mechanism	Known & simple	Novel & simple	Unclear & complex	Known & complex	Novel & complex
Results from modeling	Clear	None	Unclear & complex	Unclear & complex	Clear but complex
Dependence on osmosis	Absolute	Partial	Partial	Unclear	None
Explanation for AQP knockouts	None	None	None	Unclear	Simple
Isotonic condition robust	Yes	Unclear but doubtful	Unclear but doubtful	Unclear but doubtful	Yes
Likely future developments	Doubtful	Doubtful	Doubtful	Possible	Essential

daunting; it can only be solved numerically and is unstable. A much simpler analytical approximation has been derived (Segel, 1970) and is given by $Os = [1 - \tanh k/k]^{-1}$, where $k^2 = \pi P_{os} L^2 / rD$. Here, Os is the ratio of the tonicity of the emergent fluid to that of the source, π. As $Os \rightarrow 1.0$, the fluid becomes isotonic. L is the length of the available coupling space, r the "radius" (half-width) and D the diffusion coefficient of the salt. P_{os} is the membrane osmotic permeability, and we can see that if this is made very large, the other terms are dwarfed and k becomes large. As this occurs, $Os \rightarrow 1.0$. This is in fact the theoretical basis for assuming that with high permeabilities, e.g., due to high AQP levels, the problem is solved and the equations above do not need to be solved.

2. For those with a taxonomical frame of mind, there are two main classes of epithelia with respect to the direction of fluid transport and in which the Na pump is basolateral: forward-facing (apical to basal) and backward-facing (basal to apical). Examples include the intestine (first class) and the exocrine glands (second class). Although the Na-K pump is usually expressed on the basolateral membrane in both classes, it can be found on the apical membrane in choroid plexus and retinal pigmented epithelium. The direction of net salt transport is not dependent upon the pump location but upon the expression of cotransporters in the two membranes.
3. The P_{os} measurement problem. The osmotic theory can be salvaged by having very high permeabilities, so measurements tend to come up with high values. Many of these values have never been subjected to rigorous analysis, and calculations are now regarded as passé anyway; the apogee was reached when the failure to find a significant salt concentration in the interspace of gallbladder by ion-microelectrode penetration (Ikonomov, Simon & Fromter, 1985; experimental error 1–2 mM) was regarded by some as evidence that the osmotic permeability must be enormous to allow isotonic flow driven by such a small solute difference (!).
4. In the oocyte setup, where coupling between ion and water flow has been demonstrated for expressed SGLT1, there must be a local concentration gradient created across the oocyte membrane which would pull water into the cell by osmosis. This could be through the lipid membrane or partially through any water channels spanning the transporter itself. The buildup of this local gradient will depend upon the diffusion coefficient in the oocyte cytoplasm, which is at least five times lower than free solution for small molecules and ions. The concentrations built up by cotransporter stimulation, it has been claimed, are large enough to explain 70–100% of the water shifts attributed to water pumping (Charron et al., 2006).
5. It may be thought that if osmosis is only 50% effective (i.e., it transports only 50% of the water required to achieve isotonic flow), then a cotransporter pump, which is itself only 50% efficient, could supply the other half. However, in systems with osmotic gradients at work, the contributions do not add in a simple linear fashion. Consider osmotic flow driven by a salt pump, js, at a planar membrane of permeability, P_{os}, and with stoichiometric water pumping, jv, geared to the salt pumping. This leads to $2\pi Os = (\pi - jv/P_{os}) + \sqrt{(\pi - jv/P_{os})^2 + 4js/P_{os}}$, where Os is the ratio of the tonicity of the transported fluid to that of the source bath, π. Without the water pumping ($jv = 0$), solution of this equation for the condition $Os = 2$ (osmotic flow 50% efficient, i.e., hypertonic by 100%) leads to $js/P_{os} = 2\pi^2$. If the water pump is 50% efficient too, then $js/jv = 2\pi$, or $jv = js/2\pi$. Using these parameter values for js/P_{os} and jv in the equation leads to the result $Os = 1.4$. The overall effect of the water pumping has not produced an isotonic fluid but one which is still 40% hypertonic. The reason for this is that the cotransported water itself decreases the osmotic gradient. This example, with the intricacies of osmotic systems that it reveals, underlines the need for building a semiquantitative epithelial model for cotransport.
6. It might be claimed, with some justification, that AQP knockouts could leave the cotransporter water fluxes intact, which would explain cases like proximal tubule, airway gland and salivary gland, where there is a residual fluid secretion after knockout. There is also a decrease in salt flow which cannot be explained, however (Hill et al., 2004).
7. In systems which are backward-facing and transport salt from serosa to mucosa, the NKCC transporter may be localized on the basal membrane; and as such, it can be found in mammalian intestinal crypt cells which are apparently secreting. The two transporters have never been localized in the same cell on both membranes.
8. The equations for unidirectional fluxes, J_{ms} and J_{sm}, used here follow from a fundamental theoretical paper (Sten-Knudsen & Ussing, 1981) in which the flux ratio J_{ms}/J_{sm} is shown to be time-invariant for an element spanning the membrane. The fluxes in this element can be through a series of subelements, but they must all obey the reversible equations of electrodiffusion. Different but separate elements spanning the membrane in parallel can have their own independent flux ratio so that if two such elements are involved, the overall flux ratio will *not* be time-invariant–it will

evolve from an initial to a final constant value, depending upon the time course of each element and the magnitude of the flux that it mediates. (Readers who are interested should consult the original paper.) This is the case in an epithelium like toad intestine, where it is assumed that there are two elements involved, a cellular and a paracellular path, in the evolution of the Na flux ratio. However, there are two properties involved here which violate the original derivation. First, there is an active subelement present (the Na-K pump) which is unidirectional and irreversible in this system. Second, the two elements are not independent but interconnected via this pump. To put the matter in a nutshell, we should consider the following situation. It is quite easy to construct a simple (and realistic) model in which tracer Na^+ added to the mucosal solution enters the cell from which it is pumped into the basolateral space, the tracer also having a paracellular route between baths. This gives rise to a flux ratio that evolves over time similarly to the experimental results from intestine–but in which it is clear a priori that there is no recirculation at all.

9. These treatments are compartment models in which the reader will be surprised to see that the basolateral membrane is divided into two separate membrane systems (lateral and basal), each with different properties, and that the tight junction plays a prominent role in mediating osmotic water flows which exert solvent drag on Na. The serosal Na transporter is shown as present only in the "basal" membrane; it would, however, migrate by intramembrane lateral diffusion to the interspace unless something stops it–it is, after all, the same physical membrane. These assumptions, quite apart from the recirculation, are doubtful and not supported by any data.
10. It is difficult to conceive of e-o being localized at a transmembrane pore as these are too short and too narrow to allow appreciable ion:water coupling to occur (an ion can only drive out the maximum number of water molecules in the pore, which puts too low a ceiling on the coupling ratio). The tight junction offers a wide enough region that could allow the flow of salt solution through a matrix with apparent fixed charges. In addition, e-o coupling in the interspace itself (as opposed to the junctions) is not considered; although this may aid the exit of fluid from the interspace during fluid production by osmotic coupling, it is not per se a mechanism for *producing* that fluid (McLaughlin & Mathias, 1985).
11. In this model there is no inclusion of water flows, but they are added post hoc. Either the junctional flow of ion current is converted into an e-o flow of water using the coupling coefficient determined earlier (Sanchez et al., 2002) or the net ion flow is converted to an osmotic flow of water by assuming this to be isotonic (with saline). When e-o is operative, however, AQPs will also be mediating water flows and will contribute substantially to the volume flux. It cannot be simplified to an either–or situation.
12. We have seen that the e-o coefficient determined experimentally (Sanchez et al., 2002) is close to the salt:water ratio in basal saline (0.15 M), and it is this value that underpins the argument that an e-o mechanism can generate quasi-isotonic flows. To get near this value theoretically (Rubashkin et al., 2006), two parameters are required: β, representing the concentration ratio of mobile to fixed charge, and n_i, the electrochemical distribution of mobile ions between interspace fluid and junction. These are both concentration-dependent (*see* Fig. 5 and equations 5a and 6 in Rubashkin et al., 2006).
13. The technique used involves measurement of the flux of a range of paracellular probes (usually H^3-labeled dextrans) across the epithelium, ideally in both directions to obtain the net flux. The intercept of the flux curve at zero radius gives the flux of a probe not subject to drag coefficients, viscous or diffusive–i.e., the effective flux of water. The theory in essence is quite simple (Shachar-Hill & Hill, 2002) but can be found in greater detail in earlier studies using the technique (Hill & Shachar-Hill, 1993; Shachar-Hill & Hill, 1993).
14. The effect of the sensor-signaling pathway on JFT is given as $jv = Am(C_i - C_o) + jvo$, where jv is the resulting fluid flow rate, A and m are the gain and the amount of sensor and the Cs are the transmembrane osmotic concentrations. jvo is the sensor-independent flow rate, and this component is still there if the amount of sensor is reduced to zero (by a knockout). jv will be reduced and the fluid will no longer be quasi-isotonic, which is the case in several systems including salivary gland, airway gland and proximal tubule.
15. If there is a JFT system, then the paracellular probe studies tell us quite a bit about it (Shachar-Hill & Hill, 2002). There is cut-off at 6–8 Å for most systems. The flux at low radii is linear with probe radius, implying that convective drag is absent but there is apparent size-dependent entry to a parallel channel. Ordinary convection of probes through such a narrow channel would require enormous fluid flow rates that are impossible. Peristalsis of some kind (with closure) between parallel walls is the only remaining possibility. Ironically, this is what one might have proposed without these hydrodynamic data.

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