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A New Approach to Epithelial Isotonic Fluid Transport: An Osmosensor Feedback Model

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Abstract. A model for control of the transport rate and osmolarity of epithelial fluid (isotonic transport) is presented by using an analogy with the control of temperature and flow rate in a shower. The model brings recent findings and theory concerning the role of aquaporins in epithelia together with measurements of epithelial paracellular flow into a single scheme. It is not based upon osmotic equilibration across the epithelium but rather on the function of aquaporins as osmotic sensors that control the tonicity of the transported fluid by mixing cellular and paracellular flows, which may be regarded individually as hyper- and hypo-tonic fluids, to achieve near-isotonicity. The system is built on a simple feedback loop and the quasi-isotonic behavior is robust to the precise values of most parameters. Although the two flows are separate, the overall fluid transport rate is governed by the rate of salt pumping through the cell. The model explains many things: how cell pumping and paracellular flow can be coupled via control of the tight junctions; how osmolarity is controlled without depending upon the precise magnitude of membrane osmotic permeability; and why many epithelia have different aquaporins at the two membranes.

The model reproduces all the salient features of epithelial fluid transport seen over many years but also indicates novel behavior that may provide a subject for future research and serve to distinguish it from other schemes such as simple osmotic equilibration. Isotonic transport is freed from constraints due to limited permeability of the membranes and the precise geometry of the system. It achieves near-isotonicity in epithelia in which partial water transport by co-transporters may be present, and shows apparent electro-osmotic effects. The model has been developed with a minimum of parameters, some of which require measurement, but the model is flexible enough for the basic idea to be extended both to complex systems of water and salt transport that still await a clear explanation, such as intestine and airway, and to systems that may lack aquaporins or use other sensors.

Key words: Epithelial transport — Aquaporins — Osmosensors — Tight junctions — Water permeability — Paracellular system

Introduction

In this paper we present a concise model of a forward-facing leaky epithelium that transfers fluid in a quasi-isotonic manner from apical to basolateral bath. The model is intended to replace the one based upon osmotic equilibration of transported salt which may still be regarded by many as the 'standard model' of a fluid-transporting epithelium but which has proved inadequate to explain many features seen in experimental work. Although there is a long history of dissatisfaction with the osmotic theory we do not review that here, but rather concentrate on two more modern developments that require explanation by a more sophisticated model — one which incorporates all the elements of the standard model but has novel features that puts them into a quite different context.

The first development is that of AQP (aquaporin) studies in different epithelial systems, in particular, the effects of knocking out specific AQPs on fluid transport. This has recently been extensively reviewed [16] and may be summarized as follows: the effects of knockouts are always to decrease the osmotic permeability of the membrane involved but in the majority of cases there is no real effect on fluid transport. This indicates that osmotic equilibration cannot be the basis of the transport and it leaves open the role of the AQP. In the minority of systems

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showing a real reduction in water flow, there is almost as great a reduction in salt transport.

The second development is the emergence of evidence for paracellular fluid transport, recently reviewed [30]. Although studies are confined to a small number of different epithelia, where this has been measured with extracellular probes, it is quite clear that in virtually all of these the whole of the fluid is passing the epithelium paracellularly. If these epithelia are not extraordinary, and there is one basic mechanism at work, then it is quite probable that if these techniques were applied to other systems they would reveal paracellular fluid flow to be very widespread, if not universal.

Junctional Fluid Transfer

This flow must be across an element of the tight junction which is referred to in this paper as the 'junctional fluid transfer' or JFT system, and it is this structure that probably holds the key to the mechanism of flow. We have argued on the basis of experimental results that this active mechanism must be some form of molecular peristalsis [14, 30] rather than a simple convection which would itself require a driving force across the paracellular pathway external to the junction. It is important to stress that experiments with probes have revealed a JFT system that is not 'leaky' as a simple ion-conductive pathway would be, and there is a bi-directional passive flow of small molecules and ions that is seen in all epithelial experiments where fluxes and currents are measured, which is functioning in parallel with the JFT. Whatever the mechanism, the JFT system is impelling solution through a selective structure (determined by size, charge, or both) which allows water through more easily than solute, and the flow at this point must therefore be hypotonic (*see* the section below, Hypotonic Paracellular Flow).

It must be stressed that the JFT system is not considered here to be a property of all tight junctions in transporting epithelia, but is proposed only for isotonic fluid transporting systems. There is no evidence that such a system as JFT is operating in frog skin, urinary bladder, collecting duct or some of the strains that form confluent epithelia (e.g., MDCK cells). In this context the demonstration that in an MDCK epithelium water does not flow across the junctions [17] is compatible because this is not an isotonic fluid-transporting system: there is a very low rate of fluid transport (presumably over the lateral interspace membranes) and the ratio of salt:volume transfer shows that it is very hypertonic, about 3.5 × saline osmolarity [30]. This example, far from ruling out junctional fluid flow, leads to two important conclusions. First, this epithelium may be regarded as one that is relying solely on osmotic equilibration and shows how a system behaves without JFT; there is no detectable osmotic flow across the junction even when osmotic flow across the epithelium is induced by applying osmotic gradients between the baths. Second, it is electrically leaky (60 Ω cm^2), indicating that the shunt path for ions can exist separately from the JFT system. Isotonically transporting epithelia are qualitatively different from others and are not merely tight epithelia (in the skin model) with added osmosis.

It has been suggested that a role for AQPs in cells is that of osmosensors and a possible model of this function at a molecular scale has been based on the fact that these molecules are always tetramers and therefore capable of allosteric modification [16]. It is a natural extension of this idea that the AQP should signal to another part of the cell as part of a feedback loop. The model of epithelial fluid transport proposed here uses this central idea of AQP sensing and feedback to overcome the problem of osmotic equilibration much as the thermostat controls the water temperature in a shower (Fig. 1*a*).

Feedback Control

In a purely thermostatic system, when the hot water is turned on the thermostat regulates the cold water flow to bring the mixture to the temperature 'set point' which lies between that of the hot and cold supply. In this epithelial model the osmolarity is analogous to the temperature of the water and the set point is the tonicity of the source bath. 'Isotonic' secretion can be defined as 'isotonic to the source bath'. By comparison, the cellular route is supplying a hypertonic solution by pumping salt across the membranes, which is followed by a little water drawn osmotically across the cell — in the steady-state it will appear that this is very little, indeed, in conformity with the experimental finding that the water flow is mostly paracellular. The paracellular system is supplying a hypotonic solution. The AQP is functioning as a thermostat by sensing the transepithelial gradient and regulating — via a cell signalling system — the magnitude of the paracellular flow to approach an osmotic set point. This point is the osmolarity of the source bath and in the steady-state the system in its simplest form is an isotonic clamp operating by negative feedback (Fig. 1*b*).

This provides an explanation of why virtually all the water flows paracellularly. Although the cell membranes possess an appreciable osmotic permeability, there is no gradient to drive it through the cell because it has been abolished by the feedback system. The gradient of osmolarity across the cell is then close to, but not exactly, zero, which is a condition that cannot be attained with any clamp system. It is a common observation that the transported fluid is very slightly hypertonic, as it would be in a system of simple osmotic equilibration.

The model is presented in its simplest steady-state form, with some of its salient features presented as a

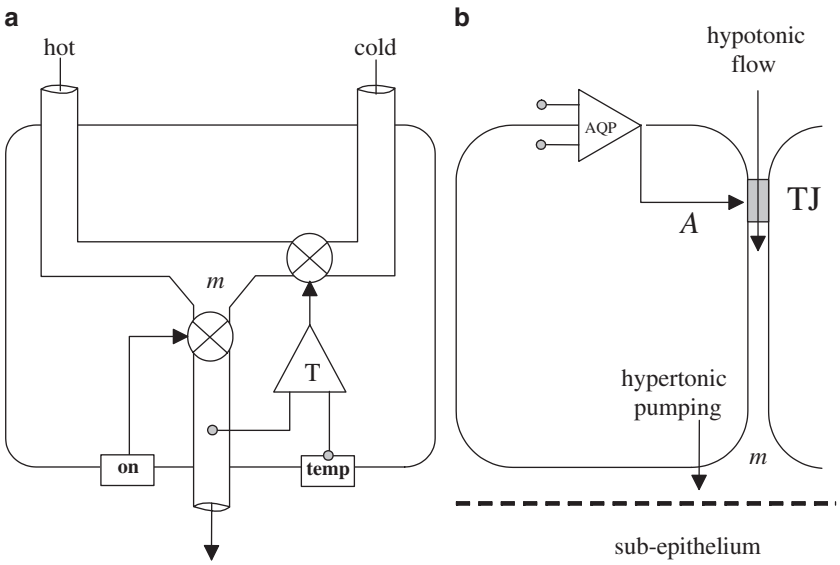

Fig. 1. *a.* The operation of an ideal (constant pressure) shower control as a feedback circuit. The temperature control (*temp*) determines the set point and the system is opened by controlling the water supply (*on*). The fluid streams mix in the region *m*. The thermostat senses the exit water temperature and the amplified difference signal reduces the flow of cold water (negative feedback). In the steady-state there will be a small input difference depending on the size of the gain *T*. *b*. A forward-facing epithelium transporting fluid quasi-isotonically. The AQP senses the osmotic difference between cell and source bath and the output signal controls the hypotonic fluid flow in the JFT system. The two fluid streams mix in the basolateral space *m*. The cell tonicity is intermediate between that of apical bath (the effective set-point) and the transported fluid and the difference in the steady-state will depend on the gain *A*.

series of graphs, much in the style of that classic exposition of the osmotic coupling theory (standing gradient osmotic flow) by Diamond & Bossert [5], which explained much of what was known about epithelial fluid transport in its day. As in their model, we treat the salt as a single solute without building in the electrophysiology of transport, but the problem of mixing the two flows, cellular and paracellular, is not treated explicitly but assumed to occur to a sufficient degree in the interspace and sub-epithelium to present a mean osmolarity to the basolateral membrane. In other words, we consider local gradients to be of secondary importance, whereas in standing gradient flow they are central to the coupling mechanism, which must achieve sufficient equilibration along the interspace system. We refer to this former paper [5] at various points to show how the present model converges on many of the same observations, but differs radically in others.

The Osmosensor Model

Equations and Parameters

Three linear simultaneous equations are sufficient to describe the behavior in the steady-state. The first describes the paracellular volume flow jv_p

$$jv_p = Am(C_c - C_a) + jvo \quad (1)$$

which is regulated by the signal input to the AQP sensor system, (C_c - C_a), where C_a is the osmolarity of the apical bath and C_c that of the cell. It is proposed that the AQP molecule is sited at the apical membrane in a forward-facing system where it uses the apical (source) bath as the set point and responds to the osmotic gradient across that membrane, i.e., between apical bath and cell. The input is transduced by the AQP molecule as a change in its conformation [16] to give a signal output where A is the gain and m is directly proportional to the sensor density in the membrane, which is set to unity in the basic model. A has the dimensions of an osmotic permeability ($cm^4 osmole^{-1} s^{-1}$) and represents the cell signaling pathway between the sensor and its target. This is the rate of operation of the JFT system, jv_p. The system may also have an offset value of *jvo*. The offset simply allows the JFT system to function per se when the sensor input is zero, which has obvious implications for knockout systems, as will be discussed later. This is the heart of the model which couples the AQP sensor to the paracellular fluid transport system by setting its rate.

The second equation represents the osmolarity of the cell, intermediate between that of the apical and basal baths C_a and C_b

$$C_c = (p_a C_a + p_b C_b)/(p_a + p_b) \quad (2)$$

and results from the fact that the rate of water entering the cell, $p_a(C_c - C_a)$, is equal to that leaving, $p_b(C_b$ - $C_c)$, in the steady state. It is the equation of continuity of water flowing across the cell. p_a and p_b

are the osmotic conductances of the two membranes, being the products of osmotic permeability P_{os} and membrane area of the apical and basolateral membranes, e.g., $p_a = (P_{os(a)}\ A_a)$ and $p_b = (P_{os(b)}\ A_b)$.

The third describes the emergent fluid osmolarity, given by

$$C_b = J_s/J_v = (jv_p C_a\theta + P_s(C_a - C_b) + js_c)/ (jv_p + p_b(C_b - C_c)) \quad (3)$$

where J_s is the transepithelial salt flow and J_v, the overall fluid transport rate. J_s is made up of three components. (i) $jv_pC_a\theta$ is the convection of salt through the JFT driven by the fluid transport mechanism with a selectivity θ. (ii) $P_s(Ca - C_b)$ is the salt diffusion across the epithelium from apical to basolateral bath where P_s is the overall permeability. This will be dominated in leaky epithelia by the passive permeability of the junction but as this is mainly permeable to only one ion it will be small and has little effect. (iii) js_c is the rate of active salt pumping through the cell. J_v is the total volume flow into the basolateral system comprising (i) the paracellular volume flow jv_p and (ii) the osmotic flow from cell to basolateral space, $p_b(C_b - C_c)$.

Eq. 3 represents what may be called a 'unilateral' solution because it assumes that the epithelium is bathed on the basolateral membrane by its own secretion. This is strictly true in the case of 'backward-facing' epithelia like exocrine glands but in other cases only partially true when there is basolateral circulation. However, the basolateral bath osmolarity must be dominated by that of the secretion, particularly in the lateral interspaces which are not subject to stirring and usually constitute a large fraction of the basolateral membrane area. As long as the system gain A is set to a reasonable value the activation of the osmosensor will be enough to drive the secretion towards isotonicity. This is not the case with osmotic fluid production [5] where the equations determine that the tonicity of the transported fluid is very sensitive to the exit concentration adjacent to the basolateral system.

It is convenient to use a tonicity ratio Os as an index of the hypertonicity of the transported fluid

$$Os = J_s/J_vC_a \quad (4)$$

and as a measure of the coupling between water and salt across the system. When $Os = 1$ the fluid is isotonic. In the osmotic theory Os cannot be exactly 1.0, as osmotic equilibration is never perfect in the steady state [5]. In the osmosensor model this is also true, but for a different reason: isotonic 'clamping' by a feedback loop is never perfect.

The feedback system then operates in the following way, which may be understood by considering a perturbation. If in the steady state the apical bath osmolarity C_a is suddenly stepped up to a new value, Os falls immediately because the cell concentrations have not yet adjusted (Eq. 4). The AQP system receives a decreased signal (C_c - C_a) and according to Eq. 1 this reduces the paracellular flow which, as discussed below, is hypotonic. This raises the osmolarity of fluid in the basolateral system (Eq. 3) which in turn raises the cell osmolarity (Eq. 2) towards the new apical value, increasing the AQP signal. The overall effect is that the system has raised the osmolarity of the transported fluid, driving it towards isotonicity again. Like all feedback systems, including the ubiquitous voltage clamp of electrophysiology, osmotic clamping is not perfect but generates an approximation to isotonicity depending on the gain A. The analytical solution of the equations for the tonicity ratio Os (too cumbersome to be shown explicitly here) indicates that, whatever the precise values of the other parameters, it converges on 1.0 as A increases. The osmosensor model, as a negative feedback system, drives the system towards quasi-isotonicity in the steady-state.

Hypotonic Paracellular Flow

At this stage it is not essential to analyze in detail the nature of the selectivity in the JFT system. It is likely that the channels which are responsible for the ion conductance of the junction and the passive permeation of small molecules are in parallel with the fluid flow system [14]. The convection term $jv_pC_a\theta$ of Eq. 3 is a general expression in which $\theta \to 1$ as the solute radius approaches that of the water and $\theta \to 0$ as the solute radius increases to that of the pathway. For ultrafiltration flow across a passive porous membrane, through which fluid is forced by a pressure difference, the selectivity is clearly due to the retardation of solute by dynamic friction with the matrix. In the case of flow in the JFT, there is no external pressure gradient driving water through the system and it has been proposed that a form of micro-peristalsis must be in operation [14, 30]. The selectivity θ then arises from the distribution of differently sized solutes between bath and micro-components of the JFT architecture [30]. However, the same term $jv_pC_a\theta$ can apply to both ultrafiltration and to the JFT system, and represents the fact that fluid leaves the bath at a concentration C_a and crosses the JFT system at a rate jv_p as a hypotonic solution, because $\theta < 1$. The selectivity turns out to be an important property of the JFT for the behavior of the model in general and some justification for choosing a value for θ is necessary.

θ must lie within the range $0 < \theta < 1$ and this fact is enough to ensure that the ratio of salt to water is hypotonic compared to C_a. As will be seen in the set of parameters that are used in this paper (Table 1)

Table 1. The basic parameters for the model

Membrane osmotic permeability	$P_{os(a,b)}$	2×10^{-1}	$cm^4/s \cdot osmole$
Apical membrane area	A_a	1	cm^2/cm^2 epithelium
Basolateral membrane area	A_b	10	cm^2/cm^2 epithelium
Concentrations	C_a,C_b	0.3×10^{-3}	$osmole/cm^3$
Salt pumping rate	js_c	1×10^{-9}	$osmole/s \cdot cm^2$ epithelium
Salt permeability	P_s	0	cm/s
Osmosensor gain	A	2	$cm^4/s \cdot osmole$
Osmosensor density	m	1	
Junctional selectivity	θ	0.8	
Feedback offset	jvo	0	cm/s

the value of θ chosen is 0.8 and this is determined from the size of the pathway through the JFT system and the effective radius of salt. The possible effects of charge on the JFT system (as opposed to the leak pathway) are not yet known. Studies with labelled paracellular probes in various epithelia have shown 'cut-off' radii of 4.2 Å and 5 Å for salivary glands [2, 23], 5–6 Å for intestine [15] and 5.5 Å for Malphigian tubule [11]. The expression for the reduced area S available for permeation through a parallel-sided channel (as may be assumed for a JFT system) is

$$S = 1 - r_s/r_{ch} \tag{5}$$

where r_s and r_{ch} are the solute radius and channel half-width and the selectivity [30] will be the ratio of S for salt and water

$$\theta = S_s/S_w \tag{6}$$

Taking a radius for small ions of 2–2.5 Å and a channel half-width of 5 Å we obtain a value of θ between 0.71 and 0.86. The exact value is not critical for the functioning of the model but, as will be discussed below, it determines the extent of the paracellular salt flow.

Basic Model Behavior

Parameters

In the description of the model behavior which follows we have chosen to use a basic set of parameters which are representative of a typical forward-facing epithelial cell system. Selected parameters are then varied to show aspects of the model behavior. In these cases all the other parameters have the initial basic values. These values are close to those that have been determined experimentally or, in the case of cell dimensions, are values obtained by microscopy, although epithelial cells vary widely. It transpires that the cell dimensions are not very important, and the system will generate isotonic fluid flow under a very wide range of conditions. A key parameter is the gain A of the feedback system. This parameter only has to be big enough to push the behavior of the system into the isotonic range—its precise value is not important beyond a certain modest size, as will become apparent below. With the model parameters used here, as A is raised from zero the system moves rapidly to an osmotically clamped state and above a value of 2 the fluid is quasi-isotonic.

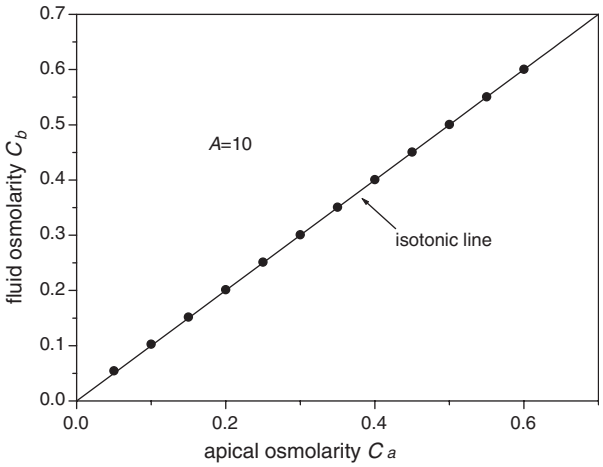

Fig. 2. The osmolarity of the transported fluid as a function of the apical osmolarity. The fluid is close to being isotonic with a slight tendency to hypertonicity at low values of C_a. Isotonicity at all values is increased by increasing the feedback gain A.

Isotonic Transport

In Fig. 2 the tonicity of the transported fluid is shown when the apical (source) bath is varied. It is apparent that the model generates isotonic fluid transport over a large range. At the lowest values of apical bath osmolarity C_a a tendency to deviate from the isotonic line can be seen which is also a feature of the osmotic equilibration model [5]. This slight hyper-osmolarity can be diminished by increasing the gain, which indicates that in this model A plays a role similar to that of P_{os} in the osmotic model. Until the cell signaling sequence is determined the expected range of A cannot be specified, but it may be that much higher values than those used here to reach isotonicity are natural. The two parameters have similar dimensions ($cm^4 osmole^{-1} s^{-1}$) and increasing them pushes the system behavior towards isotonicity. In this model, when an osmosensor feedback is operating, the system becomes insensitive to the osmotic permeabilities of the membranes and this is because only a very small fraction of the fluid is crossing the membranes by osmosis.

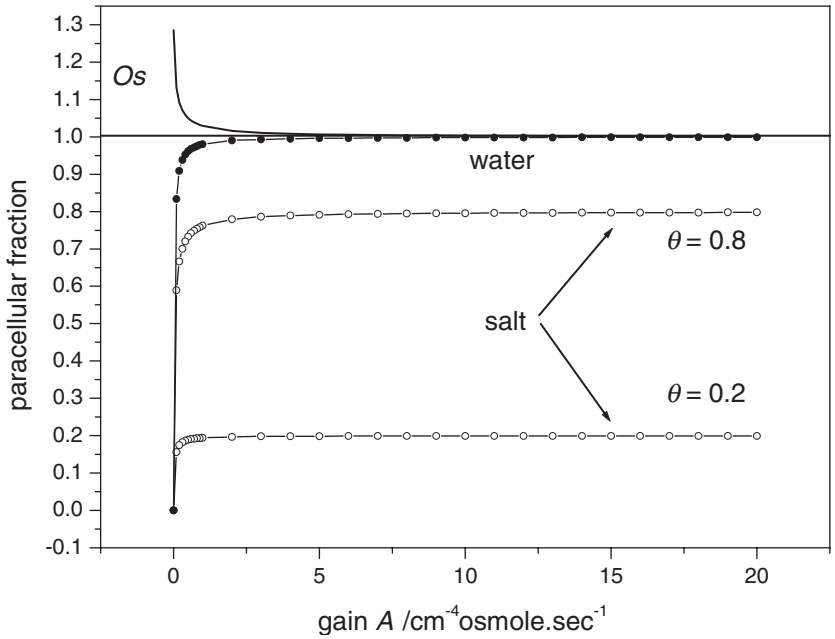

Fig. 3. The fraction of water and salt crossing the epithelium via the JFT system is determined by the osmosensor gain A. With increasing A the water flow eventually becomes entirely paracellular while the salt fraction approaches θ. The transepithelial fluid tonicity Os becomes isotonic. No offset is present ($jvo = 0$).

Water and Salt Transport in the Junction

In Fig. 3 the result of varying the gain A can be seen for salt and water transport as a fraction of that crossing the epithelium as a whole. Increasing the gain diverts a larger fraction of water and salt through the paracellular pathway. It also controls the hypertonicity Os. When A is zero, and the model only allows osmotic equilibration to occur, the transported fluid is hypertonic and all the water and salt flows are transcellular. As the gain is increased we come to a point where most of the water is flowing through the JFT system and the system is transporting isotonically. At the values of $A = 2.5$ and $\theta = 0.8$, 99% of the water and 78% of the salt flow is paracellular whilst $Os = 1.005$. When $A = 2.5$ and $\theta = 0.2$, the paracellular water flow is still 99% but the salt flow is 19% of the total.

This value for the paracellular salt flow is a natural consequence of the model and appears as a novel departure from the current osmotic theory. It can be seen that the fraction of salt crossing the JFT system is approaching the selectivity θ. This can be understood by considering the terms in Eq. 3. The transjunctional salt flux is $jv_pC_a\theta$ and the total transepithelial salt flow into the basal bath is J_vC_b. As the fluid approaches isotonicity at higher gain $C_b \rightarrow C_a$ (i.e., $Os \rightarrow 1$) and in addition all the fluid flow now becomes transjunctional in which case $J_v \rightarrow jv_p$. The fraction of salt crossing the epithelium via the JFT system is then given by

$$jv_pC_a\theta / jv_pC_a = \theta \tag{7}$$

The tonicity of the transjunctional fluid is given by Eq. 4: the ratio of salt to water crossing the JFT system relative to the source bath C_a

$$Os = \frac{(jv_pC_a\theta / jv_p)}{C_a} \tag{8}$$

Thus θ plays a role along with A as one of the two major controlling parameters of the model.

Although, as described above, we do not know the steps of the cell signaling sequence, if A is above 2 the transported fluid is isotonic. If θ is high enough, and here we have experimental data from some epithelia, the transjunctional salt flow is considerably greater than that through the cell. But when the cell pumping is reduced or inhibited ($js_c \rightarrow 0$), as shown in Fig. 4, all the salt flow is abolished, both the cellular and the paracellular. It might seem hard to understand how paracellular fluid flow can be geared to cell pumping, when there is no direct connection between these processes and they take place in different parts of the epithelium. When paracellular flow was demonstrated in epithelia [11, 15, 23, 29] it was difficult to see why inhibition of active cellular salt pumping should not leave paracellular transport intact. However, it appears as a natural consequence of the model that the feedback system couples salt and water flow across the JFT system to the rate of cell pumping and at the same time ensures virtually isotonic flow. Using the shower control analogy, if the hot water supply to the unit is choked down the thermostat will turn down the cold water flow in an attempt to maintain the preset temperature. When the hot inflow has stopped completely the cold water flow is zero also, and the total flow has ceased.

It has become virtually a truism in the epithelial field that active solute transport can only occur across cell membranes because they alone are the sites of molecular pumps and the relevant transporters.

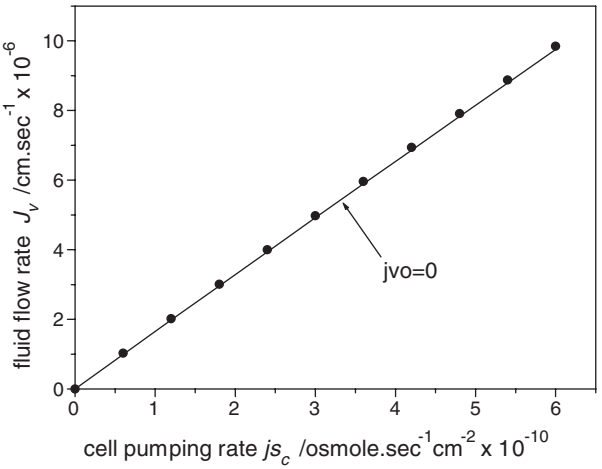

Fig. 4. The coupling of fluid transport to the cellular transport of salt. When the pump rate is decreased the fluid transport declines in parallel in a linear manner ($R^2 = 0.99$). The feedback offset *jvo* is either set to *jvo* = 0 (line) or *jvo* = 5×10^{-6} cm.s^{-1} (●), a value equal to 50% of the maximum fluid transport rate shown here.

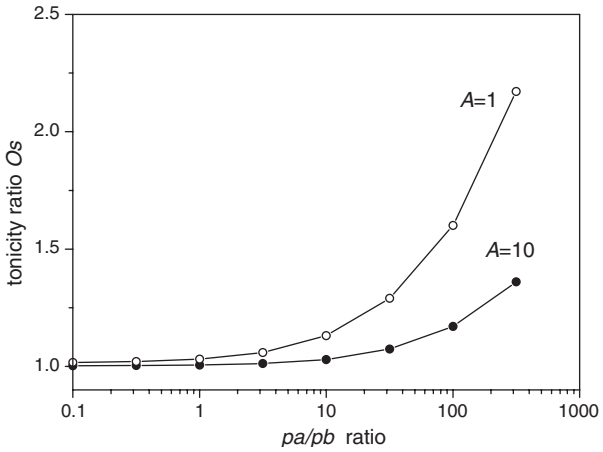

Fig. 5. The effect of membrane permeability on the tonicity of the transported fluid. For a given value of the gain A the tonicity is regulated by the permeability ratio of the apical and basal membranes not by their absolute values. At $A = 1$, 17% of the water is crossing the cell, while at $A = 10$, it is 2%.

Although this is widely accepted, it may be surprising to realize that it has little support from experiment, even after many decades. It is not a simple thing to measure the transcellular Na transport rate *per se* and compare this with the transepithelial Na flow, in the steady state and after pump inhibition. It is usually assumed that because a pump inhibitor such as ouabain exerts its effect on the Na-pump at the cell membrane, and brings transepithelial salt and water secretion (eventually) to a halt, all the Na must be crossing the cell. The model behavior developed here (Fig. 4) shows that this can be an incorrect assumption, however natural it may seem. Measurement of cell fluxes in the presence of a leaky paracellular pathway requires an analysis of time-dependent partial fluxes at both cell membranes. Where this has been done, by analyzing the partial fluxes of Na into and out of the cell at both membranes, the results show that only a fraction of the salt flow can be cellular [12, 13]. Furthermore, where ion-sensitive microelectrodes have been used in conjunction with inhibitors of active transport the results have generally shown that the rate of Na pumping by the cell is much less than the trans-epithelial rate (discussed in [13]).

When paracellular probes of a range of radii have been used to measure bi-directional fluxes in those epithelia in which this is possible [11, 14] net fluxes of probes have been recorded, indicating an 'active transport' of probes through the junctions. On the basis of characteristics of the probe transport, the mechanism proposed (micro-peristalsis, or an equivalent) could easily accommodate the transport of water and ions. There is no reason why this should be the major conductance pathway for ions and there may be a parallel system accounting for the electrical leakiness and ion selectivity through which ions pass at high rates. We have no detailed structural data yet about the molecular architecture of the junction apart from the identification of claudins and occludins in the junctional complex. We do know that the sign of the charge in the passive junctional pathway and the pattern of ion selectivity can be altered [3]. There has also been modelling of a junction with two channel types, slits and pores [9], but this has been based on earlier passive permeability experiments. We refrain from speculating upon a detailed structure for the JFT system that would be required to bring about the active fluid transport but it has to satisfy certain experimental criteria which have been discussed in a general way [14, 30].

The Role of Membrane Osmotic Permeability

The major effect of osmosensor feedback coupled to the paracellular fluid transfer is that the system becomes insensitive to the precise values of the membrane osmotic conductances p_a and p_b. In Fig. 5 results are shown taken from many model runs involving variations in p_a and p_b from which it emerges that the factor controlling the hypertonicity of the fluid is the ratio p_a/p_b. When this is small and the gain is as low as 1, the secretion is isotonic and *Os* is close to 1.0. In this case, the fraction of water crossing the cell is very small indeed. This is due to the fact that *Os* has been clamped by the feedback so near to 1.0 that there is virtually no transepithelial gradient to drive water across the cell. The osmosensor model solves the most contentious and sensitive problem that faces osmotic equilibration theories very directly, by directing the water flow through another route.

Why is the system sensitive to p_a/p_b rather than the absolute values of membrane permeability? The answer can be seen by considering Eqs. 1 and 2. The input to the osmosensor system at the apical membrane is the difference between cell and apical bath, $(C_c - C_a)$, and the value of C_c is determined by the ratio p_a/p_b. There are two extremes to be considered. From Eq. 2 it can be seen that when the apical permeability dominates i.e., when $p_a > p_b$, the cell osmolarity C_c approaches that of the apical bath, there is little input to the osmosensor and therefore feedback control is slight. When the basolateral permeability dominates, i.e., $p_b > p_a$, the cell is in closer osmotic equilibrium with the basal fluid C_b and therefore the input to the sensor approaches $(C_b - C_a)$. In this situation the feedback is maximal.

Two AQPs Are often Present in the Cell

We suggest, in terms of this model, a role for another AQP in the cell. As an AQP sensor is situated in the apical membrane, this increases p_a by virtue of its contribution to the membrane P_{os}. Thus the ratio p_a/p_b is increased and the feedback is diminished. To compensate for this, the P_{os} of the basolateral membrane has to be increased by insertion of an AQP there, to reduce p_a/p_b and to make the cell osmolarity mirror C_b. This insertion also overcomes the effect of the epithelial cell geometry because the two membranes do in general have different areas. Usually, the basolateral membrane of epithelia is much larger than that of the apical, but an increase of p_a may still need to be balanced by p_b. If this is so, then the second AQP is functioning purely osmotically to balance the effects of the first and adjust the size of the feedback input signal (C_c - C_a). In this case the two AQPs have the role of osmotic channels that control the cell osmolarity (by small water shifts) but they do not act as major water conduits for transcellular fluid flow, which is delegated to the paracellular system.

Why are there often different AQPs at apical and basolateral membranes? A possibility is that any cell signaling sequence would be initiated by interaction with one AQP only (the sensor) and would not respond to the state of another in the cell. Thus the second AQP (ideally in this model the downstream or basolateral one), inserted to expose the cell to the basolateral medium as described above, would not interfere with the primary feedback loop. It may be, however, that the signaling sequence is set up to interact with only one membrane (here the apical) into which the sensor AQP has been inserted, and the other membrane is not recognized by the cell signaling sequence. Whichever is the explanation, the model suggests a dual role for AQPs in the cell — to act in cell equilibration and to act as sensors — and these functions may well require different molecules.

Co-transporters

It has been shown that co-transporter molecules, when expressed in oocytes, can transport water along with solute [19]. This has been described as a form of active water transport ('water pumps' [18]) and suggested to be the basis for epithelial isotonic fluid transport [36]. Because the rate of water transport is not high enough to achieve isotonic flow there are questions concerning the possible hypertonicity of the transported fluid, and these have been addressed by proposing that osmotic differences are largely abolished by water flow through AQPs, the co-transporter itself and the lipid membrane. The theory is therefore in part a theory of osmotic equilibration. The experimental data showing paracellular flow on which the sensor model is based [30] are not consistent with any appreciable cellular water flow, but it may be asked how the model would deal with the presence of a certain amount of co-transporter-linked water traversing the cell. In particular, it is of interest to see whether the feedback is disrupted, leading to differences in tonicity across the epithelium. In short, how co-transporter water affects the behavior of the ratio Os.

In Fig. 6 the effects of this have been studied by introducing into Eq. 3 a term $jvco$ representing the rate of co-transporter water-pumping across the cell

$$C_b = (jv_p C_a \theta + P_s(C_a - C_b) + js_c)/ (jv_p + p_b(C_b - C_c) + jvco) \quad (9)$$

in which we do not enquire precisely how the pumping is organized. We assume that it represents an overall transport of water across the cell which is separate from both the paracellular and cell-to-bath osmotic flow, and which is proportional to the rate of salt pumping js_c. Although co-transporter water transport represents a potential contribution to epithelial water transport, no quantitative model system has ever been assembled that shows how the co-transporters at the two membranes achieve overall isotonic transport [35]. We here assume that this problem has been solved and that the inflow and efflux rates are equal.

When there is no feedback ($A = 0$) the initial values of Os for the basic parameters show that the fluid is hypertonic ($Os = 1.3$). Increasing co-transporter water $jvco$ reduces Os in a linear manner until with a high enough value it becomes hypotonic. It would seem, on this formulation, that factors are needed to ensure that the rate $jvco$ is set to a value that ensures quasi-isotonicity. However, when there is osmosensor feedback present ($A = 2$), the fluid is

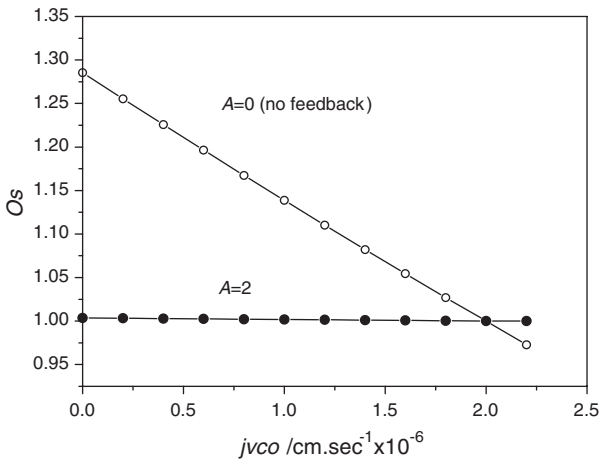

Fig. 6. The effect of a co-transporter transferring water through the cellular route. Without feedback (○) the tonicity of the transported fluid is dependent upon the value of *jvco*, becoming isotonic only at a value of 2×10^{-6} cm.s^{-1} which is 20% of the secretion rate for the parameters used here. When the feedback is operative (●) the fluid is isotonic independently of the co-transporter rate.

isotonic at all values of *jvco*. This is because the system adjusts the paracellular flow to take account of the water flux through the cell and still drives the system to isotonicity. Thus the model is independent of the presence of co-transporter water fluxes and incorporates them, irrespective of how large they may be. Co-transporter water transport therefore requires a scheme that ensures overall isotonic flow and this would be provided by the osmosensor-JFT model.

Electro-osmotic Effects

In several epithelial systems the application of current flow across the epithelium causes a change in the rate of fluid transport (*see* the review in this issue [7]). A characteristic of this flow is important — the flow is usually created by a hyper-polarization of the existing secretory potential. For this reason it has been considered as a possible mechanism underlying fluid transport across the junction because it is this secretory potential, not an externally applied one, which has to drive the junctional electro-osmotic flow *in vivo*. Usually, epithelia transport NaCl across the cell and this can be done by siting the Na-extrusion pump at either the apical or basolateral membrane (almost always the latter, but not exclusively) and arranging co-transporters to effect net salt transport in a particular transcellular direction. The net effect is that either Na^+ or Cl^- ions are extruded into the downstream or secretory bath with a secretory potential of the same sign. If the junction is selectively permeable to the counter-ion its electrodiffusion across the junction in response to the secretory potential results in net transepithelial salt transport. Therefore the sign of the secretory potential of the epithelium and the sign of the charge in the junctional leak pathway will be the same, and these are the conditions required for electro-osmosis in the steady state.

If electro-osmosis is present the only feasible pathway is through charged channels spanning the junction. This has been made the subject of detailed studies and a theoretical model [6, 26] and there is no doubt of the phenomenon in corneal endothelium. Hyper-polarizing the epithelium increases the fluid flow in the direction of the active fluid transport and therefore the secretory potential could be drawing water through the junctions by electro-osmosis. It has been customary to draw a distinction between true electro-osmosis and osmotic effects due to 'concentration-polarization'. When current is passed through a membrane that is selectively permeable to only one of the ions of (say) a uni-uni salt solution, it creates a rise of salt concentration on one side of the membrane and a fall on the other [34]. This induces an osmotic flow of water as long as the current flow is maintained. In general, opinion has tended to favor the latter effect, but the key observation would be the time-course of onset of the current-induced fluid flow. Electro-osmosis should have a fast onset (seconds) whilst concentration-polarization can be calculated to have a build-up with a half-time of minutes [26]. Experimental studies indicate onset times of a few seconds for reasonable values of the membrane osmotic permeabilities [26].

With the sensor JFT model there is another possibility. In the case of a membrane system such as the forward-facing epithelium considered here we have not considered the specific nature of either the pumps or the ion transport through the conductance channels of the junction. With a Na pump at the basolateral membrane, a positive secretory potential and a Cl^--selective junction, hyper-polarization should lead to the build-up of NaCl at the basolateral membrane and a concomitant decrease at the apical membrane by the concentration-polarization mechanism. This effect is precisely what is required to activate the AQP sensor. A rise in C_b and a fall in C_a adjacent to the epithelium will increase the input to the sensor system (Eq. 1) by decreasing C_a and increasing C_c. The extent of the rise in C_c will depend upon the values of p_a and p_b (Eq. 2). Before the full concentration difference and osmotic flow can develop to any extent the sensor input will immediately trigger a rise in paracellular fluid flow that will act to clamp out the changes.

The speed of this response cannot be easily estimated until the response of the feedback system is known. However, the time course of the input signal will depend initially upon the rise of cell osmolarity C_c when salt accumulates at the basolateral membrane, and this will be rate-limited by the time

constant of the osmotic response of the cell. Very simply, if we assume an approximation to an exponential response using a diffusive permeability of the cell membrane to water of $P_w = 10^{-3}$ cm/s, the time constant for an epithelial sheet of height h bathed on the two sides is P_w/h. For a cell of 10 μm height this comes out to be 1 s^{-1}, which is equivalent to a half-time of 0.7 s. The osmotic clamping would therefore be a fast response if it were dependent upon the response of the cell to a rise in osmolarity. The clamping by the sensor-feedback system would not allow salt to build up at the basal membrane as would be the case in simple concentration-polarization but the passage of current would act — if the junctional channels were anion selective — as a mechanism which adds to the salt pumping js_c, which is also acting to accumulate salt adjacent to the basolateral membrane. It can be seen from Fig. 4 that such an effective increment in js_c would lead to a steady-state increase in fluid flow.

The precise increase would depend upon the magnitude of the polarization, but one important effect would be that the increase in fluid flow would be isotonic. This is ensured by the fact that the feedback system adjusts the paracellular flow to cancel any potential hypertonicity at the basal membrane, however it may arise. In the corneal endothelial system it has been estimated that the increases in epithelial fluid flow induced by applied currents are quasi-isotonic [26]. To an observer the hyper-polarization of the epithelium by current-clamp would appear as a step increase in fluid secretion rate, probably with a fast onset time, that would appear to be an electro-kinetic effect, and indeed, would actually be one. We suggest that many of the flow responses to current clamp that have been seen over many years in various epithelia may originate in just this way.

AQP Knockouts

The study of AQP knockouts in epithelia (null -/- for an AQP gene) has recently been reviewed in detail [16] in the context of the osmotic equilibration hypothesis and we briefly summarize the findings here. The effect of knockouts gives no support to this theory. In almost all cases where the P_{os} of the AQP-containing membrane has been measured it falls substantially, as expected, but in most cases this does not lead to a significant reduction of active fluid flow, or one that is expected from the fall in P_{os}. In those cases where the secretion rate has been reduced the osmolarity of the fluid has not risen proportionately. For example, a fall of flow rate to near 50% in an epithelium formerly transporting isotonically should lead to a hypertonic secretion of $Os = 2$. As mentioned earlier, in some of the systems studied where there is a reduction in J_v between wild-type and knockouts, it has proved possible to measure the salt transport rate as well, and this has also been found to be reduced. The result is that the reduced fluid flow is less hypertonic than expected from the reduction in water flow rate. The sensor model has also to explain this reduction in salt and water flow which occurs after the AQP has been removed, as does the osmotic theory.

Knockouts may be simply modelled by setting the AQP density m to zero. In Eq. 1 there is an offset jvo (an intercept in the linear relation) and this has been described as the rate at which the JFT system transports in the absence of feedback. If this is set to zero, as in most of the model situations described here, the model predicts that the secretion of a knockout will be produced by osmotic equilibration and the salt flow will remain constant, being the result of cell pumping. The P_{os} of the membranes will fall to a fraction of the wild-type level (in many epithelia, an 80–90% reduction) and the resulting secretion will be small and very hypertonic.

However, there is no reason why jvo should be zero. If there is an output jvo that still controls the paracellular flow when $m = 0$ it will mean that knockouts only produce a partial reduction in fluid transport. This is equivalent to there being a constitutive component of the paracellular flow jv_p. The conductances p_a or p_b will be reduced, depending upon what AQP knockout was made and at which membrane it was situated. In either case there will be a fall in osmotic permeability of both the membrane involved and the whole epithelium. Knockouts of an AQP sited at the non-sensing membrane such as p_b in this case will reduce the signal input by restricting cell osmotic exchange with the basal bath C_b (Eq. 2) and can raise the hypertonicity of the fluid secretion by raising p_a/p_b (Fig. 5).

We have seen (Fig. 4) that the system functions isotonically when an offset is present due to the effect of osmotic clamping. With an offset value of 5×10^{-6} cm/s the behavior of the model when the AQP is removed can be seen in Fig. 7. In this figure are included the three epithelial knockouts for which data is available for salt reduction as well as water, discussed in [16]. It is apparent that the behavior is very similar between model and experiment. The model predicts a fall in both J_v and J_s with a rise in Os from an isotonic value to 1.2. The interesting point to note is that in the case of the model, the fall in the salt and water is because there has been a large fall in fluid flow traversing the JFT system, and this represents a coupled flow of water and salt. As this is hypotonic, it leaves an overall flow which is hypertonic. The experimental data show a similar effect: the wild-type was isotonic and the knockout is hypertonic, so the reduction can be explained as the apparent removal of a hypotonic flow.

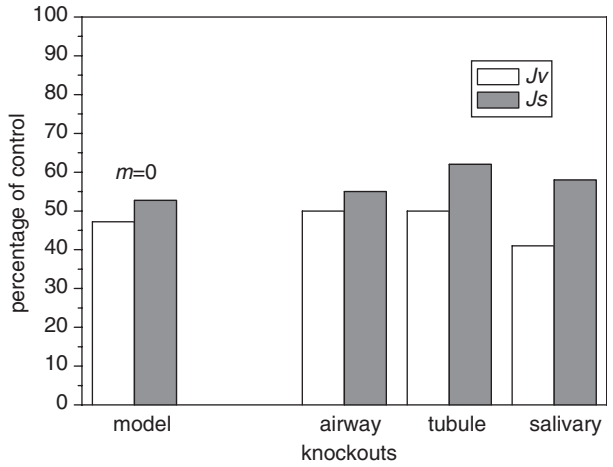

Fig. 7. The effect of knockout of AQPs in the feedback model compared with three experimental systems that have been studied where the transported water and salt can be estimated: airway (glands) [32], tubule (proximal) [27, 33], salivary glands [20], discussed in [16]. In the model the feedback offset *jvo* is set to 5×10^{-6} and the tonicity ratio *Os* of the fluid is 1.2 compared to 1.002 in the control.

Assuming that the rate of salt transport through the cell has not altered between wild-type and knockout, the selectivity of the JFT system to salt can be calculated. If the wild-type produces an isotonic fluid ($Os = 1$) then the fall is due to a coupled reduction of water and salt flow through the JFT system, caused by the removal of the osmosensor feedback. The JFT component is hypotonic (*see* Hypotonic Paracellular Flow, above) and the tonicity is controlled by θ. Thus

$$\theta \simeq \Delta Js/\Delta Jv$$

where ΔJs and ΔJv are the fractional changes in salt and water flow. The value of θ for the three datasets of Fig. 7 are 0. 9 [32], 0.76 [27, 33], and 0.71 [20] (left to right) with a mean of $\theta = 0.79$. Given that these values are estimates from the literature, they show remarkable agreement and are similar to the value of 0.8 used here as a basic parameter in the model, derived from experiments *without* knockouts (*see* above). These falls in water and salt flow represent the cessation, on this interpretation, of a coupled flow across the epithelium which is both paracellular and hypotonic.

Extension to Complex Systems

There are epithelial systems where, separated by a short distance within a tissue, two epithelial cell types are inter-dependent and transporting water or salt in opposite directions. Two examples of these tissues are (i) intestinal and (ii) respiratory epithelia. Both of them are linked by the presence of CFTR (cystic fibrosis transport regulator) in the apical membrane of the cells. It is not the purpose of this section to review in any depth the extremely complex and as yet incomplete understanding of the regulation of ion and water transport involved in these tissues. In most reviews of this subject the water flow is assumed to be osmotic in nature, but we suggest here the alternative view that the paracellular model may play a role in these systems where coupled water and salt transfer are concerned.

(i) In the small intestine the crypts are generally assumed to be sites of secretion by the 'backward-facing' ion transport model first suggested for exocrine glands [10, 31] and subsequently extended to systems in which the Na-pump is transporting Na in the opposite direction to net salt transport by the epithelium. Fluid secreted to the lumen is reabsorbed by 'forward-facing' epithelia of the villi and there is massive re-circulation on a local basis via a closely shared bath. If these two streams are to be isotonic there is no evidence that it is possible to generate them by osmotic equilibration. The crypt cells discharge salt into the crypt lumen and the expression for osmotic equilibration in a cylindrical channel [28] can be used to estimate the emergent osmolarity relative to plasma

$$Os = 1/(1 - K)$$

where K is a function of all the relevant parameters of the system, the length and radius of the channel (lumen), the membrane (apical) osmotic permeability and the diffusion coefficient of salt, L, r, P_{os} and D. Using a crypt length L and radius r of 100 μm and 1 μm, a P_{os}-value for the red cell of 10^{-2} cm.s^{-1} (one of the highest known) and the diffusion coefficient D of NaCl in solution, the value of K is 0.345 and *Os* comes out to be 1.53, which is substantially hypertonic to plasma. It is not easy to find values for normal crypt secretion, but in cholera toxin-induced secretion the concentration of the fluid, which is considered to be predominantly cryptal in origin, is close to being isotonic. Like most calculations involving the tonicity of epithelial secretion, isotonicity is rarely predicted. The osmosensor model deals with the problem faced by osmotic equilibration, operating within a particular geometry and a limited membrane permeability, by replacing it with a JFT system that achieves isotonic secretion.

The intestine of some lower vertebrates is lacking in crypts but re-circulation can be followed by using movement of paracellular probes [15]. The data for *Necturus* indicate that there must be two flows, absorptive and secretory, with different selectivities. As water and ions will have access to these probe channels it seems that isotonic fluid can recirculate on a local scale through the paracellular system. In terms of the forward-facing osmosensor model developed

here, it is possible to turn the whole scheme around and have salt driven from basal to apical bath (via a Cl^- or CFTR channel) with a JFT system transferring fluid from basal to apical (in this case, luminal) bath. This backwards-facing model would apply to secretion by the crypts and is analogous to exocrine secretion.

It may be, however, that the simple 'crypt-secretion, apical-absorption' model is not correct. In the colon there is evidence that secretion and absorption are occurring together within both crypts and apical regions [8]. If this is so, the osmotic theory faces a serious problem because secretion and absorption cannot be occurring together between the basal bath (the circulation) and the lumen in opposite directions simultaneously. The osmotic gradient driving one limb is in the wrong direction to drive the other. By using a 'push-pull' model for secretion and absorption in which the sensor JFT system is operating in two directions, secretory and absorptive, in different but adjacent groups of cells, it is possible to encompass quasi-isotonic, bi-directional flow in which virtually all the water and a large fraction of the salt is recirculating via the paracellular route.

The selectivity θ of the JFT that has been considered so far is that between salt and water. The concept can be generalized very easily to include sugars and amino-acids; indeed, the dimensions of the JFT were determined originally with small neutral probes [15, 23]. Therefore the absorptive limb of the JFT would be the natural route for paracellular sugar and amino acid absorption. This would encompass the intestinal uptake of glucose that has been postulated to occur by paracellular solvent drag across the junction, driven by osmosis [21, 24, 25]. Although we consider that the data do indeed reflect paracellular solute uptake in the intestine, the mechanism proposed for water uptake by osmosis across the junction faces great difficulties that cannot be examined here, but which have been discussed in detail elsewhere [30]. It is very important to emphasize again in this context that the JFT system which forms the basis of the sensor-feedback model, and which would be responsible for the transport of water, small solutes and ions, is not a simple osmotic or conductive channel but an active fluid transfer mechanism controlled from the cell and possessing special properties consistent with the experimental data [14, 30].

(ii) In airway epithelia there is fluid production by the submucosal glands which produce bronchial and nasal fluid which is modified by the airway surface epithelium. The primary production of fluid by the glands may be isotonic, in which case similar arguments could apply to the tonicity of the fluid as have been advanced above for intestinal crypts and exocrine glands. The subsequent homeostasis of the ASL (airway surface liquid) is a dynamic process of great importance that is poorly understood.

The main effect is that of drying out, in which case salt is left behind and the tonicity of the fluid increases. It has been suggested that this osmolarity is regulated by osmotic flow from the surface cells [1] and the total thickness of ASL, which depends upon the total salt content when the liquid is quasi-isotonic, can be regulated by salt absorption. The problem reduces to two possible functions of the airway epithelial cell: the secretion of fluid into the ASL to compensate for the evaporative loss of water, and the absorptive removal of excess remaining salt. The airway epithelium is endowed with aquaporins and while osmosis will make a contribution to the first of these, it has been demonstrated that the CFTR can react to both the Cl^- concentration and the osmotic difference across the surface membrane [4], in which case it is possible that it acts as an osmosensor. The feedback model may therefore be operative in airway epithelium. One aspect of epithelial transport that has not been explored in this paper is the production of hypotonic fluids but, as the hypotonic JFT lies at the heart of the feedback system, hypotonic secretion can actually be generated by the model and it may have a role to play in the dilution of the ASL.

We do not propose detailed suggestions at this stage about the organization of the osmosensor model in relation to these secretion-absorption systems. The model described here is quite flexible and may be modified as described below. What it would do, if introduced, is to free the general modelling of these systems from constraints due to the limited permeability of the membranes (P_{os}) upon which osmotic equilibration and flow rate depend [5] and introduce the ability of the system to change the tonicity of secretion (and absorption) in a way that osmosis cannot do at the required rate.

Model Plasticity and Other Sensors

The model described here for a forward-facing epithelium with transport of salt and water from apical to basal bath can be adapted in many ways to a great variety of situations. One obvious adaptation is to reverse the direction of fluid transport by the JFT system and re-locate the osmosensor to the basal membrane, now comparing the tonicity of the basal bath with the cytoplasm. This would turn the system into a fluid-secreting epithelium, such as an exocrine gland [22]. Other adaptations are possible to fit the two other classes of transporting epithelia which have the Na extrusion pump sited on the apical membrane (choroid plexus and retinal pigmented epithelium) but with water flows directed differently with respect to the junction.

The fluid flow does not have to be isotonic. If A is reduced or p_a/p_b increased, the fluid becomes

markedly hypertonic. More strikingly, as discussed above, it is possible for the model to produce hypotonic flows under certain conditions. This results from the fact that while osmotic flow is based upon a movement of water towards equilibrium, the JFT system is capable of *separating* water from salt, using the energy expenditure of the cell. The fluid flow rate can also be increased by increasing θ, behavior which has not been explored here. Epithelial sensor-feedback systems have a range of rich behaviors that are as yet unexplored, but which are not possible with simple osmotic schemes.

Nor is it an absolute requirement for the osmosensor to reside on the apical membrane. It has been situated there in the treatment of the forward model because that is the place most sensitive to osmotic pressure differences when the apical bath is constant, but it could be basal. The primary concern when producing isotonic secretion is that the system uses the source bath as the reference and it is therefore likely that osmosensors would be located on the apical membrane in absorptive epithelia and on the basal membrane, adjacent to the plasma, in secretory epithelia such as the acini of exocrine glands.

Most interesting, perhaps, is the possibility inherent in Eqs. 1 and 3, for input from many elements of the cell to modulate the final fluid production. First, the osmosensor input does not have to come from an AQP although we have suggested that this tetrameric molecule with its capacity for quaternary changes is a likely candidate [16]. The gain A is an overall parameter including all the elements of the proposed signalling chain which are unknown at present. Anything in the epithelial cell which can act as a receptor and which exists in two states can be involved in providing an input signal to a cell signalling sequence and there is no reason why such a receptor should not intercede to control the JFT system, independently of, or in parallel with, the osmosensor input. It would not be part of a feedback loop controlling the fluid osmolarity directly, but it could be used to control many aspects of the process such as the gain, the selectivity and the offset.

This has immediate implications for the stimulation of fluid transport by intestinal glucose and we have suggested that a co-transporter that binds glucose may be implicated in this [30]. The fact that the selectivity θ of the JFT system may also be altered during glucose uptake has already been considered in relation to its possible convection through the paracellular pathway [21, 25]. Another possible sensor is CFTR in the airway which may be responding to changes in composition of the surface liquid [4].

References

1. Boucher, R.C. 1999. Molecular insights into the physiology of the thin film of airway surface liquid. *J. Physiol.* **516:**631–638
2. Case, R.M., Cook, D.I., Hunter, M., Steward, M.C., Young, J.A. 1985. Trans-epithelial transport of nonelectrolytes in the rabbit mandibular salivary-gland. *J. Membrane Biol.* **84:**239–248
3. Colegio, O.R., Itallie, C.M., McCrea, H.J., Rahner, C., Anderson, J.M. 2002. Claudins create charge-selective channels in the paracellular pathway between epithelial cells. *Am. J. Physiol.* **283:**C142–C147
4. Davies, M.G., Geddes, D.M., Alton, E.W.F.W. 2005. The effect of varying tonicity on nasal epithelial ion transport in cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **171:**760–763
5. Diamond, J.M., Bossert, W.H. 1967. Standing-gradient osmotic flow: A mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* **50:**2061–2083
6. Fischbarg, J., Diecke, F.P.J. 2005. A mathematical model of electrolyte and fluid transport across corneal endothelium. *J. Membrane Biol.* **203:**41–56
7. Fischbarg, J., Diecke, F.P.J., Iserovich, P., Rubashkin, A. 2006. The role of the tight junction in paracellular fluid transport across corneal endothelium. Electro-osmosis as a driving force. *J. Membrane Biol.* **210:**000–000
8. Geibel, J.P. 2005. Secretion and absorption by colonic crypts. *Annu. Rev. Physiol.* **67:**471–490
9. Guo, P., Weinstein, A.M., Weinbaum, S. 2003. A dual-pathway ultrastructural model for the tight junction of rat proximal tubule epithelium. *Am. J. Physiol.* **285:**F241–F257
10. Hannafin, J., Kinne-Safran, E., Friedman, D., Kinne, R. 1983. Presence of a sodium-potassium chloride cotransport system in the rectal gland of *Squalus acanthias*. *J. Membrane Biol.* **75:**73–83
11. Hernandez, C.S., Gonzalez, E., Whittembury, G. 1995. The paracellular channel for water secretion in the upper segment of the Malpighian tubule of *Rhodnius prolixus*. *J. Membrane Biol.* **148:**233–242
12. Hill, A.E., Hill, B.S. 1987. Steady state analysis of ion fluxes in *Necturus* gall bladder epithelial cells. *J. Physiol.* **382:**15–34
13. Hill, A.E., Hill, B.S. 1987. Transcellular Na fluxes and pump activity in *Necturus* gall bladder epithelial cells. *J. Physiol.* **382:**35–49
14. Hill, A.E., Shachar-Hill, B. 1993. A mechanism for isotonic fluid flow through the tight junctions of *Necturus* gallbladder epithelium. *J. Membrane Biol.* **136:**253–262
15. Hill, A.E., Shachar-Hill, B. 1997. Fluid recirculation in *Necturus* intestine and the effect of alanine. *J. Membrane Biol.* **158:**119–126
16. Hill, A.E., Shachar-Hill, B., Shachar-Hill, Y. 2004. What are aquaporins for?. *J. Membrane Biol.* **197:**1–32
17. Kovbasnjuk, O., Leader, J.P., Weinstein, A.M., Spring, K.R. 1998. Water does not flow across the tight junctions of MDCK cell epithelium. *Proc. Nat. Acad. Sci. USA* **95:**6526–6530
18. Loo, D.D.F., Wright, E.M., Zeuthen, T. 2002. Water pumps. *J. Physiol.* **542:**53–60
19. Loo, D.D.F., Zeuthen, T., Chandy, G., Wright, E.M. 1996. Cotransport of Water By the Na^+/Glucose Cotransporter. *Proc. Natl. Acad. Sci. USA* **93:**13367–13370
20. Ma, T.H., Song, Y.L., Gillespie, A., Carlson, E.J., Epstein, C.J., Verkman, A.S. 1999. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J. Biol. Chem.* **274:**20071–20074
21. Madara, J.L., Pappenheimer, J.R. 1987. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membrane Biol.* **100:**149–164

22. Murakami, M., Murdiastuti, K., Hosoi, X., Hill, A.E. 2006. AQP and control of fluid transport in a salivary gland. *J. Membr. Biol.* **210:**91–103
23. Murakami, M., Shachar-Hill, B., Hill, A.E., Steward, M. 2001. The paracellular component of water flow in the rat submandibular gland. *J.Physiol.* **537:**899–906
24. Pappenheimer, J.R. 1993. On the coupling of membrane digestion with intestinal absorption of sugars and amino-acids. *Am. J. Physiol.* **265:**G409–G417
25. Pappenheimer, J.R., Reiss, K.Z. 1987. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membrane Biol.* **100:**123–136
26. Sanchez, J.M., Li, Y., Rubashkin, A., Iserovich, P., Wen, Q., Ruberti, J.W., Smith, R.W., Rittenband, D., Kuang, K., Diecke, F.P.J., Fischbarg, J. 2002. Evidence for a central role for electro-osmosis in fluid transport by corneal endothelium. *J. Membrane Biol.* **187:**37–50
27. Schnermann, J., Chou, C.L., Ma, T.H., Traynor, T., Knepper, M.A., Verkman, A.S. 1998. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc. Natl. Acad. Sci. USA* **95:**9660–9664
28. Segel, L.A. 1970. Standing-gradient flows driven by active solute transport. *J. Theoret. Biol.* **29:**233–250
29. Shachar-Hill, B., Hill, A.E. 1993. Convective fluid-flow through the paracellular system of *Necturus* gallbladder epithelium as revealed by dextran probes. *J. Physiol.* **468:**463–486
30. Shachar-Hill, B., Hill, A.E. 2002. Paracellular fluid transport by epithelia. *Int. Rev. Cytol.* **215:**319–350
31. Silva, P., Stoff, J., Field, M., Fine, L., Forrest, J.N., Epstein, F.H. 1977. Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. *Am. J. Physiol.* **233:**F298–F306
32. Song, Y.L., Verkman, A.S. 2001. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J. Biol. Chem.* **276:**41288–41292
33. Vallon, V., Verkman, A.S., Schnermann, J. 2000. Luminal hypotonicity in proximal tubules of aquaporin-1- knockout mice. *Am. J. Physiol.* **278:**F1030–F1033
34. Wedner, H.J., Diamond, J.M. 1969. Contributions of unstirred-layer effects to apparent electrokinetic phenomena in the gallbladder. *J. Membrane Biol.* **1:**92–108
35. Zeuthen, T. 2002. General models for water transport across leaky epithelia. *Int. Rev. Cytol.* **215:**285–317
36. Zeuthen, T., Stein, W.D. 1994. Cotransport of salt and water in membrane proteins — Membrane proteins as osmotic engines. *J. Membrane Biol.* **137:**179–195