J. Membrane Biol. 197, 1–32 (2004)
DOI: 10.1007/s00232-003-0639-6

The Journal of
Membrane
Biology

Topical Review

What Are Aquaporins For?

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Received: 10 July 2003/Revised: 22 October 2003

Abstract. The prime function of aquaporins (AQPs) is generally believed to be that of increasing water flow rates across membranes by raising their osmotic or hydraulic permeability. In addition, this applies to other small solutes of physiological importance. Notable applications of this 'simple permeability hypothesis' (SPH) have been epithelial fluid transport in animals, water exchanges associated with transpiration, growth and stress in plants, and osmoregulation in microbes. We first analyze the need for such increased permeabilities and conclude that in a range of situations at the cellular, subcellular and tissue levels the SPH cannot satisfactorily account for the presence of AQPs. The analysis includes an examination of the effects of the genetic elimination or reduction of AQPs (knockouts, antisense transgenics and null mutants). These either have no effect, or a partial effect that is difficult to explain, and we argue that they do not support the hypothesis beyond showing that AQPs are involved in the process under examination. We assume that since AQPs are ubiquitous, they must have an important function and suggest that this is the detection of osmotic and turgor pressure gradients. A mechanistic model is proposed—in terms of monomer structure and changes in the tetrameric configuration of AQPs in the membrane—for how AQPs might function as sensors. Sensors then signal within the cell to control diverse processes, probably as part of feedback loops. Finally, we examine how AQPs as sensors may serve animal, plant and microbial cells and show that this sensor hypothesis can provide an explanation of many basic processes in which AQPs are already implicated. Aquaporins are molecules in search of a function; osmotic and turgor sensors are functions in search of a molecule.

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Key words: Aquaporins — Epithelial transport — Water channels — Volume regulation — Osmotic pressure — Osmosensors — Turgor pressure

Introduction

Aquaporins (AQPs) are widespread in many membranes. They are a subset of the class of major integral proteins (MIPS), which allow water or very small solutes to pass [1, 179]. The pore is structurally created between six membrane-spanning helices. AQPs occur as homotetramers by association in the membrane and each monomer acts as a separate water channel [201]. The permeability to water of the monomers does not appear to be changed by association into a tetramer [122] and the tetramer does not appear to be entirely symmetrical, presenting a somewhat different appearance at the two membrane interfaces [201]. The tetramer packing can be affected by changes in lipid composition and magnesium concentration [140].

When present, AQPs usually make a substantial contribution to the water permeability of the membrane, often accounting for up to 90% of it as in the red cell. A feature of AQPs is that their permeability to other solutes seems to be largely on the basis of size, which suggests that their function as channels is unspecific but related to the universal solvent water-hence their name. There are a few aquaporins found in some plant, fungal or animal cells, which are permeable to glycerol or small hydroxyalkanes [41] such as Fps1p in yeast and AQP3, and even, under some circumstances, ion channels (AQP6 [191] and AQP1 [13]). We discuss here the MIPS: AQPs and aquaglyceroporins.

There is also the phenomenon of water pores that may be regions of membrane molecules, which have other functions, such as the glucose transporter [197],

but these are also not considered here for the reason that their water permeability may just be epiphenomenal and physiologically unimportant; it may well be that many membrane proteins possess regions functioning as water channels, just as many large molecules posses structural domains that can be permeated by water [129]. Strictly, they are aquapores but not aquaporins.

As frequent reference will be made in this paper to permeabilities of membranes to water, we set out here the definitions of these parameters to avoid confusion. The osmotic permeability P_{os} and the hydraulic conductance L_p are related to the volume flow J_v by

$$J_v = L_p \Delta P + P_{os} \Delta \pi \quad (1)$$

where ΔP and $\Delta \pi$ are the pressure and osmotic pressure. An additional relation is

$$P_{os} = \sigma L_p \quad (2)$$

where σ is the osmotic reflexion coefficient. The value of σ for different solutes in an AQP is an interesting topic [33, 59], but is not dealt with directly in this paper. In plant studies the permeability is often expressed in units of cm/s · Pa and designated by L_p, whether it has been measured by applying ΔP, e.g., with a pressure probe or $\Delta \pi$ (assuming $\sigma = 1$). Both usages are conventions and both symbols are used below according to context. It is a widespread practice nowadays in animal physiology to express the osmotic permeability in units of cm/s and call it P_f, essentially converting it to a diffusive permeability; we have occasionally retained this use when it is unambiguous, for convention's sake.

Although the necessity for AQPs has been questioned [175], the present consensus on the role of AQPs is that their function, in a variety of situations in animal, plant and other eukaryotic cells, in prokaryotes and in organelles, is to increase the osmotic permeabilities of membranes above that of the bilayer, which without them would not be able to sustain net water movement at a rate suitable for fulfilling certain cellular or transcellular functions. We refer to this assumption throughout as the 'simple permeability hypothesis' or SPH. There are several lines of research that have led to the SPH. One of the earliest is that water transport is pore-controlled, based on transport in the red cell [133, 150–152, 174] although the function of pore-mediated water flow in this system is unclear; another is the long-standing research on vasopressin-modulated water antidiuresis [24, 35, 91] where a clear function exists for water permeability modulation by channels; another is water transport in epithelia, where the proximal tubule received the earliest attention [134, 142] and where there already existed a significant argument about the magnitude of P_{os} required to achieve isotonic flow (*see* below). The SPH is heavily skewed in the direction of explaining epithelial water transport, particularly that of 'isotonic fluid transfer', which still remains a perplexing and complex problem, though central to animal physiology. Subsequent to the discovery of AQPs in animal systems, they began to be found in relative profusion in plants, but here the trend was reversed: functions had to be found for AQPs where none were suspected beforehand. Besides epithelial water transport, other functions that have been suggested are controlling the rate of cell volume changes during osmoregulation or cell expansion growth in plants; the facilitation of water movement across tissues such as blood vessels or plant roots; and the possible role of AQPs as conduits for small important solutes other than water.

There can be no reasonable doubt that AQPs increase the water permeability of membranes and, indeed, they do account for the major fraction of the membrane water permeability in many cases. However, there are a number of reasons to question the basic assumption of the SPH: (i) inhibition or removal of AQPs by genetic deletion often has no apparent effect or has an effect that is not in simple conformity with the SPH; (ii) in many cellular systems simple calculations show that there is no need for the enhanced water permeability that the SPH assumes is necessary for growth or osmotic adjustment; (iii) in epithelial systems it is doubtful whether most of the water is in fact crossing the cell membranes where the AQPs reside, when the SPH would claim that the very presence of these molecules indicates that they must be mediating water flow across cells; (iv) small neutral molecules like carbon dioxide (CO_2) and ammonia (NH_3) have a major parallel permeation pathway through the lipid, making the contribution of AQPs superfluous to small molecule transport. All these points form the substance of the second section of this paper.

If AQPs are not present to provide essential water conduits for flow into or across cells, they must have a different role that is both important and widespread. In the third section we present an alternative hypothesis for the function of most AQPs: that they are transmembrane sensors of differences in osmotic or turgor pressure. In support of this hypothesis we propose a mechanism by which differences of $\Delta \pi$ or ΔP across the bilayer can be reflected in a structural change in the AQP and thus signalled to other systems within the cell which use this information. Such information could then be used as part of feedback or control systems in animal and plant cells and we suggest that if AQPs are not acting in this way then there is a need for another molecule to do so in various cell systems; the principles are very general. Such functions of sensors would include signalling to the volume regulation system in animal cells or the turgor regulatory system in cells with cell walls; controlling the isotonicity of epithelial fluid transport; partici-

pating in feedback loops that regulate the size of organelles within cells; regulating extension growth in cells with walls; perceiving turgor loss in plants; and controlling changes in turgor in stomata and motor cells involved in plant movements. The possible participations of AQPs acting as sensors in these different areas are described below as applications.

As this review is quite comprehensive and covers a lot of ground, we present here an outline to guide the reader to sections of specific interest.

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Red Cell Function

Regulation of Whole-Cell Volume

Bacteria and Fungi

Cytoplasmic Volume Buffering in Plant Cells

To Act as Water Conductances in Growth Control

To Act as Water Conductances in Transepithelial Isotonic Transport

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Appendix A

Permeation of Volatiles

Permeability of Lipid Bilayers

Permeability of Gastric Gland

Appendix B

Osmosensor Model

Appendix C

Turgor Sensor Model

What AQPs Are Probably Not For

In this section we examine a number of situations in which SPH is unable to explain the presence of AQPs satisfactorily.

To Act as Water Conductances in Cellular Homeostasis

Red Cell Function

AQP1 was the first membrane protein to be identified as an AQP [133, 174] and it is the major AQP in mammalian red blood cells. AQP1 enhances the P_{os} of red cell membrane by about tenfold and its dis-

covery provided a molecular basis in this cell for water flow through a pore, which had been postulated for decades [150, 151]. The SPH requires that the role of AQP1 is to facilitate water movement across the cell membrane, but we are not aware of an explanation of how this might facilitate red cell function. The major stress the mammalian red cell is subject to under normal conditions is the osmotic gradient in the renal medulla during antidiuresis, which is largely constituted by an enhanced urea concentration. When the cell is subject to shrinkage the haemoglobin is concentrated and the shrunken dehydrated cell is subject to blockage in the vasa recta, depending on the extent of the shrinkage. To minimize this, the cell has a urea transporter that acts to equalize the urea concentrations across the membrane and abolish the osmotic gradient.

The presence of AQP1 in the membrane serves to make this potential problem worse by augmenting the osmotic flows across the membrane that shrink the red cell. Whilst it is true that the basal lipid P_{os} of the red cell membrane ($\sim 10^{-3}$ cm/s) is not low enough to prevent appreciable shrinkage during the time the red cell spends in the kidney medulla, there is no apparent advantage in increasing it. AQP1 is obviously serving some function in this membrane that remains to be elucidated, but it does not fall within the ambit of the SPH.

Regulation of Whole-Cell Volume

Animal, plant, fungal and bacterial cells need to take up water at some point in their lives to remain hydrated or to regulate their volume. It has been argued that AQPs are needed for this, but it is important to remember that lipid membranes are very permeable to water; their water permeability of 10^{-4} to 10^{-3} cm/s should be compared to that for most small solutes ($\sim 10^{-8}$) or ions ($\sim 10^{-11}$ to 10^{-12}). In terms of the SPH it has yet to be shown that their basal lipid water permeabilities are too small to allow the required water fluxes within their physiological or ecological niche.

Under extreme conditions many cells do exhibit active control of their volume, responding to hypo- or hyper-tonic challenges by unloading or loading ions or other solutes to return towards their original volume by osmosis. These are generally known as regulatory volume decrease or increase, RVD or RVI [64, 65, 159]. Like the presence of AQPs, these responses are widely distributed and are considered by many to be near-universal and essential to cell homeostasis. Animal cells are susceptible to both swelling and shrinkage, and although cells with walls are protected from swelling, they may be damaged by excessive shrinkage, e.g., breaking of the symplastic connections within plant tissues.

The enhanced water permeability conferred by AQPs actually works *against* the control of cell volume, amplifying the perturbations produced by changes in osmotic gradients and seemingly making homeostasis more difficult and volume changes more drastic within a given time span. It can be argued that solute transports drive the necessary water shifts for restoring cell volume and that the speed of these water fluxes is increased by AQPs but, as with the case of transpiration across plant cells discussed below, the problem is exacerbated by AQPs to begin with. This aspect of AQP physiology is very puzzling and constitutes a serious problem that argues for another role for AQPs in cell function.

Bacteria and Fungi

Fungi and bacteria possess MIPs. The glycerol transporters (represented by the FSP1 genes of yeast and the GlpF genes of bacteria) are effective glycerol channels but do not add significantly to membrane water permeability [30, 108]. There is evidence that the function of these is to facilitate the movement of glycerol into and out of cells and they are therefore considered in the section on small-molecule permeation. This section deals with the postulate that the function of microbial AQPs, of which AQPZ of *E. coli* and the AQY genes of *Saccharomyces cerevisiae* are the archetypes, is to facilitate transmembrane water flow during cell osmotic homeostasis.

AQPZ is the sole AQP in *E. coli* and it has been shown to have water-channel activity [18]. A role for AQPZ in water uptake during growth was proposed because deletion of AQPZ in *E. coli* caused reduced growth rates under some conditions [19]. The question of an SPH role for water uptake in cellular growth is quantitatively addressed below, where it is shown to be improbable because lipid membranes are sufficiently permeable to support growth without water channels. In the case of AQPZ this is especially so given the high surface area to volume ratio (a point that has been recognized as making a role for AQPZ in water uptake in growth unlikely [17]). Apart from the theoretical case, the observation that growth of the knockout is markedly reduced relative to wild type at low osmolarity, but is normal at high osmolarity, is not what one would expect for an SPH role in water uptake since the osmotic gradient to maintain turgor and therefore to allow expansion is likely to be maximal at low osmolarity. The observation of substantially impaired growth at 39 °C relative to growth at 25 °C (whereas wild-type cells grow significantly faster at 39 °C than at 25 °C) also suggests that another explanation is needed for the growth impairment under these conditions. However, recent work has shown no effect on growth of AQPZ

knockout in *E. coli* under any conditions and calls into question these former results [157].

In a subsequent study of AQPZ, the osmotic shrinkage and swelling of the *E. coli* cytoplasm following osmotic shock was found to be less rapid in AQPZ− than AQPZ+ cells, with AQPZ– cells taking about a minute longer to complete their volume changes [36]. This was interpreted as demonstrating a role for AQPZ in osmotic adjustment. While these observations do indeed indicate that AQPZ conducts osmotically-driven water fluxes in vivo, this does not seem to reach the level of demonstrating a function, since there is no obvious benefit for bacterial cells to distort more rapidly in response to osmotic shock.

Interestingly, most other bacterial species (including species capable of rapid growth) do not appear to have recognizable AQP genes—web-based annotation and homology searching [167] reveals that only a dozen of the more than one hundred completed bacterial genomes contain putative paralogues. About a dozen more bacterial species for which fully sequenced genomes are not available have also been found to have putative AQP genes [67]. Thus it appears that most bacteria may lack AQPs, an observation that is in contrast to glycerol permeases for which about three quarters of the sequenced bacterial genomes have genes with recognizably similar sequences to GlpF [167].

Saccharomyces cerevisiae has two AQPs (AQY1 and AQY2) that conduct water [14, 80]. Domestication of *S. cerevisiae* is associated with rapid reduction of AQP expression [86] and most laboratory strains kept in culture for many generations do not express either AQY gene, having mutations that eliminate the expression of functional AQP proteins [88]. The growth rates of domesticated *S. cerevisiae*, of long-standing laboratory strains and of AQP null mutants are the same as those of AQP-expressing yeast cells, which shows that that the basal water permeability of yeast membranes is sufficient for cellular homeostatic water movement during growth—even when growth is very rapid on rich media.

Experiments in which cells were switched rapidly between media of high and low osmolarity [14] show that the presence of AQP is not beneficial in surviving osmotic shock. Rapid volume changes induced under these conditions might be expected to be harmful, and, indeed, both *S. cerevisiae* [20] and *Candida albicans* [21] cells lacking AQP survived rapid changes between high- and low-osmolarity solutions much better than wild type. These results are inconsistent with a general role for the SPH in which AQPs might assist the homeostasis of cells during growth or osmotic adjustment.

A study of *S. cerevisiae* has shown that AQP deletion reduces freezing-tolerance and AQP overexpression increases it [164]. Evidence is presented that the causal difference between freezing-tolerant strains and freezing-sensitive ones is a higher expression of AQP and these findings have led to the suggestion that yeast AQPs function to reduce damage by the formation of intracellular ice crystals by facilitating osmotically-driven water efflux during freezing.

Intracellular water potential in *S. cerevisiae* has been shown to equilibrate with the extracellular potential within a few milliseconds after even extremely rapid osmotic shock [49]. This is because the water conductivity of the *S. cerevisiae* cell membrane is high and its cells are small, so that water fluxes driven by changes in the osmolarity of the surrounding medium are extremely rapid. It is therefore not likely that the enhanced freezing-resistance of strains overexpressing AQP is due to accelerating still further this high equilibration rate. Likewise, given that freezing-sensitive cells expressed AQP at significant (apparently several-fold lower) levels compared to freezing-tolerant ones [164], freezing-sensitive cells may be reasonably assumed to have had no more than a several-fold decrease in permeability, making it unlikely that the observation of dramatic differences in survival of freeze-thawing between freezing-tolerant and freezing-sensitive strains was due to changes in water permeability. Further doubt is raised about this interpretation by a recent study which indicates that freezing death of *S. cerevisiae* at natural meteorological rates probably occurs by a different mechanism than at the very rapid rates used [39].

Other yeast species have been shown to have AQPs [20, 164] and although almost nothing seems to have been reported on other fungal aquaporins, database searches reveal putative AQPs in the genomes of other species: *Magnoportha grizeae* appears to have three as does *Fusarium graminearum*, while *Aspergillus nidulans* appears to have two and *Neurospora crassa*, one. Based on the occurrence of sequences in databases, at least some of these are expressed. While the data on yeasts do not in our view provide good evidence for an SPH role in water movement, it is interesting that by contrast with bacteria, the fungi whose genomes are available all appear to have AQPs.

Cytoplasmic Volume Buffering in Plant Cells

It has been proposed [104, 171] that aquaporins may play an important role in moderating the effects of water stress in plant cells. When roots are subject to osmotic changes in the substrate the cells change their volume (V) and tonicity (π), ending up at a new equilibrium or steady state determined by the demands of the transpiration column higher up. Sudden changes in transpiration, which are felt down the plant as change in pressure in the vascular system, may have a similar effect. Such changes may cause

swings in volume, tonicity or pressure, which damage the cells. It is not clear what effect sudden short-lived changes in volume or tonicity would have, but disruption of the cytoskeleton or enzymatic integrity are possibilities. According to the SPH, AQPs are envisaged as relieving these potentially harmful transitions by allowing the vacuole to act as a water reservoir for buffering the cytoplasm. It is only when the P_{os} of the tonoplast is substantially higher than that of the plasmalemma that this buffering can occur and the literature indicates that tonoplast permeabilities are indeed higher than plasmalemma ones [105, 109, 125]. To examine this idea it is convenient to consider two cases: (i) the effects of external osmolarity changes; and (ii) the effect on cells outside the vascular system of changes in transpiration.

The results of modelling an osmotically coupled cytoplasm and vacuole have been presented [171] where changes in cytosolic volume V, pressure P and osmotic pressure π are brought about in response to step-changes in the external tonicity π_{ext}. When the P_{os} of the tonoplast is increased by 100-fold the changes to the cytoplasm volume and tonicity are smoothed out. However, an identical effect can be produced by smoothing the onset of the external changes (*see* Fig. 1*A*). The changes in substrate tonicity can never be rapid, limited as they are by the diffusion through the external medium. When the onset of the external change is represented by an exponential rise, for example, with a half-time of 100 s, the overshoot is absent. For changes in the substrate composition external to a plant this is still a very fast change but more realistic than a step-function, showing that a high P_{os} created by AQPs in the tonoplast is not really needed.

Increases in transpiration have their effect on the rest of the plant by lowering the hydrostatic pressure in the xylem vessels, a change that is transmitted through the apoplast. This can be modelled by a sudden lowering of P outside the cell wall. The effect is similar to raising π_{ext} and again, the overshoot can be removed by making the onset of the external pressure-drop exponential rather than instantaneous (Fig. 1*B*). In nature, pressure changes are not instantaneously established inside the plant apoplast, and rarely with a half-time of 100 s, as shown in Fig. 1*B*. We suggest that the effectiveness of aquaporins in vacuoles, as compared to their relative ineffectiveness in the plasmalemma, indicates that they are present to serve other functions in this organelle.

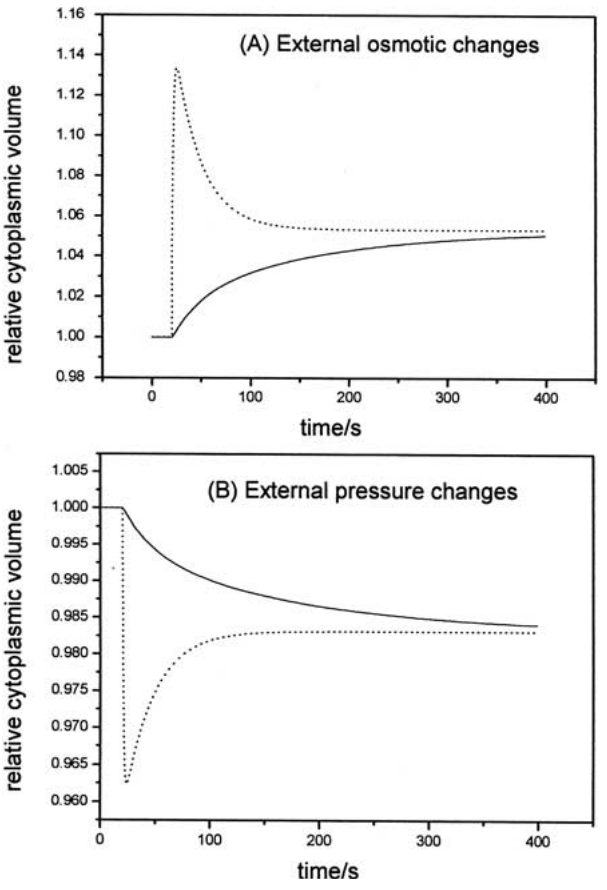

Fig. 1. Perturbations of the cytoplasmic volume after 20 s caused by external challenges to a plant cell. (*A*) Hypo-osmotic challenge from 125 to 0 mOsm. (*B*) External pressure drop from 0 to -10^5 Pa. Step challenges (*dotted line*); exponential challenges (*solid line*) have a time constant of 150 s^{-1}. The model system comprises a cytoplasm (5%) and vacuole (95%) of cell volume with a cell wall of volume modulus 4 MPa, hydraulic conductivity of the plasmalemma and tonoplast 10^{-13} m/s · Pa, hydrostatic pressure 0.8 MPa.

To Act as Water Conductances in Growth Control

When plant cells are expanding they take up water to increase their volume. There has been considerable discussion as to what 'drives' growth. However, there is a consensus that turgor is the driving force but is kept relatively constant and that the rate of expansion growth is determined by cell-wall loosening [87, 111].

Chara and related algal species have long served as informative models for measuring and understanding membrane transport processes in plants [165], including water movement and turgor regulation. Work in recent years [54, 183] has shown that characean membranes display characteristic AQP water-channel properties that include low activation energy for water conductivity and sensitivity to inhibition by mercury. The *Characae* consist of giant vacuolated cylindrical cells linked end-to-end and do not have complex differentiated tissues. AQPs do not, therefore, have a role in the movement of water *across* characean tissues, since there is none in these plants. Many of the *Characae* live in fresh water that is always very hypotonic to their intracellular compartments and are unable to tolerate more than slight increases in osmolarity or ionic strength. Osmotic

adjustment is not, therefore, a function in which AQPs might play a role.

Growth is far too slow to be limited by water permeability in these huge turgid cells. The time-constant of equilibration for a cylindrical cell of radius r is $2P_{os}/r$ per unit length. This is for an internally well-stirred cell—and giant algal cells do exhibit cyclosis. Taking r to be a millimeter and P_{os} for a lipid membrane to be about 10^{-3} cm/s, this comes out to be 0.02 s^{-1}, which corresponds to a half-time for equilibration of 35 s. The half-times for cell expansion are so much longer than this value that growth would not be limited by osmotic water fluxes in the absence of AQPs. A similar calculation applies with greater force to other plant cells that are much smaller than giant algal cells and would therefore have far shorter half-times for water equilibration.

Recent experimental work on giant algal cells, in which pressure P, extension growth and hydraulic conductance L_p were measured in the same cells of *Chara* [136] and *Nitellopsis* [200], allows more exact quantitative tests of whether AQPs might be useful for growth. In *Nitellopsis*, $L_p = 2.6 \times 10^{-5}$ cm/s · osmolar^{-1} and this means that the osmotic pressure difference required to drive the water flow across the membrane at the rate required to increase the volume is only 1 mOsm. But in fact the trans-plasmalemmal difference is 300–320 mOsm in this cell, depending on the external solution [99]. This is consistent with the measured P of 0.7 MPa, with the water being always close to equilibrium across the cell membrane. Evidently, growth can be limited by the mechanical properties of the wall, but not by L_p.

Hg^{2+} ions and a specific antibody against aquaporins in the plasmalemma inhibit extension growth in *Nitellopsis* internodes together with a proportional fall in L_p, but a fall in P is not detected [200]. Although water entry (through AQPs and across the rest of the cell membrane in parallel) is needed for growth, the observed constancy of P is not consistent with growth being *regulated* by L_p because if this were the case there would be a fall in P when L_p drops, as volume extension would initially exceed water entry. Thus, despite the sound evidence for water-channel activity in this group of plants, SPH functions in growth are not supported by the data available to date. Rather, the available data indicate that AQPs in the plasmalemma may be required for participation in a more complex sequence of events.

To Act as Water Conductances in Transepithelial Isotonic Transport

Cellular and Paracellular Models of Fluid Transfer

We consider first the role of the osmotic permeability P_{os} in isotonic fluid transfer as envisaged by the theory of osmotic coupling, in which water flow is wholly cellular. AQPs are found on both apical and basolateral membranes of epithelia and usually make a major contribution to P_{os} in animal cells. The SPH naturally regards their presence as conferring an enhanced permeability enabling water transfer to approach osmotic equilibration. AQP knockout studies (reviewed below) have given some recent support to this theory although they have raised as many problems as it has been claimed they solve.

The osmotic coupling theory [32, 38] postulates that salt is pumped over the basolateral membranes and the lateral interspace system acts as the principal region of osmotic equilibration. The value of P_{os} is a crucial parameter in determining the osmolarity of the secretion and for this to be near 1.0 (quasi-isotonic) it is doubtful whether P_{os} is high enough [58]. Such conclusions are based upon calculations that take the geometry of the membranes and interspaces into account [58, 81, 143]. Subsequent measurements of P_{os} by different methods were followed by arguments over its true magnitude, including the role of unstirred layers [37, 132] and led to divergent views concerning the real value of P_{os} and the role of osmosis, which still persist [158, 168, 186]. With the advent of AQPs, discussion of the mechanism of fluid production has now moved away from epithelial models. Direct means of measuring P_{os} using membrane vesicles and other methods [177] are now used to show that P_{os} values are dependent on the presence of AQPs and that they change when AQPs are inhibited or removed by knockout.

The SPH completely discounts the organization of the paracellular system and simply assumes that AQPs are there to provide the obvious route for osmotic flow across cells. There is emerging, however, an increasing interest in the role of the paracellular system in fluid flow. In several epithelia that have been studied with paracellular probes during secretion it is now clear that water flow in these systems is largely or entirely paracellular [56, 60, 62, 117, 128, 146, 160], results that have been recently reviewed [147]. In those systems where paracellular probe fluxes have been measured, the essential findings have been: (i) probes undergo net paracellular transport; (ii) this can only be due to convection and the magnitude of this convective flow can be calculated; (iii) when expressed as a fraction of the total transepithelial water flow the value is close to 1.0. If the technique of using junctional probes to measure water flow were more common, then paracellular flow would probably appear to be widespread and may emerge as a key component of epithelial fluid production. Such paracellular fluid flow must be due to an active junctional fluid transfer although we have as yet no concrete knowledge of how this might operate, but the mechanism may be a form of microperistalsis [61].

If volume transport is not mainly cellular this suggests other roles for epithelial AQPs. One possibility is that they are detecting and probably signalling 'osmotic' events at the membrane: this is dealt with in detail later in this paper together with a model of how this can interact with the junctional fluid transfer to bring about isotonic transport. Another is that in the epithelium they are bringing the cell into approximate osmotic equilibration with the two baths by controlling the contribution each membrane (the apical versus the basolateral) makes to the tonicity of the cell. This tonicity is intermediate between that of the two baths and this specialized function is discussed below.

The Effects of Knockouts on Fluid Transfer

One of the main interests in animal AQPs has been in epithelial fluid research. In current osmotic theories of fluid production, isotonic transport is completely dependent upon the density of AQPs in the relevant cell membranes. The development of knockout mice, null (−/−) for an AQP gene, was important in allowing permeabilities and fluid transport to be measured in AQP-deficient systems and compared to the wild type without the use of chemical inhibitors. Observations on such knockouts and mutants have shown a wide range of effects on fluid transfer rates. SPH would predict that the removal of a major pathway of water flux across membranes would have drastic effects on biological function at the cellular, tissue and whole animal level. However, these have been remarkably few, and those effects that have been observed are hard to interpret by the SPH. It is convenient to distinguish different classes of findings from null systems. In what follows, mice will be assumed to be the experimental knockout animal and other species are indicated.

Changes in the Function of the Whole Organ or Organism. Examples of these are the fact that humans lacking AQP1 (the most abundant AQP in people) have grossly normal physiology [2], the inability of AQP1-deficient mice to produce concentrated urine [98], and hearing problems in AQP4-deficient mice [92]. Interesting as these may be, they do not substantially advance our understanding of AQP action in view of the complexity and interaction of homeostatic mechanisms in the animal body. A deficiency in one function created by removal of an AQP may be compensated elsewhere in the same organ; the process is still poorly understood because of its complexity. However, any theory of AQP action has eventually to explain how the transport of water (passive or active fluid transport) can be little affected by the removal of a specific AQP.

Changes in Measured Water Permeability. As most AQPs have a high water permeability, it is not surprising that changes in P_{os} are generally the immediate effect of knockouts. These results are an important corollary to AQP presence, but they do not point to the primary cellular action of AQPs unless one has other information about the fluid-flow process. As has been observed many times, AQP knockouts show that the diminution of water permeability often has no general effect on water flows, as would be expected. Examples are endothelial AQP1 knockouts, which would be expected to severely diminish the movements of water driven by the Starling force ($P - \pi$) but do not [178], water exchange in the airways [153] and in the lung [15].

Negligible Changes in Fluid Transport Rates. There are many negative results from experiments on the deletion of AQPs, which, according to the SPH, are expected to exercise key control of active fluid transport. In some of these cases the value of P_{os} is reduced, as might be predicted, but the fluid transport is not affected. Notable cases are the following examples.

(i) The near-isosmolar fluid transport from the alveolus, where AQP5 is present on the apical membrane of the Type-1 pneumatocytes in rats and mice [95, 124], is unresponsive to AQP5 knockout, although measurements of the trans-alveolar water permeability show that P_{os} is reduced 10-fold [95]. Furthermore, knockouts of AQP3, AQP4 and AQP5 have little effect on the humidification of upper airways although these AQPs are normally present in the airway epithelium [153]. It has been concluded that although AQP5 dominates the alveolar water permeability and AQPs are widely present, they appear to play a role only in airway submucosal gland function [15] (*see* below).

(ii) The epithelium secreting bile composed of cholangiocytes has AQP1 present on the apical membrane [97, 101] and osmotic water flow is $HgCl_2$-sensitive [31]. However, the epithelium shows a hormone-stimulated fluid transport that is unaffected by AQP1 knockout; the P_{os} of the cell is also apparently unresponsive [113].

(iii) In the lachrymal gland AQP5 is present on the apical membrane, with AQP4 on the basolateral membrane [73], and in addition, AQP3 can be found on the basolateral membrane, with AQP1 in the lachrymal vasculature [114]. Knockouts of AQPs 1, 3, 4 or 5 showed no effect on the rates of tear fluid production after hormonal stimulation [114]. In addition, the tear fluid was virtually unchanged in concentration, as measured by Cl^- concentration.

(iv) In the pleural cavity AQP1 is present in vasculature and mesothelial cells [156]. Two processes can be distinguished, as in many other tissues. The

first is osmotic equilibration, which occurs when hyper- or hypo-osmotic saline is injected into the cavity and the time course of osmolarity changes in the fluid are followed. AQP1 knockouts showed a fourfold slowing of this process, presumably by lowering P_{os} of the mesothelium [156]: knockouts of AQP1 in endothelial cells of the vasculature have little effect on the rates of fluid equilibration [178].

The second process is an active fluid transport, which can be followed by measuring the rate of clearance of an isotonic volume infused into the cavity. In this case the fluid flow from this space was unaffected by AQP1 deletion [156], indicating that the water permeability seems to play little part in the secretory process.

(v) In the sweat gland epithelium AQP5 is present in the luminal membrane of rats [123] and mice [154]. In mice the rates of fluid production were measured by two methods after hormonal stimulation with pilocarpine: NMR fluid analysis and direct microscopical observation. Neither showed any difference between wild-type and AQP5-kockouts [154]. Comparison of this result with those from other glands where AQP5 is also present in the apical membrane (*see* salivary and airway glands, below) is puzzling and rules out any significant contribution of osmotic flow over the apical membrane to the fluid production.

(vi) AQP4 has been localized on the basolateral membranes of parietal (oxyntic) cells in the gastric glands [45, 85]. In human gastric cell lines transfected with rat AQP4 it appears on the basolateral membranes (as in native parietal cells) and has been shown there to contribute substantially (88%) to P_{os} [22] when on this parietal membrane. However, in AQP4 knockouts the fluid secretion from these cells after stimulation with hormones (gastrin or histamine) occurred at the same rate, having the same secretion composition and pH [182].

Apparent Changes in Rates or Osmolarity of Fluid Transport. The systems that are widely referred to as demonstrating the effectiveness of AQP knockouts in supporting the SPH are (i) the proximal tubule, (ii) the salivary glands, (iii) the upper airway glands, and (iv) epithelial systems in the eye. However, the data are far from easy to understand in terms of the SPH. We offer a description and critical analysis of these interesting results.

(i) In a study of AQP1 knockout on fluid absorption in the mouse proximal tubule [145], a measurement of P_{os} of the tubule wall showed that it fell to 22% of the +/+ control. A previous measurement on vesicles from the tubular apical membrane in these animals showed that AQP1 knockout reduced P_{os} to 11% of the wild type [98]. This difference can be reconciled by assuming that the contribution of AQP1 to P_{os} of the apical and basolateral membranes is not the same. The active fluid absorption rate fell to about 50%. In terms of simple equilibration over the cell this fall in rate is much less than expected if the value of P_{os} falls to between 11–22% and is therefore difficult to explain in terms of the SPH.

In a further study of this system [173], the luminal fluid at the end of the proximal tubule was found to be more hypotonic to plasma in the AQP1 knockout than the wild type, implying less water absorption over the length of the tubule, i.e., end-tubular osmolarity was 97% that of plasma for +/+ mice as against 89% for –/–. Although this change is in the expected direction, it is difficult to give it any precise interpretation unless we know the effects on solute transfer rates as well; these determine the osmolarity change of the absorbate and were not directly measured or calculated in this study. However, using data from these studies for the reduction of the volume absorption rate [145] and the differences between the osmolarities of the early and late tubule in the two strains [173], it is possible to calculate the active solute-uptake rates. If the glomerular filtration rate is J_{SNGFR} and the end-tubule flow rate is J_{end} then the tubular salt uptake rate J_s is given by

$$J_s = J_{SNGFR} C_{plasma} - J_{end} C_{end} \quad (3)$$

where C_{plasma} and C_{end} are the plasma and end-tubule osmolarities. Using values from these two knockout studies J_s (+/+) comes out at 1.539 nOsm/min and J_s (–/–) at 0.949 nOsm/min, a reduction of salt absorption in AQP1 knockouts to 62% of the control. In these studies, therefore, the fluid transfer rates were partially reduced (compared to expected values from the SPH) and there was a reduction of salt transport that remains unexplained and that complicates any simple interpretation of the AQP1 knockout. Since, by the SPH, halving the salt-reabsorption rate would by itself halve the water-reabsorption rate, the reduction in P_{os} would thus appear not to be having much effect.

(ii) AQP5 is the AQP present on the apical membrane of salivary acinar cells [46, 124]. The permeability of the membranes cannot easily be measured in glandular acini. In a study of general saliva production in the buccal cavity comprising the output of submandibular, sublingual and parotid glands in AQP5 knockout mice [96] the knockouts showed a fall of secretion rate over five minutes to 41% of the wild type with a rise of osmolarity of the secretion to 143%. Thus total salt transport has actually fallen to 59% of the wild type. Although this reduction in total salt transport may be due to increased ductal absorption of salt it is unlikely to account for much: the osmolarity of wild-type secretion is close to that of mouse plasma, indicating little absorption. Again, the effect of AQP

knockout is partial with a concomitant fall in solute transport.

(iii) AQP5 is also the apical membrane AQP of upper airway gland cells, which produce the airway surface liquid, with AQP4 on the basolateral membrane. A knockout study was made of various parameters of the fluid with AQP5 and AQP4 null mice [155]. Depending on the assay method for fluid volume detection, the secretion rate was reduced to 50% (±5%) with AQP5-nulls and hardly at all with AQP4 nulls. In the AQP5-null animals the Cl^- ion concentration was raised by 10% and we may take this to be an indication of the raised osmolarity if Cl^- is the main anion. In this case the salt flow after AQP5 knockout has fallen to 55% of the wild type. This is very similar to the two effects described above in proximal tubule and salivary acinar cells with only a partial reduction in volume flow and a substantial reduction in salt flow, as shown in Fig. 2.

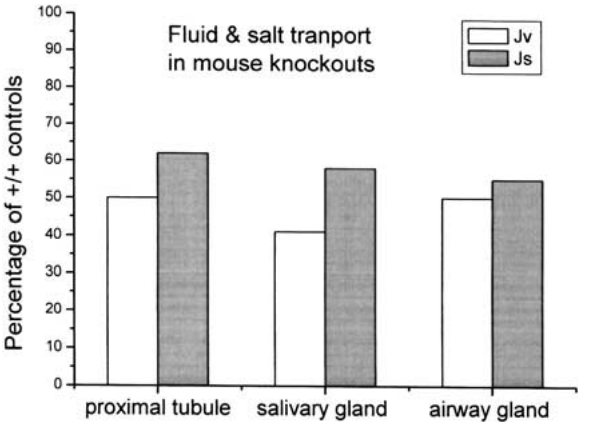

Fig. 2. The effects of AQP knockouts on three fluid transport systems (*clear bars*, fluid transfer rates J_v; *dark bars*, salt transfer rates J_s). Data for calculations (*see* text) are taken from the following sources: proximal tubule [145, 173], salivary glands [96] and airway glands [155].

(iv) AQP knockouts in the mouse eye were used to investigate fluid transport in the non-pigmented retinal epithelium (ciliary body NPE), which feeds the aqueous humour and the corneal system of outer epithelium and inner endothelium that controls hydration of the corneal stroma. In the NPE, where AQP1 and AQP4 are both present in the epithelium, studies with cultured epithelium in vitro had already shown partial inhibition of fluid transfer after treatment with $HgCl_2$ and AQP1 antisense oligonucleotides [130]. The effects of AQP1 or AQP4 knockouts on aqueous humour production were small, apparently reducing it to 75% of the wild-type rate as judged by changes in the time constant of dye clearance in the aqueous humour whose volume is fed from this epithelium [198].

In the corneal system, osmotically induced water flow across the corneal epithelium (AQP5) or corneal endothelium (AQP1) was followed by applying a hypotonic solution to one or the other and recording the rate of stromal volume increase [166]. In AQP5 nulls, hypotonic challenge to the epithelium reduced the corneal swelling to 52% of the control value and this must represent a combined water flow over both epithelium and endothelium in series. In AQP1 nulls, hypotonic perfusion of the endothelium was accompanied by simultaneous application of an oil layer to the outer surface to block epithelial water exchange; the rate fell to 21% of controls and this provides an estimate of the change in endothelial P_{os}.

Active fluid transport was measured across the endothelium by measuring the rate of stromal shrinkage after it was first swollen by application of hypotonic solution to the outer epithelium and then arresting further swelling by sealing the epithelial surface with an oil layer. The effects of AQP1 knockout reduced corneal shrinkage rates to 25% of the wild type [166]. This subsequent shrinkage must be due to two parallel processes: (a) the passive withdrawal of water to the aqueous humour dependent upon P_{os} of the corneal endothelium and (b) the active fluid transport in operation there [44]. Bearing in mind that the knockout reduced the endothelial P_{os} to 21% of the control value, it is unclear how much of the change can be attributed to changes in the active fluid transport, if any.

Thus in the mouse eye, apart from the general effect of AQP knockouts on P_{os}, the effect on active fluid transport appears to be the following: in the ciliary body (NPE) the effect is small (25% reduction), in the corneal endothelium it is dubious, and in the lachrymal gland there is none.

Conclusion. What is to be said about the effects of AQP knockouts? The attempt to establish the SPH by the use of AQP nulls represents an extensive, important and detailed body of work intended to put the theory of osmotic fluid production on a firm molecular basis. This would have settled the uncertainties and controversies of the past and also extended the role of AQPs to structures that served in many situations to alleviate the restriction placed by lipid membranes on rates of water permeation. The work has provided valuable data on transport in a wide range of cellular systems, which require interpretation by any theory claiming to describe epithelial fluid transfer. It is clear, however, that it has not been successful in confirming the SPH by proving that quasi-isotonic transfer by osmotic equilibration is correct. When knockouts have had a demonstrable effect on measured fluid-transfer rates, the results have been described as showing that "high water permeability is needed for efficient near-isosmolar fluid transport" and this may be true in these cases, but they have not shown that this occurs in the

framework of the SPH. Where fluid flow has been significantly reduced it has only been in a minority of systems; the flow reduction has only been partial where it should have been substantial; and in addition, there has been an inexplicable concomitant reduction in salt flow (where this can be deduced), which, according to the SPH and local osmotic theory, would itself be enough to explain most of the reduction in the volume flows.

To Facilitate Water Movement Through Plant Tissues

There is a remarkably large number of MIP genes in plants; 35 have been identified in *Arabidopsis* [137] and maize probably has even more [26]. Two groups of genes account for the majority of plant MIPs, including those that are most abundantly expressed. Both contain MIPs that are considered to be AQPs and the two groups are defined by the membrane location, gene structure and sequence similarities among their members. TIPs (tonoplast intrinsic proteins) are found in the tonoplast and PIPs (plasma membrane intrinsic proteins) in the plasmalemma, although this separation of location is not universal [6, 82]. The NIPS (Nod26-like intrinsic proteins) are a third group of plant MIPs similar in sequence, and probably in conductance, to Nod26—a MIP found in the peribacteroid membrane of root nodules, which shows low water conductivity and is permeable to some small solutes [141]. SIPs (small intrinsic proteins) comprise another small group of plant MIPs that are apparently not expressed much and about which little is known. The TIPs show a range of water conductivities from high to moderate, both when expressed in oocytes and when examined in tonoplast membrane vesicles, and the PIPs have moderate to minimal water conductivities (tabulated in [105]). Adding to the diversity of characteristics displayed by plant MIPs is their distribution, with some being found in many tissues, while others are found in one tissue or in one to several subsets of cells within plant tissues, and individual plant cells expressing more than one MIP.

Plant AQPs are also variable from an experimental perspective, differing widely in their sensitivity to inhibition by mercury. This makes interpreting with confidence the effects of mercury addition difficult, a difficulty that is greatly compounded in plants, since Hg^{2+} penetrates plant tissues very imperfectly [8] and by the fact that it has effects on water transport that are apparently not due to the inhibition of AQPs [199]. Another interesting pair of phenomena relating to the water conductivity of plant AQPs further complicates experiments and their interpretation. Firstly, several studies have found that plant AQPs can be phosphorylated with accompanying changes in water conductivity [78, 106]. Secondly, there is evidence that the measured water conductivity of plant AQPs may depend on the driving forces used to measure them [125, 172]. The picture is further complicated by the fact that hormones are involved in the expression of MIP genes and these may control expression levels, and thus changes in L_p, during an extended experimental period. Changes in abscisic acid (ABA) during water stress is a case in point.

This wide range of experimental and biological phenomena has stimulated much interest in plant MIPs and has been catalogued and discussed in a series of reviews in recent years [28, 77, 104, 105, 171, 172]. The large range of findings has led to more speculation about additional or alternative functions than is current in animal AQP studies. In this section our main aim is to examine the generally held view that AQPs are important to plant water relations because they facilitate water movement *through* plants and their tissues through an SPH mechanism, that is, by increasing the water permeability of membranes. According to the SPH the basal water conductivity of membranes is too low to support water movement through plant tissues and the effect of AQPs in increasing membrane permeability is their function.

Many of the observations that have been made to date do not fit with a single SPH function or set of functions for AQPs in water movement through plants. One clear example is that some MIPs have been found to be located in cells of tissues through which not much water moves—zones of cell division and expansion in particular have been found to have high AQP expression levels [105]. Another example is that the water conductivity of some PIPs is very low [25]. A third set of observations concerns the up- or down-regulation of MIP genes in response to changes in the plant environment, particularly those of changes in water stress by dehydration or osmotic challenges. Because changes in one AQP have not always been correlated with a change in L_p at a cell membrane, as they so often have been in animals, the results are usually less clear. The experimental observations that subjecting the plant to different regimes of hydration and light, etc., up- or down-regulates several of the plant aquaporins, though important, do not constitute a proof that they are there to regulate water inflow, when from a biophysical standpoint they sometimes seem to contribute little to the L_p of the plasmalemma.

Such findings are inconsistent with a major role for AQPs in water movement through plant tissues, and they have been interpreted as meaning that those MIPs have other functions. The range of functions postulated for plant MIPs has shifted since the first demonstration of AQPs in plants [107], as new observations are made and some earlier proposed

functions become unlikely. Three of the functions that are still frequently suggested as explaining the presence of so many plant MIPs are dealt with in other sections: cellular growth, buffering of cytosolic volume and permeability to CO_2 and other small solute molecules. If the arguments made in those sections are accepted then this makes functional orphans of a significant proportion of the plant MIPs. Nevertheless, the view that AQPs are important for water movement through plants appears attractive, since there are many MIPs with significant water permeability that contribute to membrane water conductivity in tissues and cells through which water moves.

Unfortunately, the general conceptual model underpinning the relation between transpiration and water conductance across the cells of leaves and roots has not been given a precise form. This makes it difficult to make testable predictions about AQPs from the SPH, but one approach would be to argue that when the plant is subject to an increase in transpiration this would result in an upregulation of L_p in the plasmalemma of the root cells to allow faster rates of water entry and thus minimize the fall in water potential in the cells. Apart from such a fall being (possibly) undesirable for the cell, this would lower the stress response and help keep the stomata open to allow photosynthesis to continue as fast as possible. In the tonoplast the same upregulation would be expected to preserve the cytoplasm by osmotic buffering [105, 171]. In leaves, the reverse should happen: increased transpiration with attendant dehydration would cause a downregulation of L_p to achieve the same result. If MIPs are inserted into plant membranes according to this hypothesis, then it can be tested.

The data obtained so far do not support this particular hypothesis. From the expression properties of MIP homologues in *Arabidopsis* and many other plant species under dehydration [105] it can be seen that regulation goes both ways about equally and irrespective of whether they are TIPs or PIPs; this observation is not new [172]. Furthermore, in those studies where plant material has been subject to desiccation [7] the changes in tonoplast MIPs (TIPs) are much slower than the expected rates of vacuolar buffering.

An alternative approach based on SPH would be to suggest that the role of AQPs in water fluxes through plants is restricted to radial water movement through roots. This is because stomata regulate evaporative losses from leaves and other surface tissues above ground and thus determine the regulation of water movement through the upper parts of the plant. Also there is little evidence that suggests an important role for trans-membrane fluxes in the leaf water movement. Indeed, since plants go to great lengths to minimize water loss it seems odd that AQPs are present to contribute significantly to the plasmalemma water permeability of leaf mesophyll cells- and therefore to evaporation rates. It is clear that the vasculature dominates long-distance water movement along roots, stems and other plant tissues and this does not involve the movement of water across membranes. The specific experimental observations which underlie the belief that AQPs are important in water transport through plant tissues mainly relate to radial movement into roots.

These observations are mostly of three sorts: reports that exposure to mercury inhibits root hydraulic conductance, a study showing alterations in the roots of *Arabidopsis* expressing an antisense sequence to a PIP [79] and the finding that some AQPs have one or more characteristics (distribution, conductance, or changes either in expression or phosphorylation in response to water stress) that are consistent with a role in radial water movement through roots (reviewed in [76]). Data of the last sort go against as often as they favor an SPH role for AQPs in water movement through plant tissues [105]. We shall consider the first two categories of findings in more detail.

Water movement through roots or whole root systems, when measured by recording exudation rates from cut root systems or net water fluxes driven by osmotic or hydrostatic pressure gradients, has been found to be sensitive to the addition of mercury (listed in [105]). This inhibition is often largely reversible with reagents designed to reduce the sulfhydryl groups with which Hg^{2+} reacts. The extent of inhibition can be very substantial, but in some cases it is only modest or so low as to be unimportant. Problems of the interpretation of mercury effects in plants have been alluded to above, and here it may be noted in addition that if mercury inhibits water movement by an SPH mechanism it should preferentially block the trans-cellular route of water movement across roots, thus inhibiting osmotically driven water fluxes much more than hydraulically driven ones. A survey of the literature on this issue offers little support for this prediction, with some of the most dramatic and often-cited observations of inhibition being driven by applied hydrostatic pressures or by transpiration [8, 100, 105].

Two other predictions can be made about the effects of mercury on inhibiting water movement through roots. Firstly, according to an SPH role, if AQPs are a major contributor to water flux, then blocking them should change the composition of root exudation, making it more concentrated in those cases where mercury reduces the exudation rate. This is very much analogous to the situation in animal tissues, and here also the observations do not match the expectation (*see*, for example, the data in [23]).

Secondly, if movement through AQPs is an important part of water flux through tissues, then the extent of mercury-induced changes in cellular permeability should be reflected quantitatively in changes in tissue hydraulic permeability. Several studies report that Hg^{2+} greatly reduces hydraulic conductivity of root cells as well as root hydraulic conductivity but we are aware of only one that places these data in the context of anatomy so as to allow the hypothesis to be tested [8]. In this case the resting cellular permeabilities are too low compared to the root conductivity and the effects of mercury on cellular permeability are too drastic compared with the mercury-inhibited root conductivity to be compatible with a major contribution of AQPs and trans-cellular water flux to radial hydraulic conductivity of the root.

One highly cited study [79] showed that the root system of *Arabidopsis* is much larger in plants expressing an antisense sequence to one of the PIPs. The overall hydraulic conductivity of the root system was reported to be the same as in control plants in spite of the greater total length of roots. This is an interesting finding and is among the most frequently cited in support of an SPH role for AQPs in plant water relations. Similar results [102] showed that plants expressing antisense sequences for PIP1 and PIP2 genes had large reductions in the levels of several PIP proteins in leaves and roots but remarkably normal water relations (transpiration rates, leaf water conductivities, etc.) despite dramatic reductions in protoplast water permeability. When expressed on a per root mass basis, however, water conductance of roots appears lower, this reduction being entirely due to the larger root mass. Differences in behavior under drought and rehydration were found for plants with low PIP levels, with longer recovery times for the transgenic plants. Those observations were taken to indicate a role for PIPs in recovery from drought. The fact that the low-AQP plants underwent more severe dehydration during the drought treatment (perhaps associated with the effects of much larger root masses in pot-grown plants) makes this conclusion uncertain in our view.

Certainly the findings of both these antisense studies is at least as suggestive of a developmental and/or sensory role for these AQPs (*see* discussion of AQPs as sensors below). A study in which water relations and conductance were examined in detail under a range of conditions [75] showed that the transgenic elimination of the expression of another PIP2 that is highly expressed in most cells of *Arabidopsis* roots had no effects on morphology or responses to water stress conditions and only a 14% decrease in the measured value of osmotically driven hydraulic conductivity of roots. Recent experiments on a much larger number of *Arabidopsis* AQPs indicate that plants having very large reductions in the expression of different AQPs fail to show phenotypes that would be expected if those genes have an SPH role in water movement across plant tissues (Bohnert, H.J. *personal communication*).

In summary, plants have many AQPs that are widely believed to be important in the conduction and regulation of water movement through plants. The location, conductivities and regulatory changes of many of these do not seem to us to fit with any coherent SPH scheme for water movement through plant tissues. Observations using mercury and transgenic manipulation to lower AQP activity have both been held to support an SPH role for some AQPs but these also fail to match SPH-based predictions in any consistent manner.

To Act as Conductances for Other Small Species

A proportion of AQPs, especially in plants and microorganisms, are permeable to small solutes as well as water. Glycerol was the first solute whose transport was shown to be AQP-mediated and the term aquaglyceroporins has been used to describe this duality [41, 57]. Other small permeant species include small polyols, urea, ions and volatiles. We shall consider small species' permeation separately from volatiles for the following reasons. Some small species, such as ions, most probably do not cross AQPs via the water channels; for others, such as glycerol and urea, not only is the permeation pathway unclear but it has been written 'the physiological relevance of such transport remains speculative because none of the small molecules involved has clear physiological relevance' [105]. This applies generally to both plant and animal cells (the role of MIPs in bacteria and yeasts has been considered above). To these molecules, cell membranes are poorly permeable or impermeable.

Neutral volatiles such as CO_2 and NH_3 are small enough to pass through the monomer water channels and these molecules by contrast do have great physiological relevance. But the control of their membrane permeability by AQP depends upon the relative permeability of the lipid bilayer operating in parallel. This crucial point is considered in detail, because the permeation of volatiles through AQPs has been regarded, unwarrantably we consider, to be an important aspect of their function.

Small Molecules and Ions

Members of the MIP family such as Fps1p in the membrane of the yeast *Saccharomyces* [41, 66], GlpF of *E. coli* [41] and PfAQP of the parasite *Plasmodium falciparum* [53] seem to serve to transport a restricted range of small solutes, including glycerol and urea. These cells either use glycerol especially as a cellular osmotic effector, releasing it to osmoregulate, or as a

metabolic substrate, exchanging it with the external medium.

The idea that AQPs have an important role in the transport of small solutes in animals and plants is problematic for two reasons. First, the location of those AQPs that conduct small solutes does not appear to correlate with environments or tissues where enhanced permeability to them might have physiological or metabolic significance. In plants these AQPs (TIPs) are found in the tonoplast [48, 105], whilst in animals they comprise several AQPs (AQP3, 7 and 9) mainly confined to epithelia [70–72, 112, 169]. In both these cases the transport of small solutes has little apparent relevance to the functioning of the cells in which they are found, especially when one considers that cells contain a plethora of specific transporters where these are required for specific purposes.

Secondly, very small solutes that can permeate the aqueous core of certain AQPs (such as formamide in NOD26 [141]) can also traverse lipid membranes with great facility in the absence of AQPs, and urea, which can permeate some AQPs, has its own highly effective transporters in animals and bacteria, the urea transporter (UT) family. In general we note that the fact that a probe molecule can traverse a channel does not mean that the channel is serving to transport it in any physiologically relevant situation. For example, it has been postulated, based on permeability to peroxide [55] that AQP(s) in *Chara* and perhaps some other plant AQPs serve as H_2O_2 channels. This is subject to the same considerations: it has been criticized as being based on experiments using unphysiologically high concentrations of peroxide [172] but in addition, peroxide is quickly scavenged inside cells so that any physiological need for a channel-assisted peroxide efflux is difficult to envisage.

It should be noted that although mutations in AQP3 can affect both the water and glycerol permeabilities, there are reports based on its physiology indicating that water and glycerol fluxes do not interact in a pore. In one study, water flows in both directions set up by osmosis did not affect the glycerol fluxes, showing that solvent drag in the pore was insignificant [196] and therefore indicating that water and solute cannot share the main pathway, or that the pathway they do share is insignificant osmotically. In another study, it proved possible to inhibit the water conductance without affecting the glycerol permeability [40], again showing that they do not interact. The measurement of a glycerol reflexion coefficient of 0.15 [196] does not indicate interaction within the aqueous pore of AQP3: the methylurea reflexion coefficient of AQP1 has been shown to be 0.54 although this molecule cannot cross the pore at all; this is due to the failure of Kedem-Katchalsky theory in pores other than straight cylinders [33]. The possibility exists that glycerol may be crossing the AQP3 through the central axis of the tetramer. It is interesting to note that in both AQP3 and AQP6 the solute permeability is gated by low pH between pH 5–6.

A small fraction of AQP1 tetramers show Na^+ permeation after interaction with cGMP [4] and this ion pathway is different from the water channels [144]. It has been suggested on the basis of structural comparisons with K^+ ion channels that the pathway is through the central hole running between the four AQP monomers [193]. There is also transport of chloride ions by the intracellular AQP6 induced below pH 5.5, which might possibly play a role in ion transport in vesicles [191] and the same mechanism of transport through the central tetramer canal has been considered for this permeation [41] rather than through the water channel in the monomers. Some AQPs are apparently permeable to other larger ions; AQP7 and AQP9 to arsenite [94] and AQP6 to nitrate [69]. This raises the distinct likelihood that ion permeation in AQPs is in parallel to the monomer channels along the fourfold symmetry axis. It also leaves uncertain to what extent the central channel may be a permeation pathway for other small solutes.

The most important point concerning these solute-conducting MIPs is that they are all AQPs in function, possessing a tetramer of water channels. Where the solute permeation can be shown to have direct physiological relevance to the cells involved, the osmotic permeability seems to be quite unrelated. We suggest that AQP function and solute permeation are distinct and that the two should be considered separately. In many contexts either the solute permeability of the AQP may be of little consequence or else the molecule has evolved as a solute channel and is no longer important as an AQP. This situation would be clarified, of course, if it could be shown that AQPs are playing a different role from that of a water conduit as envisaged by the SPH.

CO_2 and Other Gases

It has been suggested that the transport of small neutral molecules such as CO_2 and NH_3 may be facilitated by the incorporation of AQPs into the lipid membrane. There are three reasons why this may be so. (i) The molecules are small enough to traverse the central core of the AQP molecule. (ii) The permeability to CO_2 of some membranes seems quite high and might therefore indicate a special mechanism, i.e., permeation via AQP; by comparison, other membranes seem to have a low permeability and this might indicate its absence. In these cases the bilayer would be considered to be of a lower permeability to CO_2 than required for adequate transport rates into or out of the relevant cell and the presence of AQPs would relieve this. (iii)

Mercuric ions and other inhibitors of AQPs apparently affect CO_2 transport.

CO_2 can probably cross AQPs through the aqueous pore, but do AQPs have a significant effect on the membrane CO_2 permeability, P_{CO_2}? To address this question it is essential to determine the magnitude of P_{CO_2} for the lipid bilayer, and this can be estimated, depending as it does upon a few parameters in a very fundamental manner. A calculation gives 4.85×10^1 or 48 cm/s for P_{CO_2} (*see* Appendix *A*). The magnitude of this permeability is such that, if it is a reasonable estimate of cell membrane permeability, it makes measurement of P_{CO_2} in cells and vesicles very difficult due to unstirred-layer (USL) effects; these are hard to destroy when the basic membrane fluxes are high. In addition, it renders the effects of AQPs insignificant when set in parallel to the lipid membrane.

In a classic study using carbonic anhydrase to overcome the limitations of the CO_2-HCO_3 interconversion a value of 0.35 cm/s was obtained for a lecithin:cholesterol bilayer [51], which is close to the calculations above. USLs cannot have been completely absent in this study. The calculated value of the basal lipid CO_2 permeability is therefore not seriously in doubt. We suggest that in the absence of sufficient attention to USLs the measured values for P_{CO_2} in biological membranes obtained so far are underestimates [50, 161, 170, 195].

There is a report of membranes with zero CO_2 permeability, which would seem to indicate that the above theory is inadequate to describe P_{CO_2} in cell membranes [181]. This is based upon the perfusion of isolated gastric glands [180]; it is therefore important to examine this claim in some detail. The perfused gastric gland is a cylinder of epithelial tissue with the apical membranes of two cell types, parietal and chief cells, facing the central lumen and the basal membrane areas comprising the outer surface. When the outer surface is perfused with a CO_2-containing solution there is a pH change in the cells due to CO_2 entry, but when the lumen is perfused there is virtually none; this is taken to indicate near-zero CO_2 permeability of the apical membrane. There are two points to be noted in this study. (i) There is asymmetry in the system during perfusion due to the cylindrical geometry, leading to a situation where the cell receives a different CO_2 load during internal versus external perfusion (*see* Appendices). (ii) Most cells have an active buffering system determining their pH and ion composition, which is complex and when subjected to CO_2 loading this will immediately react homeostatically. The relationship between loading and pH change is almost certainly non-linear, as it is with passive buffering systems, and depending upon the intensity of the loading and the active buffering capacity there may be a pronounced pH change or very little indeed. The asymmetry of the CO_2 loading described in (i) and (ii) may be such as to create exactly this situation; during external perfusion with CO_2 solutions the cytoplasmic loading may exceed the steady-state buffering capacity of the cell, whilst during luminal perfusion the loading may be light and the buffering capacity sufficient to maintain the pH. Similar results were obtained with NH_3 permeation in this study and the same reservations apply to these. There are other studies using parietal or oxyntopeptic cells, which show that the apical membrane is clearly NH_3-permeable [90, 135, 187] although actual values may be subject to USL limitations. A calculation of P_{NH_3} similar to that on P_{CO_2} through octanol gives a value of 1.5 cm/s.

This experiment demonstrates, as do others with vesicles, the extreme difficulty of satisfying the conditions during CO_2 transport experiments whereby concentrations are maintained at known values during qualitative or quantitative estimates of P_{CO_2}. This is due to the enormous permeability of lipid membranes to CO_2. It is difficult to see how the permeability of membranes to small volatiles can be reduced by several orders of magnitude (as opposed to be varied about a characteristic value) by modifying their composition without their ceasing to be lipid bilayers at all [74], especially of the fluid mosaic type [148]. When the composition of the cholesterol-free lipid membrane was varied in artificial vesicles [89] there was a variation of 2–3 times in the value of P_{NH_3} (bearing in mind that the absolute values must be USL-limited). In high-cholesterol bilayers (40%) P_{NH_3} was an order of magnitude smaller than the most permeable value measured. Fluidity has an effect therefore, but not such as to render bilayers impermeable.

There are experimental studies whose results have been interpreted as showing a role for AQP1 in CO_2 permeation. Expression of AQP1 in oocytes followed by application of a CO_2 gradient in the presence of carbonic anhydrase showed a 40% increase in acidification, interpretable as an increased permeation of CO_2 into the egg via AQP1 channels [120]. Since this system cannot be vigorously stirred, CO_2 is very likely to be USL-controlled. There is also uncertainty about the gradient for CO_2 at the membrane surfaces in control CO_2 versus AQP1 experiments. Thus, the reported change in conductance must be viewed with considerable caution. By contrast, a more rigorous analysis found no effect of AQP1 knockout on the permeation of CO_2 into red cells [188] and lipid vesicles containing different concentrations of carbonic anhydrase showed no difference in CO_2 fluxes after AQP1 was incorporated; this indicates both that the entry of CO_2 is USL-limited and that the basal lipid membrane has such a high CO_2-permeability that the addition of AQP1 channels in parallel has no detectable effect, as would be expected. Indeed, the values obtained in those

well-mixed stop-flow experiments are completely consistent with relatively small USLs of 1–10 microns and very high membrane permeabilities. There are also studies that can show no evidence for AQP1 controlling CO_2 permeation in red cells and lung in situ after AQP inhibition [163]. It has been suggested that USLs will complicate the interpretation of AQP knockouts in CO_2 transport [29]. It is true that the relation between changes in permeability and the USL length is complicated [131] but they cannot cancel and lead to a null result.

The same arguments apply with equal force but with slightly different details to NH_3, since this is also lipophilic. NH_3 has been suggested as a permeant of the AQP Nodulin 26 in the peribacterioid membrane in nitrogen-fixing cells of legume roots [126]. Here Hg^{2+} ions caused a 40% reduction in NH_3 transport, but the results, like the effects of this inhibitor on CO_2 transport [120] or NH_3 transport [121] in oocytes expressing AQP1, are not clear cut. Again, there are conflicting reports; AQP1 reconstituted into proteoliposomes caused no increase in NH_3 permeation [194]. The activation energy for NH_3 transport is also quite high, implying domination of transport by lipid permeation. Further reducing the likelihood that MIPs play a significant role in nitrogen movement across membranes is the fact that the protonation state of NH_3 ($pK_b = 9.25$) at cytosolic pH's favors the $NH4^+$ form—for which there are known to be highly permeant, selective channels—by two orders of magnitude. This consideration is pertinent to plants where uptake by roots or across peribacteroid membranes involves the movement of ammonium from an acidic environment three to four pH units below the pK_b.

In view of the difficulty that most studies have had in taking proper account of unstirred layer effects we consider that the role of AQPs as significant physiological contributors to the transport of CO_2 and other small volatiles has yet to be experimentally established and is theoretically very unlikely.

What Aquaporins May Be For

We believe that the examination of the simple permeability hypothesis conducted in the preceding section shows that the SPH does not provide a satisfactory answer to the question posed in the title of this article. There is one outstanding exception. The presence of AQP2 in the epithelia of mammalian collecting ducts and amphibian urinary bladder is an example of AQP serving primarily as a water channel whose abundance in the membrane is hormonally controlled by the need for antidiuresis. It should be noted, however, that it functions only when an osmotic gradient exists across the tissue, which is not comparable to the situation in isotonically transporting tissues where no transepithelial osmotic gradient is measurable. This situation may be considered the exception that proves the rule: in terms of the SPH, where a clearly defined requirement for increasing P_{os} of the membrane exists (in this case dehydration), the driving forces are present and AQP levels are modulated accordingly. Mice bred to contain mutant AQP2 show failure to absorb water [189] with subsequent serious dehydration. A similar situation is not found for other AQPs in animals or plants.

We propose an alternative for the majority of AQPs, which may be termed the "sensor hypothesis" of AQP function. There are good reasons for considering such a hypothesis. We shall later apply the sensor hypothesis to various systems and show that even if AQPs are not fulfilling this function, then it would be advantageous if some other molecule did so. The sensor hypothesis includes osmotic gradient- and turgor gradient-sensing functions for AQPs.

First, there is a general need in biology for sensors of osmotic and pressure gradients, and signalling pathways for these have been shown to exist in animals [64, 65] and micro-organisms [66], yet no primary sensors have been found for these pathways. The situation in plants is probably just as crucial because changes in water status manifest as turgor are known to signal internally to control water loss by transpiration. In addition, turgor is obviously intimately involved in cell expansion and diurnal movements.

Second, the structure of AQPs and their organization in the membrane suggest a mechanism for them to detect both osmotic and hydrostatic water pressure gradients. The model of how AQPs may function as sensors meets the needs for osmo- and turgor-sensing; these are, broadly, that both osmotic gradient change and pressure change precede changes in volume and represent the earliest signal that might initiate control mechanisms.

Third, the sensor hypothesis can be extended into many areas of animal and cell function, including controlling the volumes of subcellular organelles or the rate of growth of cells where regulation is clearly needed and has often been discussed in different forms, but for which it is difficult to decide how control is initiated. Such a fundamental function is consistent with the ubiquity and antiquity of AQPs.

Under the sensor hypothesis AQPs have three central roles in biology: (i) to control fluid secretion in transporting epithelia, ensuring quasi-isotonic flow; (ii) to control cell volume as part of a feedback loop in wall-less cells (whose internal pressure is virtually equal to that outside) and cellular organelles; (iii) to monitor turgor pressure in cells with constraining walls as part of a feedback loop in mature cells and especially in cells changing their volume during growth or varying their turgor pressure diurnally. These functions are based on the same aspects of AQP structure and the interaction of these structural fea-

tures of AQP with their aqueous and lipid environments. However, it is convenient to consider these roles separately and in the two following sections we describe for each sensing function, first a mechanistic model by which AQPs may act as sensors and then consider applications in which sensing functions may serve important biological purposes.

Osmotic Pressure Sensing and Its Applications

An Osmosensor Model

AQPs occur as tetramers in membranes, and we are not aware of any compelling explanation having been made of this pronounced structural feature. These tetramers are not functioning as in ion channels where four subunits come together to form a pore although, as discussed above, ion permeation through AQPs may show this behavior and it has been suggested that there are evolutionary relationships between these two systems [193]. They seem to be more similar to enzymes where four or more independently catalytic or binding polypeptides are associated with one another to enable cooperativity, although not all oligomeric enzymes are cooperative, e.g., RUBISCO.

We propose that the structure and environment of AQPs may allow them to act as sensors of an osmotic pressure difference $\Delta\pi$ across the membrane. The quaternary structure of AQPs in membranes suggests the possibility that, as the AQP is a tetramer, it may undergo a change in its configuration if strain arises in the individual monomers, in a similar way to allosteric enzymes and hemoglobins. A consideration of the interaction of the structure of the individual AQP monomers with the adjacent baths, using simple principles of solution thermodynamics, suggests that such a strain would be the result of tension within the AQP pore, which arises by the exclusion of solutes. This mechanism has been considered as the basis of osmotic driving forces in solute-impermeable pores for many years [34, 110]. When $\Delta\pi$ is present across the membrane there would be an asymmetrical internal tension between the two ends of the AQP molecule, creating a small change in monomer shape. This would in turn favor the conversion of the tetramer to another configuration. If we envisage this as a concerted change, then it could initiate cell signalling pathways, as occurs with other receptors on the cell surface. The details of this model are set out in Appendix B, where we consider each of these aspects in more detail.

Cell signalling requires an interaction of the tetramer with other species after it is converted. At present there is no experimental evidence that this occurs but there is emerging evidence that, *vice versa*, tetramer configuration can be switched by cell molecules. AQP1 molecules can be converted to allow passage of ions after interaction with cGMP [4, 144], a change that is most probably a quaternary change that allows the four-fold symmetry axis to pass ions [13]. In pancreatic zymogen granules GTP application results in a marked potentiation of water entry via AQP1, which can be blocked by antibodies raised against the carboxyl-terminal domain of AQP1 [27]. In plants there are examples of the modulation of water permeability by phosphorylation [78] and this may apparently be brought about by ions such as Ca^{2+} and H^{+} [47]. These indicate, at the least, that changes in AQP structure can be associated with reversible cell reactions.

It is necessary to have a simple model of this interaction, which we will refer to subsequently. If an AQP is involved in giving a signal, then the total signal strength F depends on the number of AQPs in state S_{AQP} according to

$$F = AS_{AQP} + k \tag{4}$$

where A is the amplification or 'gain' of the signalling pathway and k is an 'offset'. This offset is the AQP-independent value of the function F when either the osmotic difference is abolished or the AQP is removed by knockout. Examples of this are found particularly in the area of epithelial knockouts. It is necessary to say that there is currently no direct evidence that AQPs initiate a signalling pathway although there are examples of nucleotide interactions, which will be discussed below.

Isotonically Transporting Epithelia

The concept of AQPs acting as osmosensors can be applied very profitably to the problem of isotonic fluid transport across epithelia. We have indicated in an earlier section that this central physiological process is far from being understood and that the SPH has come to exclude any other theory or finding. But experimentally there is clear evidence, where molecular probes have been used on several systems, that most of the water flows paracellularly. If this is a general phenomenon then it rules out a role for AQPs as steady-state flow conduits as envisaged by the SPH, because no water flows through the cell membranes in the steady state. It is also plain that the SPH has not been put on a sound basis by the use of knockout animals; indeed, we have argued that such experiments are not supportive of the osmotic theory of fluid production.

It has been suggested [147] that the paracellular fluid flow is mediated by a mechanism for water and salt transfer located in the junctional complex, which is an active organelle. Further, the rate of this system has to be controlled, and if this were done by an osmosensor located in the apical membrane this would solve two problems at once: it

would give AQPs an important role in the epithelium and integrate the junctional fluid transfer into the process.

Such a model has to accommodate the results from AQP knockouts on active fluid transport. It has to explain why effects range from being unobservable in many cases to being distinct but partial in a few cases. If we interpret the results using Eq.4 it becomes clear that when an AQP is removed, S_{AQP} should become zero and the function reverts to the offset value k. If this is close to the former value (i.e., A; S_{AQP} was small in the steady state) then little effect of the knockout will be observed; but if it was some way away from k, then a change will be observed. If the offset k is associated with the junctional flow, then there will be a fall in the transport of salt, which is carried by this system. These effects are clearly seen in the systems that are affected by knockout (Fig. 2).

Regulatory Volume Responses in Cells and Organelles

There is a need for a general feedback system controlling the size of all membrane-bounded spaces, such as cells without constraining walls and intercellular organelles. (Mitochondria and plastids do not, however, contain AQPs.) In these spaces there usually exists an accumulation of osmolytes against a background of exchange in particular solute species, and this has to be osmotically regulated to preserve volume (we shall treat secretory vesicles as a special case, below). In many cases there is expansion growth of the cell and some of its organelles, or in plant systems there are volume changes that occur on a diurnal basis, such as those of stomatal guard cells. In some cells, such as protozoans without contractile vacuoles, the surface:volume ratio is so high that these cells require a very aggressive method of regulating volume and they can respond to osmotic challenge very rapidly, indeed [16].

Although the phenomenon of volume regulatory decrease (RVD) after hypotonic swelling and the increase (RVI) after hypertonic shrinkage are well studied, no osmosensor system has yet been proposed. Different mechanisms have been proposed to detect volume changes such as (i) the change in concentration of an internal solute or (ii) membrane deformation of some kind (either changes in curvature or lateral stretching) acting on a mechano-sensitive channel and from a general consideration of such channels it has been concluded that osmotic forces may possibly be sensed as well [138]. These mechanisms have the disadvantage that they can only be activated after the volume change has occurred. Of these, (i) is unlikely to be very effective in the case of a large volume (such as a large plant cell vacuole) because of the time required to effect a change in concentration unless the detection mechanism is very sensitive. Mechanism (ii) suffers from the same drawback in the case of large volumes but also requires that the volume should ideally be spherical; if this is not the case then the cell or organelle can change volume by changing its shape (like a red cell) without much change in tension or overall curvature.

As a mechanism, direct osmo-sensing has neither of these drawbacks because it registers the change in driving force before much volume change has occurred. It does, however, have to be sensitive so that it can be quick to signal and effect a change. We suggest that where AQPs are found in cell and organelle membranes of volumes lacking cell walls such as the red cell, glia [5, 118, 119, 176, 184, 185, 192] and the endothelial cell [178], to name only a few in the animal body, they may be involved in osmosensing as part of a volume control system, i.e., they may constitute an osmosensor that is required to activate RVD or RVI in these cells by signalling to the channels or transporters that define the steady-state osmolyte content [64, 65, 83].

Red Cells

The red cell has a unique position in the field of osmotic and water relations because it has been the source of so much data and theory. As discussed above, the function of AQP1 in the mammalian red cell membrane is obscure although this cell is indeed subject to osmotic stresses (largely by urea) in the kidney medulla and if shrinkage is not controlled these are very deleterious by concentrating hemoglobin. Other red cells, such as those of fishes, meet osmotic challenges of a different sort in the gills. Recently, mice double knockouts for AQP1 and the urea transporter UT-B (UT3) have revealed a value for the water channel conductance P_f of UT-B that is comparable to that of AQP1 [190]. An interaction between AQP1 and the urea transporter in mice will require further study. If AQP1 is acting as an osmosensor in the red cell we can speculate that it may be interacting with UT-B at the onset of osmotic challenge and thus modulating urea fluxes across the membrane.

The situation is more striking in the case of the Cl^-/HCO_3 exchanger (Band 3) where in red cell ghosts the acidification caused by CO_2 entry (at low concentrations) is AQP1-dependent [12]. This is interpreted as being due to the inhibition of CO_2 transport by the AQP1, but, as shown above, this is not feasible due to the domination of P_{CO_2} by the lipid membrane. It would seem more likely that AQP1 is modulating the exchanger, presumably by a coupling in the membrane. A possible involvement of AQP1 relates to the fact that CO_2 entry causes an

osmotic swelling (with acidification) due to the production of ions. A feedback from AQP acting as an osmotic sensor to the exchanger would then modulate both the swelling and also the rate of acid production.

Plant Vacuoles

One organelle of special importance is the vacuole of the plant cell. This has to grow in size during cell development and at each stage stabilization of its relative volume is essential as osmolytes are added or exchanged.

If the vacuole is in osmotic equilibrium with the cytoplasm the shifts of osmotically active material from cytoplasm to vacuole will result in no overall change in cell volume but a redistribution of volume between the two spaces. This will result in a change of concentration in the cytoplasm, which would affect metabolic parameters. Regulation of these changes can be considered as a short-term effect rather than the long-term problem of setting the vacuolar volume. Viewed in this light the problem is therefore not one of stabilizing vacuolar volume per se but of regulating the *relative* volume in the interests of the cytoplasm. This process may be different from the long-term one of setting the absolute volume of the vacuole; in this context membrane tension has been invoked [115] but it is not clear how this would be transduced. The model for AQP sensing in this paper is dependent upon the pressure difference between atrium of the molecule and the lipid phase, so that changes in tension would affect the AQP states.

Furthermore, there are situations such as the diurnal phenomenon of crassulacean acid metabolism (CAM) where there is storage and removal of solutes between night and day. Plant vacuoles are rich in AQPs and as a general phenomenon the P_{os} of the tonoplast is dominated by AQPs [105]. Ultimately, the level of homeostasis in such an AQP-sensor system may be under nuclear control and is subject to up- or down-regulation depending on the events that are underway in a particular cell. In this context it is of interest to revisit Eq.4, which may be written

$$F = AM_{AQP}f(\Delta\pi) + k \qquad (5)$$

where M_{AQP} is now the amount of AQP expressed in the membrane and $f(\Delta\pi)$ is the fraction of AQP tetramers that are in a state that gives a signal, when the transmembrane gradient is $\Delta\pi$. Clearly, the degree of expression will determine the overall response. If this is small there will be little participation of AQP in a putative feedback loop. The strength of the signal and therefore the degree of control exercised by such a sensor can thus be simply altered by regulation of M_{AQP}, the expression of AQP itself, rather than changing either the signalling amplification A or the response of the AQP molecule itself. It should be stressed again that these basic parameters are introduced to give generality to the model when considering systems to which it may be applicable. The possibility exists, therefore, that the changes in AQP expression in plant systems come from the need to turn sensor systems up or down. Regulation of AQP levels may therefore be expected as the plant is subjected to environmental stimuli, as generally observed [105], although the precise reasons for regulation cannot be discussed without further information about the function of specific AQPs.

There exists an interesting case of an ion channel in the tonoplast that responds to $\Delta\pi$ rather than absolute levels of π [3]. This may be a modulated ion conductance (mainly K^+), as described above, and its possible relevance to the regulation of vacuolar volume has to be investigated further.

Secretory Granules

Another important class of organelles are secretory or zymogen granules, i.e., very small vesicles containing protein (enzymes) or mucus, which spend some time in the cytoplasm, eventually to fuse with the plasmalemma and discharge their contents. These may contain charged polymers that can generate a Gibbs-Donnan swelling, but the vesicles do not swell although they may do so momentarily at the plasmalemma during the fusion event. If there is a possibility of the granule bursting inside the cell and releasing its contents this could be disastrous. There is no possibility that the membrane can be so constituted that it is 'water impermeable' because it is a lipid bilayer and these have a P_{os} of around 10^{-3} cm/s; the half-time of swelling of a zymogen granule of radius 1 μm would be around 20 ms. Therefore, no granule can have an internal osmotic pressure different from that of the cytoplasm or it would swell quickly. Vesicles have been shown to contain AQPs, however, and this fact would make matters worse by enhancing swelling should a difference $\Delta\pi$ develop.

If there is triggering of a terminal swelling event this must involve solute particles entering the vesicle and the development of $\Delta\pi$ but the swelling would then be uncontrolled. A suitable control system would involve sensing $\Delta\pi$ and using the signal to control solute entry, which could include ion channels in a feedback loop. AQPs could therefore play a role in these vesicular systems.

Pancreatic secretory vesicles have been shown to contain AQP1 in their membranes, which is involved in a GTP-dependent swelling response [27] although the details are as yet unclear. In parotid salivary cells secretory vesicles contain AQP5 [149] and inhibition

of this with AQP5-specific antibody causes swelling in isolated vesicles which is both channel- and Cl^- ion-dependent [103]. Thus it is the AQP5 *removal* that causes swelling although it must abolish the AQP water channel conductance. This fact indicates that swelling is not rate-limited by water permeation (as the SPH would require) and strongly implicates the AQP as part of a system that controls vesicle volume in the steady-state and which requires us to consider the interaction of AQP with a process mediated by transporters. We regard the case of secretory vesicles together with the situation in red cells and the Band 3 exchanger (*see* above, [12]) to be the strongest evidence to date for the operation of AQPs as part of a control system.

Turgor Pressure Sensing and Its Applications

A Turgor Sensor Model

Cell walls are relatively inextensible and solve the problem of swelling. But for many reasons it is desirable to control and monitor the turgor pressure. Any control mechanism depends upon sensing the turgor as the first stage. Membrane flexure or stretch cannot really operate under these conditions because, with turgor pressing the membrane to the wall, the membrane is not stretched or flexed to any extent. We describe here how AQPs may serve as turgor sensors when in contact with a cell wall by an extension of the osmosensor model to situations where the water is at equilibrium and discuss how this may play an important role in both eukaryotic and prokaryotic cells. The model is described in more detail in Appendix C.

When the cytoplasm is adpressed to a cell wall a large measure of the pressure gradient between the cytoplasm and the fibres of the wall appears across the lipid membrane. The gradient is not a simple linear one but the *mean* pressure in the outer and inner leaflets is not the same. Inside the atria of the AQP there will also be pressure drops (tensions) due to solute exclusion, as described for the osmosensor model but these are now equal in each atrium because water is at equilibrium between the cell and the exterior—which is not the case when only an osmotic difference $\Delta\pi$ exists, as in animal cells that are swelling or shrinking. The strain in the AQP molecule is now due to the difference between the pressure in the atrium and that in the adjacent lipid leaflet. This strain leads to changes in the T-R ratio of the AQP tetramer, as described for the pure osmo-sensing situation and this is detected by the signalling system and transduced according to Eqs.4–5, which can be rewritten in an analogous form with ΔP replacing

$$F = AM_{\mathrm{AQP}} f(\Delta P) + k \tag{6}$$

There is an obvious relationship to other molecular systems that respond to osmotic pressure and membrane stress. To begin with, there are two-state molecules, e.g., enzymes, receptors or transporters, for which the two states possess different molar volumes and in which their ratio can be altered by changes in the osmotic pressure [129]; this is probably a near ubiquitous phenomenon in systems interacting with water or which possess water-filled cavities [139]. In the case of binding proteins like hemoglobin and ion channels we are dealing directly with either tetramers or complexes with four subunits like AQP. In the case of ion channels activated by stretching the membrane, the system is again a molecule with open and closed states of different molecular volumes, the ratio between which can be shifted by trans-membrane osmotic pressures or mechanical pressures: in this context it should be remembered that 'stretching' membranes with a patch pipette goes along with transmembrane pressure and also substantial curvature of the membrane in which the two leaflets would not have the same internal tension.

Turgor Regulation in Cells with Walls

There is an extensive literature on the problem of turgor regulation in plant cells. Much of this is slowly falling into a pattern and essentially involves the electrophysiological control of K-salt levels as osmotic agents that cause, by osmotic water shifts, the swelling or shrinkage of cells (reviewed in [42]). As far as the efflux of KCl is concerned, in response to an externally applied hypertonicity, there are obvious parallels with the RVD in many animal cells.

As reference will be made in what follows to controlling the 'pump-leak' system in relation to cell turgor, this term must be understood in the following way. Although turgor is maintained by the osmotic pressure of several solutes we assume, for simplicity of argument, that there is a master species determining the osmotic pressure at equilibrium (K^+ would be an obvious candidate) whose concentration difference ΔC_s is given by the flux equation

$$J_s = J_p - P_s \Delta C_s \tag{7}$$

where J_s is the net flux, J_p is a pump flux and P_s, the permeability. The equilibrium turgor is therefore

$$\Delta P = RT \frac{J_p}{P_s} \tag{8}$$

with the pump-leak system characterized by the ratio J_p/P_s. For ions, Eqn.7 will contain both pump kinetics and electrodiffusive terms. Either J_p or P_s could be modulated by the sensor signalling system to give higher or lower turgor pressures.

However, little is known about the link between turgor and the modulation of the overall pump-leak system because, as with volume regulation in animal cells, no turgor sensor has been identified. Marine algae have been much studied for their convenient size and for their ability to regulate turgor effectively due to the fact that they are subject to fluctuations in osmolarity in the tidal zone [52]. As an example, in the giant algal cell *Ventricaria*, when step changes are made to the osmotic pressure of the medium, causing a change in turgor pressure, there is a rapid detection and a restoration of turgor [11].

Stretch receptors have frequently been considered as candidates for sensors in the control of turgor [138], and swelling detection by an actin-based system has also been suggested [93] but these have the drawback that in cells with relatively inextensible walls little membrane stretch can be created and there is also very little flexing of the membrane because the swelling is severely limited by the wall. Obviously, AQPs may have a central role to play in these systems because if they can function in turgor sensing as described above they do not require any membrane stretching. AQP in the plasmalemma could control this probably by modulating the conductance of K^+ ion channels, involved in the rates of solute loss or accumulation.

In the sections below we shall suggest this role for AQPs not only in the control of steady-state turgor but also in the relaying of information on current turgor status to mechanisms controlling either cell wall growth or stomatal apertures.

Water Status and the Approach to Plasmolysis

Pressure sensing in the plant would signal the fall of turgor pressure towards the state of plasmolysis and, via the production of a hormone such as ABA (abscisic acid), control stomatal closure and a decrease of the transpiration rate. The sensor signal could be amplified or attenuated by up- or down-regulation of the AQPs involved. We have seen that a stress such as drought seems to upregulate some AQPs and downregulate others, either at the plasmalemma or tonoplast, in a complicated manner that is difficult to understand in terms of the SPH. At the plasmalemma there may be AQP signals required for the control of extension growth and turgor maintenance under steady conditions (discussed below), which are to some extent interacting and which may have to be adjusted in an integrated fashion. If we add to this the fact that for a systemic response to a stress like drought there has to be a contribution from different plant cell types that are not all equally important and that have to be integrated, then perhaps some of the complexity of regulatory responses might be seen in a new light (Fig. 3).

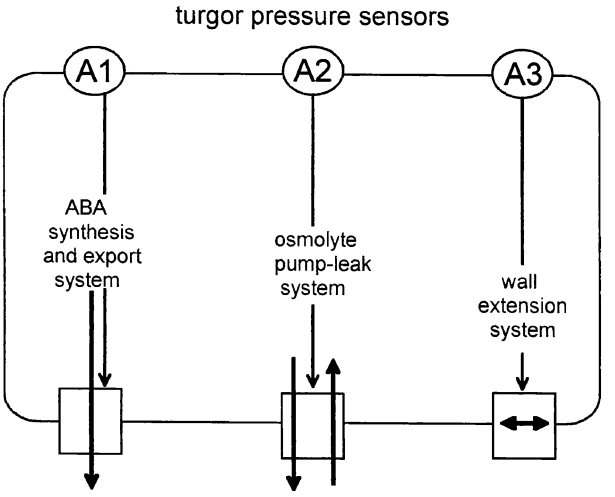

Fig. 3. Three turgor sensors (*A1–A3*) in a plant cell controlling different membrane processes. *A1*: the export of ABA (abscisic acid) in response to incipient plasmolysis. *A2*: the control of turgor by regulation of the pump-leak systems determining the osmolyte content. *A3*: interaction with the cell wall expansion system during regulation of extension growth.

Control of Extension Growth

An increase in volume of a cell occurs when cells expand, hyphae and pollen tubes extend and bacteria and yeast undergo replicative growth. In all these processes the expansion is believed to be powered by turgor, although there may not be any correlation between turgor and cell extension rate in a population of cells. However, the relation between turgor and extension growth rate in an algal cell is demonstrated by measuring increments in growth rate after fluid injections have created increments in turgor [136]. The extension process is rate-limited and geometrically directed by the complex process of cell-wall growth involving plasticizing and intercalating new material. Growth is a quasi-steady-state process, which may be described as follows. If wall growth is initiated at a constant rate this will cause an increase in wall area (and possibly elasticity) and turgor will fall. The inward driving force on water (π_i - p_i) will be increased and water will enter. At the same time, if the constants J_p and P_s of the pump-leak system are unchanged, the turgor will be driven back to its former value—the equilibrium turgor is independent of the cell area and wall elasticity.

We can recognize two possible feedback loops, as shown diagrammatically in Fig. 3:

(i) If uncontrolled, the turgor will change as a consequence of the rate of wall growth and therefore the driving force for expansion will not be constant. To make it *independent* of growth the salt accumulation should be modulated with the growth rate. Turgor sensing by AQPs may be the system which

does this and ensures that turgor is held relatively constant. This implies a similar mechanism to RVD or RVI present in animal cells where osmolytes are moved in response to osmotic challenges, but may be brought about here in response to turgor change signalled from an AQP that is controlling the generalized pump-leak ratio of the cell, as described above. The result would be the maintenance of cell turgor close to some 'local' value. This would have to involve the tonoplast (possibly indirectly) because the vacuole is the repository of most of the cell volume.

(ii) The wall expansion has to be coordinated with the turgor: if the pressure is too high the wall will be expanded at too fast a rate and could be 'thinned', or the converse. Although in some systems there is no simple correlation between turgor and growth rates [9, 136], cell expansion clearly cannot occur without turgor and small step changes created by fluid injection can produce immediate changes in growth rate [136]. This requires some feedback from a pressure sensor, which may be an AQP and which may not be the same one controlling the turgor. In growing lily pollen tubes step increases in turgor can cause wall disruption and bursting but changes in external osmolarity have quite small effects on turgor, implying turgor regulation [9]. An important finding is that in *Nitellopsis* [200] inhibition by Hg^{2+} of an AQP present in the plasmalemma of expanding internodal cells stops growth immediately and addition of an anti-RD28 AQP antibody leads to a rapid decline in linear growth although simultaneous measurements of turgor pressure show that it remains steady over the same time. This indicates that wall growth is directly coupled to turgor by an AQP. As argued above (Water Conductances in Growth Control) the growth is controlled by wall expansion and cannot simply involve restricting water entry via a fall in membrane water permeability.

Diurnal Rhythms and Stomatal Closure

Diurnal plant movements are due to slow rhythmic changes of volume in pulvinar tissue and are widely considered to occur by the deformation of the shape of cells within a localized 'motor' tissue by turgor pressure; due to the asymmetric elasticity of the walls, the cell shape changes with changes in pressure and cell volume [63]. Thus, changing turgor is essential in changing the position of the mature plant organ. If this is controlled as part of a feedback loop then some elements of the system, which determine the solute content such as rate constants, etc., have to be modulated.

Two AQPs have been characterized in the motor tissue region of *Samanea* during a diurnal plant movement and both AQPs are regulated [116]. They are associated with different groups of cells and their levels are subject to diurnal variation in phase with the movements. There are similar diurnal changes in L_p of the motor cells [116]. This might indicate that the volume changes creating the cycles of plasmolysis and turgescence in the motor cells require changes in L_p to allow the required rates of water influx or efflux. However, for a cell 10 μm in radius with an L_p equivalent to 10^{-3} cm/s (that of a lipid membrane) the half-time is about 0.5 s. For a membrane containing AQPs already inserted and about an order of magnitude more permeable it would be a tenth of this value. These times are so short compared to the swelling and shrinkage times of the motor cells during the movements that they cannot be rate limited by water permeabilities. Turgor, being an equilibrium parameter, does not depend on the value of L_p. The water shifts are not rate-limited by the L_p of lipid membranes but by solute movements. In terms of the SPH it is not clear what part the AQPs are playing in this mechanism.

In *Samanea* one of these AQP changes is apparently circadian, involving control at the level of transcription, and clocks are known to be often under nuclear control. Such regulation indicates that an AQP is involved in the movements but, as argued above, this cannot be simply to allow or restrict water entry by changing the L_p. A simple way of effecting changes whilst keeping the cell turgor under constant feedback control would be to modulate the magnitude of the feedback signal itself. If the solute pump-leak system is set so as to maintain the cell in a flaccid state near to plasmolysis with little control, but near to some high turgor state when under control (or *vice versa*), then up- or down-regulation of the turgor sensor signal would lead to changes in cell volume and movement of the plant organ. In the most general way, according to Eq.6, the magnitude of the signal F from the sensor signalling system, which acts on either the pump or leak parameters, can be controlled by regulating M_{AQP}, the amount of expressed AQP.

Concerning stomatal movements, the guard cells of *Vicia* can be shown to possess an AQP and this is expressed in the plasmalemma of the guard cells [162]. Inhibition of the AQP with antibody and Hg^{2+} greatly inhibited stomatal opening and the swelling of guard cell protoplasts induced by fusicoccin and light, and closing induced by abscisic acid [68]. Again, the time constants of volume changes predicted above (less than 1 s) are much shorter than those of stomatal movements and imply that solute exchanges are the rate-limiting factor, not changes in L_p.

We suggest that AQP turgor-sensing and feedback also apply here and in this context, opening and closing have been shown to be AQP dependent. If the movements depend upon any regulation through transcriptional control (which has not been shown to date) this may control the diurnal movements of

stomata. The direct control of stomatal aperture by fusicoccin (opening and guard cell protoplast swelling) and abscisic acid (closing and fall of protoplast turgor pressure) would seem to require the functioning of an AQP [68], but these cannot be affecting AQPs directly because there are no apparent receptor sites for these molecules on AQP itself. The hormone fusicoccin seems to be affecting solute accumulation in the cells, which would be a necessary component in the functioning of a turgor feedback loop. A possible mechanism may be the modulation of the amplification A of the signal produced by AQP acting as a sensor according to Eq.5.

Growth in Bacteria and Fungi

Some bacteria and most yeasts contain AQPs, of which AQPZ has been shown to occur in *E. coli*. The knockout of AQPZ appears to have little effect on the bacterial cell apart from its growth rate, which is inhibited when grown with wild-type cells. These cells are in no danger of bursting through uncontrolled swelling because of their cell wall system but they are in need of growth regulation as are expanding plant cells. They are simpler in this respect because they lack an internal vacuole, but their growth rate is very fast. Many other bacteria, although they have not been shown to contain AQPs by analysis of their genomic sequences, do contain glycerol transporters, which are in all major respects MIPs with tetramer structure and water channels (*see* above, To Act as Water Conductances In Cellular Homeostasis: Bacteria and Yeasts). The P_{os} of these is often low, but this does not preclude them functioning as osmo- or turgor sensors by the mechanisms described above.

They are therefore candidates for turgor regulation of cell volume expansion and its coordination with wall synthesis, as described above for plant cells.

Appendix A

Permeation of Volatiles

Permeability of Lipid Bilayers

The permeability of a membrane phase is given in accordance with Overton's rule by

$$P_{CO_2} = \frac{D_\kappa}{L} \tag{A1}$$

where D is the diffusion coefficient in the phase, κ the partition coefficient and L the membrane thickness [43, 127]. The diffusion coefficient for a small neutral solute in a solvent of viscosity 1–10 times that of water varies between about 10^{-6} and 10^{-5} cm^2/s and the partition coefficient is between 1 and 10. For a membrane of thickness 40×10^{-8} cm (40 Ångstrom), using the lowest of these values, P_{CO_2} comes out to be 2.5, i.e., between 10^0 and 10^1 cm/s—a very high value.

The diffusion and partition coefficients for lipid membranes can also be more closely estimated from measurements made in octanol (CH_3-$(CH_2)_6$-CH_2OH):water mixtures. This method is widely used to accurately model the permeability of lipid membranes and artificial phospholipid bilayers. Using the values of $D_{CO_2} = 2.87 \times 10^{-6}$ cm^2/s calculated from the Wilke-Chang equation [10] and $\kappa = 6.76$ [84] with a bilayer thickness of 40×10^{-8} cm (40 Ångstrom), the magnitude of P_{CO_2} comes out to be 4.85×10^1 or 48 cm/s. This enormous permeability arises because CO_2 permeates the bilayer as easily (or more easily) than it does an equivalent thickness of water. Indeed, the effective permeability of a 40 Å layer of water from Eq.A1 comes out to be 4.35×10^1 cm/s. This unfortunately renders any measurements of P_{CO_2} quite impossible without considering the effects of the unstirred water layers next to the membrane. We refer to the often-used equation

$$\frac{1}{P_{obs}} = \frac{1}{P_{real}} + \frac{\delta}{D} \tag{A2}$$

where P_{obs} is the observed (apparent) permeability and P_{real} the real one, δ is the unstirred-layer (USL) thickness and D is the diffusion coefficient in the bulk solution. With a value of 48 for P_{real} and δ/D of order 10^3 it becomes apparent that P_{obs} is *entirely* dominated by the USL. In the Table we show the effect of USLs on the observed permeability. Even with a USL of 10 micron, which is virtually unattainable even under vigorous stirring regimes, the value of P_{obs} is underestimated by about four orders of magnitude.

Table. Effect of unstirred layers on apparent CO_2 permeabilities

δ (μm)	1	10	100
P_{obs}/P_{real}	5.9×10^{-4}	5.9×10^{-5}	5.9×10^{-6}

Artificial planar lipid membranes cannot be stirred particularly well and other techniques such as buffering the CO_2 concentration adjacent to the membrane can be used to clamp the CO_2 gradient and destroy the USL. This technique relies on two assumptions: (i) that the buffering capacity, which depends upon the buffer concentrations and the rate constants of the buffering reaction, is sufficiently great to destroy the USL of CO_2, and (ii) that there are not gradients in buffer concentrations adjacent to

the membrane, i.e., USLs of their own, due to the fact that buffer ions have to diffuse equally through the unstirred aqueous bath to and from the membrane surface. These conditions seem to be fairly well met in a study of bilayers where very high values of P_{CO_2} were recorded [51].

If, as the above discussion indicates, membranes have high basal permeabilities to CO_2 then the effects of inserting AQPs can be simply calculated. The area (πr^2) of the AQP core of mean radius 2.5 Å is 1.96×10^{-15} cm^2 and with a D_{CO_2} of 1.74×10^{-5} in water and length of 40 Å, the permeability P_{AQP} of a single channel comes out to be 8.5×10^{-14} cm^3/s, close to that observed experimentally for water. A diffusion calculation for a similar cylinder of lipid gives the value 9.5×10^{-14} cm^3/sec. Therefore, removing a section of lipid membrane to incorporate an AQP molecule would make no difference to P_{CO_2}. Indeed, since the size of the AQP tetramer is much larger than the size of the four central channels, inserting AQPs will exclude more lipid area than it incorporates channel area, which will further lower the membrane permeability to CO_2. Thus, from simple theoretical considerations, although AQPs are probably CO_2-permeable, they cannot enhance the CO_2 permeability of the lipid membrane the way that they do for water, whose unaided permeability across lipid membranes is three orders of magnitude lower (10^{-3} cm/s).

Permeability of Gastric Gland

In the study under consideration here [181] the system is intrinsically asymmetric. The authors note that the two membrane areas are probably different but if CO_2 crosses membranes as easily as Eq.A1 would indicate, then the membranes are not rate-limiting and all that matters are the inner and outer dimensions of the perfused gland. The mean concentration C_m in the cylinder wall (i.e., the cell cytoplasm) is given by

$$C_m = \frac{(C_o b^2 - C_i a^2)\ln(b/a) + (b^2 - a^2)(C_i - C_o)/2}{(a^2 - b^2)\ln(b/a)} \quad \text{(A3)}$$

where a, b and C_i, C_o are the inner and outer radii and concentrations. When luminally perfused with a CO_2 concentration $C_i = C$ with the outer perfusate bath set to $C_o = 0$, the mean intracellular concentration will be lower than in the reverse situation of outer perfusion with $C_i = 0$ and $C_o = 0$, the ratio being due entirely to the geometry. For the dimensions of the perfused gland of $a = 10$ μm and $b = 30$ μm [180] the ratio is 1:2 or 0.5.

Another point concerns the value of C_i and whether it is possible to maintain it constant during internal perfusion. This is most unlikely if the apical membranes have high permeability to CO_2 because it will leak either out of or into the lumen. If the average value of C_i falls to $C/2$ during internal perfusion or, conversely, rises to $C/2$ during external perfusion, then this ratio of the cytoplasmic CO_2 concentrations during internal versus external perfusion falls to 1:5 or 0.2. From the data presented, judgement cannot be made with any accuracy as to the constancy of the luminal perfusion and the composition of the buffers.

Appendix B

Osmosensor Model

(i) The monomer strain may be gradual and continuous with the increasing transmembrane osmotic gradient $\Delta\pi$ but the P_f of the monomers, which are the individual water pores, may be little affected by the monomer strain. No effect of $\Delta\pi$ on the P_f of AQPs has been observed in animal AQPs to date, i.e., increasing osmotic gradients do not seem to cause 'gating' under physiological conditions (as in ion channels) although precise P_f measurements are still lacking and small changes could easily have escaped notice. In plant cells there are several reports of decreases in membrane P_{os} when $\Delta\pi$ is raised across the membrane [125, 171]; these might well be due to strain in the monomer caused by increased differences in internal tension (*see* below). P_f values obtained so far [41] are only approximations and it has not been generally recognized that measurements may be dependent on the size of the osmolytes used, which have sometimes differed between measurements [33]; some values of P_f assigned to different AQPs must necessarily be accepted as approximate.

The change in quaternary structure of the tetramer may also be gradual and continuous with increasing monomer strain. However, we shall assume in this development of a sensor model that there is a transition between two tetramer states (conventionally called 'tense', T, and 'relaxed', R) brought about by monomer strain, i.e., a T-R transition similar to hemoglobin (Fig. B1). This brings any such change in AQP into line with other membrane sensors that bind ligands or trap photons and signal to other systems in the cell by existing in two states.

(ii) The monomer, of hour-glass shape, has two atria and presents one to either bath. Salt is excluded from this atrium largely by charge effects. This is clear because physiological salines (Na^+, K^+, Cl^- etc.) give rise to the same volume flows across AQPs as do larger molecules such as sucrose, which must be sterically excluded from the atrium. This would not be the case if salt ions could enter the outer pore section or atrium [34, 59, 110]. Consider an initial situation where the two baths are at equal osmotic pressure π and hydrostatic pressure P and there is thus no water flow across the pore. As a result of

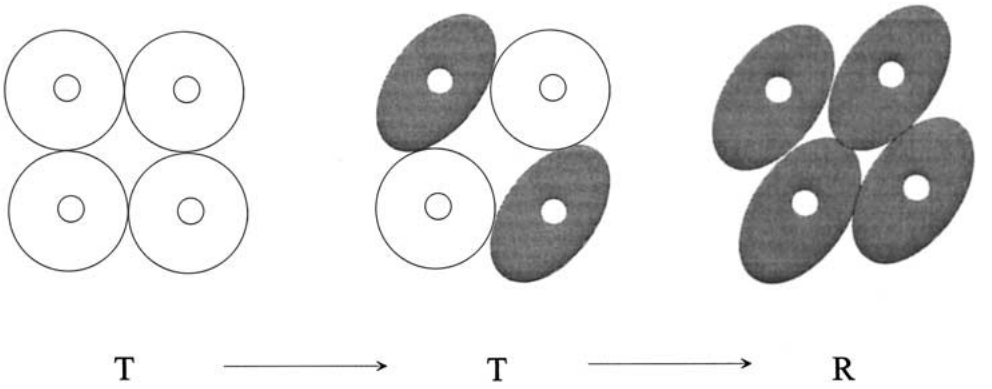

Fig. B1. Changes in a tetramer from T to R state when monomers are strained (from circular to elliptical cross-section, shown diagrammatically). In the intermediate state the tetramer is able to retain its T configuration when two monomers are strained, but with three or more there is a change to the R configuration.

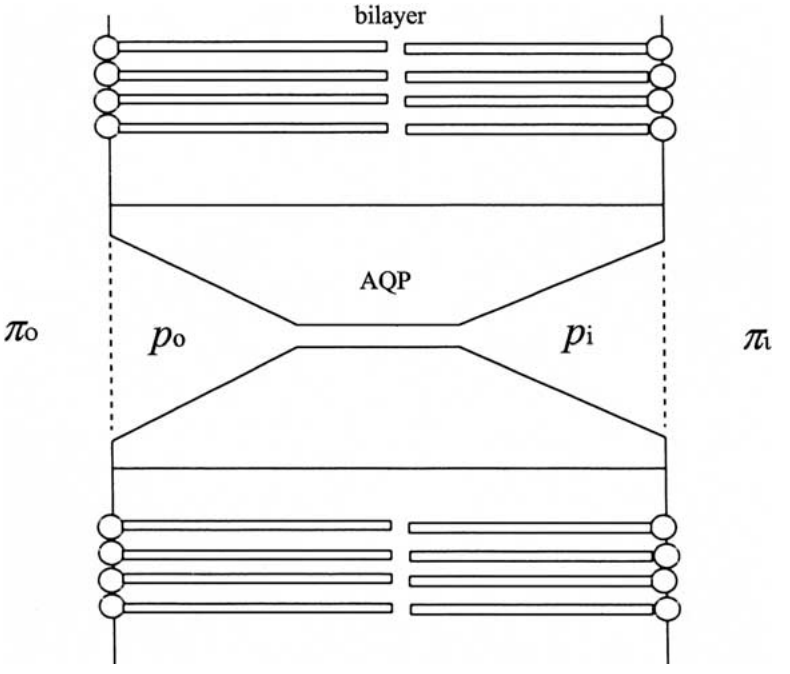

Fig. B2. The fall in hydrostatic pressure p in the outer and inner atria of the AQP hourglass configuration mirrors the osmotic pressures π in the adjacent baths according to Eq. B2.

solute exclusion, at some point on the axis just inside the pore the hydrostatic pressure p in the atria is lower than that in the baths, a pressure drop created by the thermodynamic exchange of osmotic pressure for hydrostatic pressure between an atrium and its adjacent bath at local equilibrium

$$\Delta\mu_w = 0 = \bar{V}_w(\pi + p) \tag{B1}$$

where μ_w is the water potential and $\bar{V}_w$ is the partial molar volume. The atrial pressure is

$$p = -\pi \tag{B2}$$

and this causes local strain in the molecule but it is symmetric about the mid-point of the molecule and the mid-plane of the membrane. When the baths are at different π there will be a difference in pressure between the outer and inner atria (p_o and p_i), which is responsible for the viscous flow through the pore during osmosis [59, 110]. This asymmetry causes a strain d in the molecule in the plane of the pore axis, which is a function of Δp

$$d = f(p_o - p_i) = f(\Delta p) = f(\Delta\pi) \tag{B3}$$

as shown in Fig. B2.

(iii) In the monomer spanning the membrane there will be a thermal fluctuation of water molecules in the pore (representing a change in pressure in the atria) and also a thermal fluctuation in the bilayer pressure outside the monomer. The pressure causing strain in the AQP structure is thus the difference between that in the atrium and the surrounding lipid phase in which it is

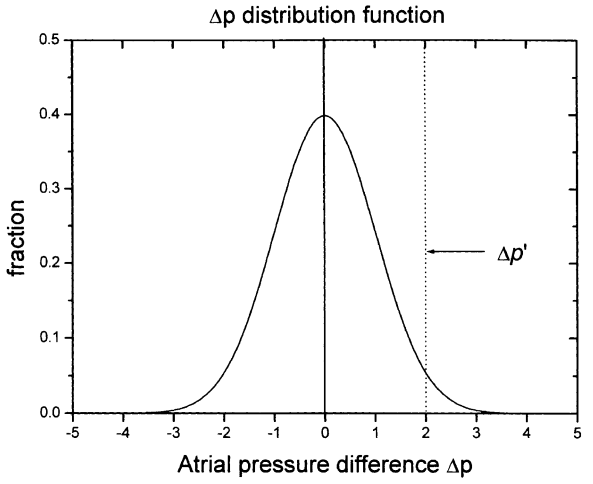

Fig. B3. The probability distribution of pressure between pore atria at zero transmembrane osmotic pressure $\Delta\pi$, shown here as a normal distribution. At $\Delta p'$ the monomer is strained to an extent such that more than two cannot be accommodated in a tetramer of configuration T. The area under the curve beyond $\Delta p'$ therefore represents the proportion of monomers in that state. As $\Delta\pi$ is raised the curve shifts to the right and the proportion.

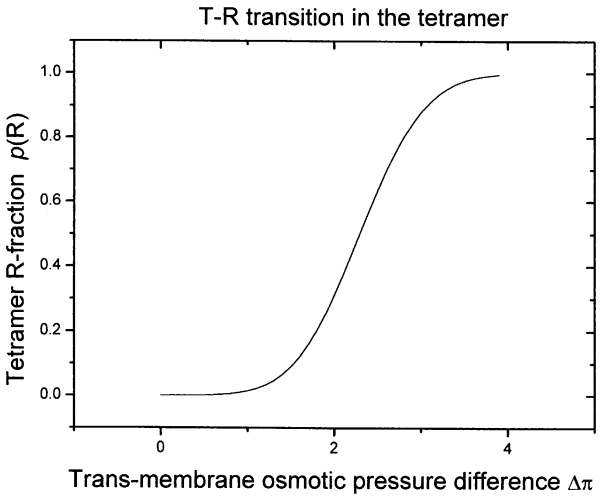

Fig. B4. The effect of changing $\Delta\pi$ on the tetramer whose monomers behave as shown in Fig. B2. As a consequence of more than two monomers being strained above the point $\Delta p'$ there is a shift from the T-state to the R-state in the tetramer. The result is that as the transmembrane osmotic pressure is raised, the proportion of tetramers in the R-state rises to 1.

embedded, i.e., the local pressure in the leaflet. The instantaneous strain produced is dependent upon the deformability of the molecule and its time constant. The result is a probability distribution curve of atrial pressure difference Δp shown in Fig. B3 where the deviation is dependent on the molecular deformability.

We assume as a simple model that at a pressure difference $\Delta p'$ the degree of asymmetric strain d' is such that two monomers showing this degree of strain can reside within the T-tetramer, but three or four cannot without there being a change to the R-tetramer. The simple result of this is that as the transmembrane osmotic pressure $\Delta\pi$ is raised the pressure difference Δp between the atria will rise and the distribution will shift to a higher mean, with the result that the probability of a monomer being in a strain state above d' becomes greater.

(iv) If the proportion of monomers that are above the strain threshold d' is q then the proportion of tetramers with more than two monomers in this state can be deduced from probability theory to be

$$p(R) = 4q^3(1-q) + q^4 \tag{B4}$$

where $p(R)$ is now the proportion of tetramers in the R-state, which is shown as a function of the trans-membrane osmotic pressure in Fig. B4. This is the familiar sigmoid curve common to allosteric enzymes or voltage-gated ion channels and is simply a consequence of cooperativity between the monomers in a tetramer to relieve strain. It is important to note, however, that, unlike allosteric enzymes where the T-R transition affects the binding constants of all the component monomers, there does not have to be an effect of the transition here on P_f, the osmotic or hydraulic conductance of the single pore, although this may occur. It may be the case that the major determinant of P_f in many AQPs is the narrow pore core whose geometry is little changed by the strain.

(v) The R-state is recognized by a cell signalling system leading to changes in membrane properties. Alternatively, the T-state is recognized and the signal therefore decreases—or both.

Appendix C

Turgor Sensor Model

In systems with cell walls the water is at equilibrium, according to Eq.B1. The osmotic pressure difference $\Delta\pi$ is balanced by the turgor pressure P_{cell} in the cell (regarding the outside medium as $P_o = 0$)

$$P_{cell} = -\Delta\pi = \pi_i - \pi_o \tag{C1}$$

where the subscripts refer to the outer and inner phases. In the outer atrium of the AQP molecule there is a pressure p_o given by

$$p_o = -\pi_o \tag{C2}$$

and in the inner atrium facing the cytoplasm a pressure p_i given by

$$p_i = -\pi_i + P_{cell} \tag{C3}$$

which means that $p_i = p_o$ and the pressures in the two atria are the same. This is different from the osmosensor case above and follows from the fact that here water is

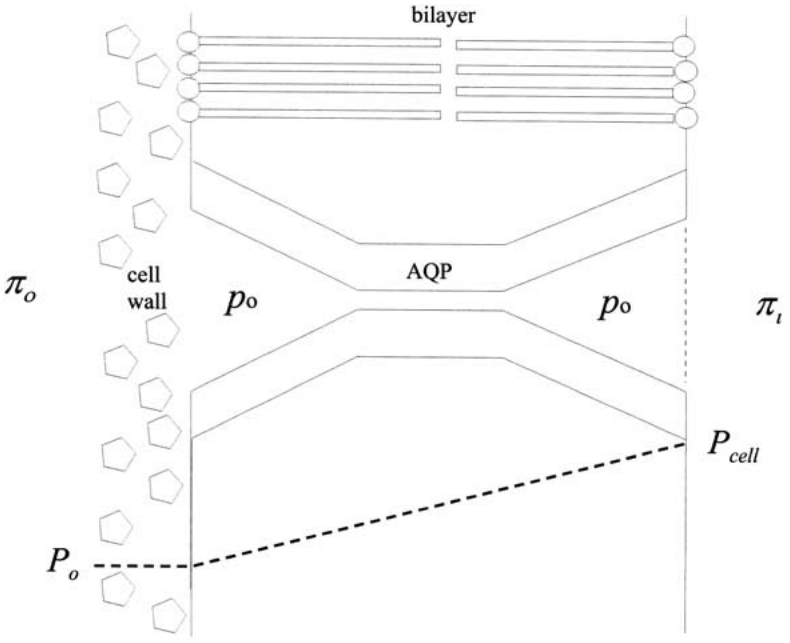

Fig. C1. Pressure relations across an AQP in a cell membrane adjacent to a wall at equilibrium. When the membrane is compressed against a rigid porous wall there is a difference of pressure $P_{cell}-P_o$ mostly across the bilayer. In the pore atria the water is at local equilibrium and at the same pressure (*see* text). There is a pressure difference between the atria and the adjacent bilayer that is different at the two sides of the AQP.

in equilibrium across the membrane, as shown in Fig. C1. The atrial pressure is the difference between that in the atrium and the surrounding lipid phase in which it is embedded, i.e., the local pressure in the leaflet. For the inner atrium this is $P_{cell}-p_i$ and for the outer atrium it is $p_o\,(=p_i)$ so that the stress and the strain that it produces are asymmetric. In the osmosensor situation, when there is a perturbation of osmotic equilibrium across the membrane, the pressure gradient is inside the AQP and there is none in the lipid; in the turgid equilibrium situation the pressure gradient is in the lipid and there is none inside the AQP. The result is the same, however: an asymmetric strain is created along the pore axis and the mechanism leading to changes in the T-R ratio of the tetramer described above is set in train. The pressures inside cells with relatively inelastic walls can be very large, in excess of 1 MPa.

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