3

Cell Water Relations

INTRODUCTION

Cells are the basic structural units of organisms, and plant organization varies from single cells to aggregations of cells to complex multicellular structures. With increasing complexity there are increasingly sophisticated systems for absorbing water, moving it large distances, and conserving it but fundamentally the cell remains the central unit that controls the plant response to water. The driving forces for water movement are generated in the cells, and growth and metabolism occur in the aqueous medium provided by the cells. The cell properties can change and result in acclimation to the water environment. As a consequence, many features of complex multicellular plants can be understood only from a knowledge of the cell properties. This chapter is concerned with those properties and how they are measured. Later chapters will consider the whole organism more fully and will use the principles described here for the cells.

STRUCTURE

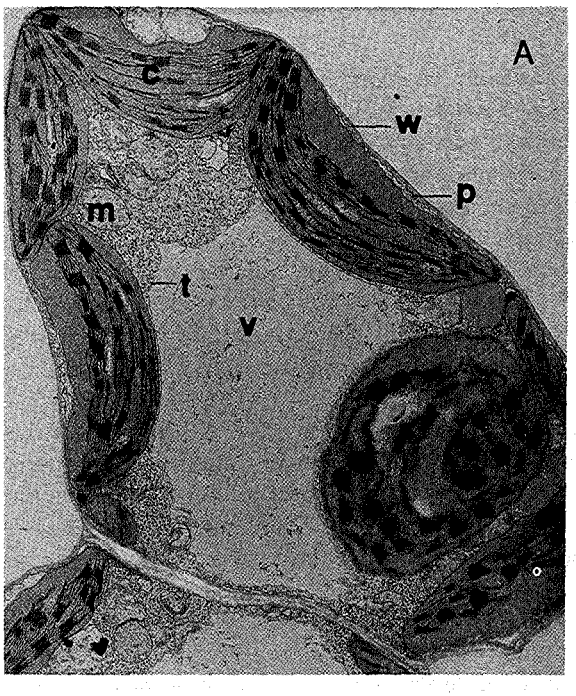
The plant cell consists of a multicompartmented cytoplasm bounded on the outside by a membrane and cell wall (Fig. 3.1A). There usually is a dilute solution on the outside, but in some instances there may be a moderately concentrated solution as in seawater or around embryonic cells. On the inside, there always is a concentrated solution that contains metabolites, inorganic salts, and macromolecules in varying concentrations, depending on the location.

The membrane bounding the outside of the cytoplasm is the plasmalemma which is highly permeable to water but only slowly and selectively permeable to solutes. The cell wall outside the plasmalemma is porous and permits water and solutes of low molecular weight to move rapidly to and from the plasmalemma. Inside the cytoplasm, there are compartments or organelles such as the vacuole, mitochondria, nucleus, and plastids, each bounded by a membrane similar to the plasmalemma and capable of exchanging water and solute with the surrounding cytosol. Each organelle contains its own unique composition of solute. The plasmalemma is thus the primary barrier controlling the molecular traffic into and out of the cell, but the cell wall and internal membranes also play a role.

The high concentration of solute inside the cell dilutes the internal water compared to that outside and water enters in response, causing the cell to swell. The plasmalemma has insufficient strength to resist the swelling but it is supported by the structurally tough and often rigid wall, which resists enlargement. As a result, the swelling causes the wall to stretch and become turgid, and turgor pressure develops inside the cell. Without the wall, the plasmalemma would rupture but with the support of the wall, the plasmalemma is pressed tightly against the wall microstructure (Figs. 3.2A and 3.2B). The wall sometimes can stretch by a considerable amount, and the membrane inside must be capable of stretching as well. Stretching of the wall will be treated in detail later but it is worth pointing out that the resistance to stretch gives structural rigidity that contributes to the form and strength of tissues. Much of the form of leaves and stems of herbaceous plants results from the turgor pressure developing in their cells.

Cells lose water when solute concentrations are high outside or when evaporation occurs, and they shrink as the volume of water decreases inside (Fig. 3.1B). The membranes cannot resist the shrinkage, and the organelles become distorted when dehydration is severe (Fig. 3.1B). The cell wall often develops folds as the cell shrinks (Fig. 3.1B), and the folding deforms the adjacent plasmalemma. In some cells, the walls are stiffened by the deposition of layers of rigid wall material and folding does not occur. In such cells, the wall resists shrinkage and the cell contents may come under tension (Boyer, 1995).

The primary walls develop in young cells and are composite porous structures consisting of cellulose microfibrils embedded in a matrix of related oligosaccharides and some structural proteins (Fig. 3.2). The microfibrils contain clusters of crystalline cellulose totaling about 10 nm in diameter. They provide much of the tensile strength of the wall. The matrix binds the microfibrils and holds them in an organized fashion. The orientation of the microfibrils may control cell growth by restricting enlargement to particular directions. The matrix probably affects the rate at which enlargement occurs (see Chapter 11). As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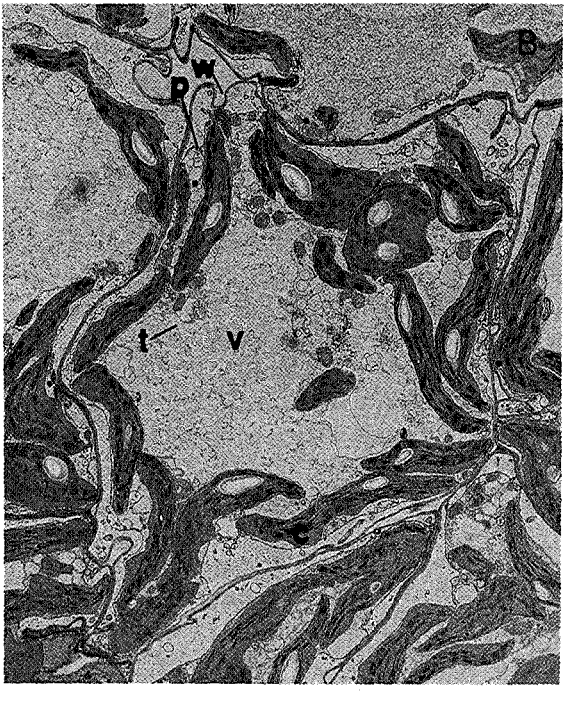
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the cell ages, growth stops and additional layers of wall are often deposited on the inside of the primary wall. These secondary wall layers may contain lignins, suberins, and other compounds that give the wall special characteristics of rigidity, imperviousness to water, and so on. The secondary walls account for most of the properties of different woods, tree bark, nutshells, and other specialized plant parts.

There are two kinds of pores in the wall. A few large pores, the plasmodesmata, are filled with protoplasm and lined with the plasmalemma (Fig. 3.2A). These pores connect the protoplasts of adjacent cells and probably transmit water and solutes directly between the protoplasts. The second kind of pore is much smaller and more numerous (Fig. 3.2B) and is not filled with protoplasm but instead is filled with the solution contacting the cell exterior. This type of pore is distributed throughout the wall and has a diameter variously estimated to be 4.0 to 6.5 nm (Baron-Epel *et al.*, 1988; Carpita, 1982; Carpita *et al.*, 1979; Tepfer and Taylor, 1981). It freely transmits water with its diameter of only about 0.4 nm, sugars and amino acids with their diameters of 1 to 1.5 nm, and smaller proteins, but the passage of molecules with weights larger than about 60,000 D (diameters larger than 8.5 nm) is generally blocked. The plasmalemma crosses the ends of these pores and is unsupported there, but it is 4.5 to 25 nm thick and thus can support itself over the small diameter of these pores (Fig. 3.2B).

Because small solutes can pass readily through the small wall pores, the solutes can move to the surface of the plasmalemma where they are selectively transported into the cell. The uptake often requires metabolic energy and, once inside, additional metabolic activity may modify them or they may be further transported into the vacuole or other organelles of the cell. Macromolecules generally do not account for much of the internal solute because they are present in comparatively small concentrations. For example, proteins typically exist in micromolar concentrations whereas the small metabolites and inorganic ions have concentrations totaling 0.5 to 1 molal in the cytoplasm. Nevertheless, many of the macromolecules are enzymes or nucleic acids that regulate the metabolites and the properties of the membranes, as well as the nature of the cell

Figure 3.1 Structure of a typical plant cell. (A) Mesophyll cell of a sunflower leaf having a high water potential (−0.44 MPa) and relative water content (99%). Cell wall (w), chloroplast (c), plasmalemma (p), mitochondrion (m), vacuole (v), and vacuolar membrane (tonoplast, t). Magnification, 6300×. (B) Same as in (A) but having a low water potential (−2.11 MPa) and relative water content (35%). Note shrunken vacuole, folded cell wall, and contorted chloroplasts in this cell. In some cells, there was evidence of plasmalemma and/or tonoplast breakage, and loss of cell contents. Magnification, 3800×. In order to preserve cell structure in these micrographs, the osmotic potential of the fixative was adjusted to equal the water potential of the cells (see Appendix 3.1). Adapted from Fellows and Boyer (1978).

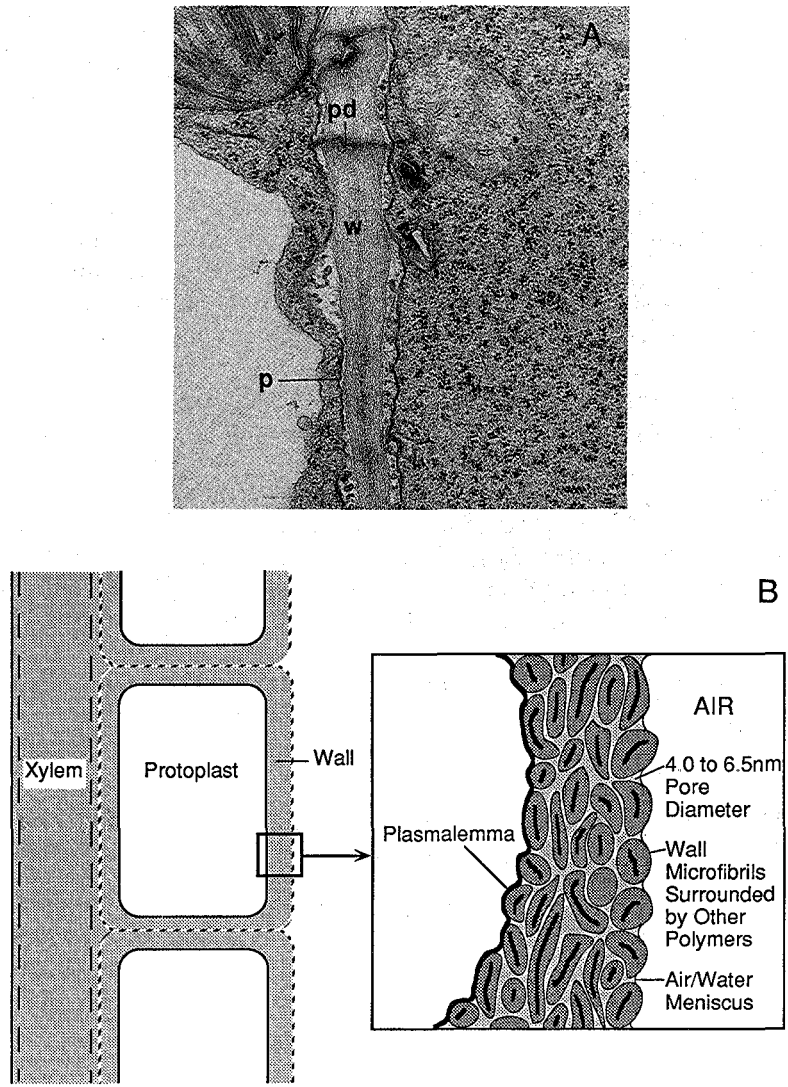

Figure 3.2 (A) Enlarged view of the cell wall (w) and plasmalemma (p) of a mesophyll cell in a sunflower leaf (37,400×). Note the plasmodesma (pd) extending through the wall to form a symplast between the adjacent protoplasts (R. J. Fellows and J. S. Boyer, unpublished). The microfibrillar structure of the wall is also apparent. (B) Diagrammatic representation of the apoplast (shaded). The microfibrillar structure of the wall is shown in the enlarged inset together with the air/water menisci between the microfibrils and matrix polymers. Not shown are cross links between the polymers. The plasmalemma is pressed against the wall substructure by the pressure inside and tension outside. The tension in the wall passes into the xylem through the 4 to 6.5 nm pores distributed throughout the wall.

walls. As a consequence, the water relations of the cell are set in motion by the macromolecules but water is affected most immediately by the small solutes and membranes.

OSMOSIS

Osmosis is the net flow of water across a differentially permeable membrane separating two solutions of differing solute concentration (also see Chapter 2). This situation occurs commonly in plant cells because of the differences in solute concentrations across the plasmalemma. The solute difference inevitably causes a corresponding but opposite difference in water concentration. Since water can cross the membrane but the solute cannot, more water molecules move toward the side with the lower water concentration than in the opposite direction. Without a compensating flow of solute, this net flow causes water to be transferred toward the side with lower water concentration and enlarges the volume on that side.

The solute concentration inside plant cells is typically 0.5 to 1 molal greater than outside, causing a strong tendency for water to enter. The resulting increase in volume of the inner solution is opposed by the resistance of the wall to stretching. Turgor pressure develops inside and can increase until it completely opposes the osmotic force causing water to enter (see Chapter 2). For a concentration of 1 molal inside the cell and 0 molal outside, the pressure calculated from the van't Hoff relation is 2.27 MPa at 273 K and 2.47 MPa at 298 K (see Chapter 2). Thus, the pressure inside equals the osmotic pressure and in this instance is about 10 times the pressure in an automobile tire!

This example is essentially that of an ideal osmometer when pure water is on one side of the membrane and a solution on the other (see Fig. 2.11). Note that the pressure is the same as is developed by 1 mol of an ideal gas (2.27 MPa at 273 K and 2.47 MPa at room temperature of 298 K). Thus, the osmotic pressure is numerically equal to the pressure calculated for an ideal gas but the mechanism is entirely different. Mainly the analogy with the gas gives us a convenient way to remember how the osmotic pressure is related to solute concentration.

Although the pressure can be large inside cells, in most circumstances it does not achieve the theoretical osmotic pressure of the cell solution for several reasons. First, the water outside normally is not pure but contains solute that reduces the internal pressure needed for balance. These concentrations in multicellular plants are in the range of 10 to 20 millimolal with few exceptions (Boyer, 1967a; Jachetta *et al.*, 1986; Klepper and Kaufmann, 1966; Nonami and Boyer, 1987; Scholander *et al.*, 1964, 1965, 1966). Second, tensions often are present in the solution outside because of the porous structure of the wall (see Appendix 2.3). These can be considerable and further diminish the pressures for balance inside. Finally, in growing cells the wall enlarges and it appears

that this can prevent the internal pressure from developing fully (see Chapter 11).

Together these effects cause the turgor pressure to vary in cells, sometimes rapidly and in large amounts, although the osmotic potential of the cell solution is relatively stable. Some confusion exists on this point because some authors use the term osmotic pressure to mean the osmotic potential of the solution (Nobel, 1974, 1983, 1991; Slatyer, 1967; Steudle, 1989). The osmotic potential is a solution property regardless of whether membranes or pressures are present but osmotic pressure depends on the presence of differentially permeable membranes and is a pressure. It is readily apparent that the osmotic pressure is more closely related to the turgor pressure than to the osmotic potential and it seems most appropriate to use the term potential to refer to the osmotic property of solutions, as Gibbs (1931) originally did (see also Chapter 2).

Typically, osmotic potentials are uniform throughout the cell. The internal compartments are bounded by membranes of negligible strength, and an increase in solute concentration in them is immediately followed by water entry from the surrounding cytosol. The compartment swells until it re-equilibrates with the cytosol. An example is the large central vacuole. In young cells, this organelle has negligible volume and most of the cell compartment is filled with cytosol containing other organelles. As the cell grows, the vacuole enlarges as it accumulates salts and some metabolites that act as reserves. A few enzymes and secondary products of metabolism also may be found in it. In response to the accumulating solute, water enters and keeps the vacuole in osmotic balance with the peripheral cytoplasm. The vacuole eventually becomes so large that it is the dominant organelle in the protoplast (Fig. 3.1A).

Osmotic balance among cells probably is enhanced by the plasmodesmata, and cells in tissues tend to behave osmotically as though there is one highly interconnected protoplasm (Fig. 3.2A). The plasmodesmatal pores are large enough in diameter to allow small solutes and even some macromolecules to pass with water so that concentration differences generally remain moderate between the cells. The plasmalemma lining the pore is continuous with the plasmalemma of the adjacent cells. Thus, it is possible for most cells in a uniform tissue to be surrounded by one continuous plasmalemma and to act as a unit, the symplast. The cell wall surrounding the symplast is termed the apoplast (Fig. 3.2B). The xylem also is part of the apoplast. For reviews of plasmodesmata and symplast function, see Lucas *et al.* (1993), Olesen and Robards (1990), and Robards and Lucas (1990).

Osmotic balance becomes more difficult when plants are subjected to dehydration or high salinity. Because water is lost but not solute, the concentration of many cell constituents increases. Cell structures are distorted (Fig. 3.1B) and the plasmalemma and vacuolar membrane (tonoplast) can break or become leaky. Fellows and Boyer (1978) observed breakage of these membranes and

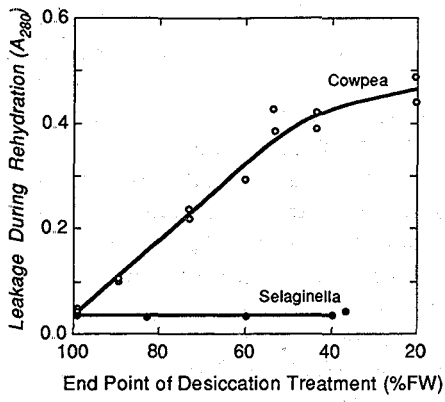

Figure 3.3 Leakage of proteins from leaf cells that had been desiccated to varying degrees and rehydrated. Proteins were detected in the rehydrating solution by measuring the absorbancy of the solution at 280 μm (A_{280}) after 20 min. Desiccation-sensitive cowpea showed a large leakage but desiccation-tolerant *Selaginella* did not. Adapted from Leopold *et al.* (1981).

loss of the cell contents. To make these measurements, special precautions were essential to preserve cell structure in the electron microscope; interested readers can find them detailed in Appendix 3.1. Leopold *et al.* (1981) showed that a species such as cowpea, which is unable to tolerate desiccation, loses cell contents to the external medium (Fig. 3.3) but a desiccation-tolerant species did not show this loss (Fig. 3.3, *Selaginella*). This suggests that desiccation tolerance may be determined at least in part by membrane properties that decrease leakage or disruption. Crowe *et al.* (1984, 1986, 1987, 1988), Crowe and Crowe (1986), Caffrey *et al.* (1988), Koster and Leopold (1988), and Madin and Crowe (1975) propose that membranes are protected by high concentrations of certain sugars such as sucrose and trehalose whose hydrogen bonding with the membrane is sterically similar to that of water. Accordingly, the bonding holds membrane constituents in an ordered fashion resembling that in water, protecting the membrane. Williams and Leopold (1989) found that certain sugars enter a glassy, candy-like state at low water contents and suggest that this could further protect the molecular structure of desiccated membranes.

WATER STATUS

It is apparent that osmosis is the central process that moves water into and through plants and that the plasmalemma is the key to the process. Indeed, if the plasmalemma is disrupted by external factors (e.g., freezing and thawing or chemical agents), water transport is abruptly diminished and the plant rapidly desiccates to the air-dry state despite the presence of concentrated solutions in

the cells. Osmosis brings about water absorption that normally maintains cell water content but the osmotic conditions vary in and around cells and it is desirable to have some way to measure their water status. As pointed out in Chapter 2, the water status is most usefully characterized in terms of the chemical potential as defined by J. Willard Gibbs (1875–1876, 1931) who applied it to membrane systems and porous media. His concepts provided much of the foundation for physical chemistry and solution thermodynamics and thus to cells. Slatyer and Taylor (1960) proposed practical expressions for the chemical potential of water in plants and soils, which gave considerable impetus to adoption of the Gibbs concepts.

The main advantage is that the water status is based on a physically defined reference rather than a biological one. This avoids some of the variation inherent in biological systems and allows the water status to be reproduced at any time or place, a great advantage for experimentation. In addition, described in this way the water status indicates the force that moves water from place to place. This permits water movement to be predicted and resistances to movement to be measured. When expressed in pressure units, the potential is termed the water potential (see Chapter 2).

The water potential is determined by several components important for cells and their surroundings. The components originate from the effects of solute, pressure, solids (especially porous solids), and gravity on the cell water potential. We will follow the practice of Gibbs (1931) and consider solutes to be all dissolved molecules whether they are aggregated or not as long as they do not precipitate, pressures to be from external forces, porous solids to cause surface effects that differ from those in the bulk medium, and gravity to be important in vertical water columns. Accordingly, the components are expressed as

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m + \Psi_g, \tag{3.1}$$

where the subscripts s, p, m, and g represent the effects of solute, pressure, porous matrices, and gravity, respectively. Each potential refers to the same point in the solution, and each component is additive algebraically according to whether it increases (positive) or decreases (negative) the Ψ_w at that point compared to the reference potential. The reference potential is pure, free water at atmospheric pressure and a defined gravitational position, at the same temperature as the system of interest.

The components affect Ψ_w in specific ways. Solute lowers the chemical potential of water by diluting the water and decreasing the number of water molecules able to move compared to the reference, pure water. In a similar way, matrices that are wettable have surface attractions that decrease the number of water molecules able to move (see Appendix 2.3). External pressure above atmospheric increases the ability of water to move but below atmospheric decreases it. Gravity similarly increases or decreases the ability of water to move depend-

ing on whether local pressure is increased or decreased by the weight of water. Pressures are high at the bottom of the ocean and tensions can develop in siphons for this reason.

In dealing with cells, gravitational potentials often can be ignored because they become significant only at heights greater than 1 m in vertical water columns, as in trees. In this case, Eq. (3.1) becomes

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m. \tag{3.2}$$

The presence of the interior and exterior of the plasmalemma in single cells and the symplast and apoplast in tissues means that Eq. (3.2) cannot be applied to cells without some consideration of structure. At its simplest, the cell consists of two compartments: the protoplast or symplast inside and the external solution or apoplast outside (Fig. 3.2). Equation (3.2) is then applied to each compartment. The protoplast contains a solution under pressure (turgor) applied by the walls. The protoplast water potential is then

$$\Psi_{w(p)} = \Psi_{s(p)} + \Psi_{p(p)}, \tag{3.3}$$

where the subscript (p) denotes the protoplast compartment. We can ignore Ψ_m because the water content generally is high and there are no air–water interfaces (Fig. 3.2).

The apoplast contains a solution in the porous cell wall subjected to local pressures generated by surface effects of the wall matrix (Fig. 3.2 and also see Appendix 2.3). The apoplast water potential is

$$\Psi_{w(a)} = \Psi_{s(a)} + \Psi_{m(a)}, \tag{3.4}$$

where the subscript (a) denotes the apoplast compartment. We can ignore Ψ_p because the external pressure is atmospheric. Figures 3.4A and 3.4B are diagrams of the potentials showing that there is a concentrated solution ($\Psi_{s(p)}$) and a turgor ($\Psi_{p(p)}$) in the protoplast but a dilute solution ($\Psi_{s(a)}$) and a matric potential ($\Psi_{m(a)}$) in the apoplast (Boyer, 1967b). The water potential is the algebraic sum of the component potentials with due regard for positive or negative quantities indicating whether the component increases or decreases the potential. In the example of Fig. 3.4A, the cell having a $\Psi_{s(p)}$ of -0.9 MPa and a $\Psi_{p(p)}$ of 0.3 MPa would have a $\Psi_{w(p)}$ of -0.6 MPa ($= (-0.9) + (+0.3)$).

In a unicellular marine alga, water surrounds the cell and saturates the porous cell wall. In this situation, the matric component can be ignored and the water potential in the apoplast is simply

$$\Psi_{w(a)} = \Psi_{s(a)}. \tag{3.5}$$

Water moves readily into and out of cells (see later) according to the water potential differences between the protoplast and apoplast compartments. The water potentials need not differ much across membranes to create large flows

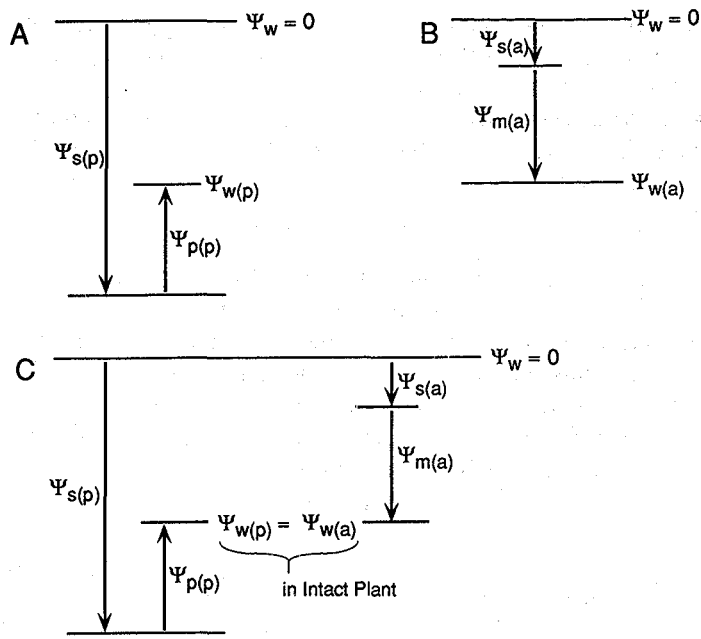

Figure 3.4 Water potentials in plant cells with component potentials shown by arrows (decreasing potentials are downward pointing, increasing potentials are upward pointing). The water potential of zero is shown by upper horizontal bar. (A) Protoplast (symplast) water potential consisting of the osmotic potential ($\Psi_{s(p)}$) and the turgor pressure ($\Psi_{p(p)}$), (B) cell wall (apoplast) water potential consisting of the osmotic potential ($\Psi_{s(a)}$) and matric potential ($\Psi_{m(a)}$), (C) equilibrium between the protoplast and apoplast water potentials. Note that the difference in osmotic potential is large across the plasmalemma ($\Psi_{s(p)} - \Psi_{s(a)}$). Also, the matric potential consists mostly of tension (negative pressure) in the pores of the apoplast. Therefore, the pressure difference across the plasmalemma also is large ($\Psi_{p(p)} - \Psi_{m(a)}$). At equilibrium, the difference in osmotic potential ($\Psi_{s(p)} - \Psi_{s(a)}$) equals the difference in pressure ($\Psi_{p(p)} - \Psi_{m(a)}$).

(see later). For flows commonly present, water potential differences across membranes are so small that a near equilibrium (local equilibrium) exists between the protoplast and its cell wall (Molz and Ferrier, 1982; Molz and Ikenberry, 1974). As a consequence, it is assumed that

$$\Psi_{w(a)} = \Psi_{w(p)} \tag{3.6}$$

in many situations, and substituting Eqs. (3.3) and (3.4) in Eq. (3.6) gives

$$\Psi_{s(a)} + \Psi_{m(a)} = \Psi_{s(p)} + \Psi_{p(p)}, \tag{3.7}$$

which shows that the components of the water potential in the protoplasts are balanced by the components in the apoplast at equilibrium. This result, shown in Fig. 3.4C, indicates that there is a large difference in the solute concentration

across the membrane with the inside being much more concentrated. Also, the turgor in the cells is positive ($\Psi_{p(p)}$) but the water in the apoplast is under tension ($\Psi_{m(a)}$) in a multicellular plant. This causes a large pressure difference across the plasmalemma. Were it not for the restraining effect of the wall, the plasmalemma would burst.

Measuring Water Status

These water potentials can be measured with a thermocouple psychrometer that detects the vapor pressure of water because there is a relationship between vapor pressure and potential (Chapter 2). A sample of cells of unknown potential is sealed into a chamber containing a droplet of solution of known vapor pressure (Fig. 3.5A). The apparatus is surrounded by a heat sink and insulation in order to keep temperatures uniform. If evaporation occurs from the water in the solution, it is detected as a cooling of the solution by using a thermocouple. The solution can be replaced by another until one is found whose vapor pressure is the same as that of the water in the cells. In this case, the droplet is neither cooled by evaporation nor warmed by condensation and equilibrium exists. The solution is isopiestic (equal in vapor pressure) with the sample and it has the same water potential (Boyer and Knipling, 1965). Since the water potential of the solution is known, the water potential of the tissue is then known.

The psychrometer measures the water potential in the cell walls because the water surface of the sample is located there and the vapor pressure develops there. The water potential of the walls is the same as the protoplasts (Fig. 3.4) and thus the potential applies to the entire cell. Figure 3.5B shows the water potential of some cells and tissues measured with this technique. The water potential of pollen is always lower than in the stigmas (silks) or leaves of the same maize plants, and it decreases through the day. The mature pollen is not attached to the vascular supply of the plant and readily dehydrates. The leaves and silks are supplied with water and do not dehydrate as much.

It is also possible to measure the osmotic potential in the apoplast by applying pressure to the cells to force water from the protoplasts into the apoplast. With tissues, a pressure chamber (Scholander *et al.*, 1965) can apply the pressure as shown in Fig. 3.6A, displacing the original wall solution into the xylem from which it exudes onto the cut surface of the xylem. The exudate is collected and its osmotic potential is measured in the thermocouple psychrometer to obtain $\Psi_{s(a)}$ of Eq. (3.4) (Fig. 3.6B). The pressure P_{gas} necessary to displace the water gives $\Psi_{m(a)}$ because it opposes the tensions pulling water into the wall pores. Thus, $-P_{gas} = \Psi_{m(a)}$ (Fig. 3.6B). The water potential measured in the wall with the psychrometer ($\Psi_{m(a)}$) can then be checked by these two additional potentials ($\Psi_{s(a)} + \Psi_{m(a)}$) according to Eq. (3.4) (Boyer, 1967a).

For the protoplast compartment, the osmotic potential can be measured by

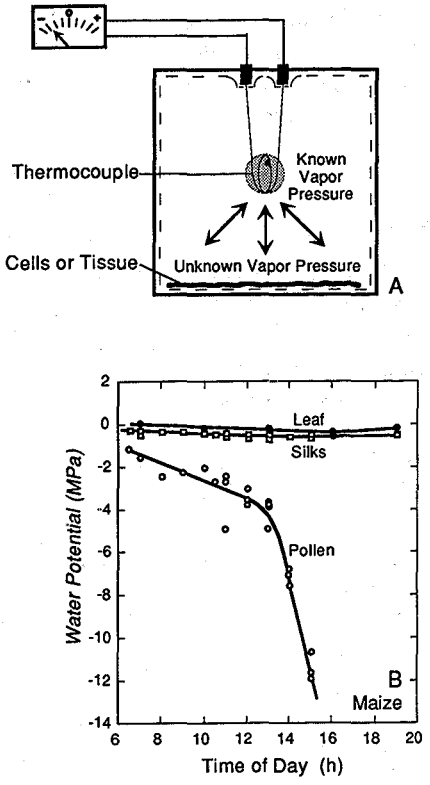

Figure 3.5 Thermocouple psychrometer (A) and measurements of water potentials in cells and tissues of maize made with a psychrometer (B). The droplet of known vapor pressure on the thermocouple can exchange water with the unknown sample on the bottom of the chamber and thus cool or warm the thermocouple. The solution neither cooling nor warming the thermocouple has a vapor pressure (water potential) equal to that of the sample. Since the solution water potential is known and is the same as that of the sample, the sample water potential is then known. The measurement is in the apoplast in equilibrium with the protoplasts. Typical measurements in maize (B) show that the water potential decreased only slightly during the day in the plant, but decreased markedly in pollen grains collected at various times from the same plant. The leaves and stigmas (silks) were connected through the xylem to the water supply in the soil but the mature pollen was not. Adapted from Westgate and Boyer (1986a).

applying pressure to cells that have been frozen and thawed to break the plasmalemma (Ehlig, 1962). The pressure removes the cell solution and the osmotic potential is measured with a psychrometer to give $\Psi_{s(p)}$ of Eq. (3.3). Since $\Psi_{w(p)}$ is known from the just-mentioned measurements in the apoplast, the turgor pressure $\Psi_{p(p)}$ can be calculated from Eq. (3.3). If necessary, the osmotic potential of the solution can be corrected for the effect of mixing with solution in the apoplast by noting the volumes of the wall and protoplast solutions and assuming complete mixing (Boyer, 1995; Boyer and Potter, 1973).

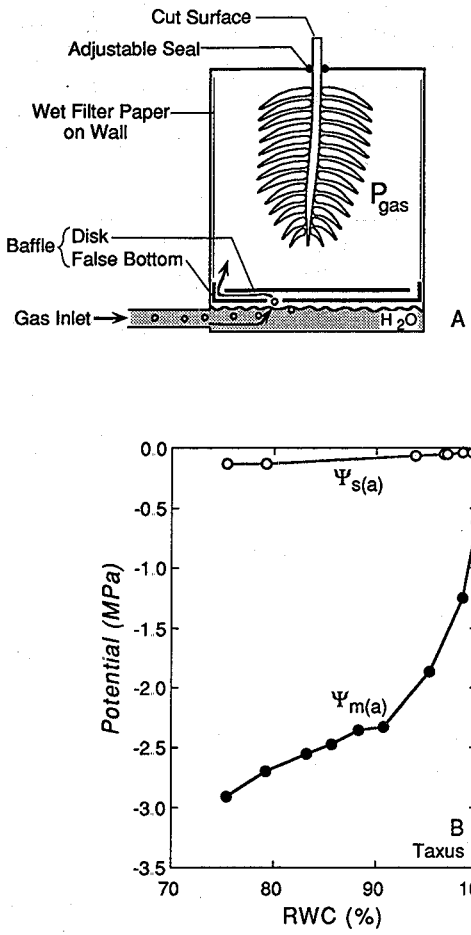

Figure 3.6 Pressure chamber (A) and measurements of the pressure in the apoplast of plant tissues using the pressure chamber (B). The incoming gas is humidified by bubbling through water, and the external pressure increases until it forces the xylem solution onto the cut surface. The pressure is adjusted to maintain the solution at the surface with no flow into or out of the tissue. This balancing pressure (P_{gas}) measures the internal pressure (tension $\Psi_{m(a)}$) on the apoplast solution according to $-P_{gas} = \Psi_{m(a)}$. In (B), the tension becomes more negative as the relative water content (RWC) decreases in the tissue (*Taxus* branch), indicating that a greater pull is being exerted by the leaves on the water in the xylem. Also shown is the osmotic potential of the apoplast solution ($\Psi_{s(a)}$) measured on xylem solution from the same *Taxus* branch. Note that $\Psi_{s(a)}$ is a small component at all RWC. The water potential of the apoplast solution is ($\Psi_{m(a)} + \Psi_{s(a)}$). Adapted from Boyer (1967b).

The $\Psi_{p(p)}$ can be checked by measuring it directly with a pressure probe (Fig. 3.7A, Hüsken *et al.*, 1978). The probe has a microcapillary whose tip can be inserted into a cell. Using a metal rod controlled by a micrometer screw, the pressure on oil in the microcapillary can be changed until the cell solution is

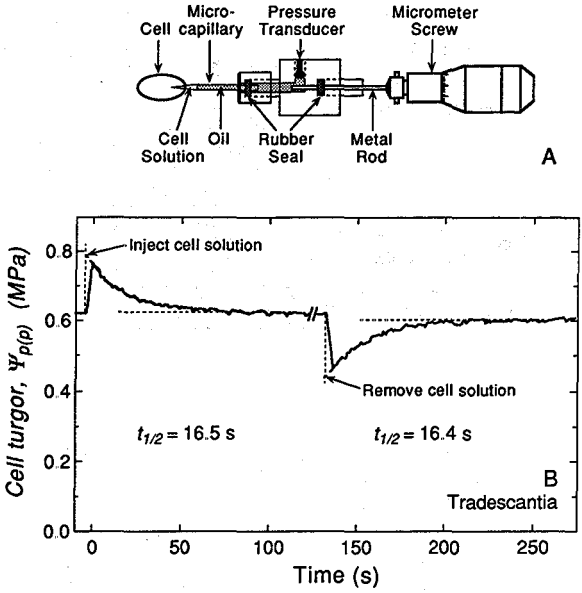

Figure 3.7 Pressure probe (A) for measuring and changing the turgor pressure inside plant cells (B). The probe is mostly filled with silicone oil (shaded), and a meniscus is visible between the cell solution and the oil in the tip of the microcapillary. When there is liquid continuity between the cell and the microcapillary, the pressure in the cell extends into the microcapillary and is sensed by the pressure transducer. The accurate measurement of cell turgor requires the meniscus to be returned to the position prior to entering the cell. Turning the micrometer screw forces the metal rod into the oil and moves the meniscus by changing the internal volume. The volume change causes the pressure to change as solution is injected into or removed from the cell (B) in a *Tradescantia* leaf. The volume of solution removed from or injected into the cell is determined from the distance the meniscus moves and the diameter of the microcapillary. Adapted from Tyerman and Steudle (1982).

returned to the original position close to the cell. The pressure inside the probe is then the same as the turgor in the cell and is measured with a pressure transducer (Fig. 3.7A).

With these methods, all of the potentials in Eqs. (3.6) and (3.7) can be measured. The methods give similar results when they are compared (Boyer, 1967a; Murphy and Smith, 1994; Nonami and Boyer, 1987, 1989, 1993; Nonami *et al.*, 1987) and can be used with a wide range of tissues. The psychrometer also can measure the water potential of soil. Boyer (1995) gives a detailed description of these methods.

In the plant cell, the protoplast and apoplast measurements are straightforward but require us to distinguish between pressures of different origins. Some authors (e.g., Nobel, 1974, 1983; Passioura, 1980b; Steudle, 1989) combine pressures such as those arising from turgor or matric potentials regardless of origin. However, matric effects are not totally explained by pressures (see Ap-

pendix 2.3). Dehydrated matrices may contain so little liquid that local pressures on the liquid molecules are meaningless. In plants, these conditions occur in desiccated seeds, dry pollen, and various tissues of desiccation-tolerant plants. They also occur in porous media such as soil, wood, or paper. Therefore, it is important to distinguish between matric potentials and external pressures and this practice is followed in this book.

Negative potentials are common in nature because water often contains solutes or is held in a matrix. To move into a cell, the potential inside the cell must be even more negative. Depending on the system, the driving force may be some component of Ψ_w or the total Ψ_w. In a cell containing viable membranes, the force usually is the difference in water potential across the plasmalemma, but not all systems contain differentially permeable membranes that can harness the osmotic potential. In soil, water moves mostly because of matric force, and solutes have little effect. Similarly in the xylem, membranes are absent at maturity and water moves because of pressure differences developed by the surrounding cells. Thus, although water always moves toward the more negative potential, the critical potential depends on the physical system. Consideration of the system often can indicate what component potentials are important.

MECHANISM OF OSMOSIS

One of the most interesting aspects of osmosis is that solutes and pressures cause equivalent flows through plant membranes. It is not intuitive why this should occur but the effect can be plainly seen with a pressure probe for single cells. Figure 3.7B shows that a pressure probe can first inject a cell solution, then remove it. When the cell solution is injected, the turgor increases above that for balancing the osmotic potential and water is driven out of the cell by the extra pressure. When the pressure is reduced and the cell solution is removed, the turgor falls below the balance point and water enters because of the excess osmotic potential. The rate (half-time $t_{1/2}$) is the same for the outward pressure-driven and inward solute-driven flows although they are opposite in direction (Fig. 3.7B).

This behavior was addressed by Ray (1960) who proposed that biological membranes contain pores inside of which pressures exist that drive water through the membranes. He reasoned that experiments had shown that osmosis could occur faster than water could diffuse across the membrane and thus water-filled pores must exist in the membrane. He also recognized that if the membrane excludes solute from the pores there must be pressures in the pores. These were simplifications because membranes transport solute at low rates, often by active processes. However, once inside the cell, the solutes do not readily leak out and he reasoned that the slow rates and lack of leakage indicated that the solutes likely were in different channels and could be ignored. His

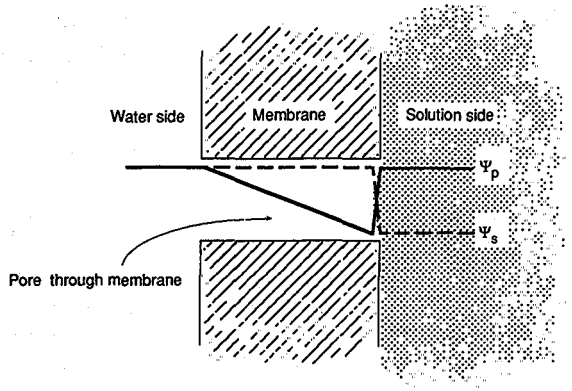

Figure 3.8 Osmotic flow through plant membranes according to Ray (1960). The osmotic potential (Ψ_s) undergoes an abrupt decrease at the pore entrance on the solution side of the membrane because no solute enters the pores. The pressure (Ψ_p) is kept at atmospheric on both sides. Because there is only water in the pore, Ray (1960) proposed that a pressure gradient exists inside the pore when osmotic flow occurs. The pressure decreases toward the solution side and the flow is driven by this pressure gradient. Adapted from Ray (1960).

concept is illustrated in Fig. 3.8 where a membrane separates a concentrated solution from pure water. The solution ends abruptly at the solution face of the membrane because solute cannot enter the pore. Water extends into the membrane pore. There is a jump downward in osmotic potential at the solution side of the membrane. A compensating pressure jump exists inside the pore to match the jump in osmotic potential at the solution side (Fig. 3.8). Because the external pressures are the same on both sides of the membrane, flow is driven by the pressure gradient in the pore.

This elegant logic received experimental support from Robbins and Mauro (1960) who used artificial membranes to measure osmotically driven flow through artificial membranes with a range of water conductances. Water was labeled with deuterium and supplied to one side to allow water diffusion to be measured. At conductances in the range for plant cells, diffusion was only a minor component of the total flow, and bulk flow predominated. This indicated that the membrane contained water-filled pores.

The presence of pressures in the pores was demonstrated by Mauro (1965) by enclosing the water on the water side of the membrane in a rigid compartment. As water moved through the membrane to the outer solution, the pressure decreased in the compartment. The pressure dropped until it prevented water from entering the membrane pores. Mauro (1965) reasoned that, in this equilibrium state, the pressure would be the same everywhere in the water. Since the water extended into the membrane pores from the water side, the pressure must also be the same inside the pores. Mauro (1965) found that large tensions developed inside the rigid container and thus in the pores (Fig. 3.9A).

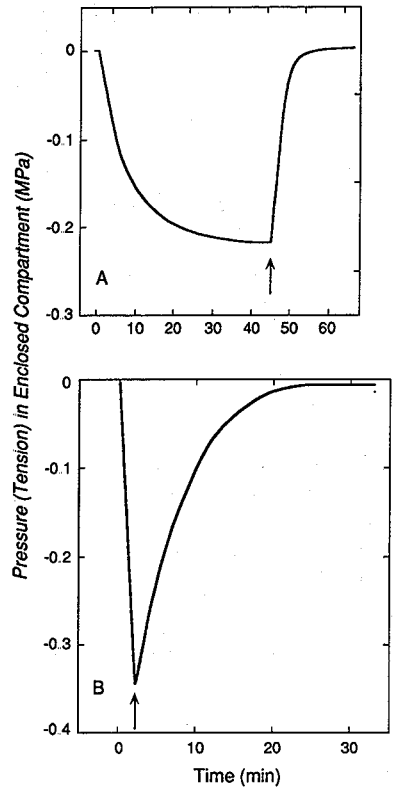

Figure 3.9 Demonstration of pressure gradient in membrane pores. The system is the same as in Fig. 3.8 except that the pressure is measured in a rigid compartment enclosing the water on the water side of the membrane (left side of Fig. 3.8). (A) As water flows into solution on the other side of the membrane, pressure in the rigid compartment falls until flow stops. The negative pressure (tension) in the compartment is the same everywhere including the membrane pores and becomes equal to the osmotic potential (−0.21 MPa) on the solution side (right side of Fig. 3.8). The solution was replaced with water at the arrow. (B) Large tensions can form rapidly in the membrane pores when a concentrated solution is present on the other side of the membrane and flow is occurring. The solution is removed at the arrow. In these graphs, zero pressure is atmospheric. After Mauro (1965).

The existence of negative pressures in the pores of membranes indicates that tensions arise in the plasmalemma and can be transmitted to various places in plants (e.g., the xylem and apoplast) much as they were transmitted to the rigid container of Mauro (1965). The pores must be very small in diameter so water is retained even under large tensions. Large tensions and rapid water movement were seen by Mauro (1965) as shown in Fig. 3.9B. In this way, the osmotic force is developed by the solute at the inner face of the plasmalemma where the pores contact the cell solution, and the force is transmitted nearly instantaneously

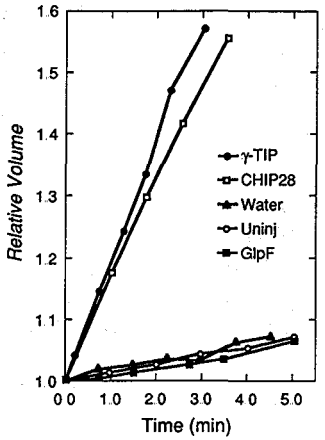

Figure 3.10 Volumes during osmotic swelling of frog (*Xenopus*) oocytes that had overproduced water channel proteins for the tonoplast membrane for 72 hr (γ-TIP) or that had the normal complement of water channel proteins (uninjected or water injected). The γ-TIP increased water transport. Also shown is the effect of a plasmalemma water channel protein from humans (CHIP28) which also increased water transport and a glycerol transport protein from bacteria called glycerol facilitator (GlpF) which did not transport water. The cells were injected individually with messenger RNA for one of the proteins and the mRNA was translated for 72 hr during which the protein was accumulated in the plasmalemma. The cells then were transferred to a dilute medium and osmotic swelling occurred as shown. Faster swelling indicates a more conductive plasmalemma. Adapted from Maurel *et al.* (1993).

as a tension through the membranes to the cell wall pores and apoplast and throughout the plant. On land, the tension can extend out of the plant and into the soil.

There is increasing evidence that special proteins form the water transport pores in plant and animal membranes. Maurel *et al.* (1993) injected messenger RNA (mRNA) for one of the plant membrane proteins (γ-TIP, tonoplast intrinsic protein) into *Xenopus* (frog) oocytes. After enough time for the oocyte to make protein and for the protein to incorporate into the plasmalemma, the conductivity of the membranes increased markedly for water (Fig. 3.10). When mRNA for the water transporting plasmalemma protein CHIP28 was injected into oocytes, there was a similar effect (Fig. 3.10, see Preston *et al.*, 1992). A membrane protein for glycerol transport (GlpF) did not have an effect on water transport (Fig. 3.10). Guerrero *et al.* (1990) and Ludevid *et al.* (1992) found evidence for variation in the amount of water transport proteins in plant membranes. Chrispeels and Maurel (1994) have also reviewed this area.

These demonstrations of protein channels in the plasmalemma and tonoplast verify the concepts of Ray (1960) that water moves primarily through membranes by bulk pressure-driven flow and explains why the flows are so fast,

reversible, and affected by pressure and solute in an equivalent manner. The membrane pores appear to be discrete structures in the membrane. As a consequence, we should not expect diffusion to play much part, and diffusion experiments with labeled water will not give an accurate view of how water moves through a membrane. In the latter case, the water moves slowly by diffusion along concentration gradients, and pressure does not change the diffusion direction in contrast to the behavior actually observed with cells.

The presence of water-transmitting pores implies that water transport should vary according to the number of pores present in the membranes. Transport also might be affected by the kinds of pores or regulatory properties of the pores. Nevertheless, at equilibrium where there is no net water flow, the water status would not be altered by the number or nature of the pores. Changes in water status would occur rapidly or slowly depending on the number and size of pores but the equilibrium finally achieved would be the same.

CHANGES IN WATER STATUS

When a cell is dehydrated, its water potential decreases because the cell contents become more concentrated and there is a smaller volume of water to extend the walls. These changes can be represented by

$$d\Psi_{w(p)} = d\Psi_{s(p)} + d\Psi_{p(p)}, \tag{3.8}$$

which shows that the change in water potential is simply the sum of the changes in the osmotic potential and the turgor pressure. This equation does not indicate the rate of change but only the size of the change between the two equilibrium states.

It is useful to know which component causes the most change in the water potential. The answer is simplest if the solute content of the cell remains constant and the $d\Psi_{w(p)}$ is caused only by water. In that case, the change $d\Psi_{s(p)}$ is proportional to the fractional change in water content dV/V (see Appendix 3.2):

$$d\Psi_{s(p)} = -\Psi_{s(p)}\frac{dV}{V}. \tag{3.9}$$

Similarly, the change $d\Psi_{p(p)}$ can be found from the tensile properties of the cell wall. These properties are described by the bulk modulus of elasticity ϵ (MPa) that relates the internal pressure to the fractional change in water content of the cell:

$$d\Psi_{p(p)} = \epsilon\frac{dV}{V}. \tag{3.10}$$

Equations (3.9) and (3.10) have a similar form and show that the effect of a

change in water content depends on whether $\Psi_{s(p)}$ or ϵ is numerically larger: the larger the $\Psi_{s(p)}$ or ϵ the larger the effect of dV/V on $d\Psi_{s(p)}$ or $d\Psi_{p(p)}$.

Substituting Eqs. (3.9) and (3.10) in Eq. (3.8) gives the total effect on the water potential

$$d\Psi_{w(p)} = -\Psi_{s(p)}\frac{dV}{V} + \epsilon\frac{dV}{V}, \tag{3.11}$$

which we can rearrange to give

$$\frac{dV}{d\Psi_{w(p)}} = \frac{V}{\epsilon - \Psi_{s(p)}}, \tag{3.12}$$

which has been called the capacitance C of the cell (Molz and Ferrier, 1982; Steudle, 1989). This is a useful expression for predicting how much the cell must dehydrate to cause a change in the water potential and also how much of the change is caused by $\Psi_{s(p)}$ or ϵ. Thus, for a cell with $\Psi_{s(p)}$ of -1 MPa and a rigid wall having ϵ of 49 MPa, the ϵ is numerically larger than $\Psi_{s(p)}$ and dehydration will cause mostly a turgor change. Equation (3.12) indicates that a decrease in water content of 2% ($dV/V = 0.02$) causes the turgor to decrease enough to decrease water potential 1 MPa in such a cell. On the other hand, the same cell with an elastic wall having ϵ of 4.9 MPa will still be dominated by the effects of turgor but the water potential decreases only 0.12 MPa for the same dehydration. Clearly, changes in water potential are caused more by changes in turgor than by changes in osmotic potential and are larger when the wall is rigid than when it is elastic.

This conclusion holds whenever there is turgor in a cell and can be demonstrated with a pressure chamber. The pressure is raised around a leaf until it is overpressured and water exudes. The new balancing pressure is measured at the new water content. A comparison of a rhododendron leaf having relatively rigid cell walls (ϵ = 97 MPa) and a sunflower leaf having relatively elastic walls (ϵ = 6.4 MPa) shows that the water potential decreases much more in rhododendron than in sunflower when water is lost from the leaves (Fig. 3.11). The larger decrease in rhododendron allows water to be extracted from the soil with only a slight dehydration of the leaf whereas sunflower requires a large dehydration before it can exert the same force on the soil water. Expressed in terms of the capacitance [Eq. (3.12)], rhododendron leaves having high water contents need to change only 1% in water content per MPa change in water potential whereas the sunflower leaves must change 13%.

Thus cells are affected by water exchange with their surroundings, and the cell water potential changes more dramatically when the wall is more rigid. Plants like rhododendron with evergreen leaves may encounter soils with little water or with frozen water during part of the year, and its rigid walls ensure that large force can be applied to extract water from the soil without excessive

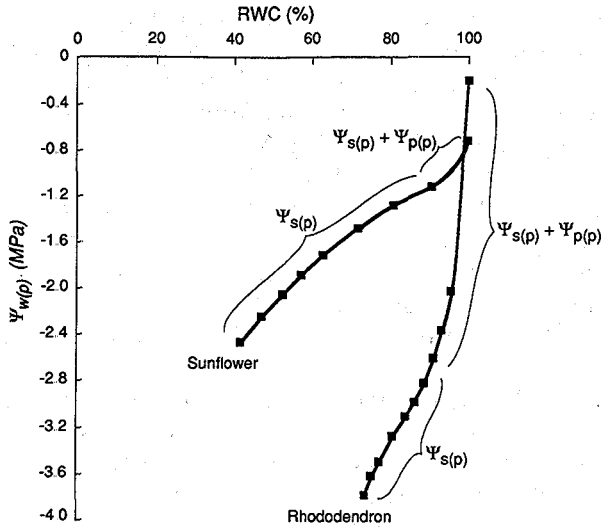

Figure 3.11 Water potential ($\Psi_{w(p)}$) at various relative water contents (RWC) in sunflower and rhododendron leaves measured with a pressure chamber. Both species show a greater decrease in $\Psi_{w(p)}$ when turgor is present ($\Psi_{s(p)} + \Psi_{p(p)}$) than when it is absent ($\Psi_{s(p)}$). However, rhododendron with thick relatively nonelastic cell walls (ϵ = 97 MPa) shows a greater decrease than sunflower with thin elastic walls (ϵ = 6.4 MPa). This results in very low $\Psi_{w(p)}$ in rhododendron with only moderate dehydration compared to sunflower. The Ψ_s was -2.6 and -1.1 MPa in hydrated rhododendron and sunflower respectively. Using ϵ, Ψ_s, and the change in RWC, the capacitance for water can be calculated for these tissues [Eq. (3.12)]. From the calculation, a decrease of 1 MPa in water potential from the fully hydrated state required a 1% decrease in RWC in rhododendron but a 13% decrease in sunflower. From J. S. Boyer (unpublished data).

leaf dehydration. The capacitance of the cells is an important physiological and ecological property.

WATER TRANSPORT

When a potential difference exists across the plasmalemma, the cell changes in water content at a rate determined by the conductivity of the plasmalemma and the size of the potential difference. The pore structure of the plasmalemma probably contributes to the conductivity and the potentials are determined not only by the external conditions but also by the turgor pressure and osmotic potential of the cell. Using the potentials of Eqs. (3.3) and (3.4) for the protoplast and apoplast, the water movement can be described by the transport equation

$$J_v = Lp(\Psi_{m(a)} - \Psi_{p(p)} + \sigma(\Psi_{s(a)} - \Psi_{s(p)})), \qquad (3.13)$$

where J_v is the steady rate at which volume crosses the membrane per unit of membrane area ($m^3 \cdot m^{-2} \cdot sec^{-1}$), Lp is the hydraulic conductivity of the membrane ($m \cdot sec^{-1} \cdot MPa^{-1}$), $(\Psi_{m(a)} - \Psi_{p(p)})$ is the pressure difference across the membrane (the matric potential on the outside minus the turgor pressure on the inside of the membrane in MPa, see Fig. 3.4C), $(\Psi_{s(a)} - \Psi_{s(p)})$ is the osmotic potential difference across the membrane (MPa, see Fig. 3.4C), and σ is the reflection coefficient of the membrane (dimensionless, see Appendix 2.2). The Lp indicates the frictional effects encountered by water as it crosses the membrane. A larger Lp shows that water more easily crosses the membrane. According to Table 3.1, Lp ranges between 10^{-6} and 10^{-8} $m \cdot sec^{-1} \cdot MPA^{-1}$ for plant cells. The range of values suggests that the plasmalemma can vary in conductivity.

For most cells and most internal solutes, there also is solute transport across the plasmalemma. Active metabolism usually is required and there is a negligibly small permeability for the passive movement of the solute. The net movement is independent of the movement of water and is much slower. Therefore, for the solutes normally present inside a cell, the plasmalemma can be considered to be an ideal differentially permeable membrane with a reflection coefficient of essentially 1, and the hydraulic conductivity can be considered to be almost entirely for water with little effect of solute transport. Table 3.2 shows that measured values for σ are near 1 for most solutes inside the cell, confirming that the plasmalemma behaves ideally. Under these conditions, Eq. (3.13) becomes simply

$$J_v = Lp(\Delta\Psi_w), \tag{3.14}$$

and water is driven across the plasmalemma by the water potential difference ($\Delta\Psi_w$) between the two sides.

In special situations, this simplification may not hold. Lipophilic solutes that are small molecules such as ethanol or isopropanol have a σ around 0.2 (Table 3.2). Other solutes can alter membrane properties and cause σ to be less than 1 in which case internal solute may leak out. Cells that are suddenly subjected to high concentrations of solutes may shrink enough to cause the plasmalemma to separate from the cell wall (plasmolysis) and disrupt the plasmodesmata. In these situations, it cannot be assumed that $\sigma = 1$.

Significance of Reflection Coefficients

If σ is less than 1, water is not the only molecule crossing the membrane, and Lp also includes the movement of some solute. The solute tends to move in a direction opposite to that of water. While the permeability of the membrane for solute can be described by a solute permeability coefficient analogous to the hydraulic conductivity, the reflection coefficient is not a permeability coefficient

but is a key parameter in Eq. (3.13) because it determines how much of the osmotic potential is used in water transport (see Appendix 2.2). When σ is less than 1, the osmotic potential is similarly less than fully effective.

Measuring the osmotic potential inside and outside of cells does not give the reflection coefficient of the membrane and thus does not indicate how much of the measured potential is contributing to the flow. Great care must be taken when placing high concentrations of solute outside of cells for this reason. Depending on how much solute enters the cell, the osmotic effect of the solute can vary dramatically. Moreover, because the reflection coefficient describes a condition of the membrane, its effects are always present and cannot be avoided by making rapid measurements or allowing only small water flows. For this reason, osmotica generally do not simulate the natural dehydration of cells and are no longer used for accurate measurements of cell water status.

The reflection coefficient for a solute can be most simply measured by determining the change in cell water potential that is caused by the solute. In the equilibrium state,

$$\sigma = \frac{d\Psi_w}{d\Psi_s}, \tag{3.15}$$

which indicates that solute supplied externally to change Ψ_s by 1 MPa will change the Ψ_w internally by 1 MPa when $\sigma = 1$. Figure 3.12 shows this kind of measurement using a pressure probe and indicates that the plasmalemma of epidermal cells of *Tradescantia* leaves had $\sigma = 1$ for sucrose but less than 1 for ethanol (Tyerman and Steudle, 1982). When $\sigma = 1$ as for sucrose, the sucrose remained outside and only water moved across the plasmalemma to give a simple shrinkage of the cell (Fig. 3.12A, left). When σ was less than 1 as for ethanol, the shrinkage was less than for sucrose even though the concentration of ethanol was greater. This indicates that the osmotic effectiveness of the ethanol was less than that of sucrose. Because the ethanol could enter the cell, Fig. 3.12 shows that the cell contracted initially as water left the cell but later swelled as ethanol entered. This two-phase contraction followed by swelling is diagnostic for a σ less than 1.

Equation (3.15) has been used to measure reflection coefficients around 1 (e.g., Tyerman and Steudle, 1982) but, for σ less than 1, the two-phase behavior of the cell causes experimental difficulties. As Tyerman and Steudle (1982) point out, permeating solutes can be dragged along by the water moving through the membrane and swept away from the membrane surface. Unstirred layers of water and solute exist next to the membrane and these can limit solute and water transfer. The results depend on how fast the solute penetrates the membrane. Thus, a σ below 1 clearly indicates that the membrane is nonideal but the actual value of σ is usually approximate.

Table 3.1 Half-time of Water Exchange ($t_{1/2}$, Hydraulic Conductivity (Lp), and Tissue Diffusivity for Water (D) in Cells as Determined from Pressure Probe Experiments

Species	Tissue/cell type	Half-time, $t_{1/2}$ (sec)	Hydraulic conductivity, Lp ($m \cdot sec^{-1} \cdot MPa^{-1}$)	Diffusivity, D ($m^2 \cdot sec^{-1}$)	Reference
Chara corallina	Internode cells	1.3–7.5	$(0.8–1.4) \times 10^{-6}$	—	*a*
Capsicum annuum	Mesophyll of fruit tissue	65–250	$(4–6) \times 10^{-8}$	$(3–6) \times 10^{-11}$	*b*
	Subepidermal bladder cells of inner pericarp of fruit	1–12	$(2–17) \times 10^{-6}$	—	*c*
	Tissue cells of inner pericarp	18–54	$(1.2–3.4) \times 10^{-7}$	—	*c*
Tradescantia virginiana	Leaf epidermis	1–35	$(0.2–11) \times 10^{-7}$	$(0.2–6) \times 10^{-10}$	*d,e,f*
	Subsidiary cells	3–34	$(2–35) \times 10^{-8}$	$10^{-11}–10^{-10}$	
	Mesophyll cells	55–95	$(4–6) \times 10^{-8}$	1×10^{-12}	
	Isolated epidermis	9–54	6×10^{-8}	$(0.5–3) \times 10^{-11}$	
Kalanchoe daigremontiana	CAM tissue of the leaf	2–9	$(0.2–1.6) \times 10^{-6}$	6×10^{-10}	*g*
Pisum sativum	Growing epicotyl	1–27 (epidermis)	$(0.2–2) \times 10^{-7}$	—	*h,i*
		0.3–1 (cortex)	$(0.4–9) \times 10^{-6}$	3.2×10^{-10}	
Glycine max	Growing hypocotyl	0.3–5.2 (epidermis)	$(0.7–17) \times 10^{-6}$	$(1–9) \times 10^{-11}$	*j*
		0.4–15.1 (cortex)	$(0.2–10) \times 10^{-6}$	$(1–55) \times 10^{-11}$	
Zea mays	Midrib tissue of leaf	1–8	$(0.3–2.5) \times 10^{-6}$	$(0.4–6.1) \times 10^{-10}$	*k*
Oxalis carnosa	Epidermal bladder cells	22–213 (adaxial)	4×10^{-7}	—	*l*
		7–38 (abaxial)	2×10^{-6}	—	
Mesembryanthemum crystallinum	Epidermal bladder cells	200–2000	2×10^{-7}	—	*m*
Salix exigua	Sieve elements of isolated bark strips	110–480	5×10^{-9} (lateral hydraulic conductivity)	—	*n*

Hordeum distichon	Root cortex and rhizodermis	1–21	1.2×10^{-7}	$(0.5–9.5) \times 10^{-11}$ (cortex) $(1–7) \times 10^{-12}$ (rhizodermis)	o
Triticum aestivum	Root hairs, rhizodermis, cortex	8–12	1.2×10^{-7}	—	*p,q*
Z. mays	Root cortex	1–28	$(0.5–9) \times 10^{-7}$	$(2–53) \times 10^{-12}$	*r*
	Root cortex, rhizodermis	—	1.2×10^{-7}	—	*q*
Phaseolus coccineus	Root cortex	0.4–2.3	2×10^{-6}	$(0.3–1.7) \times 10^{-10}$	*s*

Note. the diffusivity D refers to cell transport only.
[a]Steudle and Tyerman (1983).
[b]Hüsken *et al.* (1978).
[c]Rygol and Lüttge (1983).
[d]Tomos *et al.* (1981).
[e]Tyerman and Steudle (1982).
[f]Zimmerman *et al.* (1980).
[g]Steudle *et al.* (1980).
[h]Cosgrove and Cleland (1983b)
[i]Cosgrove and Steudle (1981).
[j]Steudle and Boyer (1985).
[k]Westgate and Steudle (1985).
[l]Steudle *et al.* (1983).
[m]Steudle *et al.* (1975).
[n]Wright and Fisher (1983).
[o]Steudle and Jeschke (1983).
[p]Jones *et al.* (1983).
[q]Jones *et al.* (1988b).
[r]Steudle *et al.* (1987).
[s]Steudle and Brinckmann (1989).

Table 3.2 Reflection Coefficients (σ) of Plant Cell Membranes for Some Nonelectrolytes

Solute	Reflection coefficients			
	Chara corallina[a]	*C. corallina*[b]	*Nitella flexilis*[c]	*Tradescantia virginiana*[d]
Sucrose	0.95	—	0.97	1.04
Mannitol	1.02	—	—	1.06
Urea	—	1	0.91	1.06
Acetamide	—	—	0.91	1.02
Formamide	0.99	1	0.79	0.99
Dimethylformamide	0.76	—	—	—
Glycerol	—	—	0.80	0.93
Ethylene glycol	—	1	0.94	0.99
n-Butanol	0.14	—	—	—
Isobutanol (2-methyl-l-propanol)	0.21	—	—	—
n-Propanol	0.24	0.22	0.17	−0.58
2-Propanol	0.45	—	0.35	0.26
Ethanol	0.40	0.27	0.34	0.25
Methanol	0.38	0.30	0.31	0.15
Acetone	0.17	—	—	—

[a] Steudle and Tyerman (1983).
[b] Dainty and Ginzberg (1964).
[c] Steudle and Zimmermann (1974).
[d] Tyerman and Steudle (1982).

These examples illustrate the central role of the plasmalemma and its reflection coefficient in the water relations of cells. Water moves at high rates because the plasmalemma allows water to pass readily, and osmotic force is generated by solutes because of the ideal nature of the membrane for the solutes normally in the cell. Without the plasmalemma, the osmotic potential could not be harnessed and water generally would not move rapidly enough to maintain cell hydration.

RATES OF DEHYDRATION AND REHYDRATION

The ease of water movement across the plasmalemma determines how readily cells dehydrate and rehydrate. Hydration changes are frequently seen in cells as algae encounter varying salinities or land plants experience evaporation (transpiration). The rate of dehydration depends on whether a water supply is present or a protective barrier exists to inhibit water loss and also on how fast individual cells lose water. Thus, the rates of dehydration at the cell level are of considerable interest.

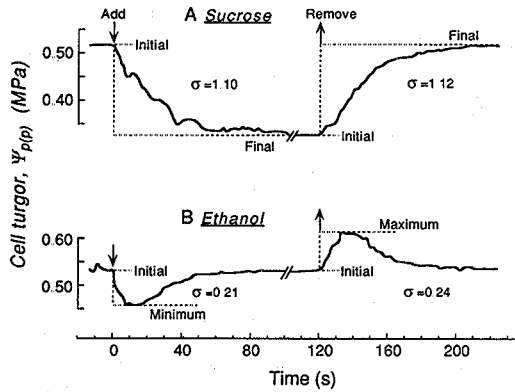

Figure 3.12 Plasmalemma reflection coefficients measured with a pressure probe in leaf epidermal cells of *Tradescantia*. The pressure probe measured the change in cell $\Psi_{p(p)}$. (A) Sucrose having Ψ_s of -0.18 MPa was added to the medium bathing the epidermis and caused water to move out. The turgor decreased by an amount that essentially equaled the Ψ_s of the sucrose, thus giving a reflection coefficient of about $-0.18/-0.18 = 1$ [see Eq. (3.15)]. At the upward arrow, the sucrose was removed and the pattern reversed as water moved in. When the reflection coefficient is 1 as for sucrose, the response is monophasic because only water moves. (B) Ethanol having Ψ_s of about -0.37 MPa caused turgor to decrease about 0.08 MPa to give a reflection coefficient of about $-0.08/-0.37 = 0.2$. Note that the ethanol caused a biphasic response. In the first phase, water moved outward and the cell shrank. In the second phase, ethanol entered and the cell swelled. At the upward arrow, the ethanol was removed and this pattern was reversed. Adapted from Tyerman and Steudle (1982).

The membrane properties in Eq. (3.14) affect the rate of dehydration, and the volume of water lost or more precisely the capacitance in Eq. (3.12) also contributes. By substituting Eq. (3.12) into Eq. (3.14), all of these factors can be combined (Appendix 3.3) for any small change in cell water potential

$$t_{1/2} = \frac{0.693V}{LpA(\epsilon - \Psi_s)} = 0.693rC, \tag{3.16}$$

where A is the surface area of the cell (m^2), r is the frictional resistance to water movement through the plasmalemma ($1/LpA$), and $t_{1/2}$ is the time for half the change in water potential.

Equation (3.16) shows that the cell acts much like an electrical circuit with a resistance and capacitance in series. The resistance r is mostly determined by the plasmalemma and controls how fast water enters the cell. The capacitance C [Eq. (3.12)] is determined by the size of the cell, the elasticity of its wall, and the internal osmotic potential, and these control how fast the potential changes for a unit change in the volume of water. The rate of dehydration is the product of the resistance and capacitance, and an increase in either resistance or capacitance makes the dehydration slower.

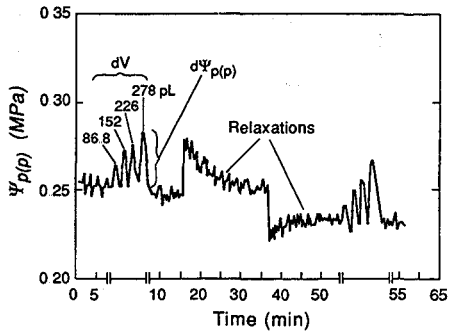

Figure 3.13 Pressure–volume relations measured with a pressure probe when a cell solution is rapidly injected and rapidly removed (left part of trace), injected and allowed to flow naturally out of the cell (relaxation in middle), or removed and allowed to flow into the cell (relaxation on right). The measurements were made in individual cells of a pepper fruit and changes in pressure ($d\Psi_{p(p)}$) and volume (dV) were used to calculate the bulk elastic modulus of the cell wall as described in the text. The relaxations on the right part of the trace measured flow through the plasmalemma and were used to calculate the hydraulic conductivity as described in the text. The small oscillations in the trace were generated to ensure that the microcapillary remained unplugged. Adapted from Hüsken *et al.* (1978).

Figure 3.7 shows the kind of measurement that can be used to determine $t_{1/2}$. A pressure probe injects or removes cell solution and changes the water potential because the turgor changes. Water leaves or enters the cell in response, and the $t_{1/2}$ is a measure of how fast dehydration or rehydration can occur.

From the $t_{1/2}$, Eq. (3.16) can be used to measure Lp as described by Steudle (1989). The method can be most simply explained by considering each term together with the measurement procedures, as shown in Fig. 3.13. The pressure probe is used to raise and lower the turgor rapidly (Fig. 3.13, left) to determine ϵ. By noting the pressure and volume of solution injected into and removed from the cell, the dP and dV are measured and ϵ can be calculated according to Eq. (3.10) (the volume V is determined from the cell dimensions). For the cell in Fig. 3.13, $d\Psi_{p(p)}/dV$ was 1.1×10^{11} MPa·m^{-3} and V was 11×10^{-12} m^3 so that ϵ was 1.2 MPa. On the other hand, if the pressure is raised and water is allowed to move out of the cell at its own pace, the $t_{1/2}$ can be measured (Fig. 3.13, relaxations) and was about 300 sec. The Ψ_s can be measured with extracts of cell solution and was -0.25 MPa. The A can be determined from the cell dimensions and was 2.97×10^{-7} m^2. The Lp can then be calculated from Eq. (3.16) to give 5.8×10^{-8} m·sec^{-1} · MPa^{-1}, which is within the range of values in Table 3.1.

This method of determining Lp, although it requires many measurements, is basically quite rapid and involves observing cell behavior for only short times. Therefore, cell properties should be quite stable while the measurements are

being performed. The only disruptive influence is the insertion of the tip of the microcapillary into the cell. This probably causes little effect because other methods that do not penetrate the cell give similar values of *Lp* (Green *et al.*, 1979; Kamiya and Tazawa, 1956; Levitt *et al.*, 1936).

In general, the *Lp* measured for plant cells show that rapid water transport occurs across the plasmalemma for small potential differences. Small cells typical of many tissues tend to have water potentials similar to those of nearby cells. Even so, the plasmalemma conductivities vary by over 100-fold (Table 3.1) and thus the plasmalemma must differ widely in its properties depending on the type of cell. Because the rate of water transport is large, the rates of hydration and dehydration tend to be rapid for plant cells. The $t_{1/2}$ are only rarely more than 5 min and then only in cells of rather large dimensions (Table 3.1). As a consequence, the rate of dehydration of plant cells depends to a large extent on other features of the plant in addition to the plasmalemma. Waxy barriers on cell surfaces can decrease evaporation, extensive connections with the soil can supply water, and so on. Also, metabolic activities within the cell can lead to changes in internal solute concentration that delay or prevent dehydration.

OSMOTIC ADJUSTMENT

Changes in the internal solute concentration will occur whenever the water content changes during hydration/dehydration or the solute content changes inside the cell. Changes in water content are passive responses resulting from absorption or loss of water and they dilute or concentrate the solute. Changes in the solute content generally result from metabolic activity and are not passive. Because the solute changes represent a change in solute content per cell and are under the regulatory control of the cell, they are termed osmotic adjustment (Bernstein, 1961). The passive responses are not actively regulated and probably should be unnamed (Munns, 1988), although they are sometimes included with osmotic adjustment and the entire response called osmoregulation (Morgan, 1984). Initially, osmotic adjustment was thought to occur only in plants subjected to high salinities (Bernstein, 1961; Eaton, 1927, 1942; Munns, 1993) but it was later found in plants in drying soils (Meyer and Boyer, 1972) and much work was done to determine the effect on plant growth. Morgan (1984) and Munns (1988) provide useful reviews of the area.

Osmotic adjustment provides a means of maintaining cell water content which is an important cell activity. Because water loss can increase the concentration of solute in the cell, molecules that regulate metabolism can be affected. Some inorganic ions such as K^+, Ca^{2+}, Mg^{2+}, and Cl^- cannot be metabolized or incorporated into cell structure significantly and they are inevitably concentrated by dehydration. Because they play regulatory roles for enzymes, enzyme activities can be affected. For example, photophosphorylation is inhibited by

Mg^{2+} concentrations slightly above the optimum of 1.5 to 3 m*M* (Pick and Bassilian, 1982; Rao *et al.,* 1987; Shahak, 1986; Younis *et al.,* 1983). Certain K^+-requiring enzymes also can be affected if K^+ concentrations become too high (Evans and Sorger, 1966; Evans and Wildes, 1971; Wilson and Evans, 1968). In addition to the concentrating effects of water loss, exposure of plants to high external salinities adds the extra problem of high concentrations of NaCl. Most enzymes are inhibited by high concentrations of NaCl even in halophytic plants (Flowers *et al.,* 1977; Wyn Jones, 1980).

Osmotic adjustment maintains cell water contents by increasing the osmotic force that can be exerted by cells on their surroundings and thus increasing water uptake. The adjustment results from compatible organic solutes accumulating in the cytoplasm which decreases the osmotic potential of the cytosol. Compatible solutes allow enzyme reactions to occur even though the solutes are in high concentration around the enzymes. Compatible solutes are sugars, glycerol, amino acids such as proline or glycinebetaine, sugar alcohols like mannitol, and other low molecular weight metabolites (Bental *et al.,* 1988b; Flowers *et al.,* 1977; Grumet and Hanson, 1986; Hanson and Hitz, 1982; Meyer and Boyer, 1981; Morgan, 1984; Munns *et al.,* 1979; Voetberg and Sharp, 1991; Wyn Jones, 1980). If large amounts of inorganic salts are present externally, they may be accumulated as well, but are stored in the vacuole which sequesters them and prevents high concentrations from occurring around cytoplasmic enzymes (Hajibagheri and Flowers, 1989). External salts used for osmotic adjustment decrease the amount of compatible solute that needs to be produced in the cytoplasm, and this keeps the energy requirement low.

Good examples of compatible solute production are seen in marine algae such as *Dunaliella* and *Oochromonas* that can withstand saturated solutions of NaCl (Bental *et al.,* 1988a,b; Kauss, 1983; Kauss and Thomson, 1982). A little NaCl enters the cells and is stored in the vacuoles (Hajibagheri *et al.,* 1986). However, the cells mostly produce large quantities of glycerol (*Dunaliella*) or galactosyl glycerol (*Oochromonas*) in the cytoplasm. The solutes are produced from reserves, mostly starch, and are returned to starch under favorable conditions (Gimmler and Möller, 1981). Figure 3.14 shows that the glycerol content nearly doubled in 4 hr in *Dunaliella* after the external salinity was increased to 3.0 *M*. There was a comparable depletion of starch. Thus, the solute was simply converted from an insoluble polymeric form to soluble small molecules. This allowed rapid osmotic adjustment and conserved carbon compounds inside the cells.

When dehydration occurs without high external salinities, similarly rapid increases in solute content can occur in cells. Typically, the growing tissues adjust throughout the plant when the soil dehydrates (Westgate and Boyer, 1985b) and concentrations of solutes can increase markedly in only a few hours. Figure 3.15B shows that cells in the growing regions of soybean stems increased in solute content sufficiently to decrease the osmotic potential by 85% in 12 hr

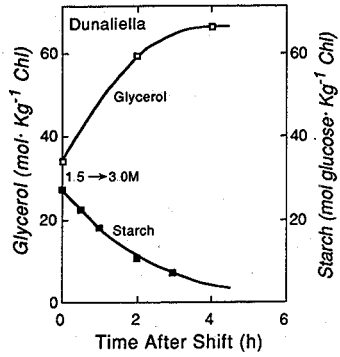

Figure 3.14 Osmotic adjustment in the marine alga *Dunaliella*. At zero time, the cells were shifted from a solution containing 1.5 *M* NaCl to a solution containing 3 *M* NaCl. The increase in cell glycerol came at the expense of cell starch (note that each glucose molecule released from starch produces two glycerol molecules). Adapted from Gimmler and Möller (1981).

after the roots were transplanted to dehydrated vermiculite (one-eighth of the water content of hydrated vermiculite). The accumulating solute was mostly glucose, fructose, sucrose, and amino acids (Meyer and Boyer, 1981). The cell water content changed only slightly (Fig. 3.15D) and turgor was maintained in these cells (Fig. 3.15C). Growth was inhibited but recovered somewhat after 48 hr (Fig. 3.15A). The mature tissues adjusted less osmotically and lost water as a result (Nonami and Boyer, 1989).

Thus, in both algae and land plants, salinity and dehydration cause metabolism to generate osmotica rapidly enough to keep pace with changes in external conditions. Most compatible solutes serve other functions in the cell and normally are produced in small quantities. Accumulation can occur simply by slowing their use in the normal reactions of the cell. In soybean stems growing with limited water, for example, Meyer and Boyer (1981) found that growth became slower and solute normally acting as substrate for growth was used less rapidly. With the slowdown in use, the solute accumulated. The unused solute accounted for most of the osmotic adjustment.

In roots, osmotic adjustment may have somewhat different origins. Sharp and his co-workers (1988, 1990) observed that maize roots decreased in diameter upon encountering dehydrated vermiculite. Fewer new cells were produced and they were smaller at maturity (Fraser *et al.*, 1990) which reduced the demand for imported solute. However, the thinner roots continued to extend at significant rates in conditions that completely eliminated stem growth.

Matyssek *et al.* (1991a,b) showed that the strong osmotic adjustment in growing regions could extract water from nearby mature tissues. Indeed, water moved backward from the shoot to the roots as the roots grew on water extracted from the mature stem (Matyssek *et al.*, 1991b). The use of internal

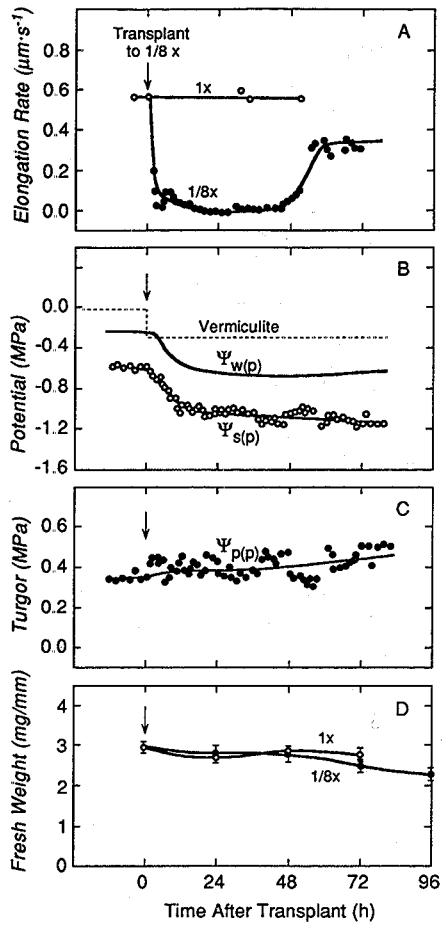

Figure 3.15 Osmotic adjustment in stems of soybean seedlings transplanted to vermiculite containing one-eighth of the water (1/8×) normally present in hydrated vermiculite (1×). (A) Stem elongation rates at 1/8× and 1×, (B) water potential of vermiculite (dashed line) and water potential ($\Psi_{w(p)}$) and osmotic potential ($\Psi_{s(p)}$) of the stem-elongating region in 1/8× plants, (C) turgor ($\Psi_{p(p)}$) of the stem-elongating region in 1/8× plants, (D) fresh weight of the stem-elongating region in 1× and 1/8× plants. Osmotic adjustment is seen in (B) as a decrease in $\Psi_{s(p)}$. The adjustment preserves turgor (C) and fresh weight (D) because the water content of the cells remains high. However, in mature cells of the stems of the same plants, osmotic adjustment was less and the turgor and water content decreased (after Bozarth *et al.*, 1987; Nonami and Boyer, 1989).

water to promote root growth is advantageous in dry soil and probably occurs frequently.

Osmotic adjustment solves several problems for cells (Morgan, 1984). Because compatible solutes accumulate in the cytoplasm, enzyme function is maintained. The water content of the cell remains high and regulatory ions do not

change concentrations. Turgor is maintained and allows a moderate amount of growth where none would occur otherwise (Meyer and Boyer, 1972; Michelena and Boyer, 1982). Continual root growth brings new water supplies to the plant (Matyssek *et al.*, 1991b; Sharp and Davies, 1985; Sharp *et al.*, 1988).

However, the process is limited by how much solute can be accumulated. Osmotic adjustment depends to a large extent on photosynthesis to supply compatible solute. As dehydration becomes severe, photosynthesis becomes inhibited. With a smaller solute supply, osmotic adjustment is curtailed. Thus, in the face of continued water limitation, osmotic adjustment delays but cannot completely prevent dehydration.

WATER RELATIONS OF CELLS IN TISSUES

Under laboratory conditions, cells may be easily subjected to large pressures or high concentrations of solute. In a tissue, however, these conditions are rare. A Ψ_w difference of 0.5 MPa might occur between leaves or perhaps across an individual leaf but almost never across a single cell. Generally, such a potential difference would be spread across at least 10 cells (20 cell wall/membrane layers) and there would be about 0.025 MPa between the inside and outside of each cell. While these small differences can drive water into the cell [see Eq. (3.14)], they are small enough so that the protoplasts in a tissue are almost always near water potential equilibrium with their own cell walls (Molz and Ikenberry, 1974; Molz *et al.*, 1979). This explains why it is possible to measure the water status of cells in a tissue by determining the vapor pressure of water in the cell walls and why cellular characteristics are distributed uniformly over considerable distances.

A cell in a tissue is not surrounded by unlimited water as an isolated cell would be when bathed in a solution. In a tissue, the cell obtains water mostly from the vascular tissue by way of other cells. Water flows through the apoplast and protoplasts or symplast (Fig. 3.16). The volume of the apoplast usually is

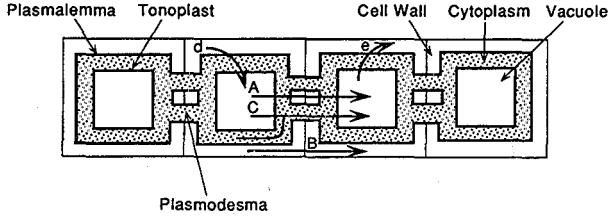

Figure 3.16 Diagrammatic representation of the water pathways in a tissue consisting of four cells. (A) Cell to cell path, (B) cell wall (apoplast) path, (C) cell to cell path through plasmodesmata (symplast), (d) water uptake by cells from apoplast, and (e) water loss by cells to apoplast. Plasmalemma lines plasmodesmata to form continuous membrane between cells. The dimensions of the various compartments are not to scale (after Molz and Ferrier, 1982).

small compared to the volume in the protoplasts or symplast. Changes in cell water potential are rapidly transmitted to the apoplast or through the symplast to affect surrounding cells. Groups of cells tend to act in concert with the immediately surrounding cells. As a consequence, gradients in Ψ_w are detectable only over distances of several cells.

In this situation, macroscopic principles involving groups of cells can be used to help understand cellular water transport. For a time in the early 1900s, water was considered to move from vacuole to vacuole in tissues (path A in Fig. 3.16), traversing the cytoplasm and membranes of each cell (Newman, 1974, 1976). Later, water was thought to move primarily in the porous cell walls (path B in Fig. 3.16), bypassing the cell membranes (Scott and Priestley, 1928). The discovery of plasmodesmata added still another pathway that allowed water to enter the first cell and then move from cell to cell without crossing another plasmalemma (path C in Fig. 3.16). In addition, water moves in and out of the protoplasts from the apoplast (paths d and e in Fig. 3.16).

Philip (1958a–d) was the first to construct a model of tissue water movement based on the characteristics of a group of cells. This seminal work has formed the basis for most of the treatments that followed, although his development did not consider reflection coefficients or water flow through the cell wall path. Molz and Hornberger (1973) extended Philip's theory to include the effects of the reflection coefficient, and Molz and Ikenberry (1974) included the cell wall path in parallel with the vacuole to vacuole path. Steudle and his co-workers conducted detailed studies of water transport in tissues using the miniature pressure probe (e.g., Steudle and Jeschke, 1983; Westgate and Steudle, 1985; Zhu and Steudle, 1991). However, no treatments have yet encompassed the plasmodesmata because their conductivity for water is not well understood (path C in Fig. 3.16). In theory, the plasmodesmata provide a means of connecting the protoplasm of adjacent cells to form a symplasm and, with a pulse of turgor from a pressure probe, one measures water transport out of the cell by both the plasmalemma and the plasmodesmata. Measurements of Lp for cells in a tissue thus include unknown contributions from the plasmodesmata.

Nevertheless, these efforts allow predictions of tissue behavior based on the characteristics of the cells, and some useful information has been obtained. In general, for mathematical purposes, the tissue is considered to be made up of infinitely small units (the cells) that collectively control the macroscopic behavior of the tissue, and equations having a form to describe diffusion are applied. The fundamental equation is

$$\frac{\partial \Psi}{\partial t} = D\frac{\partial^2 \Psi}{\partial x^2}, \tag{3.17}$$

where D is the diffusivity of water ($m^2 \cdot sec^{-1}$), t is the time, and x is the distance

for water movement. This relationship arises from a consideration of the rate of water uptake and loss by the cells and the rate of water storage by the cells applying the concept that the mass of water is conserved. It indicates that the rate at which the water potential changes in a tissue ($\partial\Psi/\partial t$) depends on the difference ($\partial/\partial x$) in the gradients of water potential ($\partial\Psi/\partial x$) along the diffusion path. The difference in the gradients is shown as ($\partial^2\Psi/\partial x^2$) in the equation. The D includes all the cell characteristics that control the development of potential gradients in the tissue.

The D can be determined by monitoring the rate of the change in water potential with time over known distances. Although the mathematics can become quite complex (Molz and Ferrier, 1982), the major goal is to compare individual cell rates with tissue rates of water transfer. If the cell rates are faster than the tissue rates, water movement is from cell to cell (paths A and C in Fig. 3.16). On the other hand, if the cell rates are the same as the tissue rates, all the cells hydrate virtually in unison at a rapid rate and water passes through a highly conductive path between them. This could occur only if water bypassed some of the protoplasts/symplast by way of the apoplast (path B in Fig. 3.16). Thus, cell/tissue kinetics can give information about which flow paths are operating in tissues.

For example, Westgate and Steudle (1985) compared the turgor relaxation of a maize leaf cell when solution was injected directly into the cell or when water was injected into the xylem (Fig. 3.17). The relaxation was much faster for water injected into the cell than into the xylem. Moreover, cells close to the xylem absorbed water faster than cells far from the xylem. This indicates that the cells were not connected to the xylem by a highly conductive path that could bypass the protoplasts. Rather, cells farther away from the xylem had to wait for water that flowed through cells nearer the xylem before they could hydrate fully. This excludes path B in Fig. 3.16 as the main flow path. Rather, water had to move primarily through the intervening cells by paths A and C with at most a modest contribution from B. It is worthwhile noting that the cell-to-cell path allows water to flow quite rapidly since the $t_{1/2}$ was only 15–18 sec (Fig. 3.17). This explains why wilted flowers recover so rapidly when their stems are cut under water and why whole plants regain turgidity soon after the soil is watered.

In several of these studies (Steudle and Boyer, 1985; Steudle and Jeschke, 1983; Westgate and Steudle, 1985; Zhu and Steudle, 1991), the tissue was pressurized on a cut surface. Steudle and Boyer (1985) pointed out the possibility of flooding the intercellular gas spaces in these kinds of experiments, which would make path B in Fig. 3.16 appear more conductive than it would ordinarily be. Therefore, methods need to be sought that do not have this complication, and studies of water transport using noninvasive methods employing nuclear magnetic resonance (Brown *et al.*, 1986, 1990; G. A. Johnson *et al.*, 1987; Kramer

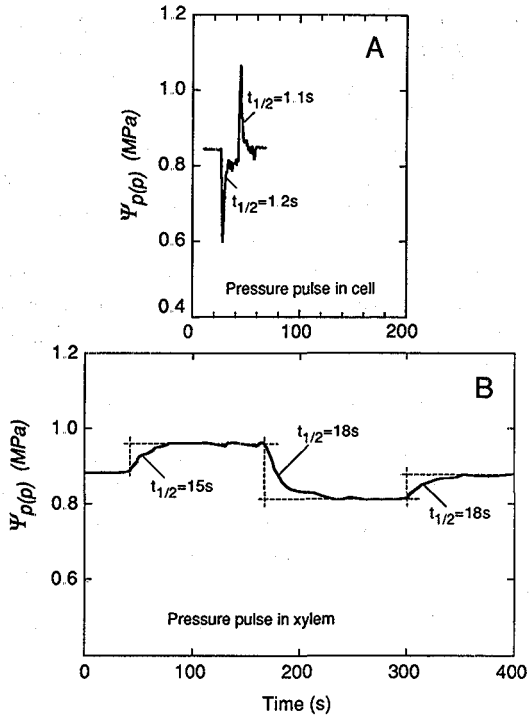

Figure 3.17 Turgor in maize cells situated in a leaf. (A) Turgor relaxation caused by a pressure pulse inside a cell using a pressure probe. (B) Turgor relaxation in the same cell when a pressure pulse was applied to water in the xylem. Relaxations are rapid in (A) where the path involves only the plasmalemma of the cell (d and e in Fig. 3.16) but slow in (B) where the path includes other cells. Note that if the apoplast was the main flow path between the cells, it would need to have a low resistance to flow. Water supplied by the xylem would be able to bypass intervening cells because of the low resistance and would reach each cell readily resulting in similar rates of relaxation in (A) and (B). However, because relaxations in (B) were much slower than in (A) for the same cell, the apoplast path was not the main contributor and water flowed mostly from cell to cell (paths A and C in Fig. 3.16). Adapted from Westgate and Steudle (1985).

et al. in Hashimoto *et al.*, 1990; MacFall *et al.*, 1990; Veres *et al.*, 1991) or mass spectrometry (Yakir *et al.*, 1989, 1990) look promising.

Molz and Boyer (1978) and Silk and Wagner (1980) used diffusion equations to model water movement in growing tissues in comparison with single cells. The models predicted local equilibrium in individual cells but significant water potential gradients over the whole growing tissue. Subsequently, measurements of the potentials of the individual cells (Nonami and Boyer, 1993) confirmed that the predicted gradients exist in growing tissue (see Chapter 11).

This work is of a fundamental nature and is necessary to understand growth

processes and water use by plants. Because it involves directly measurable parameters at the cell and tissue levels, it allows understanding to be built up from first principles that can be highly predictive and useful for tissues. Further research could be directed toward identifying the specific paths used by water and the quantitative contributions of these paths as water moves through the tissue. Particularly useful will be understanding of the paths into growing tissues and roots, and the paths leading to the evaporating surfaces in leaves.

For further information, Molz and Ferrier (1982) and Steudle (1989) provide extensive reviews of cell and tissue water transport. Background can also be gained from Kedem and Katchalsky (1958), Dainty (1963), Nobel (1974, 1983, 1991), and Zimmermann and Steudle (1978).

SUMMARY

The plasmalemma is the principal barrier controlling molecular traffic in and out of cells. For water, it functions primarily to harness the osmotic potential without which water would move into the cells too slowly to replace that which is lost by transpiration. At least in part, dehydration damage to cells can be attributed to a change in the plasmalemma and/or tonoplast.

The water status of cells is most usefully characterized in terms of the chemical potential or water potential because it involves a physically defined reference instead of a biological one and because the potential is the force moving water to and through cells. It is applied to the two major regions of the cell: the inside of the plasmalemma (the protoplast or symplast) and the outside of the plasmalemma (the apoplast), and it has different components in the two regions. The cell interior is affected mostly by the osmotic potential and turgor pressure whereas the external region is affected mostly by the osmotic potential and matric potential. These two regions are usually in equilibrium or near equilibrium with each other.

The potentials can be measured by various methods. The three most commonly used are (1) the thermocouple psychrometer which measures the vapor pressure of water (water potential) in the cell walls, (2) the pressure chamber which measures the tensions (matric potential) of water in the walls, and (3) the pressure probe which measures the turgor pressure inside the cells. By extracting the solution in the wall pores or protoplasm, the osmotic potential also can be measured in those regions.

Osmosis involves water movement across the plasmalemma because of differences in potential. Although the differences are usually small in individual cells, they can become substantial when considered over distances of many cells in tissues. Osmotic water movement appears to be driven by pressure gradients in membrane pores that are water filled. The pores appear to be located in water-transmitting proteins embedded in the membranes. The proteins have

been found in the plasmalemma and in the tonoplast. Increasing the amounts of pore-forming proteins in membranes increases rates of water transport through the membranes. Water diffusion through the membranes also occurs but is too slow to account for osmotic flow.

The dehydration of cells decreases cell water potential and thus increases the force causing water uptake. The decrease in water potential is caused more by decreases in turgor than by decreases in osmotic potential, and more turgor is lost in cells with rigid walls than in cells with elastic walls.

The dehydration can be delayed by osmotic adjustment of the cells, which increases solute contents rapidly enough to increase the osmotic force that can be applied by the cells. This allows increased water uptake and protects against changes in water content that otherwise would alter regulatory ion concentrations for the enzymes engaged in cell metabolism. The accumulating solute consists of organic molecules compatible with enzyme function that are produced by photosynthesis, released from reserves, or transported to the cells faster than they are used in metabolism.

The rates of dehydration and rehydration can give information about the conduction of water by the plasmalemma, especially when rates of hydration/dehydration are compared in cells and tissues. From differences in rates between a whole tissue and the individual cells in the tissue, conclusions can be drawn about the chief flow paths in the tissue. In those studied so far, the apoplast is rarely a dominant path and instead flow occurs mostly from cell to cell with perhaps a modest contribution from the apoplast.

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APPENDIX 3.1: Preservation of Cell Ultrastructure for Electron Microscopy

Biological observations with the electron microscope depend on the preservation of structures in the living cell so that they can be observed later in the nonliving cell. Fixatives have been developed to kill the cells rapidly so that structures are maintained. One that is frequently used is 1.5% glutaraldehyde in 0.05 *M* phosphate buffer, pH 7.2, which has an osmotic potential of -0.65 MPa that is similar to the water potential of many hydrated plant tissues. As a consequence, osmotic swelling and shrinking are minimized in the cells, and preservation of organelles is maximized.

The fixation of dehydrated cells is more difficult because the water potential of the cells is often lower than -0.65 MPa. The fixative then has a tendency to hydrate the cells because fixation is not instantaneous. Fellows and Boyer (1976, 1978) showed that abnormal cell structures are induced during this kind of fixation. Differing potentials often cause normally appressed membranes to move apart abnormally, and new vesicles and lipid droplets to appear. Several studies (Alieva *et al.*, 1971; Giles *et al.*, 1974, 1976; Kurkova and Motorina, 1974; Nir *et al.*, 1969; Vieira da Silva *et al.*, 1974) that employed this kind of fixation could have contained artifacts. On the other hand, when the osmotic potential is kept the same for fixative and tissue, most structure is preserved in dehydrated cells, as in Fig. 3.1B. Therefore, for dehydrated tissue, we recommend that fixative be prepared by adding sucrose at several concentrations to give a range of osmotic potentials, as done by Fellows and Boyer (1976, 1978). Samples of the dehydrated tissue are fixed at each osmotic potential and parallel samples are used to measure the tissue water potential. After the water potential has been determined, the fixation that most closely corresponds to the measured water potential of the tissue is chosen for microscopy, and the other fixations are discarded.

APPENDIX 3.2: Osmotic Potential and Dehydration

The effect of dehydration on Ψ_s can be calculated from the van't Hoff equation (Appendix 2.1) by replacing the concentration C with n_s/V where n_s is the number of moles of solute and V is the volume of water. Rearranging gives

$$\Psi_s V = -RTn_s \tag{3.18}$$

and, because RTn_s is constant at constant temperature, the differential gives

$$d(\Psi_s V) = 0, \tag{3.19}$$

which, when expanded, is

$$\Psi_s dV = -V d\Psi_s \tag{3.20}$$

and

$$d\Psi_s = -\Psi_s \frac{dV}{V}. \tag{3.21}$$

Equation (3.21) can be applied to small changes in cell volume caused by water loss or gain.

APPENDIX 3.3: Rates of Dehydration and Rehydration of Cells

The rates of cell dehydration/rehydration are determined by the conductivity of the plasmalemma and the capacitance of the cell. For a cell having a reflection coefficient of 1 and water potential Ψ_w in a medium having a constant water potential Ψ_o, the conductivity is described by Eq. (3.14), which in expanded form is

$$J_v = \frac{dV}{dt}\frac{1}{A} = Lp(\Psi_o - \Psi_w), \tag{3.22}$$

where V is the volume of water in the cell, t is the time, and A is the surface area of the cell (i.e., plasmalemma).

Substituting for dV the expression from Eq. (3.12) for the capacitance $dV = Vd\Psi_w/(\epsilon - \Psi_s)$ and rearranging gives the rate of change of Ψ_w

$$\frac{d\Psi_w}{dt} = \frac{-LpA}{V}(\epsilon - \Psi_s)(\Psi_w - \Psi_o), \tag{3.23}$$

which shows that the rate of change of the water potential is proportional to $-(\Psi_w - \Psi_o)$ when $LpA(\epsilon - \Psi_s)/V$ is constant, which is a reasonable approximation for small changes in Ψ_w. Since Ψ_o also is a constant, Eq. (3.23) can be integrated for $(\Psi_w - \Psi_o)$ at any time t

$$\int_{\Psi_w^o - \Psi_o}^{\Psi_w^t - \Psi_o} \frac{d\Psi_w}{\Psi_w} = -\frac{LpA}{V}(\epsilon - \Psi_s)\int_o^t dt \tag{3.24}$$

$$ln\frac{\Psi_w^t - \Psi_o}{\Psi_w^o - \Psi_o} = -\frac{LpA}{V}(\epsilon - \Psi_s)t, \tag{3.25}$$

where the superscripts o and t refer to zero time and any subsequent time, respectively. The time taken for $(\Psi_w^t - \Psi_o)/(\Psi_w^o - \Psi_o)$ to change by half $(t_{1/2})$ is

$$\ln 0.5 = -\frac{LpA}{V}(\epsilon - \Psi_s)t_{1/2} \tag{3.26}$$

$$t_{1/2} = \frac{0.693V}{LpA(\epsilon - \Psi_s)}, \tag{3.27}$$

which indicates that the half-time for a cell to change its Ψ_w depends on the properties of the wall (ϵ) and plasmalemma (Lp) in addition to the physical dimensions of the cell (A and V) and the osmotic potential (Ψ_s) of the cell solution. Because ϵ, Ψ_s, A, and V may be considered constant for small changes in water content, measuring $t_{1/2}$ for the cell allows Lp to be calculated. Of course, when changes in Ψ_w are caused by small changes in the turgor, this expression also can be used to describe the rates of the turgor change and to calculate Lp.