The Transport of Water in Higher Plants, and Ca\(^{2+}\) as Cellular Regulator (including problem set 3)

The movement of water across a cell membrane is called osmosis. This is always a passive process, as living systems have not developed pumps that directly use cellular energy to cause water movement. The passive movement of water depends on the existence of a difference in water potential, \(\Psi\), which is defined as the hydrostatic pressure minus the osmotic pressure. This quantity is discussed fully in the previous handout, The Movement of Solutes and Water Across Biological Membranes, as well as in section 37.1 of your textbook. Remember that \(\Delta \Psi = (\Psi_{pi} - \Psi_{po}) - (\Psi_{so} - \Psi_{si})\) and at water equilibrium, \(\Delta \Psi = 0\).

In order to take up water from soil by the roots, the water potential inside the root hairs must become less than that of the water in the soil. The root hairs may account for as much a 60\% of the surface area of the root. The major factor in lowering the water potential in the cells is the relatively high (compared to the soil) osmotic pressure caused by the relatively high solute concentration. You should be aware that the cytosolic osmotic pressure is something that the cells can actively control, thus permitting cells to regulate the driving force for water. As water is incompressible, the entry of water into the root cells causes them to expand, but the amount of expansion is limited because the cells are encased in a tough cell wall. The resistance of the cell wall to expansion produces hydrostatic pressure within the cell; if this hydrostatic pressure were to become large enough it could completely block further entry of water. Remember that hydrostatic pressure increases water potential, so an equilibrium condition can be reached in which the decreased water potential due to solutes is exactly balanced by the increased water potential due to hydrostatic pressure. Nearly all plant cells have positive hydrostatic pressure (compared to outside the cells) and this hydrostatic pressure is given the special name, turgor pressure.

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\Psi_o = \Psi_{po} + \Psi_{so} \\
\Delta \Psi = \Psi_i - \Psi_o = (\Psi_{pi} - \Psi_{po}) - (\Psi_{so} - \Psi_{si}) \\
(\Psi_{pi} - \Psi_{po})\) is the difference in hydrostatic pressure across the cell membrane. It is called the turgor pressure.

Animal cells do not have turgor pressure but plant cells, because of the cell wall, can have turgor. Indeed, turgor pressure is necessary for plant cell function. Turgor pressure is necessary for the support of plants and the wilting of plants is due to the loss of turgor.

Returning to roots, the hydrostatic pressure doesn’t reach the equilibrium level because the hydrostatic pressure continuously forces water into adjacent cells through the plasmodesmata. This symplast pathway eventually leads to the xylem elements in the center of the root and water is forced into the xylem. The hydrostatic pressure generated by the root hairs can force water up the xylem, but this pressure is not sufficient to drive water more than a few centimeters up the xylem.
How can the ascent of water in the xylem of trees that may reach heights of 100 m be accounted for? The start of the explanation lies in the nature of water itself. Water molecules have a strong electrical polarity, with the oxygen atom being relatively negative with respect to the two hydrogen atoms that lie within 105° of each other. The shared electrons spend, on average, more time in the vicinity of the oxygen atom, making that end of the molecule negative while the hydrogen atoms are positive. There are two important consequences of this arrangement. First, water molecules can form hydrogen bonds with other water molecules in the solution. This makes the liquid state of water very stable and a great deal of energy is required to cause a water molecule to leave the surface and enter the gas phase. Second, water molecules are electrostatically attracted to surfaces that are electrically charged or highly polar. Glass is such a material, as is cellulose. This is the origin of the phenomenon of capillarity. Water will move against gravity up the bore of a hollow glass tube a few centimeters. The smaller the bore of the tube the further water will move up. The explanation is that the polar water molecules are strongly attracted to the charged surface of the glass and tend to rise up along the walls of the tube. This creates a curved surface at the air-water interface and a negative hydrostatic pressure is produce in the water. It is this negative pressure that pulls the column of water up the tube against the force of gravity. The smaller the radius of curvature at the air-water interface, the greater the negative pressure and longer column of water that can be supported. Smaller diameter tubes have smaller radii of curvature at the air-water interface. It can also be shown that if the electrical polarity on the surface of the glass is eliminated by chemical reaction with silane, the capillary phenomenon is completely abolished.

Xylem tubes are created from the cell wall remains of dead cells and the cellulose has a high affinity for water, thus water is drawn up the xylem by capillarity. The typical diameter of xylem tubes is about 25 µm and it has been shown that xylem tubes can support a column of water that is about 60 cm high. Clearly this is not adequate to supply the water needs of even a large shrub, much less a tree. The origin of the much larger negative pressures required is found in the leaves. There, water that is still attached to the column of water in the xylem surrounds the mesophyll cells of the leaf. As this extracellular space is in contact with the atmosphere through the stomata, water can evaporate. This creates air pockets in the leaves, and the water is reduced to a thin film that is strongly attracted to the cellulose fibers in the cell walls. The water follows the irregular contours of the fibers and curve surfaces with extremely small radii of curvature (perhaps 100 nm) are formed. This produces a considerable negative pressure in the water that may average 30 atmospheres (3 MPa), which is fully sufficient to supply the water need of the tallest tree. This pressure must not only overcome gravity, it must also overcome the resistance to water flow of the delivery “pipes”, the xylem tubes. A 300 foot-tall tree requires about 10 atmospheres of pressure just to overcome gravity, so there is adequate pressure. It should be added that direct measurements of negative pressure in the tiny spaces of the cell walls is not currently possible, so this explanation of water movement is not as well established as one might hope. We can anticipate further refinement of the idea in the near future.

The interiors of leaves are in communication with the atmosphere via pores called
stomata. A single pore, a stoma, is created by the gap between two specialized epithelial cells called guard cells. The stomata allow the evaporated water to escape, which is necessary for the generation of negative pressure in the xylem, and also allow CO₂ to enter, which is necessary for photosynthesis. However, the amount of water loss must be regulated, and this is done by regulating the size of the stomatal opening.

In the fully open state, the guard cells of a stoma have a high osmotic pressure that is produced by high concentrations of K⁺ and Cl⁻, which results in water influx and high turgor pressure. As the elongated guard cells are attached to each other at their ends, the turgor pressure causes the cells to swell and enlarge the opening between them. In this state, maximum communication is established between the interior of the leaf and the atmosphere, with maximum rates of water loss and CO₂ gain. At night, when photosynthesis ceases or in periods of soil water shortage, a plant that can curtail the loss of water is more likely to survive. This is done by closing the stomatal pores. Lacking muscles, how do plants accomplish motion? In general, this is done by changing the turgor pressure of specialized cells. That is how insect-eating plants like the Venus flytrap are able to rapidly snap shut.

The special case of stomatal closing is under intense study currently by plant physiologists and the process is now fairly well understood. The closing of stomata is a result of the loss of turgor pressure by the guard cells that is in turn caused by the rapid loss of K⁺ and Cl⁻ in response to a chemical signal that is perceived by the guard cell plasma membrane. The binding of the signal molecule (in this case, a plant hormone called abscisic acid, which we will encounter again later) causes calcium channels in the plasma membrane to open, with the result that Ca²⁺ can enter the cell and dramatically increase the level of Ca²⁺ in the cytosol. This (the opening of calcium channels and the elevation of cytosolic Ca²⁺) is a common response of both plant and animal cells to external signals. It is important to understand that the calcium channels are already present in the plasma membrane of the guard cells—but they are closed when the cells are turgid and the stoma is open. The increase in Ca²⁺ causes Cl⁻ channels to open. Because of the highly negative membrane potential (say, -150 mV) and the high concentration of Cl⁻ in the cytosol, Cl⁻ rushes out of the cell, down its electrochemical gradient. The loss of negative charge from the inside of the cell causes the membrane potential to depolarize, that is, to move in the positive direction, toward the Cl⁻ equilibrium potential. As you already know, such changes in membrane potential can occur with little change in the concentration of the ions—a few ions can have a large electrical effect. However, there is also a specialized form of potassium channels in the plasma membranes of the guard cells. These channels are normally closed when the membrane potential is highly negative, but they open as the membrane potential becomes more positive. At these more positive potentials, K⁺ is driven out of the cell electrically through the newly opened channels, which tends to make the membrane potential somewhat more negative, allowing more Cl⁻ to leave. In effect, the cell is losing KCl, which is an electrically neutral event, and large amounts of the solute in chemical terms can be rapidly lost. This leads to water loss by the cells via osmosis, and the turgor pressure of the cells rapidly collapses.
While this process may seem complex, it is worth careful study as it is typical of how many cells, plant and animal, receive and respond to important communication signals. Such responses of cells to signals generated elsewhere in the organism are crucial for the functioning of the organism as an integrated whole.

**Summary from the lectures on turgor pressure in root hair cells.**

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\Psi_i = \Psi_{pi} + \Psi_{si}
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\Psi_o = \Psi_{po} + \Psi_{so}
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\Delta \Psi = \Psi_i - \Psi_o = (\Psi_{pi} - \Psi_{po}) - (\Psi_{so} - \Psi_{si})
\]
\[
(\Psi_{pi} - \Psi_{po}) \text{ is the difference in hydrostatic pressure across the cell membrane. It is called the turgor pressure.}
\]
Animal cells do not have turgor pressure but plant cells, because of the cell wall, can have turgor. Indeed, turgor pressure is necessary for plant cell function.

Suppose that water is in equilibrium across the cell membrane. That means that \(\Delta \Psi = 0\)
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\Delta \Psi = \Psi_i - \Psi_o = (\Psi_{pi} - \Psi_{po}) - (\Psi_{so} - \Psi_{si})
\]
Thus, \((\Psi_{pi} - \Psi_{po}) = (\Psi_{so} - \Psi_{si})\)

Remember that \(\Psi_i = -RT \sum c_n\)

\[
RT = 2.5 \text{ LMPa/mol at 25°C}
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This simplifies calculations if the total solute concentration is expressed in M (mol/L).

Consider a root hair cell. The total solute concentration in the soil might be 0.04 M and in the cytosol of the cell it might be 0.2 M.
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\Psi_{so} = -2.5 \frac{L \text{MPa}}{mol} \times 0.04 \frac{\text{mol}}{L} = -0.1 \text{MPa}
\]
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\Psi_{si} = -2.5 \frac{L \text{MPa}}{mol} \times 0.2 \frac{\text{mol}}{L} = -0.5 \text{MPa}
\]
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(\Psi_{pi} - \Psi_{po}) = (\Psi_{so} - \Psi_{si}) = -0.1 \text{MPa} + 0.5 \text{MPa} = 0.4 \text{MPa}
\]
The turgor pressure, \(\Psi_{pi} - \Psi_{po} = 0.4 \text{MPa} = 4 \text{atm},\)

If water is in equilibrium.

However, water is not in equilibrium because it is forced out toward the center of the root, where it enters the xylem system. The turgor pressure in the root is somewhat less than 4 atm, so water continues to enter from the soil.
A Primer on Pressure

A variety of units are used to report pressure. As a practical matter, it is important to be able to deal with this lack of standardization. Pressure is defined as a force per unit area. The proper international unit for pressure is the pascal (Pa), which is a newton/m$^2$. A newton is the MKS unit of force and is equal to a Kg m/s$^2$. In terms of energy per unit volume, one pascal equals one joule/m$^3$; this is useful in the calculation of osmotic pressure. The pressure of the atmosphere is often taken as a standard, so pressures are expressed in atmospheres (atm). 1 atm = 0.1 MPa (mega pascal). Another variant is to express pressure in terms of the height of a column of mercury that the pressure can support against gravity. 1 atm = 760 mmHg, and this same pressure of 1 atm will support a column of water of about 35 ft (because water is less dense than mercury). Finally, 1 bar is approximately equal to 1 atm.

Calcium as a Regulator of Cellular Function

One of the central problems of biology is to understand how cells translate a signal from the outside (a hormone, sperm -in the case of an egg, light, odor, a neurotransmitter, etc.) into an intracellular response. Something within the cell must change. This process is referred to as “signal transduction” and has been the subject of much research in recent years. It turns out that there are a relatively small number of cellular components that play a direct role in signal transduction. Among those, Ca$^{2+}$ is by far the most common. One example is the contraction of muscle. Muscle contraction is under the absolute control of Ca$^{2+}$. If the concentration of Ca$^{2+}$ in the cytoplasm of a muscle increases, the molecular machinery of muscle contracts and the muscle relaxes when Ca$^{2+}$ levels go down. In other words, Ca$^{2+}$ is the interpreter of signals sent by nerves to control the state of the muscle. This is only one of many examples, and in Biology 131, we will encounter a number of cases where Ca$^{2+}$ is a control element. Thus, it is important to have an understanding of the physiology of Ca$^{2+}$ and how it is regulated in cells. To understand Ca$^{2+}$ physiology, we must consider some of the features of eukaryotic cells (nucleated cells). The cytosol of the cell is in contact with two main membrane systems. By the term “cytosol”, I mean the water phase of the cell outside the nucleus and the other membrane-bound compartments. The plasma membrane separates the entire cell from the exterior and defines the limits of the cell. However, within the cell there are other membrane-delimited compartments, especially the endoplasmic reticulum. In a sense, the cytosol sits between these two compartments. With respect to Ca$^{2+}$, the cytosol is a special case. Even though Ca$^{2+}$ in the external medium and within the endoplasmic reticulum has a concentration in the millimolar range, the Ca$^{2+}$ concentration of the cytosol is much lower, typically about 100 nM. So, the cytosolic Ca$^{2+}$ concentration is some 10,000 times lower than the compartments with which it is in contact. In addition, the membrane potential across the plasma membrane is typically –70 mV (in animals), so there is an electrical driving force to bring Ca$^{2+}$ into the cytosol. Clearly, cells go to great lengths (and expend lots of energy) to keep Ca$^{2+}$ in the cytosol at such low levels. The major mechanism for this is the activity of Ca$^{2+}$ pumps in both the plasma membrane and the endoplasmic reticulum membrane. These pumps are proteins that remove Ca$^{2+}$ from the cytosol, using ATP as an energy source. Thus, they are ATPases (meaning they are enzymes that
catalyze the hydrolysis of ATP to ADP with the release of energy) and are related (but not identical) to the Na-K ATPase that you have already encountered. Cells also use secondary transport to expel excess Ca\(^{2+}\); that is, they use the energy stored in the Na\(^+\) gradient to drive Ca\(^{2+}\) efflux. This is accomplished by a particular protein called the Na-Ca exchanger, which is an integral membrane protein in the plasma membrane. In addition to the endoplasmic reticulum, plant cells typically have a large, water filled vacuole, which can act as a reservoir of Ca\(^{2+}\) that can be released into the cytosol.

As a consequence of this arrangement, the cytosolic concentration of Ca\(^{2+}\) can be rapidly increased if Ca\(^{2+}\) channels in either the plasma membrane or the endoplasmic membrane are opened. Ca\(^{2+}\) will flow down the steep electrochemical gradient into the cytosol and the concentration may increase 10 or 100-fold from the resting level.

How are increases in Ca\(^{2+}\) concentration interpreted by the cell? There are specialized proteins in cells whose activity is regulated by Ca\(^{2+}\). One important class of these proteins is the “EF-hand proteins”, so named because of the shape of their molecular configuration. An example of that class of proteins is troponin C, which is a protein that is associated with the contractile elements of skeletal muscle. When troponin C binds Ca\(^{2+}\), it changes its configuration and allows the contractile elements to interact. We will study this in some detail later, but if you are curious now, refer to p. 1049 Another member of the EF-hand family of proteins is calmodulin, which is involved in regulating vascular smooth muscle contraction, but it is also present in all eukaryotic cells and is often involved in transducing Ca\(^{2+}\) signals.

It is important that any cellular signal be limited in duration, so Ca\(^{2+}\) must be returned to its resting state shortly after the external signal is received. When cytosolic Ca\(^{2+}\) becomes elevated, the Ca\(^{2+}\) pumps in both the endoplasmic reticulum and the plasma membrane are activated (like a thermostat turning on the air conditioner when the room gets too warm) and they remove Ca\(^{2+}\) from the cytosol restoring the resting level of Ca\(^{2+}\). The cytosolic proteins that were activated by the elevated Ca\(^{2+}\) also return to their resting state, the episode is terminated, and the cell is ready for the next signal.

You may wonder how one ion can be involved in the regulation of so many cellular events. One way is by spatial limitation of the Ca\(^{2+}\) signal. A particular cell may not be uniform in regard to the distribution of plasma membrane Ca\(^{2+}\) channels or other properties, so a signal may not produce uniform influx of Ca\(^{2+}\). Because the cytoplasm has so many components that bind Ca\(^{2+}\), the diffusion of Ca\(^{2+}\) in the cytosol is slow and Ca\(^{2+}\) can be elevated in one region but not another (calcium “sparks”). This imparts a degree of specificity to Ca\(^{2+}\) signals. Another way that discrimination is achieved is by the frequency of the Ca\(^{2+}\) signal. Some Ca\(^{2+}\) response elements are tuned to specific frequencies and ignore Ca\(^{2+}\) signals that come more rapidly or more slowly. In any event, it is now understood that Ca\(^{2+}\) signaling occurs in all eukaryotic cells, and Ca\(^{2+}\) controls many cellular events.
Problem Set 3

1. Suppose that the external concentration of Ca\(^{2+}\) is 1 mM and the membrane potential of a cell is \(-87\) mV. Using the Nernst equation, determine the cytosolic concentration of Ca\(^{2+}\) that would be in equilibrium with the extracellular Ca\(^{2+}\) under these circumstances. (Remember that the valence, \(z\), of Ca\(^{2+}\) is not +1, and that at equilibrium, \(V_m = V_{Ca}\)).

2. If the actual cytosolic concentration of the cell in problem 1 is 100 nM, how many orders of magnitude from equilibrium is Ca\(^{2+}\)?

3. An alternate way of looking at the question is to start with the physiologically relevant Ca\(^{2+}\) concentrations (1 mM outside, 100 nM inside) and calculate the equilibrium potential for that distribution of Ca\(^{2+}\). What is it? If the actual membrane potential is \(-87\) mV, how many millivolts from equilibrium is Ca\(^{2+}\)?

4. Suppose a tree is 70 feet tall. How much pressure (in pascals) must be generated just to get water to the top of the tree?

5. Why is the pressure that you calculate in problem 4 not adequate to supply the water needs of the tree?

6. If a cell has a total cytosolic solute concentration of 500 mM and the total solute concentration of the extracellular medium is 200 mM, what will be the turgor (hydrostatic) pressure of the cell if water is at equilibrium across the cell membrane? Express your answer both in pascals and atmospheres. Remember that \(RT = 2.5\) L MPa/mol.

7. The trivalent cation, La\(^{3+}\), is a general blocker of calcium channels; that is, it enters an open channel in the plasma membrane and plugs it up so that the channel cannot conduct Ca\(^{2+}\). What would you expect to be the effect of La\(^{3+}\) contamination in the sap of the plant on the ability of the plant to regulate its water loss by stomata? Explain.

8. Why does water rise farther in glass tubing with a small inner diameter than in one with a larger diameter? Make a sketch of the meniscus in the two circumstances as part of your explanation. How does this relate to the accepted explanation of how water rises to the tops of the tallest trees? Try to answer this without reference to your class notes, the handout, or the textbook.

9. When the turgor pressure of a guard cell is collapsing during the closing of a stoma, what is membrane potential with respect to the equilibrium potentials of K\(^+\) and Cl\(^-\)?

10. How do deciduous trees move water in the winter when they don’t have leaves?

11. It has been pointed out that the movement of substances (the flux) can often be described by an equation of the form \(\text{Flux} = \text{Constant} \times \text{Driving Force}\), where the constant is determined by the properties of the substance and the pathway through which
it is moving. Fick’s first law was given as an example of this kind of equation. The movement of water through cellular membranes can be described by an equation like this: $J = L \Delta \Psi$. What aspect of this equation will be dependent on aquaporins (problem set 1 question 18)? According to an article published in Nature 439: 688-694, 2006 (http://www.nature.com/nature/journal/v439/n7077/suppinfo/nature04316_S1.htm), plants counteract fluctuations in water supply by regulating all aquaporins in the cell plasma membrane. Channel closure results either from the dephosphorylation of two conserved serine residues under conditions of drought stress, or from the protonation of a conserved histidine residue following a drop in cytoplasmic pH due to anoxia during flooding. How will changes in the number of open aquaporin channels affect the amount of water that moves through a cellular membrane under conditions of constant $\Delta \Psi$?