DIFFUSION AND OSMOSIS

What causes my plants to wilt if I forget to water them?

■■BACKGROUND

Cells must move materials through membranes and throughout cytoplasm in order to maintain homeostasis. The movement is regulated because cellular membranes, including the plasma and organelle membranes, are selectively permeable. Membranes are phospholipid bilayers containing embedded proteins; the phospholipid fatty acids limit the movement of water because of their hydrophobic characteristics.

The cellular environment is aqueous, meaning that the solvent in which the solutes, such as salts and organic molecules, dissolve is water. Water may pass slowly throughthe membrane by osmosis or through specialized protein channels called aquaporins. Aquaporins allow the water to move more quickly than it would through osmosis. Most other substances, such as ions, move through protein channels, while larger molecules, including carbohydrates, move through transport proteins.

The simplest form of movement is diffusion, in which solutes move from an area of high concentration to an area of low concentration; diffusion is directly related to molecular kinetic energy. Diffusion does not require energy input by cells. The movement of a solute from an area of low concentration to an area of high concentration requires energy input in the form of ATP and protein carriers called pumps.

Water moves through membranes by diffusion; the movement of water through membranes is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high free water concentration) and low solute concentration to areas of low potential (low free water concentration) and high solute concentration. Solutes decrease the concentration of free water, since water molecules surround the solute molecules. The terms *hypertonic, hypotonic,* and *isotonic* are used to describe solutions separated by selectively permeable membranes. A hypertonic solution has a higher solute concentration and a lower water potential as compared to the other solution; therefore, water will move into the hypertonic solution through the membrane by osmosis. A hypotonic solution has a lower solute concentration and a higher water potential than the solution on the other side of the membrane; water will move down its concentration gradient into the other solution. Isotonic solutions have equal water potentials.

In nonwalled cells, such as animal cells, the movement of water into and out of a cell is affected by the relative solute concentration on either side of the plasma membrane. As water moves out of the cell, the cell shrinks; if water moves into the cell, it swells and may eventually burst. In walled cells, including fungal and plant cells,

osmosis is affected not only by the solute concentration, but also by the resistance to water movement in the cell by the cell wall. This resistance is called turgor pressure. The presence of a cell wall prevents the cells from bursting as water enters; however, pressure builds up inside the cell and affects the rate of osmosis.

Water movement in plants is important in water transport from the roots into the shoots and leaves. You likely will explore this specialized movement called transpiration in another lab investigation.

■■Understanding Water Potential

Water potential predicts which way water diffuses through plant tissues and is abbreviated by the Greek letter psi (ψ). Water potential is the free energy per mole of water and is calculated from two major components: (1) the solute potential (ψ_S), which is dependent on solute concentration, and (2) the pressure potential (ψ_P), which results from the exertion of pressure—either positive or negative (tension) — on a solution. The solute potential is also called the osmotic potential.

$$\psi = \psi_P + \psi_S$$

Water Potential = Pressure Potential + Solute Potential

Water moves from an area of higher water potential or higher free energy to an area of lower water potential or lower free energy. Water potential measures the tendency of water to diffuse from one compartment to another compartment.

The water potential of pure water in an open beaker is zero ($\psi = 0$) because both the solute and pressure potentials are zero ($\psi_S = 0$; $\psi_P = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water lowers the solute potential and therefore decreases the water potential. This means that a solution at atmospheric pressure has a negative water potential due to the solute.

The solute potential (ψ_S) = – iCRT, where i is the ionization constant, C is the molar concentration, R is the pressure constant (R = 0.0831 liter bars/mole-K), and T is the temperature in K (273 + °C).

A 0.15 M solution of sucrose at atmospheric pressure ($\psi_P = 0$) and 25°C has an osmotic potential of -3.7 bars and a water potential of -3.7 bars. A bar is a metric measure of pressure and is the same as 1 atmosphere at sea level. A 0.15 M NaCl solution contains 2 ions, Na^+ and Cl^-; therefore i = 2 and the water potential = -7.4 bars.

When a cell's cytoplasm is separated from pure water by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher ($\psi = 0$), into the cell, where water potential is lower because of solutes in the cytoplasm

(ψ is negative). It is assumed that the solute is not diffusing (Figure 1a). The movement of water into the cell causes the cell to swell, and the cell membrane pushes against the cell wall to produce an increase in pressure. This pressure, which counteracts the diffusion of water into the cell, is called turgor pressure.

Over time, enough positive turgor pressure builds up to oppose the more negative solute potential of the cell. Eventually, the water potential of the cell equals the water potential of the pure water outside the cell (ψ of cell = ψ of pure water = 0). At this point, a dynamic equilibrium is reached and net water movement ceases (Figure 1b).

Figures 1a-b. Plant cell in pure water. The water potential was calculated at the beginning of the experiment (a) and after water movement reached dynamic equilibrium and the net water movement was zero (b).

If solute is added to the water surrounding the plant cell, the water potential of the solution surrounding the cell decreases. If enough solute is added, the water potential outside the cell is equal to the water potential inside the cell, and there will be no net movement of water. However, the solute concentrations inside and outside the cell are not equal, because the water potential inside the cell results from the combination of both the turgor pressure (ψ_P) and the solute pressure (ψ_S).
(See Figure 2.)

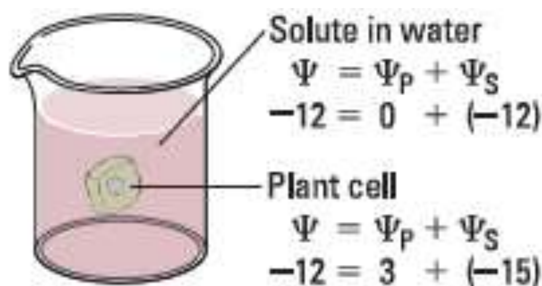

Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not equal.

If more solute is added to the water surrounding the cell, water will leave the cell, moving from an area of higher water potential to an area of lower water potential. The water loss causes the cell to lose turgor. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

PART A: DIFFUSION

Driving Question: What is the relative size of the microscopic pores in my dialysis tubing? How the heck do I find that measurement?

In this experiment, you will measure diffusion of small moleculesthrough dialsysi tubing, an example of a selectively permeable membrane. Small solute molecules and water molecules can move freely through a this selectively permeable membrane, but larger molecules will pass through more slowly, or perhaps not at all. The movement of a solute through a selectively permeable membrane is called **dialysis.** The size of the minute pores in the dialysis tubing determines which substances can pass through the membrane.

1) Obtain a piece of dialysis tubing and tie off one end.
2) Open the tubing by rubbing the untied end between your fingers
3) Pour 12 mL of glucose starch solution into the tubing.
4) Determine if glucose is present in the tubing by dipping a glucose indicator strip into the solution. Note the color of the solution also.
5) Carefully remove the excess air from the tube and tie off the end to form a bag. Be sure to leave enough space for expansion.
6) Put 125 mL of distilled water in a 250 mL beaker. Add 1mL of potassium iodide (IKI) to the beaker.
   NOTE: IKI can stain skin and clothing. Handle with caution!
7) Determine if glucose is present in the beaker by using a glucose indicator strip. Note the color of the solution.
8) Make predictions as to what will changes will occur after the bag sits in the water for 30 minutes
9) **DO IT!** Completely immerse the bag in the distilled water. Add water to cover the bag if needed and WAIT for 30 MINUTES.
10) While waiting, diagram your set-up. Create a data table to correspond with the set-up before and after it sits for 30 minutes.
11) Remove the dialysis bag and note the colors of the bag and the beaker.
12) Determine the glucose content in the beaker and in the bag using indicator strips. To test the bag, make a small cut with scissors and insert the strip through the hole.
13) Diagram your set-up after 30 minutes. Be sure to complete your data table with your new observations and tests.

ANALYSIS

1) Explain your results you obtained. What experimental evidence supports your answer? Include the concentration differences and membrane pore size in your discussion. (There are four substances in the set-up and this can be answered with 4 good sentences.)
2) Based on your observations, rank the following by relative size from smallest to largest: glucose molecules, IKI molecules, membrane pores, starch molecules, water molecules. **Explain**!
3) Quantitative data uses numbers to measure observed changes. How could this experiment be modified so that quantitative data could be collected to show that water diffused into or out of the dialysis bag?
4) Prediction Time! What results would you expect if the experiment started with a glucose and IKI solution inside the bag and only starch and water outside? Diagram your before and after set-ups and **Explain**! (Start with I predict, then explain.)

PART B: OSMOSIS

1) Obtain the proper number of beakers and label them to indicate which solutions are in the bags you are testing:
    a. Distilled water
    b. 0.2 M sucrose
    c. 0.4 M sucrose
    d. 0.6 M sucrose
    e. 0.8 M sucrose
2) Obtain the proper number presoaked dialysis tubing.
3) Tie off one end of each and fill with approximately 12 ml of the solutions that you are testing.
4) Remove the excess air by drawing the bag between two fingers. Tie off the other end of the bag. Leave sufficient space for expansion inside the bag. (The solution should fill about 1/3 to ½ of the tubing)
5) Carefully blot the bags dry and determine an initial mass for each. (Be use a weigh boat. DO NOT place the bags directly on the pan of the scale.)
6) Fill the beakers approximately ¾ full of distilled water. Immerse the bags in the proper beakers.
7) WAIT 30 minutes. Diagram your set-ups and make predictions about how the set-ups may change. Create a data table for current and future data.
8) Remove the bags, blot them as dry as possible, end determine a final mass for each.

    NOTE: WE NEED TO ALL BE ACCURATE WITH THE TIME

9) Calculate the mass difference for each bag.

    Mass difference = final mass – initial mass

10) Calculate the percent change in mass for each of the bags:

    % change in mass = mass difference / initial mass x 100

11) As a class, we will collect a class set of "% change in mass" data. GRAPH the class set of data obtained. Make a rough sketch of the data in your journal. Complete a proper graph for inclusion.
12) GRAPHING REMINDERS:
    a. Independent variable labels the x-axis
    b. Dependent Variable labels the y-axis
    c. Be sure to include units in parentheses.
    d. Title the graph using your two variables.

ANALYSIS

1) Verbalize the results of the class data table by explaining the relationship between the change in mass and the molarity of sucrose within the dialysis bags. Explain why this occurred using proper terminology.
2) Why did you calculate the percent change in mass rather than simply using the change in mass?
3) Prediction Time! Predict what would happen to the mass of each bag in this experiment if all the bags were placed in a 0.6 M sucrose solution instead of distilled water. Sketch a graph to show how the mass of each of the bags would be affected and <u>explain</u> your reasoning. (Diagrams can be helpful to think it through)

PART C: WATER POTENTIAL

<u>Driving Question</u>: What is the water potential of potato cells? (Cool, we are going to somehow measure intracellular conditions.)

1) Obtain 5 potato cores for each of the solutions you will be testing:
    a. Distilled water
    b. 0.2 M sucrose
    c. 0.4 M sucrose
    d. 0.6 M sucrose
    e. 0.8 M sucrose
2) Weigh the five cylinders together and determine an initial mass. (remember to use weigh boats!)
3) Obtain beakers and label them with the solutions you are testing.
4) Place five massed potato cores in each of your beakers and cover with the appropriate solution. (50 mL or less should be sufficient)
5) Cover with plastic wrap to prevent evaporation and colonization by spores or bacteria.
6) Let stand over night.
7) Diagram your set-ups and make predictions about how the set-ups may change.
8) Create a data table for current and tomorrow's data.
9) DAY 2 – Determine and record the temperature of the set-ups.
10) For each beaker, remove the five potato cores, blot them dry, and determine a final mass.
11) Calculate the mass difference of the potato cores for each solution.
    Mass difference = final mass – initial mass
12) Calculate the percent change in mass of the potato cores each of the solutions:
    % change in mass = mass difference / initial mass x 100
13) As a class, we will collect a class set of "% change in mass" data. GRAPH the class set of data obtained. Make a rough sketch of the data in your journal. Complete a proper graph for inclusion.
14) GRAPHING REMINDERS:
    a. Independent variable labels the x-axis
    b. Dependent Variable labels the y-axis
    c. Be sure to include units in parentheses.
    d. Title the graph using your two variables.

ANALYSIS

1) The line on your graph should show a point at which there is no change in mass. What is the value and <u>explain</u> what is occurring within a potato cell at this point. (THIS IS BIG)
2) Using the info from your graph, calculate the osmotic potential (ψ_S) of the sucrose solution at the point determined in #1. Show your work and include proper units.
3) What is the water potential (ψ) of the potato cells? Explain how you know this.
4) PREDICTION TIME! If a potato core is allowed to dehydrate by sitting in the open air, would the water potential of the potato cells decrease or increase? <u>Explain</u>.
5) A lab tech is analyzing blood cells under the scope. He has lost his solutions for making a proper wet mount, so he decides to use distilled water instead. <u>Explain</u> what would happen to the red blood cells and what would the lab tech most likely see.
6) Why can't humans drink salt water for hydration?

DIFFUSION / OSMOSIS: Turgor & Plasmolysis

DRIVING QUESTION:
In the winter, why does grass often die near roads that have been salted to remove ice?

1) Prepare a mount wet mount of red onion epidermal cells. Observe under magnification. Sketch and describe a single cells appearance.
Label: Cell Wall, Plasma Membrane

2) Create an environment that would resemble the question above.
What will you add to your cells? Explain why
Predict what you expect to see.
Do it.
Sketch and describe a single cells appearance.
Label: Cell Wall, Plasma Membrane
Explain what has happened and why.
If your observation does not match your prediction, explain why that might be, make adjustments, make a new prediction (if needed), and try again. Document this!!!!!!

3) Create an environment to return your cells to their original state.
What will you add to your cells? Explain why
Predict what you expect to see.
Do it.
Sketch and describe a single cells appearance.
Label: Cell Wall, Plasma Membrane
Explain what has happened and why.
If your observation does not match your prediction, explain why that might be, make adjustments, make a new prediction (if needed), and try again. Document this!!!!!!

4) Answer the driving question!