INVESTIGATION 4

DIFFUSION AND OSMOSIS

What causes plants to wilt if they are not watered?

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 solutes (e.g., salts, organic molecules) dissolve in water, which is the solvent. Water may pass freely through the membrane by osmosis or through specialized protein channels called aquaporins. Most ions move through protein channels, while larger molecules, such as carbohydrates, are carried by 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. 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; this process is called osmosis. Like solutes, water moves down its concentration gradient. Water moves from areas of high potential (high water concentration) and low solute concentration to areas of low potential (low water concentration) and high solute concentration. In walled 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 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. 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 potential.

The movement of solutes and water across cellular membranes is an overarching concept. Cells must maintain their internal environments and control solute movement. These concepts can be illustrated using model systems and living cells. Students will revisit the concepts of osmosis and water potential when they investigate transpiration in plants.

This investigation consists of three parts. It is recommended that students work through all three sections. In Procedure 1, students use artificial cells to study the relationship of surface area and volume. In Procedure 2, they create models of living cells to explore osmosis and diffusion. Students finish by observing osmosis in living cells (Procedure 3). All three sections of the investigation provide opportunities for students to design and conduct their own experiments.

Understanding Water Potential

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 or undergoes crenation; if water moves into the cell, it swells and may eventually burst or lyse. In walled cells, including fungal and plant cells, 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 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.

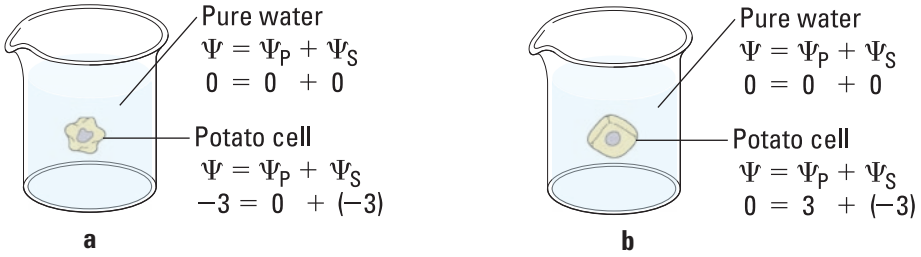
The water potential of pure water in an open beaker is zero ($\psi = 0$) because both the solute and pressure potentials are zero ($\psi_S = 0$; $\psi_P = 0$). An increase in positive pressure raises the pressure potential and the water potential. The addition of solute to the water lowers the solute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

The solute potential (ψ_S) = – iCRT, where i = the ionization constant, C = the molar concentration, R = the pressure constant (R = 0.0831 liter bars/mole-K), and T = 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 then 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), as shown in Figure 2.

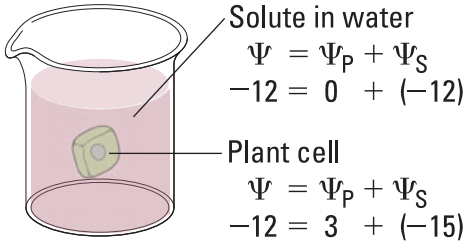


Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not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

Have students read the information about water potential and answer the following questions. You likely will have to guide students with insufficient mathematical skills through the calculations.

- Calculate the solute potential of a 0.1 M NaCl solution at 25°C. If the concentration of NaCl inside the plant cell is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M NaCl solutions?
- What must the turgor pressure equal if there is no net diffusion between the solution and the cell?

PREPARATION

Materials and Equipment

The materials and equipment are listed for each separate experiment.

Timing and Length of the Lab

This investigation requires a minimum of four laboratory periods of about 45 minutes each, plus time for discussions and measurements. There are three subparts, each requiring one class period. An additional class period will be needed for discussion. You may also assign the prelab questions or online activities/tutorials for homework. You will need to set aside time to prepare the solutions and the agar for your students.

Safety and Housekeeping

The HCl and NaOH solutions will cause chemical burns. Students must wear safety goggles or glasses, gloves, and aprons and prepare the NaOH and HCl solutions in a hood. Have the students use these solutions in spill-proof plastic trays or pans.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM FRAMEWORK

This investigation can be conducted during the study of concepts pertaining to cell structure and function, modeling cellular processes, and the movement of materials through biological membranes (big idea 2).

Enduring Understandings

- 2B Growth, reproduction, and dynamic homeostasis require that cells create and maintain internal environments that are different from their external environments.
- 2B1: Cell membranes are selectively permeable due to their structure.
- 2B2: Growth and dynamic homeostasis are maintained by the constant movement of molecules across membranes.

Learning Objectives

- The student is able to use calculated surface area-to-volume ratios to predict which cell(s) might eliminate wastes or procure nutrients faster by diffusion (2A3 & SP 2.2).
- The student is able to explain how cell size and shape affect the overall rate of nutrient intake and the rate of waste elimination (2A3 & SP 2.2).
- The student is able to use representations and models to pose scientific questions about the properties of cell membranes and selective permeability based on molecular structure (2B1 & SP 4.2, SP 4.3, SP 4.4).

- The student is able to construct models that connect the movement of molecules across membranes with membrane structure and function (2B2 & SP 2.1, SP 2.2, SP 5.1).
- The student is able to use representations and models to analyze situations or solve problems qualitatively and quantitatively to investigate whether dynamic homeostasis is maintained by the active movement of molecules across membranes (2B1 & 2B2 & SP 2.2, SP 5.2, SP 5.3).

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

The form and function of cells, organelles, and organisms is a central concept in biology. You can help students think about cell shape in relation to its function by providing examples. Epithelial cells in the small intestine have many microvilli that serve to increase the surface area. These cells take up nutrients from food and move the nutrients into the capillaries. An erythrocyte's concave shape increases the rate of oxygen diffusion out of the cell and into the tissues. The elongated projection of the root hair — and the large number of them — greatly increases the surface area through which water and minerals pass into the root of a plant.

Students should understand that temperature influences molecular kinetic energy directly. They have made observations but may not have made the connections. Ask them to remember what happened to the sugar they added when they prepared an iced coffee drink versus hot coffee.

A more difficult concept for students to grasp is that molecular weight is inversely related to the rate of diffusion. Have students think about 10 dump trucks and 10 small cars at the opposite end zones of a football field. Then ask them to predict which vehicles will scatter faster across the field. Most will understand that large dump trucks move more slowly (have a lower kinetic energy) than the smaller cars.

Skills Development

Students will develop the following skills, which are reinforced in the transpiration investigation:

- Calculating surface area and volume of a model cell
- Designing experiments to measure the rate of osmosis in model cells
- Designing experiments to measure water potential in plant cells

Potential Challenges

Students struggle with the concepts of the random nature of diffusion (nondirectional) and kinetic energy. A good demonstration is to drop some coins over a table to show that they spread out in all directions; as the height increases (more kinetic energy), the coins spread out farther from each other.

Most students will comprehend that substances move from an area of high concentration to an area of low concentration but fail to realize that the diffusion rate is based on the concentration differences. Many students confuse concentration with the amount of a solution. A human skin cell in normal (isotonic) saline will not change its shape regardless of the amount of saline around it.

Students also think that molecules stop moving at equilibrium. This is not the case. Diffusion reaches a dynamic equilibrium, not a static equilibrium.

Water potential is a difficult concept for students to grasp. For students lacking sufficient mathematical skills, you likely will need to provide guidance as they work through the information and calculations on water potential. Solutes effectively prevent water from diffusing because the water is no longer "free." Solutes are surrounded by hydration shells and reduce the concentration of water. Students have difficulty understanding how walled cells, such as plant cells, control their internal pressure using the central vacuole. They will need help comprehending how solutes reduce the free water in a system and therefore act to reduce the water potential.

The terms *hypotonic, hypertonic,* and *isotonic* are confusing until students realize that these are relative terms and refer to the solute concentration, rather than water concentration. Use the following online tutorials to help guide your students:

http://mw.concord.org/modeler/
http://www.phschool.com/science/biology_place/labbench/lab1/intro.html

THE INVESTIGATIONS

Getting Started: Prelab Assessment

You may assign the following questions for homework; as a think, pair/group, share activity, in which pairs or small groups of students brainstorm ideas and then share them with other groups; or as a whole-class discussion to assess students' understanding of key concepts pertaining to kinetic energy, osmosis, and diffusion:

- What is kinetic energy and how does it differ from potential energy?
- What environmental factors affect kinetic energy and diffusion?
- Why do these factors alter diffusion rates? How do they affect rates?
- How are gradients important in diffusion and osmosis?
- What is the explanation for the fact that most cells are small and have cell membranes with many convolutions?
- Will water move into or out of a plant cell if the cell has a higher water potential than the surrounding environment?
- What would happen if you applied saltwater to a plant?
- How does a plant cell control its internal (turgor) pressure?

Procedure 1: Surface Area and Cell Size

Because cell size and shape are important factors in determining the rate of diffusion, students begin their investigation of the movement of molecules across cell membranes by exploring the relationship between surface area and volume. Ask students to consider cell shapes, especially those involved with nutrient uptake; good examples include intestinal villi and root hair cells. Students should predict which measurement — surface area or volume — has the greater influence on the rate of diffusion. They should then calculate surface area-to-volume ratios and determine the diffusion depth and rate in agar.

Materials

- 2% agar containing the pH-indicator dye phenolphthalein
- 1% phenolphthalein solution
- 0.1M NaOH
- 0.1M HCl
- Squares of hard, thin plastic (from disposable plates); unserrated knives; or scalpels from dissection kits
- Metric rulers
- Petri dishes or test tubes to hold the agar cubes

Preparation

Wear safety goggles or glasses when preparing these materials.

To prepare 2% agar with phenolphthalein, do the following:

1. Dissolve 1 g phenolphthalein in 100 mL of 95% ethanol to make a 1% solution.
2. Mix 20 g of agar with 1 L water; heat to near boiling or until solution is clear.
3. Cool the agar to ~55°C and add 10 mL 1% phenolphthalein; if the agar solution is clear, add dilute NaOH until the agar is bright pink.
4. Pour the agar into baking pans or shallow trays to 3 cm deep; let the agar cool overnight.

Have students make cubes (1 cm per side, 2 cm per side). Some students will think to make the pieces long and thin.

Tip: If the agar loses its color, simply place into dilute NaOH for a few hours.

To prepare NaOH and HCl, do the following:

1. 0.1 M NaOH: Add 0.4 g of NaOH to 80 mL of H_2O. Stir to dissolve and add water to 100 mL total volume. Store NaOH solutions in plastic bottles. Label *Hazardous-Caustic Solution*.
2. 0.1 M HCl: Add 0.83 mL of concentrated HCl (12.1 M) to H_2O to bring to 100 mL total volume. Label *Hazardous-Strong Acid*.

An alternative method calls for mixing one packet of unflavored gelatin with 237 mL of water and adding 2.5 mL 1% phenolphthalein and a few drops of 0.1 M NaOH.

The solution should be bright pink. Pour the gelatin mixture into shallow pans, and refrigerate overnight. You may use white vinegar in place of the 0.1 M HCl.

Data Analysis

From the data, students should consider several questions:

- Which surface area-to-volume ratio gave the fastest diffusion rate?
- Which surface area-to-volume ratio had the greatest diffusion depth?
- How might a cell's shape influence the rate of diffusion?
- What factors affect the rate of diffusion and how can these be tested?

Designing and Conducting Independent Investigations

Using the provided materials, students design and conduct an experiment(s) to test the predictions they made regarding the relationship of surface area and volume in artificial cells to the diffusion rate using the phenolphthalein–NaOH agar and HCl solution.

Procedure 2: Modeling Diffusion and Osmosis

Students create models of living cells using dialysis tubing. Dialysis tubing contains pores that permit the passage of small ions and molecules, including water and glucose, but not larger molecules such as starch and proteins. Like cell membranes, dialysis tubing is selectively permeable. Students fill their model cells with different solutions and determine diffusion rates. Students then can investigate questions about the movement of water across cell membranes and use their model cells to explore osmosis in more depth.

Materials

- 1 M sucrose
- 1 M NaCl
- 1 M glucose
- 5% ovalbumin (egg white protein)
- Dialysis tubing (5 pieces per group)
- Balances
- 8 or 10 oz. drinking cups or beakers
- Distilled water, volumetric pipettes, and graduated cylinders for preparing dilutions

Note: 5% ovalbumin = 5 g/100 mL = 50 g/liter. The MW of ovalbumin is 45,000 g/mole. The molarity of a 5% solution = mole/45,000 g × 50 g/liter = 0.0011 M.

Preparation

1. 1M sucrose: Dissolve 342.3 g of sucrose in 500 mL of H_20; bring to 1 L total volume.
2. 1 M NaCl: Dissolve 58.4 g of NaCl in 500 mL of H_20; bring to 1 L total volume.
3. 1 M glucose (dextrose): Dissolve 180.2 g of glucose in 500 mL of H_20; bring to 1 L total volume.

4. 5% ovalbumin (if possible, store powder in the refrigerator to prevent clumping): Mix 50 g of ovalbumin with 500 mL of H_20; bring to 1 L total volume.

To prepare dialysis tubing, cut dialysis tubing into 20-cm pieces; soak pieces in water. Extra dialysis tubing can be kept in 20% ethanol in the refrigerator to prevent bacterial growth.

Students use the dialysis tubing to model cells. The dialysis tubing is knotted in one end, filled with 10 mL solution, and knotted to close the tube. Make sure students leave enough space for water to diffuse into the tube. Tell students to keep the dialysis tubing moist.

Data Analysis

From the data, students should consider several questions.

- What factors determine the rate and direction of osmosis?
- What would you predict if you used a starch solution instead of the protein?
- Can you diagram the flow of water based upon the contents of your model cell and the surrounding solution?
- When will the net osmosis rate equal zero in your model cells?
- Based upon your observations, can you predict the direction of osmosis in living cells when the cells are placed in various solutions?
- How is the dialysis tubing functionally different from a cellular membrane?

Designing and Conducting Independent Investigations

Have students design five different pairs of solutions and make a prediction about diffusion; one pair — water in the dialysis tube placed into water — is the control. Have groups of students do replicate experiments. Students are surprised that the tube containing 5% albumin has no weight change when placed in water.

Procedure 3: Observing Osmosis in Living Cells

It is important that students observe and understand osmosis in living cells. A quick demonstration is to soak celery sticks in water and in 1 M NaCl and have students break the sticks. The sticks in water have high turgor pressure and break with a "snap," and those in saltwater are limp and difficult to break. Ask students to explain how the sound (snap) is produced.

Materials

- *Elodea* tips or *Mnium hornum* (moss)
- Microscope slides and cover slips
- Microscopes
- Solutions from Procedure 2

Preparation

Elodea tips can be purchased from biological supply companies; however, some states have restricted its use because of *Elodea's* invasive nature. Moss (*Mniun hornum*) can be obtained from a greenhouse or from the woods. Have students observe and draw the cells at 400 X total magnification. The cell membrane shrinks away from the cell wall, and the central vacuole collapses when a high concentration of either sugar or salt is added; this process is called plasmolysis.

Ask students how they would measure the water potential in the different types of plants. This can be done by measuring/calculating the change in weight, change in length, or change in volume. The laboratory should be set up on one day and measured the next day.

Designing and Conducting Independent Investigations

Ask students how they could measure water potential in plant cells. This can be done by measuring/calculating change in mass, change in length, or change in volume over time in plant sections from potatoes. You will prepare several solutions with different concentrations of sucrose; however, you will color-code the solutions with food coloring instead of labeling the concentrations for students. Students design an experiment to identify the concentrations of the sucrose solutions and then use the solutions to determine the water potential of the plant tissues.

Students should set up their investigations in one period (45–60 minutes) and conduct them the next day. Waiting too long causes the potato cores to become mushy.

Materials

- Potatoes, sweet potatoes, or yams
- Cork borers or french fry cutter
- Balances
- Metric rulers
- 8 or10 oz. drinking cups
- Sucrose solutions of different concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0 M)

Preparation

Prepare 2,000 mL of 1.0 M sucrose. Use this 1.0 M stock solution to make 1,000 mL dilutions (0.2, 0.4, 0.6, and 0.8 M). Use a drop of food coloring to give each solution a different color. The quantities are sufficient for 24 students and can be adjusted for smaller classes.

Do not label the solutions for students!

1.0 M: 684.6 g sucrose/2000 mL d-H_20

0.8 M: 800 mL of 1.0 M to 200 mL d-H_2O

0.6 M: 600 mL of 1.0 M to 400 mL d-H_2O

0.4 M: 400 mL of 1.0 M to 600 mL d-H_2O

0.2 M: 200 mL of 1.0 M to 800 mL d-H_2O

Alternative Experiments

This investigation consists of three parts. It is recommended that students work through all three sections. However, if time is an issue, the investigations can be modified.

Procedure 1 can be skipped; instead, ask your students to consider cell structure and function and have them calculate the surface area and volume of model cells. Procedure 1 can be integrated into the water potential experiment (Procedure 3) by cutting potato pieces into different sizes and comparing the size/weight changes.

Procedure 2 can by modified by having students choose among water, protein, and 1 M sucrose. The model cells can be prepared and left overnight in the second solution and weighed the next day, as long as there is sufficient space in the dialysis tubing bags. An alternative is to place thin celery sticks in the solutions overnight and ask students to measure how far the celery can bend without breaking the next day.

You can ask students to view videos that show the effect of salt or sugar solutions on plant cells. For example, see the following:
http://www.csun.edu/scied/7-microscopy/elodea_plasmolysis/index.htm
http://www.teachertube.com/viewVideo.php?video_id=135394

Summative Assessment

1. Review the learning objectives. You can use the learning objectives to generate analysis questions. Do the students' answers to your questions suggest that they understand the concepts?

2. Review students' experimental evidence. Did students make the appropriate measurements and graphs to analyze the data? Were they able to make simple volume and surface area calculations?

3. Have your students prepare laboratory notebooks; keep the first two pages blank for a table of contents. Students should record their experimental designs, data, graphs, results, and conclusions. They may use Excel to prepare the graphs.

4. Ask your students to use the principles of osmosis to explain how foods are preserved. For example, foods are prepared using high concentrations of salt or sugar (e.g., preserves, jams, jellies). The high solute potential in the solution prevents microbial growth.

5. Review water potential with your students; they will revisit the concept when exploring transpiration in plants.

Where Can Students Go from Here?

Ask students if they think that fungal cells have turgor pressure. Then ask them to design an experiment to test their hypothesis.

SUPPLEMENTAL RESOURCES

Molecular Movement and Membranes: Osmosis and Diffusion

Taiz, Lincoln and Eduardo Zeiger. 2010. Unit One: Transport and Translocation of Water and Solutes in *Plant Physiology, 5th ed*., pp. 67–159. Sinauer Associates, Inc., Sunderland, MA.

The unit covers water and cells, water balance in plants, and solute transport. The book is an excellent reference on plant physiology.

http://mw.concord.org/modeler/

http://www.phschool.com/science/biology_place/labbench/lab1/intro.html

The Molecular Workbench and Lab Bench laboratory online resources about diffusion and osmosis are excellent prelaboratory resources. Both provide feedback with hints when students answer the questions.

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2003

The Nobel Prize in Chemistry 2003 was awarded to Peter Agre and Roderick MacKinnon for their work on aquaporins. The Nobel Prize website provides information about these protein channels and their roles in osmosis.

Kowles, Richard V. 2010. Regulation of water in plant cells. *Bioscene: Journal of College Biology* 36(1): 34–42.

This reference reviews water movement in plant cells and describes an experiment to measure water potential.

Additional Experiments and Demonstrations

Concannon, James P. and Patrick L. Brown. 2008. Transforming Osmosis: Labs to address standards for inquiry. *Science Activities: Classroom Projects and Curriculum Ideas:* 45 (3): 23–25.

Sweeney, Ryan M., Lisa Martin-Hansen, Geeta Verma, and John Dunkhase. 2009. Embracing learners' ideas about diffusion and osmosis: A Coupled-inquiry approach. Science Scope: 33(1): 38–45.

Friedrichsen, Patricia Meis and Amy Pallant. 2007. French fries, dialysis tubing & computer models: Teaching diffusion & osmosis through inquiry & modeling. *The American Biology Teacher* online February 2007, pp. 22–27.

http://www.nabt.org/websites/institution/File/pdfs/publications/abt/2007/069-02-0031.pdf

This resource has questions to help students apply their knowledge about osmosis to everyday questions, such as how sorbitol affects the human digestive system.

Instructional Videos

http://www.youtube.com/watch?v=2Th0PuORsWY&feature=related

This video demonstrates the diffusion of iodine through dialysis membrane into starch.

http://www.youtube.com/watch?v=xQ9DWem9l_8&feature=related

http://www.youtube.com/watch?v=DRHKq0piN0M&feature=related

These videos show the diffusion of glucose through dialysis membrane with explanation.

http://www.youtube.com/watch?v=VK-_YHakvho

http://www.youtube.com/watch?v=zHyfDGVNdvM&feature=related

These resources show plasmolysis in *Elodea* cells under the microscope.

http://www.youtube.com/watch?v=DpVbcJY4amA

http://www.youtube.com/watch?v=1vQzqk2hzj8&feature=related

These videos show an osmosis experiment in decalcified eggs (2 parts) using water and corn syrup. They reveal ways to measure the egg sizes and make good observations about the water and corn syrup before and after the incubation period.

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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 solutes (e.g., salts, organic molecules) dissolve in water, which is the solvent. Water may pass slowly through the 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.)

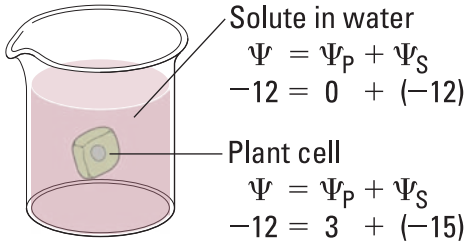

Figure 2. Plant cell in an aqueous solution. The water potential of the cell equals that of surrounding solution at dynamic equilibrium. The cell's water potential equals the sum of the turgor pressure potential plus the solute potential. The solute potentials of the solution and of the cell are not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

- Calculate the solute potential of a 0.1 M NaCl solution at 25°C. If the concentration of NaCl inside the plant cell is 0.15 M, which way will the water diffuse if the cell is placed into the 0.1 M NaCl solutions?
- What must the turgor pressure equal if there is no net diffusion between the solution and the cell?

Learning Objectives

- To investigate the relationship among surface area, volume, and the rate of diffusion
- To design experiments to measure the rate of osmosis in a model system
- To investigate osmosis in plant cells
- To design an experiment to measure water potential in plant cells
- To analyze the data collected in the experiments and make predictions about molecular movement through cellular membranes
- To work collaboratively to design experiments and analyze results
- To connect the concepts of diffusion and osmosis to the cell structure and function

General Safety Precautions

You must wear safety glasses or goggles, aprons, and gloves because you will be working with acids and caustic chemicals. The HCl and NaOH solutions will cause chemical burns, and you should use these solutions in spill-proof trays or pans. Follow your teacher's instructions carefully. Do not work in the laboratory without your teacher's supervision. Talk to your teacher if you have any questions or concerns about the experiments.

THE INVESTIGATIONS

This investigation consists of three parts. In Procedure 1, you use artificial cells to study the relationship of surface area and volume. In Procedure 2, you create models of living cells to explore osmosis and diffusion. You finish by observing osmosis in living cells (Procedure 3). All three sections of the investigation provide opportunities for you to design and conduct your own experiments.

Getting Started

These questions are designed to help you understand kinetic energy, osmosis, and diffusion and to prepare for your investigations.

- What is kinetic energy, and how does it differ from potential energy?
- What environmental factors affect kinetic energy and diffusion?

- How do these factors alter diffusion rates?
- Why are gradients important in diffusion and osmosis?
- What is the explanation for the fact that most cells are small and have cell membranes with many convolutions?
- Will water move into or out of a plant cell if the cell has a higher water potential than the surrounding environment?
- What would happen if you applied saltwater to a plant?
- How does a plant cell control its internal (turgor) pressure?

Procedure 1: Surface Area and Cell Size

Cell size and shape are important factors in determining the rate of diffusion. Think about cells with specialized functions, such as the epithelial cells that line the small intestine or plant root hairs.

- What is the shape of these cells?
- What size are the cells?
- How do small intestinal epithelial and root hair cells function in nutrient procurement?

Materials

- 2% agar containing NaOH and the pH-indicator dye phenolphthalein
- 1% phenolphthalein solution
- 0.1M HCl
- 0.1 M NaOH
- Squares of hard, thin plastic (from disposable plates); unserrated knives; or scalpels from dissection kits
- Metric rulers
- Petri dishes and test tubes
- 2% agar with phenolphthalein preparation

Step 1 Place some phenolphthalein in two test tubes. Add 0.1 M HCl to one test tube, swirl to mix the solutions, and observe the color. Using the same procedure, add 0.1 M NaOH to the other test tube. Remember to record your observations as you were instructed.

- Which solution is an acid?
- Which solution is a base?
- What color is the dye in the base? In the acid?
- What color is the dye when mixed with the base?

Step 2 Using a dull knife or a thin strip of hard plastic, cut three blocks of agar of different sizes.

These three blocks will be your models for cells.

- What is the surface area of each of your three cells?
- What is the total volume of each of your cells?
- If you put each of the blocks into a solution, into which block would that solution diffuse throughout the entire block fastest? Slowest? How do you explain the difference?

Alternative Method

Mix one packet of unflavored gelatin with 237 mL of water: add 2.5 mL 1% phenolphthalein and a few drops of 0.1 M NaOH. The solution should be bright pink. Pour the gelatin mixture into shallow pans and refrigerate overnight.

You may use white vinegar in place of the 0.1 M HCl.

Designing and Conducting Your Investigation

Using the materials listed earlier, design an experiment to test the predictions you just made regarding the relationship of surface area and volume in the artificial cells to the diffusion rate using the phenolphthalein–NaOH agar and the HCl solution. Once you have finished planning your experiment, have your teacher check your design. When you have an approved design, run your experiment and record your results. Do your experimental results support your predictions?

Procedure 2: Modeling Diffusion and Osmosis

You are in the hospital and need intravenous fluids. You read the label on the IV bag, which lists all of the solutes in the water.

- Why is it important for an IV solution to have salts in it?
- What would happen if you were given pure water in an IV?
- How would you determine the best concentration of solutes to give a patient in need of fluids *before* you introduced the fluids into the patient's body?

In this experiment, you will create models of living cells using dialysis tubing. Like cell membranes, dialysis tubing is made from a material that is selectively permeable to water and some solutes. You will fill your model cells with different solutions and determine the rate of diffusion.

- How can you use weights of the filled cell models to determine the rate and direction of diffusion? What would be an appropriate control for the procedure you just described?
- Suppose you could test other things besides weights of the dialysis tubes. How could you determine the rates and directions of diffusion of water, sucrose, NaCl, glucose, and ovalbumin?
- Will protein diffuse? Will it affect the rate of diffusion of other molecules?

Materials

- Distilled or tap water
- 1 M sucrose
- 1 M NaCl
- 1 M glucose
- 5% ovalbumin (egg white protein)
- 20 cm-long dialysis tubing
- Cups
- Balances

Step 1 Choose up to four pairs of different solutions. One solution from each pair will be in the model cell of dialysis tubing, and the other will be outside the cell in the cup. Your fifth model cell will have water inside and outside; this is your control. Before starting, use your knowledge about solute gradients to predict whether the water will diffuse into or out of the cell. Make sure you label the cups to indicate what solution is inside the cell and inside the cup.

Step 2 Make dialysis tubing cells by tying a knot in one end of five pieces of dialysis tubing. Fill each "cell" with 10 mL of the solution you chose for the inside, and knot the other end, leaving enough space for water to diffuse into the cell.

Step 3 Weigh each cell, record the initial weight, and then place it into a cup filled with the second solution for that pair. Weigh the cell after 30 minutes and record the final weight.

Step 4 Calculate the percent change in weight using the following formula: (final – initial)/initial X 100. Record your results.

- Which pair(s) that you tested did not have a change in weight? How can you explain this?
- If you compared 1 M solutions, was a 1 M NaCl solution more or less hypertonic than a 1 M sucrose solution? What is your evidence? What about 1 M NaCl and 1 M glucose and 1 M sucrose?
- Does the protein solution have a high molarity? What is evidence for your conclusion?
- How could you test for the diffusion of glucose?
- Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell?

Designing and Conducting Your Investigation

Living cell membranes are selectively permeable and contain protein channels that permit the passage of water and molecules. In some respects, the dialysis tubing you used is similar to a cell membrane, and you can use it to explore osmosis in greater depth. Think about the questions that came up as you worked through the investigation. What unanswered questions do you still have about osmosis that you could investigate further?

Using the available materials, design an investigation to answer one of your questions. Have your teacher check your design first. Remember to record your results, and be sure to use appropriate controls.

These questions can help jump-start your thinking.

- What factors determine the rate and direction of osmosis?
- What would you predict if you used a starch solution instead of the protein?
- Can you diagram the flow of water based upon the contents of your model cell and the surrounding solution?
- When will the net osmosis rate equal zero in your model cells? Will it ever truly be zero?
- Based upon your observations, can you predict the direction of osmosis in living cells when the cells are placed in various solutions?
- How is the dialysis tubing functionally different from a cellular membrane?

Procedure 3: Observing Osmosis in Living Cells

The interactions between selectively permeable membranes, water, and solutes are important in cellular and organismal functions. For example, water and nutrients move from plant roots to the leaves and shoots because of differences in water potentials. Based upon what you know and what you have learned about osmosis, diffusion, and water potential in the course of your investigations, think about these questions.

- What would happen if you applied saltwater to the roots of a plant? Why?
- What are two different ways a plant could control turgor pressure, a name for internal water potential within its cells? Is this a sufficient definition for turgor pressure?
- Will water move into or out of a plant cell if the cell has a higher water potential than its surrounding environment?

Step 1 Start by looking at a single leaf blade from either *Elodea* (a water plant) or a leaf-like structure from *Mnium hornum* (a moss) under the light microscope. If you need assistance, your teacher will show you how to place specimens on a slide.

- Where is the cell membrane in relation to the cell wall? Can you see the two structures easily? Why or why not?
- What parts of the cell that you see control the water concentration inside the cell?

Back in Procedure 2 you tested diffusion and osmosis properties of several solutions. Now you are going to determine how they affect plant cell turgor pressure.

- What changes do you expect to see when the cells are exposed to the solutions?
- How will you know if a particular treatment is increasing turgor pressure? If it is reducing turgor pressure?
- How could you determine which solution is isotonic to the cells?

Step 2 Test one of the four solutions from Procedure 2 and find out if what you predicted is what happens. When you are done, ask other students what they saw. Be sure to record all of your procedures, calculations, and observations.

Designing and Conducting Your Investigation

Materials

- Potatoes, sweet potatoes, or yams
- Cork borers or french fry cutter
- Balances
- Metric rulers
- Cups
- Color-coded sucrose solutions of different, but unlabeled, concentrations prepared by your teacher

Design an experiment to identify the concentrations of the sucrose solutions and use the solutions to determine the water potential of the plant tissues. (You might want to review the information on water potential described in Understanding Water Potential.) Use the following questions to guide your investigation:

- How can you measure the plant pieces to determine the rate of osmosis?
- How would you calculate the water potential in the cells?
- Which solution had a water potential equal to that of the plant cells? How do you know?
- Was the water potential in the different plants the same?
- How does this compare to your previous determinations in the *Elodea* cells?
- What would your results be if the potato were placed in a dry area for several days before your experiment?
- When potatoes are in the ground, do they swell with water when it rains? If not, how do you explain that, and if so, what would be the advantage or disadvantage?

Analyzing Results

1. Why are most cells small, and why do they have cell membranes with many convolutions?
2. What organelles inside the cell have membranes with many convolutions? Why?
3. Do you think osmosis occurs when a cell is in an isotonic solution? Explain your reasoning.

Where Can You Go from Here?

Do you think that fungal cells have turgor pressure? Design an experiment to test your hypothesis.