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Diffusion and Osmosis

AP* Biology — Big Idea 2, Investigation 4
An Advanced Inquiry Lab

Introduction

How do cell membranes help regulate internal cellular makeup? The purpose of this laboratory activity is to observe, measure, and identify factors that influence diffusion and osmosis in model cells.

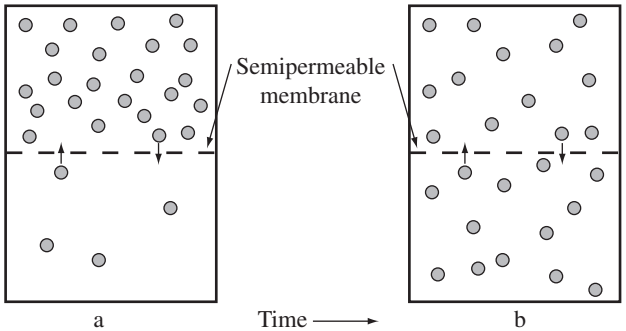
Concepts

- Diffusion
- Cell size
- Concentration gradient
- Hypotonic, hypertonic, and isotonic solutions
- Osmosis
- Surface area/volume ratio
- Semipermeable membrane

Background

A cell must be able to transport materials back and forth across its membrane to maintain homeostasis. This movement is regulated because cell membranes are selectively permeable. *Selective permeability* means that some substances can pass through the membrane while others cannot. Both solutes and solvents may cross the cell membrane.

Diffusion is the movement of solute from an area of higher concentration to an area of lower concentration. The mechanism of diffusion is quite simple. Molecules and ions are in constant motion. Since they are always moving they will eventually collide with one another. The higher the concentration of molecules, the greater the number of collisions (see Figure 1a). These collisions cause the molecules to change direction and to spread out until they eventually become uniformly distributed (See Figure 1b). Even after the molecules are evenly distributed, they are still moving, causing them to collide and redistribute. Molecular motion does not cease when uniform distribution is reached. Consequently, uniform distribution is called a *dynamic equilibrium* because there is no further net movement of the molecules down a concentration gradient. The term concentration gradient simply describes a difference in concentration across a physical distance. Diffusion is one of the key processes involved in the movement of materials into and out of cells and throughout living systems.



Diffusion across a Membrane

Figure 1.

Water travels through membranes by a diffusion process known as osmosis. *Osmosis* is the diffusion of water through a selectively permeable membrane from an area where it is more concentrated to an area where it is less concentrated. The terms hypotonic, hypertonic, and isotonic are used to describe the relative concentrations of different solutions. A *hypotonic* solution has a higher concentration of water and a lower solute concentration than a reference solution, while a *hypertonic* solution contains a

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lower concentration of water and a higher concentration of solute. Hypotonic and hypertonic solutions therefore represent unequal concentrations of molecules on either side of a permeable membrane. There will be a net flow of water via osmosis from the hypotonic side to the hypertonic side to equalize the water concentration or water potential. Water will continue to move across the membrane in equal amounts creating dynamic equilibrium. Two solutions are considered isotonic when equal concentrations of solute and water exist on either side of the cell membrane. See Table 1 for a comparison of hypotonic, hypertonic, and isotonic solutions

Table 1.

Solution Type	Water Concentration	Solute Concentration
Hypotonic	Higher	Lower
Hypertonic	Lower	Higher
Isotonic	Equal	Equal

Experimental Overview

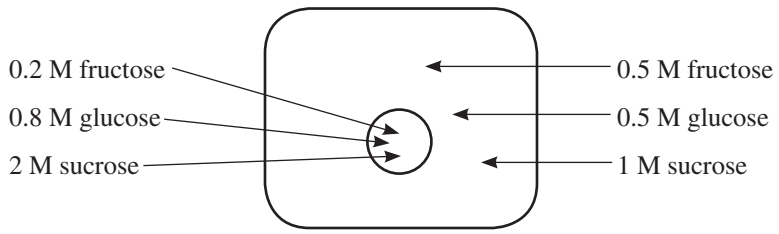
This advanced inquiry lab consists of two activities, each with a control or baseline experiment as well as opportunities for student inquiry.

In Part 1, diffusion of ions into a model cell is investigated using dilute hydrochloric acid solution and agar blocks containing phenolphthalein, an acid–base indicator. Results from the baseline activity will provide a procedure and model for guided inquiry to investigate the relationship between the volume and surface area of a model cell and the rate of diffusion. What happens to the rate of diffusion as cells get larger? How can the surface area of a cell be increased while keeping the overall volume constant?

In Part 2, model cells will be constructed using dialysis tubing to enclose sample solutions containing water, sodium chloride, glucose, sucrose or albumin. The ability of various substances to diffuse across a semipermeable dialysis membrane is investigated in the *Baseline Activity* by measuring the net direction of osmosis for five different pairs of solutions representing model cells and the surrounding cytoplasm, respectively. The results are analyzed to identify the mutual relationships among the solutions as hypotonic, hypertonic or isotonic. Opportunities for inquiry include the effect of solution concentration on the net direction of osmosis and the permeability of the membrane with respect to starch and other large biological molecules.

Pre-Lab Questions

1. Look up the acid–base properties of phenolphthalein indicator, including its expected color changes and the pH range for each color form.
2. The phenolphthalein–agar model cells used in Part 1 are initially pink. (a) Predict the observations when these cells are placed in dilute hydrochloric acid, assuming H_3O^+ ions are able to diffuse into the agar. (b) How can these observations be used to measure the *rate of diffusion*?
3. Calculate the volume and surface area of a 2-cm cubic unit cell used in the Baseline Activity in Part 1.
4. *Consider the following scenario:* An artificial membrane that is selectively permeable encloses an aqueous solution. The solution surrounding the model cell contains a different aqueous solution. Assume that monosaccharides such as glucose and fructose are able to cross the membrane but that larger disaccharides, such as sucrose, do not. The exact concentrations of each solute in the cell and surroundings are shown in the figure below.
   a. Which solute(s) will exhibit a net diffusion into the cell?
   b. Which solute(s) will exhibit net diffusion out of the cell?
   c. With respect to glucose, is the surrounding environment hypertonic or hypotonic to the cell?



5. Review the possible solutions listed for Part 2 in the *Materials* section. a) Using those materials, select four different pairs of solutions, including water, to study in the *Baseline Activity*. One solution of each pair will go inside a model cell, and the other solution will be placed in the surrounding environment. Enter your choices in Table 2. b) What is the purpose of Trial No. 5, with water in both compartments?

Table 2.

No.	**Model Cell** (inside dialysis tubing)	**Surrounding Environment** (plastic cup)	**Net Diffusion** (into or out of cell)
1			
2			
3			
4			
5	Water	Water	

6. Using your knowledge of concentration gradients and the permeability of the membrane, predict whether there will be net diffusion of water by osmosis into or out of each model cell. Enter your prediction in the table.

Materials*

Part 1. The Rate of Diffusion and Cell Size

Hydrochloric acid, HCl, 0.1 M, 150 mL
Phenolphthalein agar block
Sodium hydroxide, NaOH, 0.1 M
Beaker, 150-mL
Metric ruler
Paper towel
Plastic knife
Plastic spoon

Part 2. Modeling Osmosis and Diffusion

Albumin, 5% solution
Glucose solution, $C_6H_{12}O_6$, 1 M
Sodium chloride solution, NaCl, 1 M
Sucrose solution, $C_{12}H_{22}O_{11}$, 1 M
Water, distilled or deionized
Balance, 0.01-g precision
Cups, plastic, 9-oz
Dialysis tubing, 18 cm
Funnel
Graduated cylinder, 25-mL
Permanent marker
Weighing dish, large

**Amounts will vary based on the experimental design for the guided-inquiry experiments.*

Safety Precautions

Hydrochloric acid is toxic by ingestion and inhalation. Sodium hydroxide and hydrochloric acid solutions are corrosive to skin and eyes. Phenolphthalein solution contains alcohol and is a flammable liquid. Avoid contact of all chemicals with eyes and skin. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Follow all normal laboratory safety guidelines and wash hands thoroughly with soap and water before leaving the laboratory.

Part 1. The Rate of Diffusion and Cell Size

Baseline Activity

1. Using a plastic knife and a metric ruler, cut the phenolphthalein agar block into a 2-cm cube.
2. Pour 100 mL of 0.1 M hydrochloric acid into a 150-mL beaker.
3. Using a plastic spoon, carefully place the agar cube in the beaker of hydrochloric acid.
4. Gently agitate the solution and turn the cube with a spoon occasionally while soaking.
5. After 10 minutes, gently remove the agar cube using the plastic spoon. Blot the cube dry using a paper towel.
6. Using a plastic knife, cut the cube in half and measure the depth to which acid penetrated the cube.
7. Record observations and results.

Analysis of Results

Calculate the rate of diffusion of hydrochloric acid into the agar–phenolphthalein cube and compare the surface area-to-volume ratio of this agar "model cell."

Guided Inquiry

1. Consider the following questions while reflecting upon your knowledge of cell size, diffusion, and nutrient transfer.
   a. Why are most cells so small? Why aren't cells larger?
   b. How does the rate of diffusion influence the ability of a cell to obtain needed nutrients?
   c. Calculate the expected surface area-to-volume ratios for smaller and larger model cells.
   d. Predict how the surface area-to-volume ratio might affect the rate of diffusion into a cell.
   e. Many cells or organelles that play a key role in nutrient absorption or energy transfer have highly "convoluted" membranes with many folds. How does this affect the surface area of the cell or organelle and the rate of diffusion?
2. Design a controlled experiment to investigate the effects of surface area and cell volume on the rate of diffusion in agar model cells.
3. List any potential physical or chemical hazards that may arise in the experiment and identify the safety precautions that must be followed to reduce these hazards.
4. Review your hypothesis, safety precautions, procedure, data table and proposed analysis with your instructor prior to doing the experiment.
5. Analyze and explain the results in terms of the metabolic requirements of cells in both larger organisms and in specialized tissues, such as the small intestine.

Part 2. Modeling Osmosis and Diffusion

Baseline Activity

1. Obtain five plastic cups and label them 1–5 using a permanent marker.
2. Obtain five 18-cm pieces of pre-soaked dialysis tubing.
3. Twist approximately 3 cm of tubing at one end and tie into a knot.
4. Using a graduated cylinder, measure 15 mL of the specified solution listed for Model Cell 1 in Table 2 (see *PreLab Question* #5).
5. Open the opposite end of the dialysis tubing by rubbing it together between your fingertips.
6. Place a funnel in the open end of the dialysis tubing and transfer the solution into the model cell.

7. Twist and knot the open end of the tubing to seal the bag of dialysis tubing.
8. Gently rinse the dialysis tube bag with DI water to make sure none of the solution within the bag has dripped on the outside. *Note:* This step may be omitted if the bag contains DI water.
9. Place the dialysis tube bag on a paper towel and gently roll it back and forth to remove any excess liquid.
10. Place a weighing dish on the balance and zero the balance.
11. Measure and record the mass of the dialysis tube.
12. Fill cup number one with approximately 100 mL of the solution listed in the Surrounding Environment column, row 1 of Table 2.
13. Place the dialysis tube bag in cup 1.
14. After 30 minutes remove the dialysis tube bag from the cup. Gently roll it back and forth on a paper towel to remove excess liquid. Measure and record the final mass of the dialysis tube bag.
15. Repeat steps 3–14 with the remaining solutions chosen in Table 2.

Analysis of Results

Calculate the percent change in mass for each model cell and explain the results in terms of membrane permeability, the nature of the solutes, and solution concentration. Identify the solutions as hypotonic, hypertonic or isotonic. Discuss whether any of the experiments provide evidence for the permeability of the membrane with respect to specific solutes.

$$\text{Percent change} = \frac{(\text{Final mass} - \text{Initial Mass})}{\text{Initial mass}} \times 100\%$$

Guided Inquiry

1. Living cell membranes are selectively permeable and contain protein channels that permit the passage of water and various molecules. Dialysis tubing is similar to a cell membrane in many ways. Consider the following questions while reflecting upon your knowledge of membrane permeability, osmosis, and diffusion.
   a. Is the rate of diffusion directly proportional to the solute concentration?
   b. What other variables might influence the rate and direction of osmosis?
   c. How would diffusion of a starch solution be different than that of a protein?
   d. How could you prove or disprove that a specific solute, such as sucrose, was able to diffuse through a semipermeable membrane?
2. Design a controlled experiment to answer one of these questions or investigate a variable that might affect the exchange of nutrients between a model dialysis cell and its surrounding environment.
3. List any potential physical or chemical hazards that may arise in the experiment and identify the safety precautions that must be followed to reduce these hazards.
4. Review your hypothesis, safety precautions, procedure, data table and proposed analysis with your instructor prior to doing the experiment.
5. Analyze the results and explain how you might use a procedure such as this to determine the solute concentration inside a living cell.

Teacher's Notes

Diffusion and Osmosis

Materials Included in Kit (for 8 groups of students)

- Agar, 40 g
- Albumin, 50 g
- Dextrose (glucose), $C_6H_{12}O_6 \cdot H_2O$, 200 g
- Hydrochloric acid solution, HCl, 0.1 M, 1500 mL
- Phenolphthalein, 1% alcoholic, 30 mL
- Sodium chloride, NaCl, 75 g
- Sodium hydroxide solution, NaOH, 0.1 M, 150 mL
- Sucrose, $C_{12}H_{22}O_{11}$, 1000 g
- Agar trays, 2
- Cups, plastic, 10-oz, 40
- Dialysis tubing, 12 meters
- Knife, plastic, 8
- Ruler, metric, 8
- Spoon, plastic, 8

Additional Materials Needed (for each lab group)

- Balance, 0.01-g precision (shared)
- Beaker, 150-mL
- Funnel
- Graduated cylinder, 25-mL
- Paper towels
- Permanent marker
- Weighing dishes, large, 8

Additional Materials Needed (for *Pre-Lab Preparation*)

- Beaker, 1-L
- Heat-resistant gloves
- Magnetic stirrer/hot plate, 7″ × 7″
- Scissors
- Thermometer
- Volumetric flask, 1-L
- Weighing dishes, large, 4

Pre-Lab Preparation

Part 1. Preparation of Phenolphthalein Agar

1. Heat 1 L of water almost to a boil and slowly add 20 g of agar. Stir frequently—if agar is added too quickly, lumps will form.
2. Continue heating the solution to a slow boil until it appears translucent.
3. Remove the agar mixture from the heat source.
4. Once the agar has cooled to approximately 55 ºC, add 10 mL of 1% phenolphthalein solution. Stir the mixture and add a small volume (typically 8–10 mL) of 0.1 M sodium hydroxide to achieve a uniform, bright pink color.
5. Pour the entire mixture into agar trays to a depth of at least 3 cm.
6. Allow the agar to set overnight to completely cool and solidify. Prior to class, cut the agar into blocks so that each group can trim their block down to the specified cube sizes. If the agar is prepared more than a day ahead of time, CO_2 from the air will diffuse into the blocks and react to become carbonic acid which will turn the agar colorless. The agar will need to be soaked in a dilute sodium hydroxide to return to the desired pink color in Part 1.

Part 2. Baseline Activity

1. Cut dialysis tubing into 20-cm long pieces.
2. Soak in DI water for at least 10 minutes prior to class. *Note:* It is okay if the tubing soaks longer than 10 minutes.
3. Prepare 1 L of 5% albumin by dissolving 50 g of albumin in 1 L of DI water.
4. Prepare 1 L of 1 M glucose solution by dissolving 198 g of dextrose (glucose) in 1 L of DI water.

5. Prepare 1 L of 1 M sodium chloride solution by dissolving 58.5 g of sodium chloride in 1 L of DI water.
6. Prepare 800 mL of 1 M sucrose by dissolving 342 g of sucrose in 1 L DI water.

Part 2. Guided Inquiry

The following chart summarizes the amounts of sucrose needed to prepare 500 mL of different sucrose solutions to investigate the effect of solute concentration on the net amount or rate of diffusion into artificial dialysis cells.

Table 3. Preparation of Sucrose Solutions

Sucrose Solution	Sucrose required per 500 mL
0.2 M	34 g
0.4 M	69 g
0.6 M	103 g
0.8 M	137 g
1.0 M	171 g

Safety Precautions

Hydrochloric acid is toxic by ingestion and inhalation. Sodium hydroxide and hydrochloric acid solutions are corrosive to skin and eyes. Phenolphthalein solution contains alcohol and is a flammable liquid. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Remind students to wash their hands thoroughly with soap and water before leaving the laboratory. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Hydrochloric acid may be neutralized and disposed of according to Flinn Suggested Disposal Method #24b. Sodium hydroxide may be neutralized and disposed of according to Flinn Suggested Disposal Method #10.

Alignment with AP Biology Concepts and Curriculum Framework

Big Idea 2: Biological systems utilize free energy and molecular building blocks to grow, to reproduce, and to maintain dynamic homeostasis.

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 waste or procure nutrients faster by diffusion.
- The student is able to explain how cell size and shape affect the overall rate of nutrient intake and the rate of waste elimination.
- 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.
- The student is able to construct models that connect the movement of molecules across membranes with membrane structure and function.

Lab Hints

- Enough materials are provided in this kit for 24 students working in groups of three, or for 8 groups of students. The hydrochloric acid "diffusion" solution used in Part 1 may be recycled for the baseline activity and guided inquiry portions of the experiment. Two or more blocks may also be placed in the same hydrochloric acid solution at one time, as long as they can be totally submerged in the process.
- When preparing the phenolphthalein agar, the amount of 0.1 M of sodium hydroxide required varies based on the pH of distilled water. Add just enough to achieve the bright pink color. If too much is added, the agar will not solidify.
- Students might want to experiment with diffusion of hydroxide ions into colorless phenolphthalein–agar cubes in Part 1 to test if the rate of diffusion is the same or different for hydronium and hydroxide ions. This experiment will also allow them to explore diffusion of the indicator dye from the agar cube into the surrounding aqueous solution. The rate of diffusion is traditionally slower for sodium hydroxide, 3–4 cm in 10 minutes.
- If students decide to soak the agar cubes in water virtually no visible diffusion takes place.
- Securing the ends properly on the dialysis tubing and rinsing the outside of the bag are key steps in this experiment. Demonstrate proper technique and emphasize the importance of these steps in obtaining accurate results.
- It may be appropriate to perform the guided inquiry experiments in Part 2 as a cooperative classroom activity. Different student groups can test different concentrations of sucrose to explore whether net diffusion of water (osmosis), as measured by the change in mass of an artificial dialysis cell, is proportional to the solute concentration. The amount of sucrose provided in this kit is sufficient for preparing 500 mL each of the sucrose solutions listed in the table.

Teaching Tips

- Students struggle with the concepts of the random nature of diffusion (nondirectional) and kinetic energy. An ideal demonstration to introduce this concept is to drop a handful of hex nuts or washers over a table to show that they spread out in all directions. As the height the objects are dropped from increases (increased kinetic energy) the hex nuts will spread out further from each other when they are released.
- Equilibrium can also be tricky for students to accurately comprehend. Reinforce that molecules do not stop moving at equilibrium. Even if net equilibrium is zero the molecules still move but their relative concentration stays the same.
- The terms hypotonic, hypertonic, and isotonic are relative terms and always refer to the solute concentration rather than the water concentration.
- The College Board chose to incorporate surface area and cell size, modeling diffusion and osmosis, and water potential into one investigation. Flinn Scientific decided to divide this investigation into two labs to enhance student comprehension of fundamental concepts.

Answers to Pre-Lab Questions

1. Look up the acid–base properties of phenolphthalein indicator, including its expected color changes and the pH range for each color form.

   Phenolphthalein is a pH indicator that turns from colorless to pink at a pH range of 8.2 to 10.0.

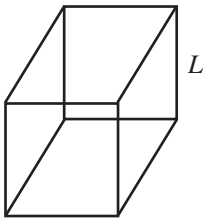
2. The phenolphthalein–agar model cells used in Part 1 are initially pink. (a) Predict the observations when these cells are placed in dilute hydrochloric acid, assuming H_3O^+ ions are able to diffuse into the agar. (b) How can these observations be used to measure the *rate of diffusion*?

   a. *The pink color will disappear and the agar cubes will decolonize as H_3O^+ ions diffuse into the agar, decreasing the pH of the agar.*

   b. *Measure the depth of the decolonization after a defined period of time.*

3. Calculate the volume and surface area of a 2-cm cubic unit cell used in the Baseline Activity in Part 1.

   V (cube) = L^3, where L is the length of one edge (side) of the cube. SA = $6L^2$



4. *Consider the following scenario:* An artificial membrane that is selectively permeable encloses an aqueous solution. The solution surrounding the model cell contains a different aqueous solution. Assume that monosaccharides such as glucose and fructose are able to cross the membrane but that larger disaccharides, such as sucrose, do not. The exact concentrations of each solute in the cell and surroundings are shown in the figure below.

   a. Which solute(s) will exhibit a net diffusion into the cell?

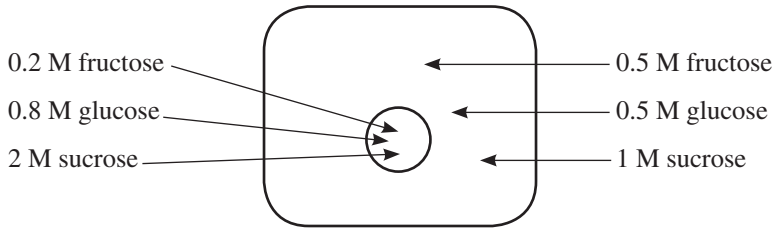
   Fructose.

   b. Which solute(s) will exhibit net diffusion out of the cell?

   Glucose.

   c. With respect to glucose, is the surrounding environment hypertonic or hypotonic to the cell?

   Hypotonic.



5. Review the possible solutions listed for Part 2 in the *Materials* section. a) Using those materials, select four different pairs of solutions, including water, to study in the *Baseline Activity*. One solution of each pair will go inside a model cell, and the other solution will be placed in the surrounding environment. Enter your choices in Table 2. b) What is the purpose of Trial No. 5, with water in both compartments?

Table 2.

No.	**Model Cell** (inside dialysis tubing)	**Surrounding Environment** (plastic cup)	**Net Diffusion** (into or out of cell)
1	*NaCl, 1 M*	*Water, DI*	*Into the cell*
2	*Water, DI*	*Glucose, 1 M*	*Out of the cell*
3	*Sucrose, 1 M*	*Water, DI*	*Into the cell*
4	*Albumin*	*Sucrose, 1 M*	*?*
5	Water	Water	*Net movement= 0*

6. Using your knowledge of concentration gradients and the permeability of the membrane, predict whether there will be net diffusion of water by osmosis into or out of each model cell. Enter your prediction in the table.

Sample Results — Part 1

Baseline Activity

Cube	**Surface Area (cm^2)**	**Volume (cm^3)**	**Surface Area-to-Volume Ratio**	**Diffusion Depth (mm)***	**Diffusion rate (mm/min)**
2 cm	*24 cm^2*	*8 cm^3*	*3:1*	*5 mm*	*mm/min*

*After 10 minutes

Guided Inquiry—Effect of Cell Size on the Rate of Diffusion

Students are likely to come up with a wide variety of predictions and experiments to answer questions about the effect of surface area and cell volume on the rate of diffusion. Below is an example of one possible investigation. The hypothesis is that the diffusion depth into a model agar cell is independent of the overall cell size or volume. If the diffusion depth is the same for different size cells of the same shape then the linear diffusion rate in mm/min will be the same. How would this affect the rate of diffusion calculated in volume (cm^3) per minute? Three different sized cubes were tested. Extrapolating from these results to the metabolic requirements of large vs. small cells we would conclude that a smaller portion of the overall volume of the cell is accessible by diffusion in a larger cell than in a smaller cell for the same length of time. This is a disadvantage for a larger cell because of the need for constant exchange of nutrients throughout the cell.

Cube	Surface Area (cm^2)	Volume (cm^3)	Surface Area-to-Volume Ratio	Diffusion Depth (mm)*	Diffusion rate (mm/min)
1 cm	6 cm^2	1 cm^3	6:1	5 mm	.5 mm/min
2 cm	24 cm^2	8 cm^3	3:1	5 mm	.5 mm/min
3 cm	54 cm^2	27 cm^3	2:1	5 mm	.5 mm/min

Sample Results — Part 2

Baseline Activity

No.	Model Cell (inside dialysis tubing)	Surrounding Environment (plastic cup)	Prediction—Water will diffuse into or out of cell	Percent Change in Mass
1	*NaCl, 1 M*	*Water, DI*	*Into the cell*	*4.4%*
2	*Water, DI*	*Glucose, 1 M*	*Out of the cell*	*12.8%*
3	*Sucrose, 1 M*	*Water, DI*	*Into the cell*	*16.5%*
4	*Albumin*	*Sucrose, 1 M*	*?*	*18.6%*
5	Water	Water	*Net movement = 0*	*0.98%*

Guided Inquiry—Effect of Sucrose Concentration on the Rate of Diffusion into a Model Cell

Sucrose Concentration in Dialysis Bag	Initial Mass (of cell)	Final Mass (of cell)	Percent Change in Mass*
0.2 M	15.55 g	16.87 g	+8.5%
0.4 M	14.96 g	16.86 g	+12.7%
0.6 M	15.76 g	18.25 g	+15.8%
0.8 M	16.85 g	20.88 g	+23.9%
1.0 M	15.42 g	19.44 g	+26.1%

*After 30 minutes.

References

AP Biology Investigative Labs: An Inquiry-Based Approach, College Entrance Examination Board: New York; 2012.

Campbell, N.A. *Biology;* Benjamin Cummings: San Francisco, CA; 2004; 6th Edition.

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