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Catalog No. FB2030
Publication No. 11117

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.

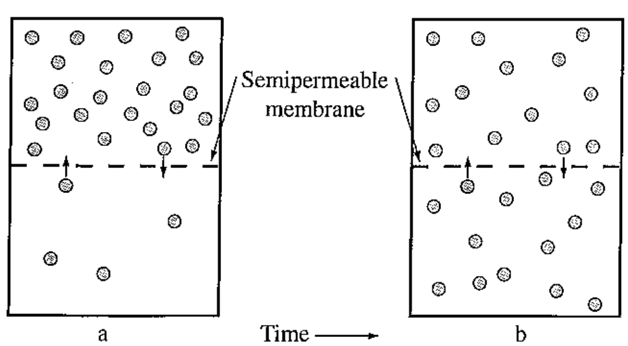
Concepts

- 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

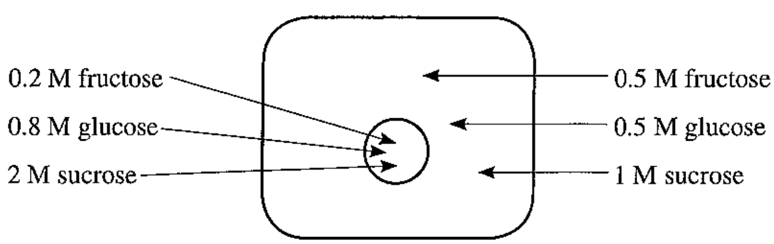
This advanced inquiry lab consists of two activities, each with a control or baseline experiment as well as opportunities for student inquiry.

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.