

AP Biology Lab #1: Osmosis and Diffusion

Principles of Enzyme Catalysis

Materials and Equipment:

1. Orange Indicator Dye, low molecular weight
2. Violet Dye, high molecular weight
3. 1 M Sucrose – 1 M NaCl solution
4. Powdered sucrose
5. Dialysis tubing
6. Beakers (300 to 400 ml) *
 - 20 part A
 - 50 Part B
 - 16 Part C
7. 1 ml, 5ml, and 10 ml pipets
8. Graph paper
9. Scales
10. Distilled Water
11. Thermometers (10)
12. Potatoes (4)
13. Cork borers, or small kitchen knives

Principles and Practice of Diffusion and Osmosis

Molecules undergo constant thermal motion. This motion enables them to move from one region to another with a velocity that depends on their mass, shape and the temperature and viscosity of the medium. The average speed of a water molecule at 37° C is approximately 1500 miles per hour. However, the motion of a molecule in solution is constantly affected by collisions with other molecules. Each collision alters the molecular trajectory so that the net result is random motion. Increases in the temperature of the medium increases the molecular velocity. Increases in the molecular mass decreases the velocity. The shape of a molecule affects its frictional drag with the solvent. Generally, longer rod-like molecules have increased drag.

Passive diffusion always results in the net flow of molecules from a region of high concentration to a region of low concentration. The magnitude of this flow of matter is determined by the size of the concentration difference between two regions over a given distance. At equilibrium, the net transfer of material is zero and the concentration is the same in all regions of the system. A system is most stable when it has attained equilibrium. A system will tend to go to equilibrium (lowest, accessible energy state) in the absence of added energy.

The diffusion of molecules in response to a concentration gradient is of fundamental importance to living systems. Examples include the oxygenation of blood in the lungs, the exchange of nutrients and waste products in extracellular compartments, and the transmission of biochemical signals at neural synapses. Individual molecular velocities are very high, but the frequency of collisions limits linear trajectories to very small distances.

The overall diffusion rate of nutrient molecules to a cell or system of cells is critical. Cells that are very close to a blood vessel (1 cell diameter, approximately 10 microns) will be in a region of higher oxygen and nutrient concentration more rapidly than cells several hundred microns away. The rate of diffusion of molecules in and out of a cell limits the cell growth. The larger the cell, the larger the cytoplasmic volume that must be supplied by diffusional processes. These factors help determine the density of tissue vascularization and the size and shape of cells. Muscle cells are several centimeters in length, which is much larger than the majority of cells. Since muscle cells are very thin, the diffusional exchange rate of nutrients and waste is not a problem. Consequently, molecules only have to travel a short distance to reach the interior. The surface to volume ratio of a cell must be within certain values to sustain its required metabolic rates by diffusional processes.

BACKGROUND INFORMATION

Background Information, continued

The cell membrane is a highly selective barrier consisting of two layers of lipid. Embedded in these layers are a wide variety of proteins, glycoproteins and glycolipids. The membrane components are always in a dynamic state of flux which may create transient pores. In the context of passive, random diffusion those molecules that are less polar (more lipid soluble) will generally penetrate the membrane more rapidly. However, in certain cases, highly polar molecules such as water, sodium, potassium and chloride ions penetrate the membrane far more rapidly than would be predicted by their low lipid solubility. These small, polar molecules pass directly through the membrane pores.

Passive diffusion of larger molecules possessing high polarity and charge such as amino acids, sugars, water soluble vitamins, proteins and nucleic acids have no appreciable rate of penetration under normal conditions. Glucose and amino acids enter the cell via mediated transport mechanisms. The transporters are membrane associated proteins that specifically bind these molecules and carry them across the membrane.

Mediated transport can be facilitated or active. Facilitated transport responds to concentration gradients, as does passive diffusion, but it is specific for the type of molecules being carried. Chemical energy is not required for facilitated mechanisms. Active transport carries molecules against a concentration gradient by the utilization of chemical energy (ATP, for example). Active transport can create intracellular concentrations of sugars and amino acids 2 to 50 times higher than extracellular concentrations.

A solute occupies a certain volume formerly occupied by pure solvent. The concentration of solvent is decreased due to the displacement of some of the solvent molecules by solute. This effect is caused only by the number of solute molecules added and not their chemical nature. The total solute concentration is the osmolarity. One osmole is equal to 1 mole of a non-ionizing molecule. A 1M solution of sodium chloride contains 2 osmoles of particles, since this salt completely ionizes into sodium and chloride ions.

A net diffusion of water molecules occurs across a semi-permeable membrane when the concentration of water on the two sides of the membrane is different. This process is termed osmosis. For example, a closed sack composed of a membrane permeable only to water molecules and containing a 1 M aqueous sucrose solution is placed in a bath of pure water. Since the concentration of water is higher on the outside of the membrane there will be a net flux of water into the sack. The volume of liquid in the sack will increase and it will swell.

BACKGROUND INFORMATION

Background Information,
continued

The water bath is hypotonic relative to the sucrose solution. If the liquid in the sack is pure water and the bath consists of a 1 M sucrose solution, then the bath is hypertonic relative to the water. There is a net flux of water out of the sack and the sack shrinks. When the concentration of water is equal on both sides of the membrane, the bath is isotonic and no change in volume occurs. The freezing point, boiling point, and osmotic pressure of a solvent can be dramatically changed by the concentration of solute, independent of its chemical composition. These properties are termed colligative.

A cell is enclosed by a semi-permeable membrane through which water can readily penetrate. The cytoplasm contains many types of molecules that cannot penetrate the membrane. The cell can shrink or swell depending on the water concentration in the surrounding medium. The cellular response to water concentration is very rapid. There is essentially no significant delay in water flow when a cell is placed in a hypotonic or hypertonic medium. Extremes in shrinkage or swelling can cause cell death. Plant and bacterial cells can withstand extremes in osmotic flow since their membranes are covered with a reinforcing, rigid cell wall.

The maintenance of the proper physiological osmolarity and extracellular fluid volume in humans is, in part, coordinated by osmoreceptors in the hypothalamus. These receptors influence the secretion of antidiuretic hormone (ADH). ADH governs renal reabsorption of water and salts. On a molar basis, sodium is the most important factor in determining plasma osmolarity. The extracellular concentration of sodium is much greater than its intracellular concentration because it is pumped out by active transport. Chloride, bicarbonate, calcium and albumin proteins are other major components that determine plasma osmolarity.

The water potential of a system, Ψ , is the chemical potential of water at specified conditions of temperature, pressure and volume. The chemical potential is the amount of free energy, or energy available to do work, per mole of water. The chemical potential is a measure of how the energy of a phase changes with the number of moles of a component while other components, such as temperature and pressure, are held constant. As an example, one phase of a system could be a 1 M sucrose solution and the other a closed sack of dialysis membrane containing pure water. This assumes conditions before equilibrium and that the membrane is only permeable to water. Water moves from a region of higher water potential to a region of lower water potential. At constant atmospheric pressure the water potential can be expressed as:

$$\Psi = \Psi_p$$

BACKGROUND INFORMATION

Background Information, continued

Ψ_p is the osmotic pressure. The osmotic pressure is the change in pressure caused by the diffusion of water due to solute concentration, i.e. osmosis. The osmotic pressure for a dilute, non-ionizing solute is approximated by the equation of van't Hoff:

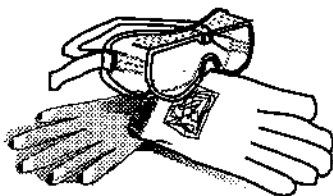
$$\Psi_p = -cRT$$

where c is the molar concentration of solute, R is the gas constant (0.0821 liter x atmosphere/ K° x mole) and T is degrees Kelvin ($C^\circ + 273$). The addition of solute to pure water lowers the concentration of water, which lowers the osmotic pressure (makes it more negative). Lower osmotic pressure lowers the water potential.

If potato cells are placed in pure water there will be a net influx of water into the cells, since Ψ_{cell} is lower due to the cytoplasmic solutes. The cell will swell and gain mass. The water flow stops when Ψ_{cell} equals Ψ_{water} . If the potato cells are placed in sucrose solutions where Ψ_{cell} is higher than Ψ_{solution} , there will be a flow of water out of the cells. The cells will shrink and lose mass. At some intermediate concentration of sucrose, Ψ_{cell} and Ψ_{solution} will be equivalent and no net change in cellular mass or volume will occur. At this concentration of sucrose, the water potential of the cell can be determined by calculating Ψ_p .

In Part A, dialysis experiments will be done with two dyes of different molecular weights to visually demonstrate the size selectivity of membranes. The experiments will also demonstrate changes in the equilibrium of the diffusible dye as it is removed from the system. Part B of the experiment will approximate an osmotic system by demonstrating net flow of water through a dialysis membrane. Part C involves the calculation of potato cell water potential from experimental data.

EXPERIMENTAL PROCEDURES



EXPERIMENT OBJECTIVE:

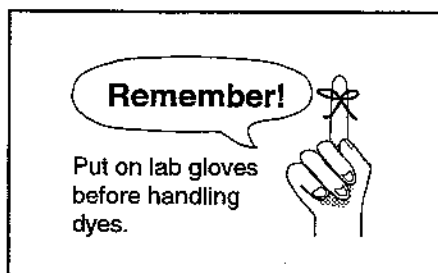
The objective of this experiment is to develop an understanding of the molecular basis of diffusion and osmosis and its physiological importance. Students will analyze how solute size and concentration affect diffusion across semi-permeable membranes and how these processes affect water potential. Students will also calculate water potential of plant cells.

LABORATORY SAFETY

Gloves and safety goggles should be worn routinely as good laboratory practice.

Student Experimental Procedures

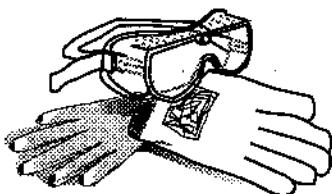
PART A. DIFFUSION AND DIALYSIS



Dialysis membranes are made of purified cellulose containing microscopic pores. The pore size is controlled during manufacture. The pore size determines the membrane's permeability to solutes of different sizes. Increasing size generally corresponds to increasing molecular weight when molecules have similar shapes. The dialysis tubing being used in this experiment has a molecular cut off of approximately 10,000, which means that molecules having molecular weights greater than 10,000 cannot penetrate the membrane. The orange dye has a molecular weight of about 300 and the violet dye has a molecular weight in excess of 100,000.

1. Tie a knot at one end of each piece of dialysis tubing. Start approximately one inch from the end. **DO NOT TIE THE KNOT TOO TIGHTLY**, otherwise tubing may rip or puncture. Keep tubing moist but avoid having too much water inside.
2. Fill each of 2 beakers (300 to 400 ml in size) with 250 ml of distilled water.
3. Add 1 ml distilled water to an empty test tube with 1 ml pipet.
4. Add 1 ml of violet dye to the water in the test tube (Step 3) with the 1 ml pipet. Mix.
5. Transfer all the 2 ml of diluted violet dye with a pipet to one of the dialysis tubes.
6. Tie a knot at the open end of the tubing as instructed in Step 1.
7. Place the tubing in one of the beakers of water.

EXPERIMENTAL PROCEDURES

Student Experimental
Procedures, continued

**WEAR SAFETY GOGGLES
AND GLOVES**

Quick Reference:

Evidence of diffusion and dialysis should be observable within 10 to 15 minutes after beginning the experiment. However, dialysis is a slow process and can take several hours or days before equilibrium of the diffusible compound is achieved. In Steps 11-13, some diffusible dye was removed from the system. The system will now tend towards equilibrium as before. Equilibrium of the diffusible dye will be achieved when its concentration is the same inside and outside the dialysis tubing. By repeatedly removing dye from the dialysis bath, the majority will be removed from the system.

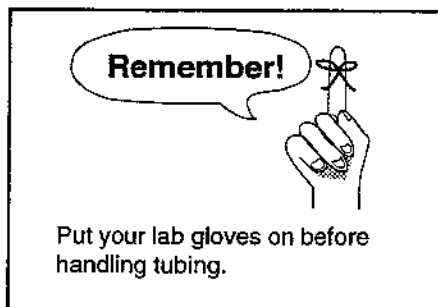
8. Transfer 1 ml of the violet dye to the test tube containing the orange dye with the 1 ml pipet. Mix. Note the color.
9. Transfer all the 2 ml of mixed dye to the other dialysis tubing with a pipet and tie the open end as instructed in Step 1.
10. Place the tubing in the second beaker of water. Occasionally mix by stirring the water in the beakers with a stirring rod or by swirling. Note any changes.
11. After 30 minutes briefly stir the beakers. Note any color changes.
12. Remove the dialysis tubing containing the dye mixture from the beaker and pour out the water.
13. Fill the beaker with 250 ml of fresh distilled water and put the dialysis tubing back in the beaker.
14. Do Step 15 during your next lab period (next day if possible).
15. Remove the dialysis tubing containing the mixed dyes from the beaker and pour out the water.
16. Fill the beaker with 250 ml of distilled water and put the dialysis tubing back into the beaker.
17. During your next lab period remove the tubing and observe the color. Compare with the tubing that only contained the violet dye.

PART B. OSMOSIS

Osmosis is the net flow of water across a semi-permeable membrane due to changes in solute concentrations. Increases in solute concentrations decrease the concentration of water. Water diffuses from a region of higher concentration to a region of lower concentration. In this experiment, solutions containing different concentrations of sucrose and salt will be transferred to dialysis tubing which will then be placed in distilled water. This arrangement behaves like an osmotic system. It is not a true osmotic system because the sucrose and the salt also penetrate the membrane. However, the dialysis tubing will only be in the water bath for 30 minutes. As this is not long enough for the system to come to equilibrium, a net change in water distribution should be observed.

1. Tie a knot at one end of each of 5 pieces of dialysis tubing. Start to tie the knot approximately 1 inch from the end. **DO NOT TIE THE KNOT TOO TIGHTLY**, otherwise the tubing may rip or puncture as you pull.

EXPERIMENTAL PROCEDURES

Student Experimental
Procedures, continued

- Consecutively label 5 dry beakers (300 to 400 ml) 0 M, 0.25 M, 0.5 M, 0.75 M and 1 M.
- Transfer 5 ml of distilled water to a piece of tubing with a 5 ml pipet and tie the open end as described in Step 1. Place the tubing in the beaker labeled 0 M.
- Transfer 5 ml of 0.25 M sucrose-salt solution to a piece of tubing and tie the open end as described. Place the tubing in the beaker labeled 0.25 M.
- Transfer 5 ml of 0.5 M sucrose-salt solution to a piece of tubing and tie the open end. Place tubing in the beaker labeled 0.5 M.
- Transfer 5 ml of 0.75 M sucrose-salt solution to a piece of tubing and tie the open end. Place in beaker labeled 0.75 M.
- Transfer 5 ml of 1 M sucrose-salt solution to a piece of tubing and tie the open end. Place tubing in the beaker labeled 1 M.
- Briefly blot each piece of tubing with paper towel to dry. Weigh each piece to the nearest tenth of a gram. Record the mass as the initial mass in the chart at left.
- Immerse the dialysis tubing by filling each beaker with 250 ml of distilled water.
- After 30 minutes remove the tubing and blot dry with paper towel. Weigh each piece and record the mass as final mass in the chart.
- Determine percent change between initial mass and final mass:

MOLARITY	INITIAL MASS	FINAL MASS	%
0.25			
0.50			
0.75			
1.00			

$$\frac{\text{FINAL MASS} - \text{INITIAL MASS}}{\text{INITIAL MASS}} \times 100$$

Enter these values in the table under %. Do not be concerned if the tubing containing the distilled water has a slightly smaller or larger final mass. This is due to experimental error.

- Graph the percent change on the Y-axis versus the molarity on the X-axis.

EXPERIMENTAL PROCEDURES

Experimental Procedures,
continued

PART C. WATER POTENTIAL

In this experiment, the water potential of plant tissue will be determined. The cytoplasm has a lower water potential, Ψ , than pure water because of high concentrations of dissolved solutes (e.g., metabolites, salts, proteins, nucleic acids). Many of these solutes cannot penetrate the membrane and leave the cell by passive diffusion. A lower value of Ψ indicates a lower concentration of water. At some concentration of sucrose in solution, the water potential will equal the water potential of the cell. The chemical nature of the solute does not matter (assuming it is not toxic), since water potential is only determined by the number of solute molecules. Potato cells will be placed into solutions containing different concentrations of sucrose. Some of the solutions will have lower water potentials (higher solute concentration) than the cells and are hypertonic. Others will have higher potentials (lower solute concentration) and are hypotonic relative to the cells. Changes in cell mass will occur by osmosis. Graphical extrapolation of the initial and final masses of the potato cells versus the sucrose concentration will yield the approximate sucrose concentration that is isotonic relative to the cells. This value will be used to calculate the osmotic pressure Ψ_p , which equals Ψ , at constant atmospheric pressure.

Quick Reference:

$$\frac{\text{Final Mass} - \text{Initial Mass}}{\text{Final Mass}} \times 100$$

1. Obtain a beaker containing a specified sucrose solution or distilled water.
2. Remove any residual skin that may be present on a portion of potato.
3. Cut out three sections that are approximately 3 to 4 cm in length with a cork borer.

Alternatively, cut out three sections that are approximately 0.5 cm wide, 3 to 4 cm long, and 3 - 4 cm deep with a knife.

4. Place the pieces in a beaker or weigh dish. Cover to avoid drying.
5. Determine the mass of the potato sample to the nearest tenth of a gram and record this value as initial mass after obtaining your solution. (See Data Table)
6. Immerse the potato sample in the solution and cover the beaker. Let the samples sit overnight.
7. Determine the temperature of the solution in °C and convert to °K by adding 273 to this value. Record in the table under T.
8. Remove the potato samples from the solution and blot dry with a paper towel. Keep the sample covered in a beaker or weigh dish to avoid drying.

EXPERIMENTAL PROCEDURES

Student Experimental
Procedures, continued

9. Determine the mass of the sample and record as Final Mass in the Data Table.
10. Determine the percent difference between the initial and final masses. Record this value in the table (See Quick Reference at left for calculation formula).
11. Evenly divide a piece of graph paper by drawing a horizontal line (starting at the midpoint of the Y-axis). Label this line as zero (0) on the Y-axis. This axis will represent the percent change in mass.
12. Label the Y-axis in increments of negative 5% below the zero (0) point, and in increments of positive 5% above the zero (0) point.
13. Plot the percent change in mass versus the sucrose concentration (molarity) on the X-axis.
14. Draw the best average line through the data points. The point of intersection (the line determined by the data points and the horizontal line corresponding to 0% change in mass) represents the approximate molarity of sucrose that is isotonic relative to the potato cells.
15. Calculate Ψ at the isotonic sucrose concentration by determining Ψ_p :

$$\Psi_p = -cRT$$

The units will be in atmospheres.

$R = 0.0821$ liter \times atmosp/ $K^\circ \times$ mol.

$c =$ sucrose molarity.

Data Table

MOLARITY	INITIAL MASS	FINAL MASS	($^\circ K$) T	%
0				
0.1				
0.2				
0.3				
0.4				
0.5				
0.6				
0.7				

Study Questions

1. Which dye penetrated the membrane in the dialysis experiment? Why?
2. What molecular weight cut off value would allow both dyes to penetrate the membrane?
3. In the idealized case involving non-charged solutes, the equilibrium constant (K_{eq}) for the diffusible dye in the dialysis experiment, is

$$K_{eq} = \frac{[DYE]_{in}}{[DYE]_{out}}$$

[] is the molar concentration. If K_{eq} is 1, then, at equilibrium, the concentration of dyes on both sides of the membrane are equal, as expected for a passive diffusion experiment. Assuming constant pressure and temperature, answer the following:

- a. Does changing the water bath alter the K_{eq} ?
 - b. Does changing the water bath change the amount of time it takes the system to reach equilibrium?
 - c. Assume the K_{eq} of the diffusible dye in the mixed dye experiment is 10. What could account for a value greater than 1?
4. How could the rate of dialysis of the dye be increased?
 5. How did the mass of the dialysis tubing change with the concentration of sucrose-salt solution? Why? What would you expect if the experiment were reversed, i.e., the bath contained increasing concentrations of sucrose-salt and the tubing initially contained pure water?
 6. A protein having a molecular weight of 65,000 has been purified. The protein solution has a high concentration of sodium chloride as a result of the last purification step. Suggest a method for removing the salt from the protein solution.