**AP Biology Lab 4:**

**Diffusion and Osmosis**

What causes plants to wilt if they are not watered?

**I. ■ 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.

**II. ■ Water Potential (Ψ)**

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 (ψs), which is dependent on solute concentration, and (2) the pressure potential (ψp), which results from the exertion of pressure — either positive or negative (tension) — on a solution. The solute potential is also called the osmotic potential.

ψ = ψs + ψp Water Potential = Solute Potential + Pressure 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 (ψ = 0) because both the solute and pressure potentials are zero (ψs = 0; ψp = 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 **(ψs) = – 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 (ψp = 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 (ψ = 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 (ψp) and the solute pressure (ψs). **(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.

1. 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? **(1 pt)**

1. What must the turgor pressure equal if there is no net diffusion between the solution and the cell? **(1 pt)**

**■ 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.

**III. ■ Pre-lab assessment**

These questions are designed to help you understand kinetic energy, osmosis, and diffusion and to prepare for your investigations. All lab questions should be answered in thorough, complete sentences.

• 1) What is kinetic energy, and how does it differ from potential energy? **(2 pt)**

• 2) What environmental factors affect kinetic energy and diffusion? **(2 pt)**

• 3) How do these factors alter diffusion rates? **(1 pt)**

• 4) Why are gradients important in diffusion and osmosis? **(1 pt)**

• 5) What is the explanation for the fact that most cells are small and have cell membranes with

 many convolutions? **(2 pt)**

• 6) Will water move into or out of a plant cell if the cell has a higher water potential than the

 surrounding environment? **(1 pt)**

• 7) What would happen if you applied saltwater to a plant? Why? **(2 pt)**

• 8) How does a plant cell control its internal (turgor) pressure? **(2 pt)**

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Cell Transport Lab Part 1: Cell Size and Diffusion

## **Introduction**

 You have learned that virtually all living cells are dependent on the process of diffusion in order to obtain the essential nutrients they need in order to survive. As cells take in these nutrients, they break them down and use the resulting energy and molecular building blocks to make more cellular components. This causes a cell to grow by increasing in size. However, cells never get too big, even if the organism is rather large. Cells are always small. In other words, the cells of an ant and a horse, are, on average, the same size; a horse just has a lot more of them.

These observations raise an interesting **INVESTIGATION QUESTION**:

**Why are cells so small?**

Here are two potential answers to this question:

* **Explanation 1:** Cells that have a larger surface area to volume ratio are more efficient at diffusing essential nutrients. (This means that materials would diffuse to a set point faster with large SA:Volume)
* **Explanation 2:** The rate of diffusion is related to cell size. Nutrients diffuse at a faster rate through small cells than they do through large cells. (This means that materials would diffuse further with large SA:Volume)

## **Methods**

You can test the validity of these different explanations by constructing a model cell using agar. Agar is a gel-like substance that is easy to cut into a variety of shapes. Chemicals diffuse through agar. Bromthymol blue, a chemical indicator, has been added to this agar. When the indicator comes into contact with an acid, it changes color. This allows you to see how far an acid diffuses into your model cell over time.

You will have the following materials available to use during your investigation:

* Indicator-agar cubes of 3 different sizes
* Weak acid (vinegar)
* Beakers
* Stopwatch or clock
* Ruler

##

## **Procedure**

1. With your group, determine which explanation above (#1 OR #2) provides the best answer to the research question (Why are cells so small?). Record the Investigation Question. Write your chosen Explanation as your Hypothesis. **(5 pts)**
2. Select 1 “cell” agar block of each of 3 sizes. (1 cm3, 2cm3 and 3cm3)
3. Set up a data table like the one below.
4. Place each cell block in its own separate container (beaker or cup) and add just enough acid (vinegar) to fully cover (submerge) the cubes. Start your timer.
5. Let the cubes sit for your assigned time interval. (5 min, 10 min, 15 min, 20 min, 30 min, and 40 min).
6. After the allotted time is up, carefully remove each block from its container with a spoon. Dip it briefly in water. Place on a white paper or plat, and cut in half with a knife. Observe any color changes. Measure the distance (in cm) of the color change has penetrated form the exterior toward the interior. Use this number to estimate the percentage of the block that the treatment has penetrated. *(Ex: .3 cm / 3 cm = 10% penetrance*) Record % penetrance in the data table. **(3 pts)**
	1. For observations, draw appropriately scaled cubes of each of your 3 “cells” in your lab notebook. **(3 pts)**
	2. Calculate the surface area of each of your 3 “cells.” Show your equation and calculations for surface area in your lab notebook. **(3 pts)**
	3. Calculate the volume of each of your 3 “cells.” Show your equation and calculations in your lab notebook. **(3 pts)**
	4. Label/write the Surface Area to Volume ratios for each of your 3 “cells.” **(3 pts)**
	5. Gather data from other groups to complete your data table.

## **Data Table:**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cell Dimensions** | **Surface Area (cm2)** | **Volume (cm3)** | **Ratio of surface area :****volume** | **% penetrance after 5 min** | **% penetrance after 10 min** | **% penetrance after 15 min** | **% penetrance after 20 min** | **% penetrance after 30 min** | **% penetrance after 40 min** |
| 1 cm3 |  |  |  |  |  |  |  |  |  |
| 2 cm3 |  |  |  |  |  |  |  |  |  |
| 3 cm3 |  |  |  |  |  |  |  |  |  |

**Graphs:**

1. Create an appropriately sized and scaled line graph to show the rate of diffusion in the 3 different sized “cell” cubes. Rate should be plotted as **distance** (cm) **/ time** (min). The different time intervals should go on the x-axis and the distance should go on the Y-axis. Use 3 different colors for each sized cube. Give your graph an appropriate title. **(10 pts)**
	1. Use an appropriate equation to calculate a rate of change for each cell size. Show your equation and your work in your lab notebook **(6 pts)**
2. Create an appropriately sized and scaled line graph to show the rate of penetrance in the 3 different sized “cell” cubes. Rate of penetrance should be plotted as **% penetrance** **/ time** (min). The different time intervals should go on the x-axis and the % penetrance should go on the Y-axis. Use 3 different colors for each sized cube. Give your graph an appropriate title. **(10 pts)**

## **Data Analysis – Answer these questions in your lab notebook in complete sentences. Do not write the question but answer it sufficiently as to clearly indicate the question you are answering.**

1. Which surface area-to-volume ratio gave the fastest diffusion rate? Support your answer with evidence. **(2 pt)**
2. Which surface area-to-volume ratio had the greatest diffusion depth? Support your answer with evidence. **(2 pt)**
3. How might a cell’s size influence the rate of diffusion? Do you think the shape matters too? Why or why not? **(3 pt)**
4. Other than cell surface area and cell volume what factors (give at least 2) might affect the rate of diffusion through cells? How could you test these? **(4 pts)**
5. Which graph is more helpful toward proving your claim? Explain how/why. **(2 pt)**

## **Conclusion**:

State a claim (using one of the 2 explanations – this does NOT have to be your original hypothesis). Use your evidence (observations, data, calculations, graphs gathered from your experiment) to support and justify your claim. Provide reasoning (scientific concepts/explanations/principals) to show how your data supports your claim**.** Use correct grammar, spelling, and punctuation. **(6 pts)**

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**Lab Part 2: Modeling Diffusion and Osmosis**

**Pre-Lab Discussion:**

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. With your group members ***discuss*** these questions before moving on with the lab.

1. Why is it important for an IV solution to have salts in it?
2. What would happen if you were given pure water in an IV?
3. 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.

**Pre-Lab Questions:** *(Write answers in thorough sentence format in your lab notebook. You do not need to copy the question)*

1. How is dialysis tubing similar to living cells? **(1 pt)**
2. How can you use weights of the filled cell models to determine the rate and direction of diffusion? (Propose a brief procedure for testing this) What would be an appropriate control for the procedure you just described? **(3 pts)**
3. Suppose you could test other things besides weights of the dialysis tubes. What other ways could you determine the rates and directions of diffusion of water, sucrose, NaCl, dextrose, and albumin? **(2 pts)**
4. Will protein diffuse? Why or why not? Will it affect the rate of diffusion of other molecules? **(2 pts)**

**Materials**:

* Distilled or tap water
* 1 M sucrose
* 1 M NaCl
* 1 M Dextrose
* 5% albumin (egg white protein)
* 20 cm-long dialysis tubing
* Cups
* Balance
* Graduated cylinder

**Procedure:**

**Step 1** You will need 5 dialysis tubes.Choose 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. Draw diagrams and label your predictions ***or*** create a table to organize your predictions. **(10 pts)**
* Make sure you label the cups to indicate what solution is *inside the cell* and what solution is *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. You only need enough solution to cover the “cell.” After 30 minutes, remove the “cell,” gently dry off excess fluid, mass and record the final weight. You will need to create a data table to organize this information. Include an appropriate title and units. **(10 pts)**

**Step 4** Calculate the **percent change in weight** using the following formula:

(final – initial)

 initial X 100.

Record your results in your data table. **(5 pts)**

**Analysis**: *(Write answers in thorough sentence format in your lab notebook. You do not need to copy the question)*

1. Which pair(s) that you tested did not have a change in weight? How can you explain this? **(2 pts)**
2. Rank or order your solutions from greatest diffusion (osmosis) rate to least. Include water in your sequence. **(2 pts)**
3. Does the protein solution have a high or low molarity? What is evidence for your conclusion? **(2 pts)**
4. How could you test for the diffusion of dextrose (glucose)? **(1 pts)**
5. When will the net osmosis rate equal zero in your model cells? **(1 pt)**
6. Based on your observations, can you predict the direction of osmosis in living cells when the cells are placed in various solutions? **(1 pt)**
7. Based on what you learned from your experiment, how could you determine the solute concentration inside a living cell? **(1 pts)**

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**Lab Part 3: Experimental Determination of the Water Potential of a Vegetable Sample**

**Task 1:** You will be given six unlabeled sucrose solutions of concentrations spanning from 0.0M to 1.0M in 0.2M increments (0.0M, 0.2M, 0.4M, 0.6M, 0.8M, 1.0M). You will use your understanding of water potential and tonicity to determine the molarity of each solution.

## **Task 2:** You will need to determine the water potential of a sample of a vegetable.

**Materials**

* Potatoes, sweet potatoes, or yams cut into equal sized pieces
* Balances
* Metric rulers
* Cups
* Color-coded sucrose solutions of different, but unlabeled, concentrations prepared by your teacher

**Procedure**: (**30 pts** for well presented data and analysis)

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.) Record your data in appropriately constructed data tables, charts, or drawings. Respond to all lab questions in complete sentences in your lab notebook.

**Pre-Lab Questions:**

1. How can you measure the plant pieces to determine the rate of osmosis? What 2 ways will we be using for this lab? **(2 pts)**

**Data Analysis Questions:**

1. What was the molarity of each solution? **(3 pts)**
2. Which solution had a water potential equal to that of the plant cells? How do you know? **(2 pts)**
3. Use the graph provided to construct a labeled graph of % Change in Mass vs Sucrose Molarity (we will discuss this further as a class) **(5 pts)**
	1. What is the molar concentration of the potato cell? Label it on your graph. **(2 pts)**
	2. How would you calculate the water potential in the potato cells? **(2 pts)**
4. What would your results be if the potato were placed in a dry area for several days before your experiment? Explain. **(2 pts)**
5. 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? **(4 pts)**

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*Use this graph for Part 3 of Lab #4 Diffusion/Osmosis to find water potential of Potatoes*

**Percent Change in Mass of Potato Cores at Different Molarities of Sucrose**



Determine the molar concentration of the potato core. This would be the sucrose molarity in which the mass of the potato core does not change. To find this, draw a “line of best fit” through your data points. **The point at which this line crosses the x-axis represents the molar concentration of sucrose with a water potential that is equal to the potato tissue water potential.** At this concentration there is no net gain or loss of water from the tissue. Indicate this concentration of sucrose both on the graph above *and* in the space below.

Molar Concentration of Sucrose = \_\_\_\_\_\_\_\_\_M