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**AP BIOLOGY LAB: DIFFUSION AND OSMOSIS**
OBJECTIVES

* Investigate the processes of osmosis and diffusion in a model of a membrane system
* Investigate the effect of solute concentration on water potential as it relates to living plant tissues

**Background:**

Many aspects of the life of a cell depend on the fact that atoms and molecules are constantly in motion (the concept of kinetic energy). This kinetic energy results in molecules bumping into and rebounding off each other and moving in new directions. One result of this molecular motion is the process of diffusion.

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 solvent is water, in which the solutes, such as salts and organic molecules, are dissolved. 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 physical pressure exerted on the cell).

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.

**Understanding Water Potential (****)**

In non-walled 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 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 (). Water potential is the free energy per mole of water and is calculated from two major components: (1) the solute potential (S) – also called the osmotic potential – is dependent on solute concentration, and (2) the pressure potential  (P), which results from the exertion of pressure — either positive or negative (tension) — on a solution.

**Water Potential = Pressure Potential + Osmotic Potential**

 **=** **P +** s

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 ( = 0) because both the solute and pressure potentials are zero (s = 0;  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 () = – iCRT, where i = the ionization constant, C = the molar concentration (a.k.a. osmolarity), 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 (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- (where sucrose stays as one particle); therefore i = 2, and the water potential = -7.4 bars.

When a cell‘s cytoplasm is separated from pure water (e.g. distilled water) by a selectively permeable membrane, water moves from the surrounding area, where the water potential is higher ( = 0), into the cell, where water potential is lower because of solutes in the cytoplasm (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 (not just osmotic potential!) equals the water potential of the pure water outside the cell ( of cell =  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 (P) and the solute pressure (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 pressure. A continued loss of water will cause the cell membrane to shrink away from the cell wall, and the cell will plasmolyze.

**Part A - Osmosis Across a Membrane**

1. Obtain 6 strips of dialysis tubing and tie an knot in one end of each.
2. Pour approxiamately 25 mL of each of the following solutions into separate bags.

|  |  |  |
| --- | --- | --- |
| Clear | Blue | Green |
| Red | Yellow | Purple |

3. Remove most of the air from the bag (but leave a little bit of space) and tie the baggie.
4. Rinse the baggie carefully in distilled water to remove any sucrose that may have spilled and carefully blot.
5. Record the mass of each baggie.
6. Fill six cups two-thirds full with distilled water and place a bag in each of them. Make sure that you record which baggie is which.
7. Let the bag sit for 20-30 minutes. (While this is running, you may want to begin Part 2)
8. After 20-30 minutes, remove the baggies from the water, and carefully blot dry.

9. Measure the mass of each baggie and record.

10. To calculate: percent change in mass= (final mass-initial mass)/ initial mass. Then multiply answer by 100.

11. Gather the data from the other groups in the class and find the class average for each baggie.

12. Determine the molar concentration of each bag.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Contents in bag | Initial mass | Final mass | Mass difference | % change in mass | Average | Molar concentration |
| Clear |  |  |  |  |  |  |
| Red |  |  |  |  |  |  |
| Blue |  |  |  |  |  |  |
| Yellow |  |  |  |  |  |  |
| Green |  |  |  |  |  |  |
| Purple |  |  |  |  |  |  |

12. Graph the results for both your **individiual data** and **class average** on on graph. The independent variable is on the X axis, and the dependent variable is on the Y axis



**ANALYSIS**

1. Explain the relationship between the change in mass and the molarity of sucrose within the dialysis bag.

2. Predict what would happen to the mass of each bag in this experiment if all the bags were placed in 0.4 M sucrose solution instead of distilled water. Explain your response.

3. Why did you calculate the percent change in mass rather than simply using the change in mass?

4. A dialysis bag is filled with distilled water and then placed in a sucrose solution. The bag's initial mass is 20 g, and its final mass is 18 g. Calculate the percent change of mass, showing your calculations.

**Part B - Determining the Water Potential of Carrot Cells**

Directions:
1. Pour 100 mL of each sucrose solution into a separate cup. Label each cup.
2. Determine the mass of six carrots and record the mass in Table 1. Put each carrot into the appropriate cup of solution. Label which carrot went into each solution.
3. Cover each cup to prevent evaporation. Let stand overnight

5. The next day, remove the carrots from each cup, blot dry, and determine each mass.
6. Record each mass, calculate the % mass change, and share class data to get averages.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Contents in Bag**/color** | Initial Mass | Final Mass | Mass Difference | %Change in Mass | Class average |
| Distilled Water/ |   |   |   |   |   |
| 0.2 M/ |   |   |   |   |   |
| 0.4 M/ |   |   |   |   |   |
| 0.6 M/ |   |   |   |   |   |
| 0.8 M/ |   |   |   |   |   |
| 1.0 M/ |   |   |   |   |   |

7. Graph the results for both your individiual data and class average on on graph. In order to do so, the 0 axis line should actually be in the middle of your graph. The y axis above this line should be labeled % increase in mass while the y axis below this line should be labeled % decrease. The x axis is the sucrose molarity within the beaker.



8. Determine the molar concentration of the carrot. This would be the sucrose molarity in which the mass of the potato core does not change. To find this, draw the straight line on your graph that best fits your data. **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.

What is the Molar concentration of the carrots?\_\_\_\_

9. Calculate the solute potential for the sucose solution: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_



10. Water potential of carrot = \_\_\_\_\_\_\_\_\_\_

11 Explain water potential and describe how it affects osmosis.

**Part C – Plant cell plasmolysis**

Cells lose or gain water due to the difference in solute concentrations between the

cytoplasm (the intracellular fluid) and the solution surrounding the cell (the extracellular

fluid). The movement of water in and out of a cell is governed by the laws of diffusion:

water flows from a region of higher water concentration to a region of lower

concentration.

When a cell is in a hypertonic solution, it will experience a net loss of water. A

hypertonic solution contains a higher concentration of solutes than the cell and

therefore a lower concentration of water. Consequently, water will flow out of the cell

from the region of higher water concentration to the region of lower concentration.

When a cell is in a hypotonic solution, it will experience a net gain of water. A

hypotonic solution contains a lower concentration of solutes than the cell and therefore

a higher concentration of water. Consequently, water will flow into the cell from the

region of higher water concentration to the region of lower concentration.

When a cell is in an isotonic solution, it will experience neither a net gain or loss of

water. A isotonic solution contains an equal concentration of solutes as the cell and

therefore an equal concentration of water. Consequently, water will flow equally into

and out of the cell.

Plasmolysis is the shrinking of the cytoplasm of a plant cell in response to diffusion of

water out of the cell and into a hypertonic solution surrounding the cell as shown below

in Figure 1. During plasmolysis the cell membrane pulls away from the cell wall. In this

lab exercise, you will examine this process by observing the effects of a highly

concentrated salt solution on plant cells.



Procedure: Observe various plant cells under the microscope.

Questions for Part C

1. Describe what you see happening to the plant cells. Include the terms plasmolysis, central

vacuole, diffusion, hypertonic and hypotonic.

2. In the winter, icy roads are often salted to remove the ice and make them less slippery.

Grasses and other herbaceous plants often die near the side of these roads. What causes

this to happen?

3. When a person is given fluid intravenously (an I.V.) in the hospital, the fluid is typically a

saline solution isotonic to human body tissues. Explain why this is necessary.

4. What if the unthinkable happened at the hospital! A patient was given an I.V. bag with

distilled water in it rather than saline solution. Describe what would happen to their red blood

cells and explain why this would occur.

5. Many freshwater one-celled organisms, like Paramecium, have contractile vacuoles. These

structures collect and pump out excess water that accumulates in the cell. Explain why

these organisms needs such a structure.

6. In each case, label the

diagrams as to whether the

red blood cells are in a hypotonic, isotonic, or hypertonic solution

 

**Part D: Determining the Water Potential of Various Vegetables or**

**Fruits- An Inquiry Activity**

Using the knowledge that you have gained from the above investigations, work with your group

to develop a procedure to determine the water potential of other vegetables or fruits of your

choosing. Do different types of apples or potatoes have different water potentials?

**Lab Notebook Guidelines**

* **Title**
* **Objectives**
* **Background information**
* **Materials**
* **Part A:**
	+ **Procedure, data table, graph, answers to questions**
* **Part B:**
	+ **Procedure, data table, graph, answers to questions**
* **Part C:**
	+ **Sketch of microscopic cells and answers to questions**
* **Part D:**

**1.Your hypothesis**

**2. Your groups procedure**

**3. A table of your individual results**

**4. A graph to show determination of the isotonic point of your vegetables/fruits**

**5. A calculation of water potential for your vegetables/fruits**

**6. A table of class data on isotonic point and water potential for all vegetables & fruits investigated.**

**7. A discussion of the results of this part of the laboratory, in keeping with normal conclusion expectations**