AP BIOLOGY
CELL UNIT
ACTIVITY #6

NAME____________________

DATE___________HOUR_____

# DIFFUSION AND OSMOSIS LAB

## INTRODUCTION:

In this laboratory you will investigate the processes of diffusion and osmosis in a model membrane system. You will also investigate the effect of solute concentration on water potential as it relates to living plant tissues.

Many aspects of the life of a cell depend on the fact that atoms and molecules have kinetic energy and are constantly in motion. This kinetic energy causes molecules to bump into each other and move in new directions. One result of this molecular motion is the process of diffusion.

**Diffusion** is the random movement of molecules from an area of higher concentration to an area of lower concentration. For example, if one were to open a bottle of hydrogen sulfide ($H_2S$ has the odor of rotten eggs) in one corner of a room it would not be long before someone in the opposite corner would perceive the smell of rotten eggs. The bottle contains a higher concentration of $H_2S$ molecules than the room does and, therefore, the $H_2S$ gas diffuses from the area of higher concentration to the area of lower concentration. Eventually a dynamic equilibrium will be reached; the concentration of $H_2S$ will be approximately equal throughout the room and no **net** movement of $H_2S$ will occur from one area to the other.

**Osmosis** is a special case of diffusion. Osmosis is the diffusion of water through a selectively permeable membrane (a membrane that allows for diffusion of certain solutes and water) from a region of higher water potential to a region of lower water potential. Water potential is the measure of free energy of water in a solution.

Diffusion and osmosis do not entirely explain the movement of ions or molecules into and out of cells. One property of a living system is active transport. This process uses energy from ATP to move substances through the cell membrane. Active transport usually moves substances against a concentration gradient, from regions of lower concentration of that substance into regions of higher concentration.

## PART I: DIFFUSION

In this experiment you will measure diffusion of small molecules through dialysis tubing, an example of a selectively permeable membrane. Small solute molecules and water molecules can move freely through a selectively permeable membrane, but large molecules will pass through more slowly, or perhaps not at all. The movement of a solute through a selectively permeable membrane is called **dialysis**. The size of the minute pores in the dialysis tubing determines which substances can pass through the membrane.

A solution of glucose and starch will be placed inside a bag of dialysis tubing. Distilled water will be placed in a beaker, outside the dialysis bag. After 30 minutes have passed, the solution inside the dialysis tubing and in the beaker will be tested for glucose and starch. The presence of glucose will be tested with Diastix or Testape. The presence of starch will be tested with Lugol's solution (Iodine Potassium-Iodide or IKI.)

### PROCEDURE:

1. Obtain a 30-cm piece of 2.5-cm dialysis tubing that has been soaking in water. Tie off one end of the tubing to form a bag. To open the other end of the bag, rub the end between your fingers until he edges separate.

2. Place 15 mL of the 15% glucose/1% starch solution in the bag. Tie off the other end of the bag leaving sufficient space for the expansion of the contents in the bag. Record the color of the solution in the Data Table for Part I.

3. Use the Diastix or Testape to test the 15% glucose/1% starch solution for the presence of glucose. Record your results in the Data Table for Part I

4. Fill a 250-mL beaker or cup two-thirds full with distilled water. Add approximately 4 mL of Lugol's solution to the distilled water and record the color of the solution in Data Table for Part I. Test the solution for glucose and record the results in the Data Table for Part I.

5. Immerse the bag in the beaker of solution.

6. Allow your set-up to stand for approximately 30 minutes or until you see a distinct color change in the bag or in the beaker. Record the final color of the solution in the bag, and of the solution in the beaker, in the Data Table for Part I.

7. Test the liquid in the beaker and in the bag for the presence of glucose. Record your results in the Data Table for Part I.

**DATA TABLE FOR PART I**

| | | Bag | Beaker |
|---|---|---|---|
| **Initial Contents** | | | |
| **Sol'n Color** | **Initial** | | |
| | **Final** | | |
| **Presence of Glucose** | **Initial** | | |
| | **Final** | | |

**ANALYSIS OF RESULTS**

8. Which substance(s) is(are) entering the bag? What evidence supports your answer?

________________________________________________________

________________________________________________________

9. Which substance(s) is(are) leaving the bag? What evidence supports your answer?

________________________________________________________

________________________________________________________

10. Explain the results you obtained. Include the concentration differences and membrane pore size in your discussion.

| Results | Explanation |
|---|---|
| **Glucose** | |

| Results | Explanation |
| --- | --- |
| **IKI** | |
| **Starch** | |
| **Water** | |

11. Quantitative data uses numbers to measure observed changes. How could this experiment be modified so that quantitative data could be collected to show that water diffuses into the dialysis bag?

____________________________________________

____________________________________________

12. Based on your observations, rank the following by relative size, beginning with the smallest: glucose molecules, water molecules, IKI molecules, membrane pores, starch molecules.

| **Smallest** | | | | **Largest** |
| --- | --- | --- | --- | --- |
| | | | | |

13. What results would you expect if the experiment started with a glucose and IKI solution inside the bag and only starch and water outside? Why?

Expected Results: ____________________________________________

____________________________________________

Explanation of Results: ____________________________________________

____________________________________________

____________________________________________

**PART II: OSMOSIS**

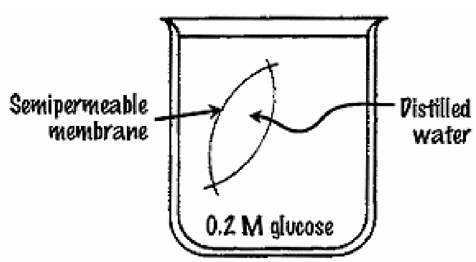
In this experiment you will use dialysis tubing to investigate the relationship between solute concentration and the movement of water through a selectively permeable membrane by the process of osmosis.

When two solutions have the same concentration of solutes, they are said to be **isotonic** to each other (iso means same, -ton means condition, -ic means pertaining to.) If the two solutions are separated by a selectively permeable membrane, water will move between the two solutions, but there will be no **net** change in the amount of water in either solution.

If two solutions differ in the concentration of solutes that each has, the one with more solute is **hypertonic** to the one with less solute (hyper means over or more than.) The solution that has less solute is **hypotonic** to the one with more solute (hypo means under or less than.) These words can only be used to compare solutions.

Now consider two solutions separated by a selectively permeable membrane. The solution that is hypertonic to the other must have more solute and therefore less water. At standard atmospheric pressure, the water potential of the hypertonic solution is less than the water potential of the hypotonic solution, so the **net** movement of water will be from the hypotonic solution into the hypertonic solution.

14. Label the sketch below to indicate which solution is hypertonic, which is hypotonic, and use arrows to show the initial **net** movement of water.




**PROCEDURE:**

15. Each group will be assigned a different solution to investigate. What solution has your group been assigned?

    Assigned solution: ________________

16. Obtain three 30-cm strips of presoaked dialysis tubing.

17. Tie a knot in one end of each piece of dialysis tubing to form three bags.

18. Pour approximately 25 mL of your assigned solution into each bag.

19. Remove most of the air from each bag by drawing the dialysis bag between two fingers. Tie off the other end of the bag. Leave sufficient space for the expansion of the contents in the bag. (The solution should fill only about one-third to one-half of the piece of tubing.)

20. Rinse each bag gently with distilled water to remove any sucrose spilled during filling.

21. Carefully blot the outside of each bag and record in the Data Table for Part II Group Results the initial mass (in grams) of each bag.

22. Fill three plastic cups two-thirds full with distilled water.

23. Immerse each bag in one of the cups of distilled water and number each cup (1, 2, or 3.) Be sure to completely submerge each bag.

24. Let the bags and cups stand for 15 minutes.

25. At the end of 15 minutes remove the bags from the water. Carefully blot the outside of each bag.

26. Determine the mass of each bag and record the mass in the Data Table for Part II Group Results.

27. Calculate the difference in mass for each bag and record your answers in the Data Table for Part II Group Results.

28. Calculate the percent change in mass for each bag and record your answers in the Data Table for Part II Group Results. Use the formula below to calculate the percent change in mass:

$$\text{Percent Change in Mass} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

29. Record the class' results in the Data Table for Part II Class Results.

**Data Table for Part II Group Results**

| **Solution Tested:** | |
|---|---|

| **Trial** | **Initial Mass** | **Final Mass** | **Mass Difference** | **% Change in Mass** | **Ave. % Change in Mass** |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |

**Data Table for Part II Class Results**

| **Solution** | **Distilled Water** | **0.2 M** | **0.4 M** | **0.6 M** | **0.8 M** | **1.0 M** |
|---|---|---|---|---|---|---|
| **Ave. % Change in Mass** | | | | | | |

30. Graph the class results for Part II.

The **independent** variable is ________________________________________.

The independent variable should be used to label the ________ axis.

The **dependent** variable is __________________________________________.

The dependent variable should be used to label the _________ axis.

Graph Title: _________________________________________________________

## ANALYSIS OF RESULTS

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

______________________________________________________________

______________________________________________________________

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

| Solution | Predicted change | Explanation |
|---|---|---|
| **Distilled Water** | | |
| **0.2 M** | | |
| **0.4 M** | | |
| **0.6 M** | | |
| **0.8 M** | | |
| **1.0 M** | | |

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

______________________________________________________________

______________________________________________________________

34. 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 in mass. Show your work.

| |
|---|
| |

35. The sucrose solution in the beaker would have been ______ to the distilled water in the bag. (Circle the word that best completes the sentence.)

**isotonic** **hypertonic** **hypotonic**

## PART III: WATER POTENTIAL

In this part of the exercise you will use potato chunks indifferent molar concentrations of sucrose in order to determine the water potential of potato cells. First, however, we will explore what is meant by the term "water potential."

Botanists use the term water potential when predicting the movement of water into or out of plant cells. Water potential is abbreviated by the Greek letter psi ($\psi$) and it has two components; a physical pressure component, pressure potential $\psi_p$, and the effects of solutes, solute potential $\psi_s$.

| $\psi$ | = | $\psi_p$ | + | $\psi_s$ |
|---|---|---|---|---|
| **Water potential** | **=** | **Pressure potential** | **+** | **Solute potential** |

**Water will always move from an area of higher water potential** (higher free energy; more water molecules) **to an area of lower water potential** (lower free energy; fewer water molecules.) Water potential, then, measures the tendency of water to leave one place in favor of another place. You can picture the water diffusing "down" a water potential gradient.

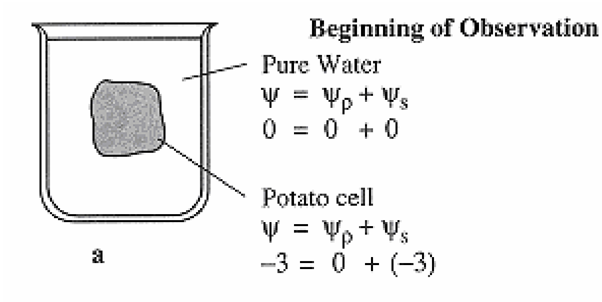
Water potential is affected by two physical factors. One factor is the addition of solute which lowers water potential (fewer water molecules.) The other factor is pressure potential (physical pressure.) An increase in pressure raises the water potential. By convention, the water potential of pure water at atmospheric pressure is defined as being zero ($\psi$ = 0.) For instance, it can be calculated that a 0.1 M solution of sucrose at atmospheric pressure ($\psi_p$ = 0) has a water potential of –2.3 bars due to the solute ($\psi$ = -2.3). (Note: A bar is a metric measure of pressure, measured with a barometer and is about the same as 1 atmosphere. Another measure of pressure is the megapascal (MPa.) 1 MPa = 10 bars.)

Movement of water into and out of a cell is influenced by the solute potential (relative concentration of solute) on either side of the cell membrane. If water moves out of the cell, the cell will shrink. If water moves into an animal cell, it will swell and may even burst. In plant cells, the presence of a cell wall prevents cells from bursting as water enters the cells, but pressure eventually builds up inside the cell and affects the net movement of water. As water enters a dialysis bag or a cell with a cell wall, pressure will develop inside the bag or cell as water pushes against the bag or cell wall. The pressure would cause, for example, the water to rise in an osmometer tube or increase the pressure on a cell wall. It is important to realized that water potential and solute concentration are inversely related. The addition of solutes lowers the water potential of the system. In summary, solute potential is the effect that solutes have on a solution's overall water potential.

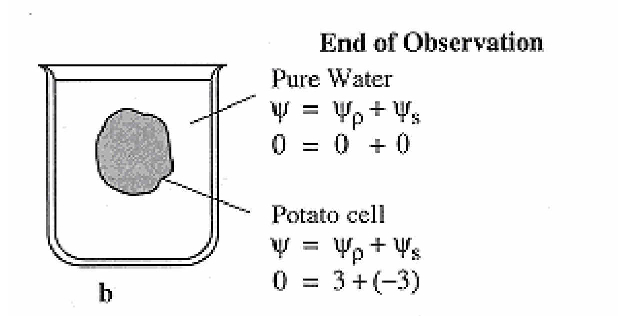
Movement of water into and out of a cell is also influenced by the pressure potential (physical pressure) on either side of the cell membrane. Water movement is directly proportional to the pressure on a system. For example, pressing on the plunger of a water-filled syringe causes the water to exit via any opening. In plant cells this physical pressure can be exerted by the cell pressing against the partially elastic cell wall. Pressure potential is usually positive in living cells: in dead xylem elements it is often negative.

It is important for you to be clear about the numerical relationships between water potential and its components, pressure potential and solute potential. The water potential value can be positive, zero, or negative. Remember that water will move across a membrane in the direction of lower water potential. An increase in pressure potential results in a more positive value, and a decrease in pressure potential (tension or pulling) results in a more negative value. In contrast to pressure potential, solute potential is always negative; since pure water has a water potential of zero, any solutes will make the solution have a lower (more negative) water potential. Generally, an increase in solute potential makes the water potential value more negative and an increase in pressure potential makes the water potential more positive.

To illustrate the concepts discussed above, we will look at a sample system using the figures below. When a solution, such as that inside a potato cell, is separated from pure water by a selectively permeable cell membrane, water will move (by osmosis) from the surrounding water where water potential is higher, into the cell where water potential is lower (more negative) due to the solute potential ($\psi_s$). In the picture at the right (picture a) the pure water potential is 0 ($\psi$=0) and the solute potential is –3 ($\psi_s$ = -3.) We will assume, for purposes of explanation, that the solute is not diffusing out of the cell.

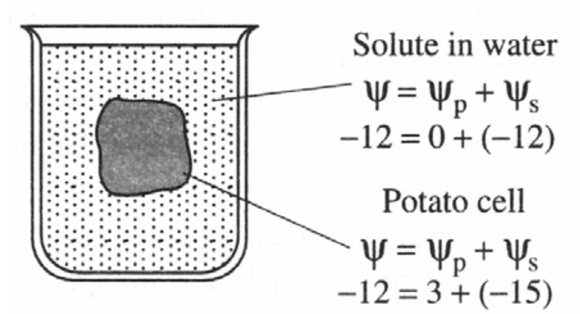


By the end of the observation, the movement of water into the cell causes the cell to swell and the cell contents push against the cell wall to produce an increase in pressure potential (turgor) ($\psi$=3.)




Eventually, enough turgor pressure builds up to balance the negative solute potential of the cell. When the water potential of the cell equals the water potential of the pure water outside the cell ($\psi$ of cell = $\psi$ of pure water = 0), a dynamic equilibrium is reached and there will be no NET movement of water (picture b.)

If you were to add solute to the water outside the potato cells, the water potential of the solution surrounding the cells would decrease. It is possible to add just enough solute to the water so that the water potential outside the cell is the same as the water potential inside the cell. In this case, there will be no net movement of water. This does not mean, however, that the solute concentrations inside and outside the cell are equal, because water potential inside the cell results from the combination of both pressure potential and solute potential.




If enough solute is added to the water outside the cells, water will leave the cells, moving from an area of higher water potential to an area of lower water potential. The loss of water from the cells will cause the cells to lose turgor. A continued loss of water will eventually cause the cell membrane to shrink away from the cell wall (plasmolysis.)

**Procedure**

36. Each group will be assigned a different solution to investigate. What solution has your group been assigned?

    Assigned solution: _______________

37. Obtain three plastic cups and place 100 mL of your assigned solution in each of the three cups.

38. Obtain six potato chunks from the supply area. Keep the potato chunks in a covered beaker or cup until you are ready to mass the chunks.

39. Determine the mass of two potato chunks. Place these two chunks in a cup of solution. Write the mass of the potato chunks on the cup. Also record the mass of the chunks in the Data Table for Part III Group Results.

40. Repeat step 39 for each of the other cups. (Mass two potato chunks, place the chunks in a cup, write the mass of the chunks on the cup, and record the mass in the data table.)

41. Cover each cup with plastic wrap and let them stand overnight.

42. Use forceps or a sharp probe to remove the potato chunks from the first cup. Tap the probe or forceps on the side of the cup several times to remove the excess solution from the potato chunks.

43. Determine the mass of the potato chunks from the first cup. Record the mass on the cup and in the Data table for Part III Group Results.

44. Repeat steps 42 and 43 for the potato chunks in the other two cups.

45. Calculate the change in mass for the potato chunks in each cup. Record your results in the Data Table for Part III Group Results.

46. Calculate the percent change in mass for the potato chunks in each cup. Record your results in the Data Table for Part III Group Results.

$$\text{Percent Change in Mass} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

47. Calculate the average percent change in mass and record your answer in the data table.

48. Record the average percent change in mass for your solution and potato chunks in the Data Table for Part III Class Results.

**Data Table for Part III Group Results**

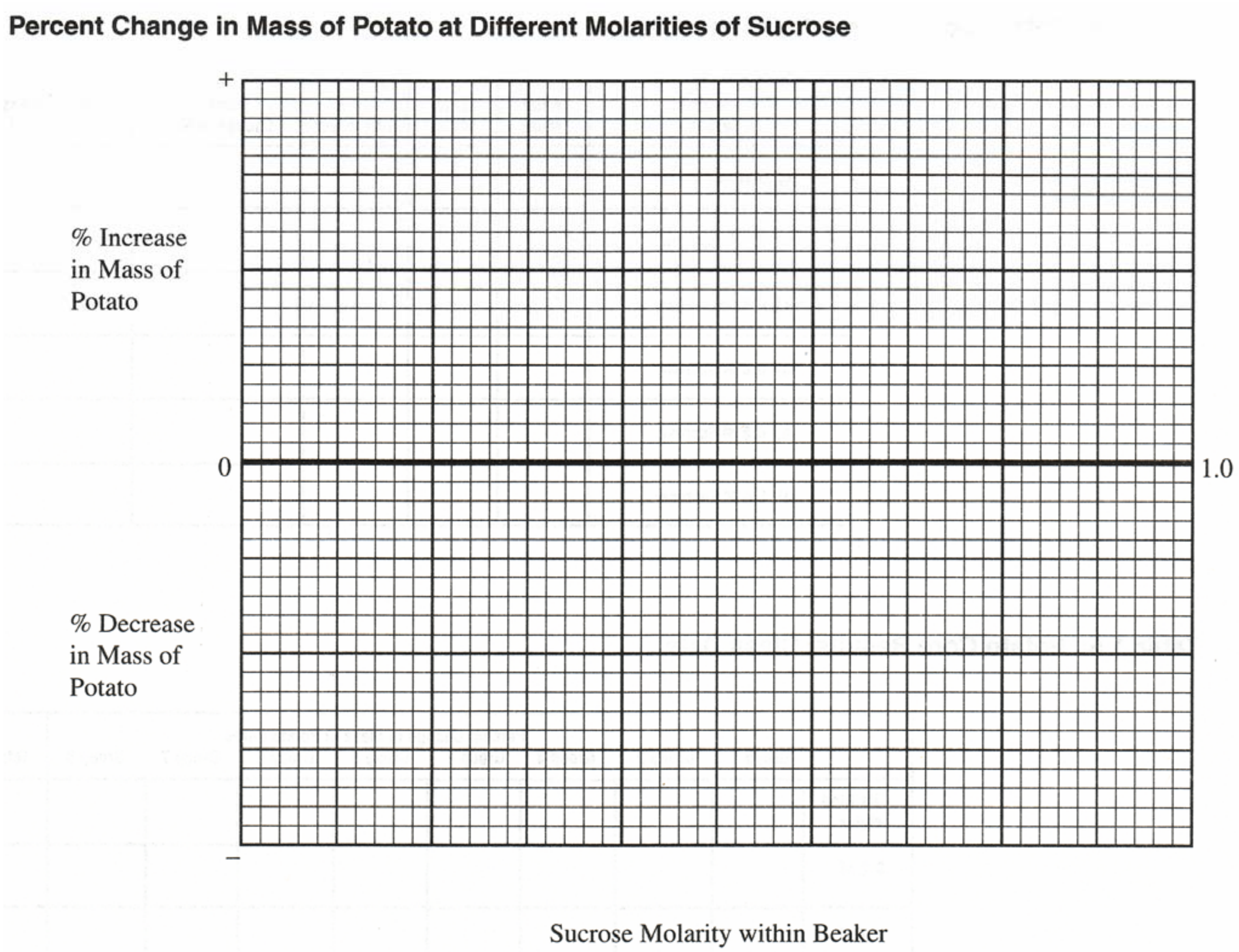
| **Solution Tested:** | |
|---|---|

| **Trial** | **Initial Mass** | **Final Mass** | **Mass Difference** | **% Change in Mass** | **Ave. % Change in Mass** |
|---|---|---|---|---|---|
| **1** | | | | | |
| **2** | | | | | |
| **3** | | | | | |

**Data Table for Part III Class Results**

| Solution | Distilled Water | 0.2 M | 0.4 M | 0.6 M | 0.8 M | 1.0 M |
|---|---|---|---|---|---|---|
| Ave. % Change in Mass | | | | | | |

49. Graph the class results for the percent change in mass.




50. Determine the molar concentration of the potato chunks. This would be the sucrose molarity in which the mass of the potato chunks does not change. To find this, draw the straight line on the 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. Indicate this concentration of sucrose in the space provided below.

    Molar concentration of sucrose = ____________ M

## PART IV: CALCULATION OF WATER POTENTIAL FROM EXPERIMENTAL DATA

51. The solute potential of this sucrose solution can be calculated using the following formula:

$$\psi_s = -iCRT$$

i = Ionization constant (for sucrose this is 1.0 because sucrose does not ionize in water)
C = Molar concentration (determined from the graph on page 13)
R = Pressure constant (R = 0.0831 liter bars/mole K)
T = Temperature in K (273 + $^{o}$C of the solution)

The units of measure will cancel as in the following example:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $\psi_s$ | = | **-i** | x | **C** | x | **R** | x | **T** |
| $\psi_s$ | = | -1 | x | 1.0 mole/L | x | 0.0831 liter bar/ mole K | x | 295 K |

$\psi_s$ = -24.51 bars

52. Knowing the solute potential of the solution ($\psi_s$) and knowing that the pressure potential of the solution is zero ($\psi_p$ = 0) allows you to calculate the water potential of the solution. The water potential will be equal to the solute potential of the solution.

$$\psi = 0 + \psi_s$$
$$\psi = \psi_s$$

The water potential of the solution at equilibrium will be equal to the water potential of the potato cells. What is the water potential of the potato cells from Part III? Show your work.

53. Water potential values are useful because they allow us to predict the direction of the flow of water. Recall from the discussion that water flows from an area of higher water potential to an area of lower water potential. For the sake of discussion, suppose that a student calculates that the water potential of a solution inside a bag is –6.25 bar ($\psi_s$ = -6.25, $\psi_p$ = 0) and the water potential of a solution surrounding the bag is –3.25 bar ($\psi_s$ = -3.25, $\psi_p$ = 0.) In which direction will the water flow?

______________________________________________________________

Explain your answer.

______________________________________________________________

______________________________________________________________

54. If a potato is allowed to dehydrate by sitting in the open air, would the water potential of the potato cells decrease or increase?

______________________________________________________________

Why? _________________________________________________________

______________________________________________________________

55. If a plant cell has a lower water potential than its surrounding environment and if pressure is equal to zero, is the cell hypertonic (in terms of solute concentration) or hypotonic to its environment?

______________________________________________________________

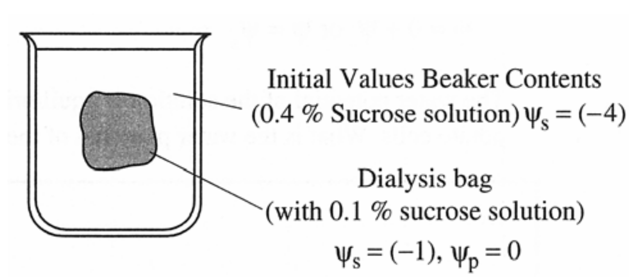
Will the cell gain or lose water? ____________________________________

Explain your answer. _______________________________________________

______________________________________________________________

56. The beaker pictured at the right is open to the atmosphere. What is the pressure potential ($\psi_p$) of the system?

______________________




57. Where (the beaker or the dialysis bag) is the water potential greatest?

______________________________________________________________

58. Will water diffuse into or out of the bag? ______________________________

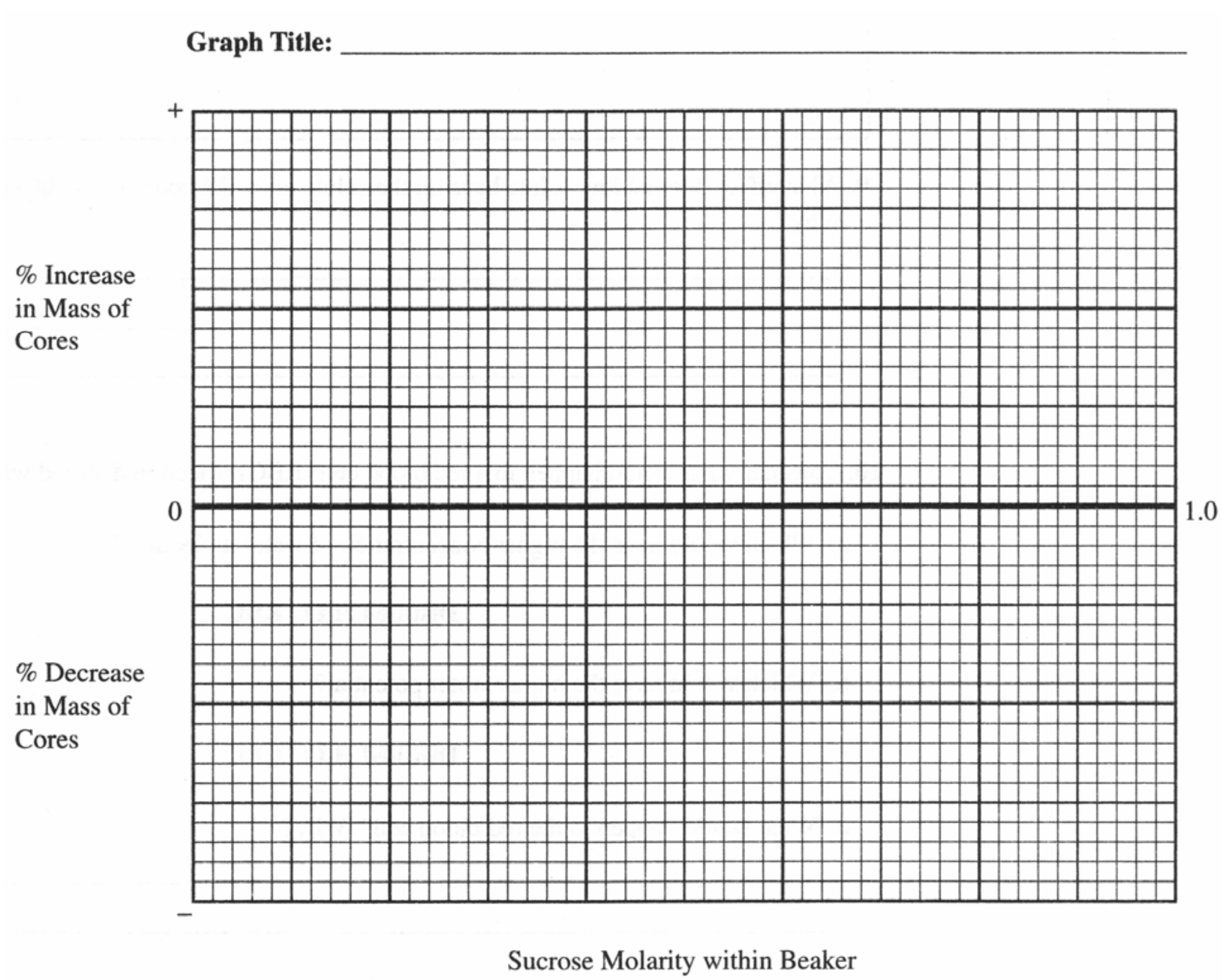
Why? ____________________________________________________________

____________________________________________________________________

59. Zucchini cores placed in sucrose solutions at 27°C resulted in the following percent changes after 24 hours:

| Sucrose Molarity | % Change in Mass |
|---|---|
| Distilled water | 20 % |
| 0.2 M | 10% |
| 0.4 M | -3% |
| 0.6 M | -17% |
| 0.8 M | -25% |
| 1.0 M | -30 % |

Graph the above data on the graph below.



60. What is the molar concentration of solutes within the zucchini cells?

______________________________________________________________

61. Refer to the calculations in #51 to calculate the water potential for the zucchini data.

a. Calculate the solute potential ($\psi_s$) of the sucrose solution in which the mass of the zucchini cores does not change. Show work below.

| |
|---|
| |

b. Calculate the water potential ($\psi$) of the solutes within zucchini cores. Show your work below.

| |
|---|
| |

62. What effect does adding solute have on the solute potential component ($\psi_s$) of that solution?

______________________________________________________________

Why? ________________________________________________________

______________________________________________________________

63. Consider what would happen to a red blood cell (RBC) placed in distilled water.

a. Which (RBC or distilled water) would have the higher concentration of water molecules?

________________________________________________________

b. Which (RBC or distilled water) would have the higher water potential?

________________________________________________________

c. What would happen to the red blood cell?

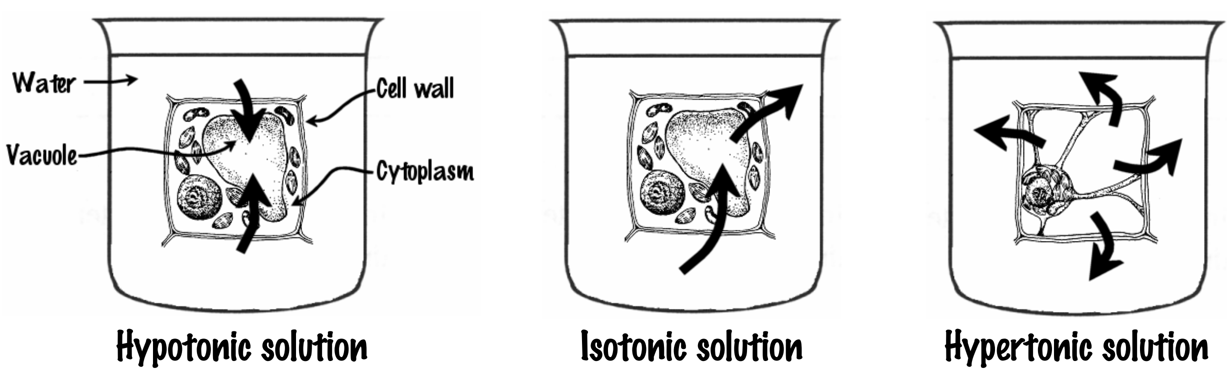
______________________________________________

Why? __________________________________________

______________________________________________

## PART V: ELODEA CELL PLASMOLYSIS

**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 (high solute concentration) surrounding the cell as shown in the figure below. During plasmolysis the cellular membrane pulls away from the cell wall. In the next lab exercise you will examine the details of the effects of highly concentrated solutions on diffusion and cellular contents.




64. Prepare a wet mount of an Elodea leaf. Observe the leaf under 100X magnification. Sketch and describe the appearance of the cells in the leaf.

| Sketch | Description |
|---|---|
| | |

65. Add two drops of 15% NaCl to one edge of the cover slip. Draw this salt solution across the slide by touching a piece of paper towel to the fluid under the opposite edge of the cover slip. Sketch and describe the leaf cells. Explain what has happened.

| **Sketch** | **Description** |
| --- | --- |
| **Explanation** | |

66. Remove the cover slip and flood the leaf with fresh water. Observe under 100X. Describe and explain what happened.

| **Description** | **Explanation** |
| --- | --- |
| | |

## Analysis of Results

67. What is plasmolysis?

________________________________________________________

________________________________________________________

68. Why did the leaf cells plasmolyze?

__________________________________________________

__________________________________________________

69. In winter, grass often dies near roads that have been salted to remove ice. What causes this to happen?

__________________________________________________

__________________________________________________