13. What was the experimental variable?

14. What was the measured variable?

GRAPH YOUR RESULTS BELOW USING A LINE or COLUMN/BAR GRAPH: *(don't forget to identify the independent and dependent variables and label the x and y axes appropriately)*

Figure 1. _____(*title your fig*ure)

PART II: Diffusion of solvent (OSMOSIS) and Diffusion of solutes (DIALYSIS) across a semi-permeable membrane

A membrane, whether it is a cellophane dialysis membrane or the membrane of a living cell, may be thought of as having small holes or pores. Some substances, if they are small enough, can pass through these membrane pores. Others, whose molecular sizes are too large, cannot pass through these holes. Non-living membranes that allow some molecules to pass while preventing others, due to size limitations, are said to be **semi-permeable**. Plasma membranes surrounding live cells contain transport molecules and electrically charged channels which, in addition to pore size, affect the ability of some molecules to pass, regardless of size; therefore, plasma membranes are said to be **selectively permeable**.

It was noted in the introduction of this exercise that molecules are all in a constant state of random motion. Picture, in your mind, these molecules randomly bouncing against the side of a semi-permeable membrane (semi-permeable membranes have tiny holes in them). If the molecule that strikes the membrane is smaller than the holes in the membrane, the molecule will simply pass through the membrane. However, if the molecule is larger than the holes in the membrane, the free passage of the molecule will be blocked and the molecule will remain "trapped" on one side of the membrane. *This is the concept behind the use of semi permeable dialysis tubing.* (Dialysis tubing can be obtained with different pore sizes.) The size of the pores in dialysis tubing dictates which solutes may cross the membrane.

The diffusion of any solute across a semi-permeable membrane is called **dialysis**. Within the human body, the greatest amount of dialysis occurs in the kidneys. The blood is dialyzed within the nephron capillaries. Water and small solutes are forced out of the blood through pores in the capillaries and empty into the nephron tubules. Some solutes are then reabsorbed into the blood by dialysis, while water (the biological solvent) is reabsorbed by a

form of diffusion called **osmosis** (the diffusion of water across a selectively permeable membrane). Dialysis and osmosis do not require any energy expenditure by the cell; they are forms of **passive transport**. Furthermore, they do not usually require any carrier proteins to aid their movement across the membrane. You may want to view the following brief video online: http://www.youtube.com/watch?v=sdiJtDRJQEc

In order to complete this exercise, it will be necessary for you to prepare a section of dialysis tubing. Read the procedure carefully and then follow the directions to prepare the dialysis tubing for your experiment.

DIALYSIS PROCEDURE*: READ ALL INSTRUCTIONS BEFORE BEGINNING!*

PROTECTIVE EYEWEAR (GOGGLES) MUST BE WORN WHEN WORKING WITH THE LUGOL'S, BENEDICTS AND BIURET REAGENTS.

15. Obtain a cut piece of pre-soaked dialysis tubing.
16. Wet the dialysis tubing with distilled water from the squeeze bottle found at your table. Carefully grasp one end of the tubing between your thumb and index finger and, with a sliding motion back and forth, attempt to separate the two sides of the tubing. ***Note***: these clamps are not disposable, please save them when done with experiment. The tube can now be filled like a bag.
17. **Mix the bottle of dialysis solution** found in your tray and fill the dialysis bag about three-fourths (3/4) full with the solution. The dialysis solution contains approximately **10% glucose (Mol. Wt.= 180), 0.5% starch (Mol Wt. = ~200,000) and 1.5% protein (egg albumin, Mol. Wt. = 45,000**). Be careful not to allow the solution to spill over onto the exterior sides of the bag. When 3/4 full, the bag should hold **about 10 ml of dialysis solution (*you don't need to measure the volume*). This is called the "Experimental Bag" (EB).**
18. Remove some air from the open end of the Experimental Bag and seal the open end of the dialysis bag with another clamp**. The bag should be flexible/flaccid so that it may gain water by osmosis.** It should also fit into the dish without folding it. Trapped air will not harm the results of the experiment.
19. Carefully rinse the ends and the outside of the bag with tap water to remove any excess dialysis solution. ***Note potential source of human error:*** *excess water on the outside of your dialysis bag will affect the weight.*
20. Use a weigh boat to weigh your bag on a top loading balance and record its mass as the "Experimental.bag" weight at time "0", in the table below [**Note**: *be sure that the electronic balance is measuring in correct units (g). Your instructor will demonstrate this if necessary*]. Do not discard your weigh boat, as you will use it later.

Table 1. Pre & Post Incubation Data on the Dialysis Bags

Time (minutes)	Your Experimental Bag		Control Bags (instructor will post on the chalk board))			
	Weight Exptal bag (g)	Color of contents	Weight of Water bag (g)	Water bag color	Weight of Glucose bag (g)	Color of contents
0						
30						

Table 2. Weight change in the Dialysis Bags

Calculated % weight change (refer to p. 6)	Your Experimental Bag	Water Control Bag (negative control)	Glucose Control Bag (positive control)

21. Obtain a clean glass dialysis dish from your lab bench. Place your dialysis tubing containing the dialysis solution into the dish and use a graduated cylinder to add approximately 100ml of the prepared Lugol's iodine solution to just cover the dialysis bag [0.4% Iodine Potassium Iodide = **I_2KI = a solution of elemental iodine (I_2) and potassium iodide (KI)].** Like other iodide salts, K**I** forms I_3 when combined with elemental iodine.

    $KI(aq) + I_2(s) \rightarrow KI_3(aq)$ (aq=aqueous; s=solid) (also known as I2KI)

22. **CALCULATE THE MOLECULAR WEIGHT OF I_2KI: _____.**

    Allow the experimental dish to remain **undisturbed** for 30 minutes at your lab bench.

23. Your instructor will prepare 2 other pieces of dialysis tubing filled with a) distilled water as a **negative control** and b) glucose solution, as a **positive control for the Benedict's Test**. These bags will also be rinsed, blotted dry & weighed and then placed in 2 dishes of Lugol's iodine solution for 30 minutes. Briefly explain the purpose of a **negative control and of a positive control**:

_____

_____

24. Your instructor will weigh the control bags and post this data. Record this 'time zero' data, in Table 1 above.
25. While waiting for the incubation period (30 minutes), prepare 6 glass test tubes (as described in the table for the PROTEIN and SUGAR TESTS). Also label a 7th test tube 'ED' to collect the sample from the experimental dish. **Wear goggles when handling Benedicts and Biuret solutions**. These reagents contain copper, which is poisonous. Discard into chemical collection container in the hood when finished.
26. Upon conclusion of the 30-minute period, remove 10 ml of the dish solution from right next to your dialysis bag. Transfer it to an empty test tube labeled 'ED'. Then remove your dialysis bag from the dish with fingers, forceps or a clamp. Be careful not to puncture the bag. Very gently blot the bag with a paper towel, removing any solution clinging to the outer surface. Record the color of the bag contents (note that the color of the contents will change slowly after removing from Lugols solution). Using the same balance that you previously used, weigh the bag. Record your data in your table (above) along with the color of the experimental bag solution and the control bag solutions.
27. Also, indicate the color of the solution in your dialysis DISH after 30 min: (record here:) _____
28. Was there a change in the mass over the 30 minute period? If so, did the weight increase or decrease? _____
29. What was the change in weight after 30 minute incubation?_____
30. Calculate the % change (or %) in weight using the following formula and enter this data into Table 2 (above) (this calculation was introduced in Lab Ex.1)

$$\left[\frac{\text{30 min. wt.} - \text{time 0 wt.}}{\text{time 0 wt.}}\right] \times 100 = \%\triangle$$

31. To what do you attribute this change in mass? Explain: _____

_____

32. Your instructor will weigh the *control* dialysis bags at 30 minutes and post the data on the chalkboard or overhead projector. Record the weights in Table 1 on the preceding page.
33. To what do you attribute the change in mass in the *control* bags, if any? Describe the degree to which water

osmosis and solute dialysis played in the change in weight.

_____

_____

34. After noting your observations, empty the contents of your dialysis bag into your weigh boat as done for the PROTEIN and SUGAR TESTS. Dispose of the tubing in the trash. **DO NOT DISCARD THE CLAMPS!! Be sure to return the clamps to the tray on your lab bench.**

Lugol's Iodine, which is an amber (orange-yellow) color, reacts with starch to produce a color change to dark purple/black. Was there a color change in the contents of the bag containing dialysis solution? ___Yes / No___

If so, to what do you attribute this change? _____

Do you think that the starch molecules diffused out of the bag? Explain your answer:

_____

PROTEIN TEST: Use the **Biuret reagent** to determine the presence of protein. Follow the steps in the table below (reading left to right)

Table 3. Biuret Test procedure

1st Step	2nd	3rd	4th	5th
Label 3 Test tubes as below*:	Add 3 ml Biuret Reagent to each tube (use a 10 ml graduated cylinder)	Using a disposable pipet**, add 5 ml of post-dialysis solution taken from:	Cover each tube with parafilm*** * and mix briefly	Record color of tube contents immediately. + = positive - = negative
"PEB" *		Dialysis bag solution		PEB:
"PED"		'ED'*** tube Exptal dish		PED:
"PCD"		Instructor negative Control dish		PCD:

* PEB abbreviation for Protein test of Experimental Bag contents; PED=Protein test of Experimental Dish contents; PCD= Protein test of Control Dish (on instructor's front table)
** Your lab instructor should demonstrate the use of disposable pipets.
***this is the solution that you transferred from the experimental dish before you removed the bag from the dish.
*** Parafilm is a stretchable sealer that looks like wax paper. Remove the paper backing prior to use and stretch the film slightly over the glassware to be sealed. Invert the tube several times to mx thoroughly.

WHAT THE BIURET TEST RESULTS MEAN: *A lavender/purple color indicates the presence of protein; blue color is negative for the presence of protein.*

35. Did your test results indicate the presence of protein in the experimental dish? _____

36. Did your test results indicate that protein was still inside the experimental bag? _____

37. What conclusions can you make regarding the size of the protein molecules in relation to the size of the dialysis membrane pores?

SUGAR TEST:

You will also use **Benedict's solution** to detect the presence of monosaccharides & disaccharides (sugars). Benedict's solution is a blue liquid containing copper that oxidizes when incubated with sugars to form a yellow, orange or reddish precipitate (solid/insoluble particles). The more sugar present, the more precipitate and more orange-red the solution becomes when reacted with Benedicts solution.

Use the **Benedict's reagent** to determine the presence of sugar (glucose). Follow the steps in the table below (reading left to right). *You may prepare steps 1 & 2 during the 30-minute incubation of your dialysis bag.*

Table 4. Benedict's test procedure

1st Step	2nd Step	3rd Step	4th Step	5th Step	6th Step
Label 3 Test Tubes as below*:	Using a graduated cylinder, Add 5 mL of Benedicts reagent to each tube	Use a different disposable pipet to add 5 ml of post-dialysis solution taken from:	Cover each tube with parafilm and mix briefly by inverting several times	Incubate the 3 tubes in hot water bath for 10 minutes at 70-80C Parafilm may stay on	Remove tubes from bath and record color of contents and amount of precipitate (++/+/-) below: ++ = strong positive result for sugar + = weak positive - = no sugar
SEB		Dialysis bag solution			SEB:
SED		'ED'*** tube Exptal dish			SED:
SCD		Instructor negative Control dish			SCD:

*SEB: abbreviation for Sugar test of Experimental Bag contents; SED=Sugar test of Experimental Dish contents; (-) SCD= Sugar test of negative Control Dish (on instructor's front table)

Your instructor will perform a Benedict' test on the contents of the positive Control Dish: "Glucose Control Bag" (+SCD)

WHAT THE BENEDICT'S TEST RESULTS MEAN: *Green cloudy solution indicates the presence of a trace amount of sugar, yellow or coral-colored solution indicates moderate amount, while an orange-red, cloudy solution indicates the presence of a large quantity of sugar in the test solution. Also note the amount of precipitate, which indicates strength of the reaction.*

38. Did your test results indicate the presence of sugar in the experimental dish? _____

39. Did your test results indicate that sugar was still inside the experimental bag? _____

40. If sugar was found in both the bag and dish, were the test results identical with respect to the color and density of precipitate in the test results? YES/NO Describe the results.

41. Your instructor ran a sugar (glucose) control bag in Lugol's Iodine solution. What were the Benedict's test results in both the Positive Control dish and the Positive Control dialysis bag? (Get the results from your instructor)

CLEAN UP: Empty all Benedict's and Biuret test tubes into the plastic container in the fume hood. Rinse the tubes in the sink and dispose of the tubes in glassware boxes near sinks or hood. Agar tubes from Part I may be discarded in these boxes also. Rinse other dishes, clamps and leave them on your lab bench in the tray. Thanks.

QUESTIONS FOR FURTHER REVIEW:

1. *Physiological Application*: (a) How will the temperature of a muscle change during exercise? Will it (i) increase or (ii) decrease? (circle the correct choice)

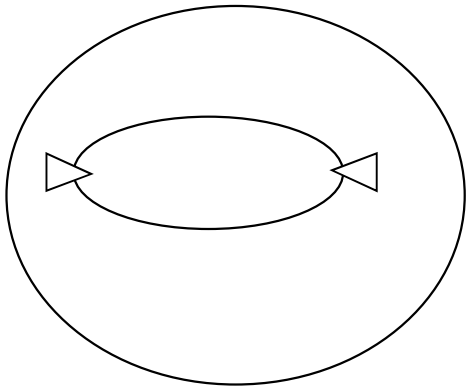
   (b) Why will the temperature in that muscle change? (there are actually 2 or more reasons for this)

   (c) How will the change in temperature affect the rate of diffusion of molecules within the cytoplasm of the muscle cells? Why?

   (d) How will it affect the rate of (i) dialysis and (ii) carrier-mediated transport across the plasma membrane between the ISF & ICF? Explain.

2. How would the Benedict's test results compare (between SED & SEB), if you allowed the experimental dialysis bag to remain in the dish overnight? (i.e. would **all** the sugar dialyze out of the bag into the dish?, etc.) Explain your answer.

3. In the diagram (below) of the dialysis bag set up in the dish, list the contents of the dish and bag with % concentrations of each solute and the % of water in each solution at the start of the experiment. U*se the information provided on p 5, step #17*). The bag simulates a cell filled with ICF and the dish solution represents the ECF. List the contents of each to the left of the diagram and use arrows to designate where each solute is located.



4. When a chemical formula is written for glucose ($C_6H_{12}O_6$), what do the letters and numbers mean?

5. Did it appear that there was more glucose in your experimental dish than remained in your dialysis bag at the end of your experiment? _____**.**

    - **I**s this a physical possibility? If so, why? If not, why not? Your instructor will discuss this with you by relating the results of the positive glucose control to your results.

6. In today's experiment, glucose was a penetrating solute, which simply diffused across the dialysis membrane. But in a living cell, glucose is considered a non-penetrating solute. Why the difference in penetrability?

7. The concentration of glucose ($C_6H_{12}O_6$) in the dialysis solution was given as a 10 percent (%). **This is also known as "grams percent" which is equivalent to the number of grams of solute per 100 ml of solution. (***note: you should memorize what g% means***).** *Reference= figure 2.7 (6th & 7th ed. Textbook)*

    (a) Based on the information above, how many grams of glucose would be found in 100 ml of dialysis solution? (note: this is known as grams percent)

    (b) Now determine the number of grams of glucose you would use if you were making one liter of 10% glucose solution (hint: use part 'a' of this question to help you, after calculating how many ml are in 1 liter)

    c) MOLAR CONCENTRATION: Your instructor will review how to determine molar concentration of solutions. Then you should calculate the molarity (moles/liter) of the glucose ($C_6H_{12}O_6$) in the dialysis solution. (show your work in the space below for future reference)