Name ____________________________ **Period _________**

Regents Biology **Date ______________________**

LAB _____. DIFFUSION THROUGH A MEMBRANE

INTRODUCTION

Your cells — actually all cells — need sugars and oxygen to make energy to fuel daily life. Cells also need raw materials to be able to repair themselves and to build new cells. And of course cells always need water to remain healthy. All these materials have to move into a cell to feed it.

On the other hand, cells produce waste products during their daily activities. Cells make the waste product, carbon dioxide, when they make energy. They also make the waste product ammonia when they digest proteins. Both of these waste products have to be removed or the cell will be poisoned and die.

All of these materials enter or leave the cell through the **cell membrane**. In most cases, they move through the cell membrane through a process called **diffusion**. ***Diffusion is the movement of molecules from an area of higher concentration of those molecules to an area of lower concentration.*** A good metaphor for this movement of molecules is what happens if you were to open a bottle of perfume in one corner of a room. It would not be long before someone in the opposite corner of the room would smell the perfume. The molecules moved from an area of higher concentration of perfume where the open bottle was to an area of lower concentration of perfume like the opposite corner of the room. Eventually a balance, or **equilibrium**, is reached. In other words, the concentration of perfume will be approximately equal throughout the room and no **net** movement of perfume will occur from one area to the other.

Diffusion is how most materials move into and out of a cell. When there is more sugar outside of a cell, the sugar automatically moves into a cell — moving from high concentration to low concentration. When there is more water in a cell, then water automatically moves out of the cell — moving from high concentration to low concentration. This movement of molecules does not require any energy because it is an automatic process. We refer to this as **passive transport** because it doesn't cost the cell any energy.

Sometimes a cell needs to move material against this automatic direction of movement. They need to move materials from areas of low concentration to areas of higher concentration. This would be for materials that are very valuable to the cell, like sugars, that the cell would use to make energy. That would be like swimming upstream against the current, so it requires energy. The only way for cells to do this is to use energy to pump the material "upstream" across the cell membrane. We call this process, **active transport**, because it actively uses energy to move molecules either into or out of the cell. The energy that the cell uses is in the form called ATP. ATP is used to move material from areas of low concentration of that substance into regions of higher concentration.

In this lab, we will investigate diffusion of sugars, starches, water and other materials across a membrane.

A. Chemical Testing

Before we start our actual experiment, we will perform a series of chemical tests to become familiar with our **indicator solutions**. We will use these indicator solutions throughout this lab so we need to be sure they are working properly and to see what a positive and a negative results looks like for each indicator solution.

HOW TO TEST FOR STARCH	1. 4 drops **Lugol's Iodine indicator** 2. Solution **immediately** turns from amber to blue-black
HOW TO TEST FOR GLUCOSE	1. 4 drops **Benedict's solution indicator** 2. Solution turns from blue to bright orange when **heated**

1. Obtain 6 clean test tubes and 6 clean pipettes.

2. **TEST GLUCOSE with BENEDICT'S**: Place a 2mL sample of **glucose solution** in a test tube. Label the test tube with tape. Test the solution with Benedict's indicator (remember to heat it). Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

3. **TEST STARCH with BENEDICT'S**: Place a 2mL sample of the **starch solution** in a test tube. Label the test tube with tape. Test the solution with Benedict's indicator (remember to heat it). Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

4. **TEST WATER with BENEDICT'S (CONTROL):** Place a 2mL sample of **water** in a test tube. Label the test tube with tape. Test the solution with Benedict's indicator (remember to heat it). Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

5. **TEST GLUCOSE with IODINE**: Place a 2mL sample of **glucose solution** in a test tube. Label the test tube with tape. Test the solution with iodine indicator. Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

6. **TEST STARCH with IODINE**: Place a 2mL sample of **starch solution** in a test tube. Label the test tube with tape. Test the solution with iodine indicator. Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

7. **TEST WATER with IODINE (CONTROL):** Place a 2mL sample of **water** in a test tube. Label the test tube with tape. Test the solution with iodine indicator. Record the **color** observed after the test in **Table 1** (**Chemical Tests**).

Table 1. Chemical Tests

Indicator	Material Tested		
	Water	Starch Solution	Glucose Solution
Benedict's Solution			
Iodine Solution			

B. Diffusion Experiment

In this experiment, you will measure the diffusion of small molecules through dialysis tubing, an example of a **semi-permeable membrane**. A semi-permeable membrane is a barrier that allows some materials through, but stops others. Small dissolved molecules and water molecules can move freely through a semi-permeable membrane, but larger molecules will pass through more slowly, or perhaps not at all. The size of the tiny pores in the dialysis tubing determines which substances can pass through the membrane. We will explore the process of diffusion through a semi-permeable membrane in this activity.

Procedure: DAY 1

1. Measure 20mL of the sugar-starch solution (15% glucose/1% starch solution). Before you use it, you need to test it to verify that it has both starch and glucose in it. Take a 2mL sample of the solution from your 20mL and perform your **starch test** in a test tube. Take another 2mL sample of the solution and perform your **glucose test** in a test tube. Record your results in **Table 2** (**Observations & Predictions: Contents**). If starch or glucose is present write "Yes" in the appropriate box in the data table.

2. Take a 30cm piece of 2.5cm dialysis tubing that has been soaking in water. Tie off one end of the tubing to form a bag. To open the other end, rub the end between your fingers until the edges separate. Leave the tubing soaking while you complete the next steps.

3. Pour plain water into a 250mL beaker until it is about two-thirds full. Before you use it, you need to test it to verify that it does NOT have either starch or glucose in it. Perform your starch and glucose tests on 2mL samples of the water. Record your results in **Table 2** (**Observations & Predictions: Contents**). If starch or glucose is not present write "No" in the appropriate box in the data table.

4. Now we need to get our beaker ready for the experiment. Add iodine to the water in the beaker. Add only enough iodine to turn the water a medium amber color. Record the **color** of the solution in **Table 3** (**Observations & Predictions: Color of Solutions**).

5. Now pour your remaining sugar-starch solution (~15mL) into the dialysis bag using a funnel. Tie off the other end of the bag, leaving space for expansion of the contents in the bag. In case any solution spilled on the outside, rinse off the model "cell" you just made by holding it under running water.

6. Record the **color** of the solution inside the "cell" in **Table 3** (**Observations & Predictions: Color of Solutions**).

7. Place the "cell" in the beaker of iodine solution. Be sure the "cell" is covered. Cover the beaker with plastic wrap. Set the beaker aside to sit undisturbed overnight.

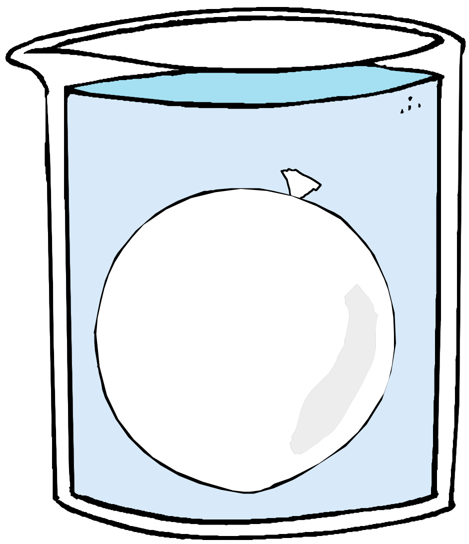
8. Label the contents of your set up in the "**Initial State**" diagram in **Figure 1**.

9. Color the "**Initial State**" diagram in **Figure 2** to accurately illustrate the contents of the beaker and the cell.

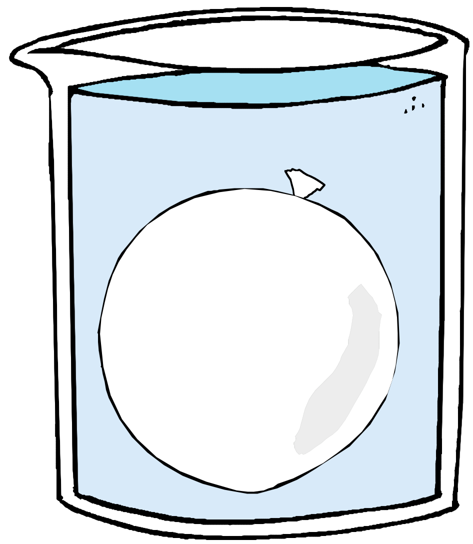
10. Now let's make some predictions in **Table 2** and **Table 3**. What do you think will happen by tomorrow?

FIGURE 1. IDENTIFY CONTENTS & MOVEMENT

Label the diagrams below using — **GLUCOSE, STARCH, WATER, IODINE** — to identify the contents of the "cell" and the beaker in the initial state and in the final state of the experiment. Also in the "Final State" diagram, clearly draw **arrows** to indicate the movement of molecules during the experiment.



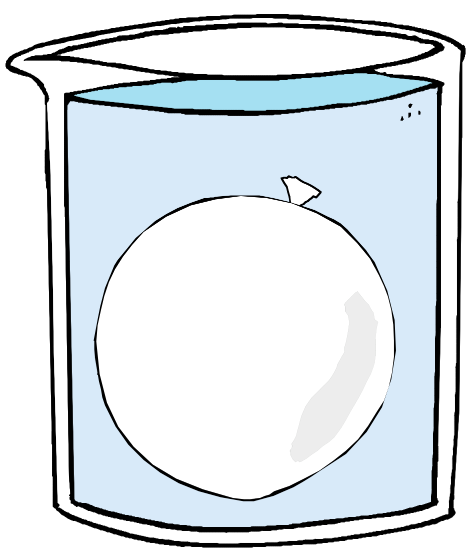
INITIAL STATE



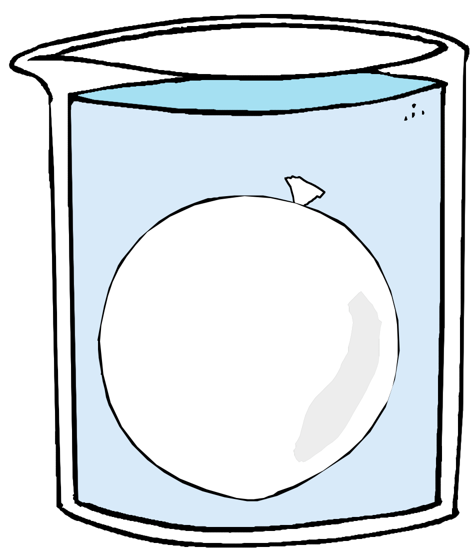
FINAL STATE

FIGURE 2. IDENTIFY COLORS OF CONTENTS

Color the diagrams below to illustrate the colors of the solutions of the "cell" and the beaker in the "Initial State" and in the "Final State" of the experiment.



INITIAL STATE



FINAL STATE

DIFFUSION DATA TABLES

TABLE 2. OBSERVATIONS & PREDICTIONS: CONTENTS

	Initial contents	1 Presence of Glucose			2 Presence of Starch		
		A Initial	B Predicted Final	C Final	A Initial	B Predicted Final	C Final
"Cell"	15% glucose 1% starch						
Beaker	H_2O & Iodine						

TABLE 3. OBSERVATIONS & PREDICTIONS: COLOR OF SOLUTIONS

	Initial contents	A Initial State	B Predicted Final State	C Final State
"Cell"	15% glucose 1% starch			
Beaker	H_2O & Iodine			

PROCEDURE: DAY 2

1. Carefully exam your beaker and the "cell". It is now in its "**Final State**"

2. Take note of the color changes that have occurred. Record the final colors of the solution in the beaker and in the "cell" on the "**Final State**" diagram in **Figure 2**.

3. Use a pipette to transfer a 2mL sample of the solution from the beaker to a clean test tube. Test the solution for the presence of **glucose** using Benedict's solution (remember to heat it). Record the results of your test in **Table 2** **(Observations & Predictions: Contents**).

4. You do ***not*** need to take a sample of the solution from the beaker to test for the presence of **starch**, because the iodine indicator is already in the beaker, so this already served as a test for starch. Record your results of this test in **Table 2** **(Observations & Predictions: Contents**).

5. Carefully take the "cell" out of the beaker and place it in a separate clean beaker. Carefully cut open one end and pour the contents into the new beaker. Use a pipette to transfer a 2mL sample of the solution from the "cell" to a clean test tube. Test the solution for the presence of **glucose** using Benedict's solution (remember to heat it). Record your results of this test in **Table 2** **(Observations & Predictions: Contents**).

6. You do ***not*** need to take a sample of the solution from the "cell" to test for the presence of **starch**, because the iodine indicator already served as a test for starch. Record your results of this test in **Table 2** **(Observations & Predictions: Contents**).

7. Compare your **predictions** to your actual data. Any surprises? If so, discuss with the class.

8. Complete the **"Final State"** diagram in **Figure 1** by labeling the contents of both the solution in the beaker and in the "cell". Using arrows, be sure to indicate the movement of each of the materials involved:

   - glucose
   - starch
   - iodine

9. Complete the **"Final State"** diagram in **Figure 2** by coloring the contents of both the solution in the beaker and in the "cell".

10. Complete the **Summary Questions**.

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SUMMARY QUESTIONS

1. Did glucose diffuse out of the cell? ____________ Explain how you can tell.

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2. Did starch diffuse out of the cell? ____________ Explain how you can tell.

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3. What is the best explanation for the color change that occurred inside the "cell"?

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4. Based on your tests, which substance(s) diffused through the dialysis "cell" membrane?

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5. Based on your tests, which substance(s) did **not** diffuse through the dialysis "cell" membrane?

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6. Explain why some substances were able to pass through the membrane while others were not able to.

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7. Although we didn't measure it, what other molecule can we assume also moved across the membrane?

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8. How could we have accurately measured whether water moved into or out of the dialysis tubing "cell" in this experiment?

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9. Why did the glucose flow out of the cell?

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10. Why did the iodine flow into the cell?

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11. Based on your observations, compare the size of each of the following molecules (glucose molecules, water molecules, iodine molecules, starch molecules) with the <u>size of the pores in the membrane</u> of our model cell. Complete each of the following sentences with the one of the following phrases (**<u>greater than</u>**, **<u>less than</u>**):

The size of glucose molecules are ________________________ the membrane pore size.

The size of water molecules are__________________________ the membrane pore size.

The size of iodine molecules are _________________________ the membrane pore size.

The size of starch molecules are _________________________ the membrane pore size.

12. What results would you expect if the experiment was set up incorrectly: the water and iodine solution was placed inside the dialysis bag and the starch and glucose solution was placed in the beaker.

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13. Animals (including humans) often eat starches in the form of fruits and vegetables and breads and baked goods The starches are then digested into glucose in the small intestines. This is done by the enzyme **amylase**. The glucose then diffuses from the cells of the small intestines to the blood stream and is then transported from the digestive system by the blood through the body to all cells, so they can use it to make energy. Based on the findings of this lab, explain why the digestion of starch to glucose is necessary.

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14. Summarize the process of diffusion.

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15. Explain the difference between simple diffusion and facilitated diffusion.

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