Prelab Information

Movement is one of the characteristics of life. The ability to control the movement of material across the cell membrane is an incredibly important aspect of a cell’s ability to maintain homeostasis. In this lab we will examine the process involved with moving molecules across the cell membrane, looking at the concepts of passive transport – which requires no cellular energy and active transport – which requires cellular energy. Movement across the cell membrane is necessary for the cell to remove wastes and take in nutrients and molecules that it needs to synthesize products and materials needed by the cell, or simply to maintain the correct balance of ions between the inside environment (intracellular environment) and outside environment (extracellular environment).

Movement across the cell membrane must be controlled, and the cell membrane controls this movement by the nature of its structure. The cell membrane is a phospholipid bilayer membrane complete with proteins, carbohydrates and cholesterol. See figure 1 below for a simple diagram of the phospholipid bilayer membrane. This creates what is referred to as a selectively permeable membrane. This means that there is some control (selection) over what goes into and out of the cell. Because of the membranes structure, movement across the membrane is determined by shape, size and charge of molecules. For example, hormones such as testosterone will diffuse through due to its hydrophobic nature (water fearing), while a large molecule such as starch will not pass through the plasma membrane simply because it is too large.

If a molecule is able to move across the membrane without requiring ATP, relying rather on its own kinetic energy and gradient, it is said to be moving passively. Gradients may be established by concentration differences (concentration gradient), by pressure differences (pressure gradient) or even electrical differences (electrochemical gradient). When a gradient is established, molecules move from
an area of high to an area of low in a process called passive transport. If this movement involves material other than water it is referred to as **diffusion**. If the movement involves water it is referred to as **osmosis**. In some instances, there is a gradient established, but a molecule can’t move across the cell membrane (because of size, or charge) without the help of a membrane channel. This type of diffusion is referred to as **facilitated diffusion**, and still does not rely on ATP. See figure 2 below for diagrams of the different types of diffusion processes.

Active transport is the process by which cells can move material across the cell membrane regardless of whether a gradient is present or not. If there are more molecules of something inside the cell than there is outside the cell, but the cell still needs more to maintain homeostasis, then energy is expended to move material against the concentration gradient. Active transport commonly involves transport proteins to move substances across the membrane in the required direction (See Fig. 3). Some substances may be too large to fit through a membrane channel – in these cases, the cell may actively bring substances into the cell by wrapping it in a portion of cell membrane, it is referred to as **endocytosis**. If the cell actively removes substances from the cell by wrapping it in a portion of cell membrane, it is referred to as **exocytosis** (see diagrams below). Exocytosis may also be used by cells to place new structures into the cell membrane. Both endocytosis and exocytosis require the cell to expend cellular energy in the form of ATP.
Pre-lab Questions:

1. What is the function of the cell membrane?

2. Why does having the ability to move materials into and out of the cell help the cell to maintain homeostasis?

3. What is the difference between passive and active transport? What types of energy are involved with each?

4. Osmosis specifically deals with the movement of what molecule?

5. Based on what you know about the cell membrane; why do polar molecules typically require transporters (channels or carriers) to move from one side of the cell membrane to the other?
Lab Exercise 1: Diffusion

Rates of diffusion depend on the size and charge of the molecules diffusing through the membrane. For this experiment we will be using agar plates to determine rates of diffusion for molecules of different sizes.

Materials:

- agar plates
- two different molecular weight molecules
- plastic ruler

Procedure:

1. Obtain an agar plate.
2. Place a small amount of one compound (A) as shown in the figure to the right. Try to make your spots as round as possible.
3. Place a small amount of a different compound (B) as shown in the figure to the right, again trying to make your spots as round as possible.
4. As soon as you have placed the compounds, record your starting time.
5. Every 5 minutes measure the diameter of each compound and record in the table below.
6. Write the name of each compound (material) in their respective spots in the table below.

<table>
<thead>
<tr>
<th>Start Time:</th>
<th>Material A</th>
<th>Material B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol. weight:</td>
<td>mol. weight:</td>
</tr>
<tr>
<td></td>
<td>diameter (mm)</td>
<td>diameter (mm)</td>
</tr>
<tr>
<td>0 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min.</td>
<td></td>
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<tr>
<td>10 min.</td>
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</tr>
<tr>
<td>15 min.</td>
<td></td>
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<tr>
<td>20 min.</td>
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</tr>
<tr>
<td>25 min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min.</td>
<td></td>
<td></td>
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</tbody>
</table>
Lab Exercise 2: Diffusion & Osmosis

How can we determine if something is diffusing or osmosing if we can’t actually see the process occurring? In biology we can use different tests to determine where certain molecules are and where they are not. In this exercise we are going to set up an experiment that is designed to mimic the behavior of the plasma membrane of our cells. The artificial membrane we will be using is called dialysis tubing. Recall that diffusion and osmosis both rely on gradients and the inherent kinetic energy of molecules to move. What we want to determine by experimentation is what exactly is moving in which direction.

Materials (per group of 4):

- dialysis tubing
- string
- distilled water
- 10% sucrose solution
- 20% sucrose solution
- 10% starch solution
- scale & weigh boat
- 4 – 400 mL beakers
- 1 – 100 mL beaker
- 100 mL graduated cylinder
- Lugol’s iodine (dropper bottle)

Procedure:

Your instructor will demonstrate how to handle and prepare the dialysis tubing.

1. Obtain your materials (either from the back counter or your individual group tray).

2. Label and set up each of your four beakers (A, B, C & D) in the following manner.
   a. In beaker A, place 200 mL of distilled water.
   b. In beaker B & D, place 200 mL of 10% sucrose solution
   c. In beaker C, place 200 mL of 20% sucrose solution

3. Prepare four dialysis tubes by filling three tubes with the 10% sucrose solution, and the fourth dialysis tube with the 10% starch solution.
   a. Weigh each of the three 10% sucrose solution tubes and the 10% starch solution dialysis tubes.
   b. Record their starting weights in table 2.

4. Place a single 10% sucrose solution filled dialysis tube in beaker A, another in beaker B and another in beaker C, and place the 10% starch solution filled dialysis tube in beaker D.
   a. Record the start time for each tube in table 2.

5. After approximately 30 minutes, remove the tubes from their beakers one at a time. Do not remove them all at once, as you may mix the dialysis tubes up.
   a. Remove and record the ending weights of the dialysis tubes from beakers A, B and C in table 2.

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b. Remove the dialysis tube from beaker D, record its weight in table 2.
   i. Open the dialysis tubing and empty its contents into the 100 mL beaker
   ii. Add a few drops of Lugol’s Iodine to the solution now in the 100 mL beaker, as well as into beaker D.
   iii. A positive (+) indication for starch will result in the solution turning a dark purple when Lugol’s Iodine is added, a negative (-) indication for starch will not produce a color that is different from the test solution itself. Indicate the start test result in table 2.

6. Dispose of the solutions in the appropriate waste containers. Ask if you are unsure.

7.  Clean, dry and return your glassware.

Table 2

<table>
<thead>
<tr>
<th>Dialysis Setup</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning Weight (grams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ending Weight (grams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference in Weight (grams)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Test Results for Starch: (+) for positive (-) for negative</td>
<td>Beaker:</td>
<td></td>
<td></td>
<td>Dialysis Tube:</td>
</tr>
</tbody>
</table>
Lab Exercise 3: Observing Active Transport

In this experiment we will be observing a live *Amoeba sp.*, these are single celled organisms (protists) and you will be able to observe them moving around on the slide. Active transport (endocytosis) occurs when small food particles are actively engulfed by the amoeba wrapping its pseudopods around a food object and forming a food vacuole. The contractile vacuole helps to actively maintain the osmotic (water) balance between the intracellular and extracellular environment, while the nucleus controls the minute by minute operations of the cell.

By creating a wet-mount of a living amoeba, we should be able to observe endocytosis.

Materials:
- microscope slides & coverslips
- stained and fixed slide of *Ameoba sp.*
- culture of live *Amoeba sp.*
- stained yeast
- plastic pipettes
- binocular microscopes

Procedure:
1. Obtain and observe a stained and fixed slide of an ameoba. Ask your instructor to check and verify that what you are looking at is an ameoba. After you are given the okay, return the slide and proceed to step 2.
2. Use a pipette to remove a drop of culture water that contains some amoeba. You should agitate the water gently first to increase the chances of getting some amoeba into your drop.
3. Place a drop of the amoeba culture on your slide. If you have excess fluid still in your pipette, return it to the culture jar.
4. Add a very small drop of stained yeast to the drop on your slide.
5. Place a coverslip over your drops.
6. Using proper microscopy technique, find and observe your amoeba. The goal is to observe them ingesting via endocytosis the small stained yeast.
   - *Amoeba may be difficult to see as they are relatively transparent. Make sure you are in focus on the correct plane, and adjust your lighting, condenser and iris to increase the contrast as much as possible.*

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1. What is the molecular weights of the two compounds that you tested during the simple diffusion of a chemical through agar experiment?
   
   Mol. Weight Chemical A: _______________
   
   Mol. Weight Chemical B: _______________

2. State your hypothesis regarding diffusion rates here.

3. Which chemical compound had a faster rate of diffusion? Was your hypothesis supported or rejected?

4. Why do you think the chemical compound which had a faster rate of diffusion was faster?

5. If we dropped the same two chemicals into water, do you think the rates of diffusion would be faster or slower? Why or why not?

6. If we dropped the same two chemicals into water, do you think the chemical that diffused faster in the agar would still diffuse faster in water? Why or why not?
7. Graph the diffusion rates for each of your chemicals from exercise 1 on the following graph.

8. Based on your graph, what relationship (positive, negative or neutral) exists between the size of a molecule and the rate at which it diffuses through its environment?

9. The fluid environment that living cells are in exists in one of three different states: 1 – hypertonic, 2- isotonic and 3 – hypotonic. In **hypertonic** solutions there are a greater number of solutes outside of the cell than inside. This also means that there must be less water outside the cell than inside the cell. This gradient will cause water to move from the inside of the cell to the outside of the cell, shrinking it. In an **isotonic solution** there are equal numbers of solutes (and therefore equal numbers of water molecules) inside and outside of the cell. Because it is already at an equilibrium there is no net movement of water into or out of the cell and the cell maintains its shape. In a **hypotonic solution**, there are less solutes and therefore more water outside of the cell than inside, as a result, water will move by osmosis into the cell, causing it to swell, and potentially burst or lyse. Based on this information, what is the tonicity of solutions in beakers A, B and C?

   Beaker A = _____________________ solution
   Beaker B = _____________________ solution
   Beaker C = _____________________ solution
10. State a possible hypothesis for the experiment with beakers and dialysis tubes A through C.

11. Did your experimental results support your hypothesis? If not, propose a new hypothesis.

12. What is your hypothesis for the experiment with beaker D and the starch solution in the dialysis tube?

13. What were your results, and did the data support your hypothesis for Beaker D?

14. Explain your results based on what you know about the cell membrane.