Diffusion & Osmosis

Background:
I. Membrane Structure.

One of the most important organelles found in both prokaryotic and eukaryotic cells is the plasma membrane. The plasma membrane is found on the outside of animal cells and beneath the cell wall in bacteria, plants, fungi, and some protists. It is a selectively permeable membrane, which means that it has the ability to regulate which molecules can cross the membrane, allowing some to cross and preventing others. Part of this ability comes from the structure of the phospholipid molecules, which make up the bulk of the plasma membrane.

Phospholipids are made up of a glycerol, two fatty acids, and a polar, phosphate head. These parts are loosely organized into two regions. The phosphate group and the glycerol portion of the molecule form the “head” region. Since the phosphate group is polar, the heads are hydrophilic (water soluble). Because the fatty acids, which comprise the “tail” region, are non-polar, they are hydrophobic (lipid soluble). The figure at right outlines the structure of phospholipid molecules.

The plasma membrane consists of two layers of phospholipid molecules arranged with their tails pointing inward, as in the figure below.
a hydrophobic region in the center of the membrane which blocks the movement of polar molecules across the membrane. Non-polar molecules, however, are free to pass across the membrane.

II. Transport Mechanisms.

Why do living organisms need a selectively permeable membrane? 1. They need to be able to transport water and required nutrients into the cell. 2. They need to be able to let wastes and secretory proteins out of the cell. 3. They need to be able to somewhat regulate how much and what type of chemical enters the cell – for metabolism and protection. In short, things have to pass across the membrane on a regular basis.

Molecules can either move across the membrane via passive transport or active transport. Passive transport means that the process occurs naturally, without any energy output from the cell. Small molecules may move directly through the membrane, in between phospholipids, or may diffuse through protein channels embedded in the membrane. Active processes require the cell to expend energy.

The 2 most common passive transport mechanisms by which chemicals, nutrients, gases, or water molecules are into the cell are Diffusion and Osmosis.

Diffusion describes the tendency for molecules (any molecules), which are constantly in motion, to move from areas where they are most concentrated to areas where they are least concentrated. If someone spilled a bottle of perfume in one corner of a room, then scent molecules would spread from the point of the spill to the opposite side of the room – until equilibrium was reached.

Remember that since diffusion down this concentration gradient is a passive process, it occurs naturally, without any energy output from the cell. This is important from the cell’s standpoint because it doesn’t have to allot any of its energy reserves to passive processes.

Osmosis can be thought of as a special case of diffusion, the diffusion of water across a differentially or selectively permeable membrane. Remember that the center of cell membranes contains the nonpolar fatty acid tails of phospholipid molecules. Because of this large nonpolar area, charged particles and large polar molecules cannot diffuse across the membrane. Water molecules, even thought they are polar, can diffuse across
Osmosis occurs when a solute (example: salt, sugar, protein, etc.) cannot pass through a membrane but the solvent (water) can. Water always moves from where it is most concentrated (has less solute) to where it is less concentrated (has more solute).

In general, water moves toward the area with a higher solute concentration because it has a lower water concentration. The diagram below depicts what will happen to red blood cells (internal solute conc. = 0.9%) in solutions of different tonicity. Because water will always move from the area of higher water concentration (lower solute conc.) to the area of lower water concentration (higher solute conc.), identifying these areas will help you determine the direction that water should move.

In the container on the left side of the diagram, the region within of lower water concentration is within the cell, so the water will enter the cell. Because of this, the cell will begin to swell as water enters it and it will eventually burst. Since the cells are in a solution with lower solute concentration, they are said to be in a hypotonic solution. In the center drawing, water is more concentrated inside the cell, so the water will move out, causing the cell to shrivel, or crenate as the volume of water in the cell decreases. In this case, because the water has a higher solute concentration, they are in a hypertonic solution. If the solute concentration is the same inside as it is out (the container on the right side of the diagram), the amount of water that moves out will be approximately to the amount that moves in. Since the two areas have equal solute concentrations, this is an isotonic solution. Osmotic pressure is the force of osmosis (driving the movement of water). The greater the concentration gradient, the greater the osmotic pressure.

Prelab Questions

1) What type of molecule makes up the bulk of a plasma membrane?
2) What is significant about the head and tail regions of that molecule?
3) What is the difference between active and passive transport mechanisms?
4) Define diffusion.
5) Define osmosis.
6) In part A of this experiment, what was the reason for measuring the volume of liquid in the pillow following its immersion?
7) In part B of this experiment, what is the significance of the first (distilled water) pillow?
8) In part B of this experiment, identify the dependant and independent variables.
**Experimental Procedure**

This experiment is designed to test the ability of water and solutes to move across a semi-permeable membrane (artificial cell membrane) and the effects of variation in solute concentration on the movement of water. We will be using dialysis tubing to simulate our cell membranes. Dialysis tubing is a semi-permeable membrane with microscopic pores in the membrane of a specific size. Any molecules that are small enough to pass through the holes may pass across the dialysis tubing, where as larger molecules will be blocked. Using this knowledge as well as what you learned about starch and glucose during the last lab, perform the following experimental procedures.

**Part A – Diffusion in a liquid.**

**Write a hypothesis about the ability of starch, glucose and water molecules to pass across the dialysis tubing membrane.**

**Procedure:**

1) Take a piece of dialysis tubing and close one end using a clamp, leaving about 1cm of dialysis tubing at the end. You do not need to tie a knot in the tubing – the clamps create sufficient seals.

2) Using a graduated cylinder and the funnel provided, measure and add 20ml of the starch/glucose solution to the dialysis tubing bag you have just made.

3) Leaving approximately 1/3 of the bag empty and unfilled, clamp the opposite end closed as you did in step 1 to make a little pillow.

4) Find the beaker labeled “starch/glucose” and fill it with approximately 200mL of distilled water. Immerse the pillow in the beaker for 30 minutes.

5) While the pillow is in the distilled water, set up part B of the experiment.

6) After 30 minutes, remove the pillow from the beaker of distilled water. Trying not to spill any of the contents, unclamp one end of the pillow and, using the funnel, pour the contents back into the graduated cylinder to measure the volume. Did it increase, decrease or stay approximately the same? What does this imply about the direction that water moved?

7) Test the contents of your beaker of (initially) distilled water for the presence of starch and glucose using the Iodine and Benedicts solution provided (method provided at the end of the lab if your class did not perform the enzyme lab). Is there any glucose or starch in the surrounding water? What does this imply about the ability of these two molecules to pass across the dialysis tubing membrane?

8) Discard of the starch/glucose tests in the designated container(s), thoroughly wash and rinse your tubes, and return them to your test tube rack.

9) Record your results below.

<table>
<thead>
<tr>
<th>Pillow Volume (ml)</th>
<th>Beaker – Starch</th>
<th>Beaker – Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Part B – Affects of varied solute concentration on osmosis.

Write a hypothesis about the affects of varied solute concentrations on osmosis.

Procedure:
1) During this activity, you will create 6 dialysis bags using the same procedure that you used in part A of the experiment.
2) Add 200ml of distilled water to the six 250ml beakers that are labeled as follows: distilled H2O (or dH2O), 0.2M sucrose, 0.4M sucrose, 0.6M sucrose, 0.8M sucrose, and 1.0M sucrose.
3) Using the same method used in Part A, add 20ml of distilled water to the first bag, clamp the opposite end, leaving approximately the same volume empty & unfilled in the pillow. Place the pillow on the lab bench in front of the distilled water beaker.
4) There are 5 sucrose solutions provided with different concentrations of sucrose measured by their molarity (molarity is actually a measure of the number of moles of a substance per Liter of water. A .2M solution has a low concentration of sucrose, while a 1.0M solution has a high concentration). As in step 2, Add 20ml of the 0.2M glucose solution the second bag and place it on the lab bench in front of the 0.2M beaker.
5) Repeat step 4 for the remaining sucrose solutions. Be sure that your graduated cylinder is completely emptied of the previous solution before measuring the next one.
6) Immerse all 6 pillows in their respective beakers for 30 minutes.
7) After 30 minutes, remove the pillows and measure the volume of their contents with the graduated cylinder – again, ensure that you empty your cylinder completely before measuring each solution – you can empty your solutions into their respective test beakers as you perform this step.
8) When you are finished with the activity, please rinse all of your test beakers, the graduated, cylinder, and the funnel. Please leave the labels on your group’s beakers. Please DO NOT empty the starch/glucose solution or the sucrose solutions provided in your group’s tray. They will be used by other classes.
9) Record your results below.

<table>
<thead>
<tr>
<th>Sucrose Molarity</th>
<th>Initial Volume (ml)</th>
<th>Final Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0M (Distilled H2O)</td>
<td>20ml</td>
<td></td>
</tr>
<tr>
<td>0.2M</td>
<td>20ml</td>
<td></td>
</tr>
<tr>
<td>0.4M</td>
<td>20ml</td>
<td></td>
</tr>
<tr>
<td>0.6M</td>
<td>20ml</td>
<td></td>
</tr>
<tr>
<td>0.8M</td>
<td>20ml</td>
<td></td>
</tr>
<tr>
<td>1.0M</td>
<td>20ml</td>
<td></td>
</tr>
</tbody>
</table>

Write your Lab Report after you have completed the experimental procedure (including any tables &/or graphs) using the guidelines provided and turn it in at the beginning of the next lab.
Iodine and Benedict’s Reagent Tests for Starch and Glucose

We will be using the Iodine Test to test for the presence of starch (polysaccharides). Iodine turns blue-black in the presence of starch (but not in the presence of monosaccharides). The darker the solution, the more starch is present (yellow=0, brown=1+, blue=2+, black=3+). **Use the following procedure to test for starch:**

1) Add 3 drops of iodine reagent into each tube to be tested for starch.
2) Interpret the color of the solution and record your results.

We will be using the Benedict’s Test to test for the presence of glucose (monosaccharides). It tests for monosaccharides (can sometimes also pick up a few disaccharides), but doesn’t detect polysaccharides. Benedict’s reagent (normally a bright blue color) will turn from blue to yellow to orange to red in the presence of glucose (the greater the color change, the more sugar is present: blue=0, green=1+, yellow=2+, orange=3+, red/rust=4+). **Use the following is a procedure to test for glucose:**

1) Add about 10 drops of Benedict’s reagent to each test tube to be tested for glucose (enough to turn the solution light blue)
2) Place the test tubes in the boiling water bath for 3 minutes. (NOTE: our hotplates do not heat to the temperature indicated on the LED display – turn the Heat dial all the way up to boil the water in your water bath beaker.)
3) After 3 minutes, remove the tubes from the boiling water bath using the test tube holders and allow the tubes to cool.
4) Interpret the color of the solution and record your results.