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BIOL 243L HUMAN ANATOMY AND PHYSIOLOGY LABORATORY
OSMOSIS AND SOLUTE CHEMISTRY

This laboratory exercise will demonstrate some of the principles of osmosis and diffusion. As you work through the demonstrations and consider the questions asked, refer to the text sections on movement of materials across plasma membranes (Chapter 3, permeability across cell membranes). Above all, think about what might be going on with things as small as atoms and molecules to produce the results we see.

I. Osmosis

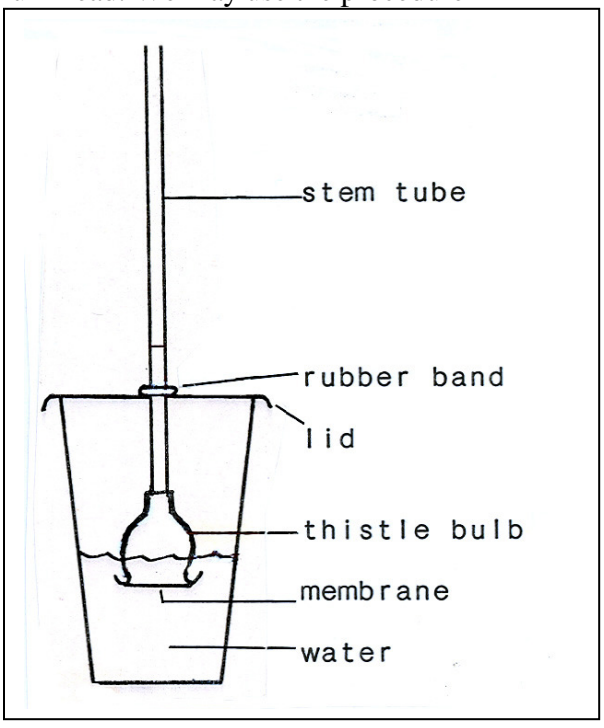
A solution is a mixture of one kind of compound called the **solute** dissolved into a larger volume of a liquid compound called the **solvent**. We will observe the movement of a solvent (water) across a semi-permeable membrane in response to differences in concentration of some dissolved solutes, in this case the sugars, glucose and sucrose. This process of movement of compounds across semi-permeable membranes (also known as selectively permeable membranes) is called osmosis.

Osmotic processes can take some time. The results of one of our experiments require at least an hour to detect well. We will do the initial set up of this lab exercise first, or it may be already set up in front of the laboratory as a single preparation demonstration. We can then observe the progress after doing the other lab exercises.

The osmometer includes the following parts:

a. a disc of animal membrane secured to a plastic chamber
b. a detachable plastic stem tube
c. a plastic cup
d. a plastic lid

"Animal membrane" is a polite way of saying "gut." You may have heard of traditional uses of gut such as strings for bows of musical instruments, drum heads, and as dissolving suture thread to close wounds. It all boils down, literally and figuratively, to the connective tissues of the gut tube which are loaded with collagen. Processing this tissue results in a membrane that is both semi-permeable when immersed in water and tough enough for a drum head. We may use the procedure below and in the diagram. Alternatively, we may instead use a cellophane plastic membrane tube called dialysis tubing instead of animal membrane and set the experiment up as a demonstration at the front of the laboratory. The principles demonstrated will be the same.

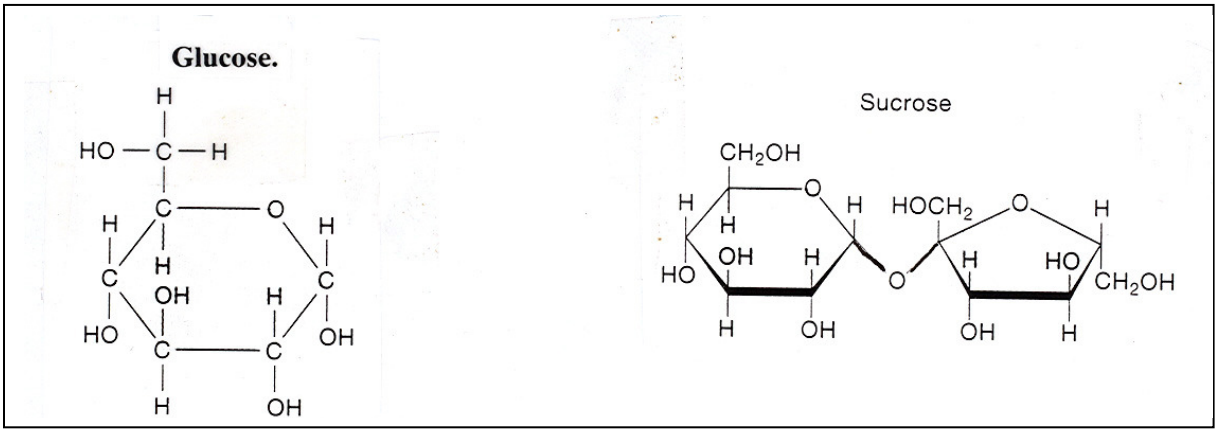


The procedure for the setup or demonstration is as follows:

1. Moisten the membrane by squirting some water into the bulb onto the membrane, using a dropper. Then set the bulb membrane-side-down on a piece of wet paper towel for 5 minutes.
2. Pour, or shake, the water out of the chamber.
3. Put about 4 oz. (120 ml.) of plain water into the plastic cup.
4. Use a dropper to fill the chamber completely full of 20%

glucose or 20% sucrose solution. **Note: choose one of the droppers with the smooth tapered tip that fits into the chamber.** The two sugars have different molecular weights. A 20% solution of each means the same total mass of each sugar is dissolved in the same volume of solution.

What does this mean for the number of molecules or the number of moles dissolved in the same volume of solution?



It is a bit tricky to get the apparatus finally assembled without air bubbles, but this is essential. Please read the directions for steps 5-8 first and understand the procedure before launching into the steps.

5. Load the 20% sugar solution into the detached stem tube using the following procedure:
   a. Place the latex rubber dropper bulb on the end of the stem tube.
   b. Place the lower end of the stem tube into the sugar solution stock bottle.
   c. Suck the sugar solution about halfway up the stem tube.
   d. Block the LOWER end of the stem tube with your finger and then remove the dropper bulb from the top end of the stem tube.

6. Place a finger at the TOP end of the stem tube to keep the liquid from running out of the stem tube when you remove your finger from the bottom of the tube.

7. While keeping your finger on the top of the stem tube, slide the detached cup lid up the stem tube from below, until it comes up to the rubber band. The proper location is shown on the diagram on the previous page.

8. With your finger still on the top of the stem tube, and while still holding the lid on the tube; insert the bottom end of the stem tube tightly into the completely full bulb. (Keep in mind that the membrane is delicate, while you apply pressure on the bulb with the stem.)

9. Look for bubbles in the stem. There must not be any bubbles there!
If there are bubbles in the stem tube:
   a. Detach the stem tube from the bulb.
   b. Refill the bulb, if any fluid was lost.
   c. Do steps 5, 6, 7, 8, & 9 again.
   d. Keep trying until there are no bubbles and the fluid level in the stem tube is about halfway up the tube.

10. If there are no bubbles in the stem tube, place the end of the membrane-covered bulb into the plastic cup so the membrane is beneath the surface, and secure the lid onto the top of the cup. (check the diagram again)

11. Adjust the rubber band so that it holds the bottom of the membrane-covered bulb at least a short distance above the bottom of the cup. The fluid must be above the lid of the cup and above the rubber band! (see diagram.)

12. Make a mark with a red marking pencil (red grease pencil) on the stem tube to show where the fluid is at the beginning of the hour. Record the time here, so you'll know when the hour is over. Time begin_____ Time end_____

13. After an hour, make a second mark on the stem tube to show the fluid level at the end of the hour. Then measure how far the fluid rose in the stem tube. Record the rise in millimeters. (Millimeters are the smallest divisions on the metric ruler. Note that there are ten millimeters for each number printed on the ruler.)

14. When finished:

LEAVE THE MEMBRANE ON THE BULB!

a. Detach the stem from the bulb.
b. Empty the sugar solution from the bulb.
c. Rinse the inside of the bulb by squirting water inside (using a dropper) then emptying it out again.
d. Rinse the outside of the bulb-and-membrane and the stem.
e. Place the bulb-and-membrane on its side on the tray where you first got it.

If the osmometer is a demonstration with dialysis tubing, look over the results at the front of the laboratory. There will be two tubes and membrane containing 20% glucose and 20% sucrose at the front of the classroom.
Why is there a difference between the lengths of fluid rise in the glucose osmometer and the sucrose osmometer?

What would happen if we used a 10% solution of glucose instead of 20%?

At equilibrium, the height the solution rose is proportional to the rise in pressure just inside the membrane. Pressures in physiology are often expressed as heights of columns of water (eg 35 cm of water). If you exerted additional pressure on the solution in the bulb, you could force water back across the membrane filtering water from the sugar solutions. What is this process is called?

II. Differential Permeability

Semipermeable membranes may be permeable to some solute molecules, not just to the solvent molecules.

1. Obtain a piece of flat cellophane dialysis tubing. Sections of tubing a few inches long are

soaking in jars of water.
2. Remove the soaked tubing from the water, and tie a knot with string tightly near one end.
3. Rub the other end of the tubing between your thumb and finger to separate its two sides so you can open the tube.
4. Using the dropper, fill the tube with 1% STARCH solution, but leave enough cellophane so you can tie off the end and leave the tube loosely filled, but not tight like a balloon under pressure.
5. Tie a tight knot just above the starch solution, but again, not to place liquid under pressure.
6. Rinse the outside of the tubing and the region of the knots, to remove any starch solution that might still be there.
7. Lay the filled, rinsed tube into a beaker that contains about an **inch** of water. It is all right if one end of the tube is above water. Add 4 drops of iodine solution. (This solution is iodine dissolved in potassium iodide solution, I_2KI.)
8. Look for color to appear along the portion of the tubing that contains the starch solution. (It takes 5 or 10 minutes for the color to appear, as the iodine solution reacts with the starch.)
Where is the color located, inside or outside the tubing?
What has been transported across the tubing membrane?
9. You may cut the tube, and "bleed" it into the sink before discarding it.

III. Dialysis

Although dialysis is now known as the mechanism for "artificial kidneys", it had been in use for many decades as a means to separate small molecules from large molecules. Our setup will demonstrate that process. (Read the CAUTION, below, before starting!)

1. Prepare a cellophane tube as described in steps 1 through 3 in the exercise above.
2. Pour enough Starch-salt (starch and sodium chloride) solution into the tube to fill 2-3 inches of it.
3. Tie a tight knot just above the starch-salt solution.
4. Rinse the outside of the tubing and the region of the knots, to remove any starch-salt solution that might still be there.
5. Lay the filled, rinsed tube into a beaker that contains about an inch of plain water.
6. Wait at least 1/2 hour, then pour off some of the water from the beaker into 2 test tubes.
7. Add a drop or two of the I_2KI solution to one of the test tubes, adding it to the water. Look for the indication of starch as you saw in the exercise above. Is starch present?
8. Silver nitrate is highly soluble and is a clear solution, but silver with chloride will form a cloudy precipitate. Add a drop or two of SILVER NITRATE solution to the other test tube.
Does a cloudiness occur?
What does this mean?

(CAUTION! Silver nitrate solution, although it looks like just water, will stain your skin or clothing dark brown! The color doesn't appear until the next day, so you won't know until it is too late! SO WASH YOUR HANDS IMMEDIATELY AFTER USE!)

Is there evidence that either the starch or one of the salt ions has left the cellophane tube?
How do the starch and salt molecules differ?

10. You may cut the tube, and "bleed" it into the sink before discarding it.

IV. Brownian Motion

This is an opportunity to see the action of moving atoms and molecules directly.

1. Briefly shake the bottle of Carmine Red suspension holding your finger on the dropper to keep it and the solution from flying out. Place a single drop of the suspension on a microscope slide.
2. Put a plastic cover-slip on the drop.
3. Focus first with the low power (10X) objective lens, to find the edge of the cover slip, then the red particles. (Usually the largest, heaviest, particles settle on the glass slide; so focus up a bit to see particles that are still suspended in the water.)

Once you can see the suspended particles with low power, change to the high power (40X objective).

Examine the small, suspended particles carefully. Can you see that some of the smallest ones have a jiggling motion? This shaking motion is Brownian Motion. This is evidence of collisions of these fine particles with even finer particles.

Blot-dry the slide and cover slip, and put them into the large beaker in the sink.

V. Preparation of an Emulsion

An emulsion is a liquid-in-liquid suspension. The liquids do not completely dissolve into one another (i.e. it is not a molecular solution). However, if the liquid particles are sufficiently fine, they will be held in suspension.

1. Place about 15 drops of Oil into the bottom of a test tube.
2. Add 15 drops of water to the tube.
3. Shake the tube vigorously, with it in a "lying-down" position, for about 5 seconds.
4. Add enough additional water to make the mixture at least one inch deep, and swirl the contents to mix.

What happened to the drops of oil?
Does all the oil rise to the top, or is the water cloudy from tiny suspended drops?

5. Empty the tube.
6. Place one drop of Detergent into the bottom of the test tube.
7. Place 15 drops of Oil into the bottom of the test tube.
8. Add 15 drops of water to the tube.
9. Shake the tube vigorously, with it in a "lying-down" position, for about 5 seconds.

Add enough additional water to make it at least one inch deep, and swirl the contents to mix.

What happened to the oil?
Can you see the droplets into which it has broken?
Are they smaller than in the first tube?
Does the oil rise to the top, or is the water cloudy from tiny suspended drops?

A suspension of liquid droplets too small to separate from the suspension is called an EMULSION. Emulsions are necessary for digestion of fatty foods in our small intestine.

BEFORE YOU LEAVE:

1. BE SURE ALL YOUR GLASSWARE IS CLEAN AND PLACED UPSIDE DOWN TO DRAIN.
3. PLACE SLIDES AND PLASTIC DROPPERS IN THE LARGE BEAKERS IN THE SINKS.
4. PLACE MICROSCOPES BACK ON THE RACK.