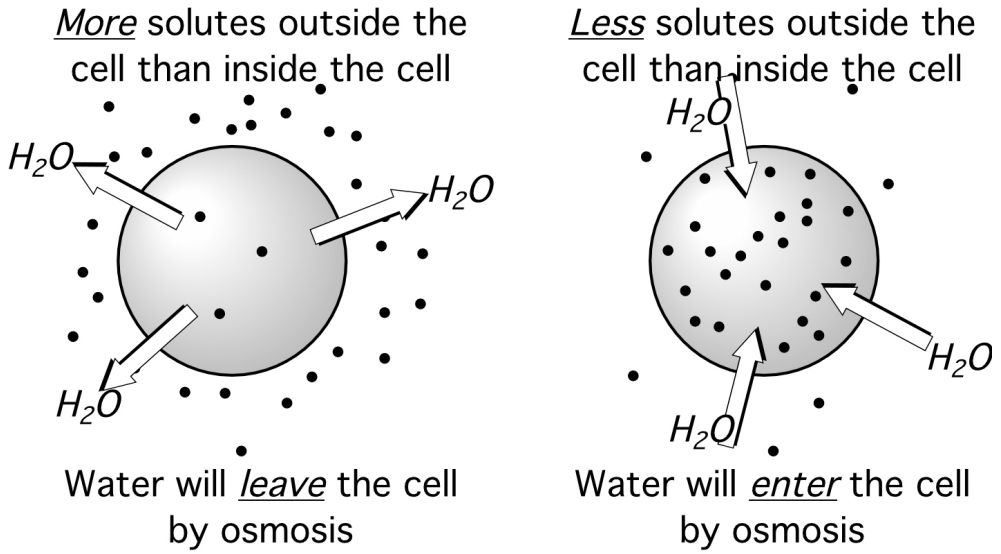
Diffusion and Osmosis (lab 2.5)

The background information for understanding this lab is in lab manual experiment 2.5.

a) Introduction to osmosis

Osmosis is the movement of water through a membrane. To be more exact, osmosis is defined as the movement of water across a semi-permeable membrane towards whichever side of the membrane has the highest solute concentration. (A semi-permeable membrane is one that allows water molecules to pass through but not solute molecules). Water molecules are attracted to solutes. So in osmosis water always moves across the membrane toward the higher solute concentration.

The membranes of cells are semi-permeable. As a consequence, a cell will lose water by osmosis if the cell is placed in a solution that has a higher solute concentration than the cell, and a cell will gain water by osmosis if the cell is placed in a solution that has a lowerer solute concentration than the cell. These concepts are illutrated below.



To predict whether water will move into or out of a cell by osmosis, you need to compare the total solute concentration inside the cell to the total solute concentration outside the cell. The total concentration of solute particles is called the osmolarity (OsM).

Osmolarity (OsM) = The total concentration of solute particles

Any liquid that has a higher osmolarity (a higher total solute concentration) than a cell is called a hypertonic solution. Cells will always lose water by osmosis when the cell is in a hypertonic solution. Any liquid that has a lower osmolarity (a lower total solute concentration) than a cell is called a hypotonic solution. Cells will always gain water by osmosis when the cell is in a hypotonic solution. A liquid that has the same osmolarity as a cell is called an isotonic solution. Cells don't gain or lose water in isotonic solutions.

Procedure B) Osmosis demonstration using dialysis tubing simulated cells

In this activity, you will demonstrate osmosis on cells by constructing three "cells" using dialysis membranes. Although you can't see it with the naked eye, the dialysis membrane has millions of tiny holes. Water molecules are small enough to easily pass through, but larger solute molecules (such as sucrose) can't pass. Thus, the membrane functions as a semi-permeable membrane (similar to a real cell's membrane).

1) Obtain three 5.5 inch sections of dry dialysis tubing, six plastic clamps for the tubes, and three 300 ml beakers. Using a wax pencil, label the beakers:

- Water
- 0.44 OsM sucrose
- 0.88 OsM sucrose.

2) Fill the Water beaker with 200 ml water. For now, don't add anything to the other two beakers. Place all three dry dialysis tubes into the "Water" beaker. Let them soak for 5 minutes.

3) After 5 minutes, remove one of wet dialysis tubes. By gently massaging one end of the tube with your fingers, open one end. Clamp the other end of the tube shut by first folding that end in half **sideways** and then applying a plastic clamp to the sideways fold. Your instructor has prepared a demonstration clamped dialysis tube on the front desk for you to check that you have prepared yours correctly.

4) Carefully fill the tube with 25 ml of 0.44 OsM sucrose (sugar) solution. The dialysis tube in this experiment represents a cell in the body. The 0.44 OsM sucrose inside the tube represents the solutes in the cytoplasm (the fluid inside the cell).

5) Seal the cell shut by folding the top of dialysis tube in half (expelling as much air as possible) then clamping the top end shut. **Leave some empty room at the top of the tube for the tube to expand.** If sealed properly, the cells should not leak and should look the same as the instructor's demonstration tube on the front desk.

Now repeat steps 3 - 5 with the other two wet dialysis tubes. You should end up with three sealed dialysis tube "cells", with each cell filled with 25 mL of 0.44 OsM sucrose.

6) Using paper towels, gently dry the three cells. Try to remove all drops of water from the surface of each cell and from the plastic clamps.

7) Fill the 0.44 OsM sucrose beaker with 200 ml of 0.44 OsM sucrose, and fill the 0.88 OsM sucrose beaker with 200 ml of 0.88 OsM sucrose.

8) Tare (zero) an electronic balance. Place the first cell on the balance and record its mass in the first row of the results table below. Next, place this cell in the 0.88 OsM sucrose beaker. Be sure that the cell is fully submerged under the 0.88 OsM sucrose.

9) Tare (zero) an electronic balance. Place the second cell on the balance and record its mass in the second row of the results table. Next, place this cell in the 0.44 OsM sucrose beaker. Be sure that the cell is fully submerged under the 0.44 OsM sucrose.

10) Tare (zero) an electronic balance. Place the third cell on the balance and record its mass in the third row of the results table. Next, place this cell in the water beaker. Be sure that the cell is fully submerged under the water.

11) Let the three dialysis tube cells soak in the beakers for an hour. While the cells are soaking, you can start section (c) of this lab.

12) After 1 hour, remove the cell in the 0.88 OsM sucrose. Use paper towels to completely dry the cell. Again, try to remove all drops of water from the surface of the tube and from the plastic clamps.

13) Tare (zero) an electronic balance. Place the cell on the balance and record its mass in the results table.

14) Repeat steps 12 and 13 with the cell in the 0.44 OsM sucrose and then with the cell in the water.

15) After you have shown your instructor the completed data table, clean up your materials: Wash all beakers and dialysis clips thoroughly with water, then return them to where you obtained them. The membranes and solutions can be disposed of in the garbage and the sink.

Data Table for Osmosis with dialysis tubing cells:

	Weight at start:	Weight at end:	+/- grams weight change:
Cell containing 0.44 OsM sucrose soaked in a 0.88 OsM sucrose solution:	_____	_____	_____
Cell containing 0.44 OsM sucrose soaked in a 0.44 OsM sucrose solution:	_____	_____	_____
Cell containing 0.44 OsM sucrose soaked in a water:	_____	_____	_____

Procedure C) Solubility of compounds in Polar and Non-polar solvents

1) Obtain a test tube from your physiology lab basket.

2) Add 10 ml water and 2 ml corn oil to the test tube. They do not mix because one is hydrophilic and one is hydrophobic.

Which substance forms the top layer? ___ Which forms the bottom layer? ___

The molecular formula for water is H_2O. A typical molecular formula for corn oil triglyceride is $C_{57}H_{105}O_6$. Judging from the molecular formulas, which substance is hydrophilic? _____ Which is hydrophobic? _____

3) Add 2 drops of Methylene blue dye and one rice-grain size amount of Sudan red dye. One of the dyes is hydrophobic and one is hydrophilic. Put a rubber stopper into the test tube and then mix the tube well by shaking it vigorously for a minute.

At first the tube will be maroon color (red + blue), but as the oil and water slowly separate, you will see the two colored dyes separate also. This is because of the "like mixes with like" principle: The hydrophobic dye moves into the hydrophobic solvent and the hydrophilic dye moves into the hydrophilic solvent.

4) Let the test tube stand until end of period. This will allow the dyes to fully separate. When they have fully separated, answer these questions:
Which dye is hydrophobic? _____ Which is hydrophilic? _____

5) When done, wash the test tube using test tube brush in your basket and the Dawn™ soap on the counter. Remember, it's always clear and bright when it's Dawn™

Procedure D) Concentration and tonicity

Read section C of lab 2.5 for background information on this activity.

1) On the countertop are three test tubes (A, B, and C). Each tube contains a drop of sheep's blood in salt solution. The salt solution in one tube is hypotonic, the salt solution in one tube is isotonic, and the salt solution in one tube is hypertonic.

2) Before the lab started, a sample from each of the three test tubes was placed on a microscope slide for viewing. The samples from each of the test tubes can be viewed under microscopes A, B, and C.

3) View each slide under the microscopes. Because the cells were in different osmotic solutions, their shapes will be different (due to water loss or water gain), as described below:

   Normal shape = isotonic solution
   Crenated (shriveled shape) = hypertonic
   No cells = hypotonic (due to hemolysis (blood cell tearing)).

4) Judging from the shapes of the cells, fill in the following table with either A, B, or C. Show your instructor your results when done.

   Hypotonic solution = _____
   Isotonic solution = _____
   Hypertonic solution = _____