LABORATORY #4 -- BIOL 111
Osmosis
Lecture 10 and Section 5.4 are good reviews for this lab! Bring your text to lab, if possible!

One of the most obvious and prominent processes in the universe is **diffusion**, which is defined as "the movement of a substance from an area of high concentration to an area of low concentration." For example, when you exhale air, it is composed principally of carbon dioxide. As soon as it leaves your mouth, the carbon dioxide is at a high concentration (relative to the surrounding air) and so diffuses away from your mouth to all the surrounding air. It is important to understand the distinction between **concentration** and **abundance/amount**. When we consider concentration, the substance must be contained—in a container of some type—like a balloon, or beaker or cell. Concentration is the amount of something in the (per) total volume of the container. For example, a salt concentration of 5% in a beaker of water of 100 milliliters (ml) would be 5 grams of NaCl dissolved in the 100 ml of water. (5 g of NaCl in a swimming pool, would be a much lower concentration, despite being the same abundance/amount/mass.)

As you heard/read/learned in class this week, everything, including water, is subject to diffusion. **Osmosis** is a specific type of diffusion. **Osmosis is the diffusion of <u>water</u> across a semi-permeable membrane**. A "semi-permeable membrane" is any object that allows free passage of only some substances. The prime example of a semi-permeable membrane is the covering around every cell in your body, i.e., the plasma membrane. The plasma membrane allows free passage of water, oxygen, carbon dioxide and other small compounds, but resists the crossing of larger molecules like sugars & salts.

Because many solutions contain different amount of compounds (e.g., pure freshwater has nothing in it; saltwater is water <u>and</u> salt), osmosis is a very important biological process. For example, a walleye has saline (i.e., salty) solution coursing through its body, yet it swims in freshwater that has very little salt. This means that the salt is more concentrated in the walleye's body than in the environment that it swims in. Conversely, the water in the walleye is less concentrated than it is in the lake (i.e., salt is taking up some of the space in the walleye where there could be water). Because the walleye is composed of cells (which have cell membranes that are semi-permeable; they allow free passage of water but prevent the passage of salts), the water will move from the area of high concentration (the lake) to an area of low concentration (within the walleye's body). The result would be a walleye that gains water and swells. Eventually the walleye would burst from all the excess water flowing in by osmosis. Fortunately, the walleye has kidneys that prevent this; walleyes urinate often and their urine in very dilute (i.e., rich in water and low in salts). The opposite process occurs in fishes that live in the ocean.

You may not realize that your cells do the same…they allow water in and out via the laws of osmosis. Understanding today's lab requires some careful thought…and basic chemistry. Remember, the higher the concentration of "stuff" dissolved in water, the lower the concentration of water in that solution…Test yourself---which has a higher **water** concentration 10% NaCl or 20% NaCl? **When considering osmosis, think of the water concentration!**

You will also build on your last lab, in which you got to look at plant cells. Recall the structure of plant cells. Remember they tend to be rigid and strong due to the cell wall, that is absent in animal cells. Cell walls do not restrict osmosis!

Procedure

1. Each pair of students will work as a team. Each team should obtain 2 vials, 1 carrot, 1 metric ruler, a digital scale and a small knife. Sorry, no stabbing allowed!!

2. Fill each of the 2 vials 3/4 full of the appropriate solution (either H2O/distilled water or 1 M sodium chloride/NaCl). Note: the "M" refers to how much stuff there is in the water. The larger the number, the more stuff there is in the water. "M" stands for "molarity." **By the way, typical molarity of NaCl *inside* a plant cell is 0.15 M**. This means that 1 Molar salt water is really salty, compared to plant cells!

3. Prepare 2 carrot sticks from the same carrot. Slice narrow rectangular strips as close to 100mm long x 10mm wide x 5mm thick as possible.

4. Measure each strip's length in millimeters and weigh each stick in grams. Place them in the vials according to the table (question 2). Be careful to keep track of which stick is in which vial! Leave the carrot sticks in the vials for at least 20 minutes.

5. Meanwhile, answer questions 1, 5, & 6 from the requirements page.

6. After 20 minutes, remove the carrot sticks, blot dry, re-measure & re-weigh them. Record the final length and weight for each stick. Compute the change (final length minus initial length). Be sure to record changes with "+" or "−", and the number!

7. There are lots of random factors which influence our results. This is why we need **statistics**. Your instructor will lead you through a statistical analysis to determine if the group data represent significant changes.

For each set of before & after length data, we will calculate the probability of the null hypothesis ("Carrots strips *did not* change in length") using so-called *t-test*. We will use an on-line t-test calculator (http://graphpad.com). Search for t-test calculator. Enterinitial data on the left and final data on the right. Make sure that "groups are matched" and "two tails" are selected. Then press "calculate". You will be given the p-value, which reflects the *probability of the null hypothesis*. This is the probability that there was not a change. Record the p-value.

8. Address question 3. If the p-value is less than a threshold (0.05), this is an indication that the null hypothesis ("Carrots strips *did not* change in length") is unlikely. Consequently, if p-value < 0.05 it's likely there was a significant change. We should reject the null hypothesis, and fail-to-reject the hypothesis indicating change!

9. Clean up as instructed.

Name________________________

Requirements Lab 3 (page 1)

1. Based on the background you have learned on osmosis, provide a hypothesis for the movement of water into/out of the carrot cells in each experiment you set up. As always, provide an explanation for your hypothesis. Use complete sentences! *(3pt)*

2. Based on your hypothesis, above, predict what will occur in the carrots in the different NaCl solutions. Specifically, will the carrot gain length/weight, lose length/weight, or remain the same in weight/length. *(2 pts)*

A. freshwater experiment (weight)

B. freshwater experiment (length)

C. saltwater experiment (weight)

D. saltwater experiment (length)

2. Collect your data. Fill out the table below. Don't forget units—what did you measure in your carrots? Your instructor will help you with the p-value and significance determination *(3 pt)*

A. Freshwater Experiment

Initial weight______ Initial length_______

Final weight______ Final length_______

Change__________ **Change**___________

p-value__________ **p-value**___________

Significant?_______ **Significant?**___________

B. Saltwater Experiment

Initial weight______ Initial length_______

Final weight______ Final length________

Change__________ **Change**___________

p-value__________ **p-value**___________

Significant?__________ **Significant??**______________

Requirements Lab 3 (page 2)

4. Does the <u>class data</u> cause you to reject or fail-to-reject each hypothesis? Explain why or why not using p-values and common sense. *(2pts)*

A. freshwater experiment (weight)

B. freshwater experiment (length)

C. saltwater experiment (weight)

D. saltwater experiment (length)

3. Thinking about your two measurements the change you noticed… is change in <u>weight</u> a good indicator of osmosis in these cells? Is change in <u>length</u> a good indicator of osmosis in these cells? Explain why or why not—Think about the cells you used! Note: Significant results do not always mean an indicator is good! *(2pts)*

5. In medical labs, when blood is drawn from a patient, the blood is typically placed in a dilute salt solution---about 0.9% NaCl. Why not pure water? What does this tell you about your red blood cells? What would happen to your red blood cells in pure water? *(3pts)*