Challenging Misconceptions about Osmosis

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Biography

Robert J. Kosinski is a professor of Biology at Clemson University, where he is the sole lecturer in the Introductory Biology course for majors and also the coordinator of the labs for that course. He received his B.S. degree from Seton Hall University and his Ph.D. in Ecology from Rutgers University. His interests include laboratory development, investigative laboratories, and the educational use of computer simulations, all in introductory biology. He was chosen as the Alumni Master Teacher of Clemson University in 2007. He has attended every ABLE meeting since 1989, has presented at 13 of those meetings, and acted as the chair of the host committee for the 2000 ABLE meeting at Clemson University.

Charles Kaighn Morlok is an undergraduate Biological Sciences major at Clemson University, with plans for graduation in 2010. After graduation he plans to go to South Africa to work with orphans for a year or two, and then return to the US and attend medical school, where he plans a specialty in emergency medicine. Kaighn gave great assistance with the development of this exercise while serving as a work-study student in the Department of Biological Sciences. 2008 was his first ABLE meeting, but he hopes it will not be his last.

Introduction

Osmosis is a traditional topic in introductory biology courses, but incorrect explanations of it are common. This laboratory allows students to estimate the water potential of potato cores using the weight change method and three different solutes: sucrose, glucose, and NaCl. The use of the three solutes does not interfere with the water potential determination, but does allow the students to assess three theories of the cause of osmosis—that it is caused by water concentration differences, by the binding of water to hydrophilic solutes, and by the simple *number* of solute particles (van't Hoff's Law).

Wet Lab Component

The "wet lab" portion of the exercise is simple but time-consuming. Students punch out potato cores, remove excess water with a paper towel, and weigh them (takes about 30 minutes), soak them in a variety of solutions (about 1.5 hr), and then weigh them again and do some calculations (another 30 minutes). It would be best to combine this exercise with other exercises related to diffusion and osmosis that could fill up the 90-minute soaking period.

The results are usually clear. Equimolar concentrations of glucose and sucrose produce identical weight changes in the cores (despite their different molecular masses of 180 and 342 g/mole) because each molecule only produces one solute particle. NaCl (despite its small molecular mass of 58 g/mole) produces twice the osmotic effect per mole because it ionizes into two *particles* per molecule. A sodium or chloride ion and a large sucrose molecule produce the same osmotic effect on the potato cores.

Instructor's Data Analysis and Distribution Requirement

Because every student only works with one solute but must have the results from all three solutes, there is an analysis and data distribution burden placed on the instructor. At Clemson, the data sheets at the end of the student exercise are handed into the lab instructor, and the course coordinator then compiles the results and distributes them on a Web site. This involves computing the average percent weight change in the whole course for each concentration of sucrose, glucose, and NaCl. Also, at Clemson the lab coordinator uses the chi-square median test to determine is the three solutes estimate different potato water potentials, and also whether the no-weight-change osmolality (number of solute particles/L), water concentration, and g/L solute differs between solutes. A Web site at <http://biology.clemson.edu/bpc/bp/Lab/110/osmosis.htm> shows the most recent results of this analysis.

Interpretation

The wet lab portion of this exercise is easy for students, but the interpretation phase is difficult. Estimating the water potential of the potato cores and comparing the estimate with literature values is straightforward, explained in the student outline, and does not cause the students any trouble. However, the students do have problems with the comparison of the osmosis theories. The simplest way for the instructor to approach this is to make the exercise a test of van't Hoff's Law by asking if the predictions of van't Hoff's Law are supported or not. Table 4 in the Student Outline organizes these results for the students. The van't Hoff predictions are:

a) The plot of core weight change vs. osmolality (Fig. 10) will produce *the same* line for all three solutes (because osmosis is determined by the osmolality, or number of solute particles/L);
b) The plot of core weight change vs. g solute/L (Fig. 9) will produce *different* lines for all three solutes;
c) The plot of core weight change vs. water concentration (Fig. 8) will also produce *different* lines for the three solutes;
d) The potato water potentials estimated by the three solutes will be *the same*;
e) The osmolality that produces no weight change in the potato cores will be *the same* for the three solutes;
f) The g solute/L that produces no weight change in the potato cores will be *different* for the three solutes;
g) The water concentration that produces no weight change in the potato cores will be *different* for the three solutes.

Again, the reasoning behind all these predictions is the van't Hoff's Law prediction what really matters for osmosis is solute particles/L.

Finally, at Clemson we ask the students to write a paper on the exercise. We cannot anticipate the requirements at different institutions, so the student outline gives the Clemson directions. Clemson has the students write a full scientific paper with literature review (assisted by a potato osmosis literature review Web site at the URL mentioned above). The paper must address both the water potential of potato cores and the success of the van't Hoff hypothesis at explaining the results. In the spirit of building student skills, this rather complex paper follows a simpler paper on reaction time.

Student Outline

Objectives

In this exercise, we will examine the effect of NaCl, glucose, and sucrose solutions of various concentrations on white potato tissue. Our objectives will be:

a) To use our data to estimate the water potential of white potato tissue, and to compare that estimate with literature values;
b) To determine whether the rate of osmotic water movement is more determined by the water concentration of the solution, by the mass of solute in the solution, or by the number of solute particles, irrespective of their size or characteristics.

Introduction

Osmosis and Water Potential. **Osmosis** is the movement of water through a **semipermeable** membrane (defined as a membrane that allows water but not solute molecules to pass through it). When a cell is placed in a solution that is osmotically more concentrated than its protoplasm (the outside solution is **hypertonic**), the cell loses water. Both animal cells and plant cells may **plasmolyze** (drastically shrink) if they are put in a very concentrated solution. If the cell is placed in a solution that is osmotically less concentrated than its protoplasm (the outside solution is **hypotonic**), the cell gains water. Animal cells usually burst if this cannot be controlled (e.g., by bailing water out with a contractile vacuole). Plant cells, with their thick cell walls, can resist the influx of water by building up **turgor pressure**. Eventually they come to equilibrium in a hypotonic solution because as much water is leaving due to turgor pressure as is coming in due to osmosis.

The fact that building turgor pressure can precisely balance osmotic inflow implies that the magnitude of these two forces can be expressed on the same scale. Turgor pressure and osmosis have been summarized into one concept called **water potential**. In the experiments in this laboratory, we can describe water potential with the following equation:

$$Y = Y_p + Y_s \qquad (1)$$

where Y is water potential, Y_p is pressure potential, and Y_s is osmotic potential. All of these are expressed in units of pressure. The pressure units used here are bars (0.987 atmospheres or 0.1 megapascals). Water always moves from a higher (more positive) water potential to a lower water potential. Looking at equation 1, we can see that even if a cell is very hypertonic to its surroundings and Y_s is a large negative number, overall water potential can be brought to zero if Y_p is a sufficiently large positive number. In other words, a high turgor pressure can balance a brisk osmotic inflow of water.

Any measure has to have a standard with which it is compared. For pressure potential, atmospheric pressure is defined as a pressure potential of zero. Therefore, any pressure less than atmospheric will give a negative pressure potential, and any pressure greater than atmospheric will give a positive pressure potential. Osmotic potential uses distilled water as a standard—the osmotic potential of distilled water is said to be zero. Any solutes in distilled water will make the osmotic potential negative. There is no such thing as positive *osmotic* potential because water cannot be purer than distilled water.

When we add the pressure potential standard (atmospheric pressure) and the osmotic potential standard (distilled water) together, we find a fact worth remembering: **The water potential of distilled water at atmospheric pressure is zero.**

Let's say our plant cell above is in distilled water at atmospheric pressure. The outside environment has a water potential of zero bars. The cell has solutes in it, making its osmotic potential negative, but it also has turgor pressure greater than atmospheric pressure, making its pressure potential positive. Provided the cell wall can stand it, the pressure potential will rise until it is equal and opposite in sign to the osmotic potential. At that point, the sum of the pressure potential and osmotic potential (the cell's water potential) will be zero, and the cell will be in equilibrium with its environment.

Estimation of the Water Potential of Potato Tissue. We will punch out some cores of potato tissue and weigh them. Then we will soak the cores in distilled water, and in sucrose, glucose, and NaCl solutions ranging from 0.1-0.5 molal. A 1 molal solution has 1 mole of solute per 1000 g of solvent. After 1.5 hours of soaking, we will weigh the potato cores again. We expect that the cores in the distilled water and the dilute solutions will gain weight because their water potential is less than that of the soaking solutions. We expect that the potato cores will lose weight to the concentrated solutions because this time the solutions have the lower water potential. In other words, we expect a pattern like this:

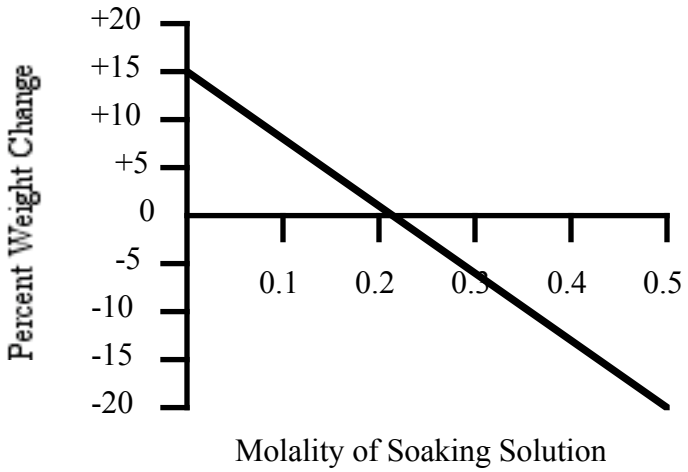

Figure 1. Potato cores should lose water to concentrated solutions and gain water from dilute solutions.

The point where the line crosses the x axis is the molality of the soaking solution that has the same water potential as our potato cores. This soak solution (0.22 molal in this example) has a water potential equal to that of the potato cores. The soak solutions are at atmospheric pressure, so their pressure potential is zero. Therefore, the solution water potential is caused by its osmotic potential only. We can compute a solution's osmotic potential (and therefore its water potential) from its concentration and temperature. This will give us the *water* potential of the soaking solution at equilibrium, and this will the same as the water potential of the potato cores. We will never know the *osmotic* potential of the cores because the cores probably have a nonzero pressure potential. We will compare this water potential estimate with literature values. Literature summarized at

http://biology.clemson.edu/bpc/bp/Lab/110/osmosis.htm

indicates that most estimates of white potato tuber water potential are around -5 to -10 bars if the potatoes are not stressed by drought.

What Controls Osmosis? Our second objective is to support or reject three alternative hypotheses for why osmosis occurs. This seems *like* a nineteenth-century question, but the answer is still in dispute today. A simple and appealing explanation for osmosis is the **concentration of water explanation**--water in pure water is simply more concentrated than water in solutions because the solute has to take up some room in the solution. According to this idea, water diffuses into a hypertonic solution because it is diffusing down its concentration gradient. In Figure 2 below, the circles represent water molecules, and the dotted line is a semipermeable membrane. If the water molecules are in random motion, more water molecules will be hit the membrane and pass through it from left to right.

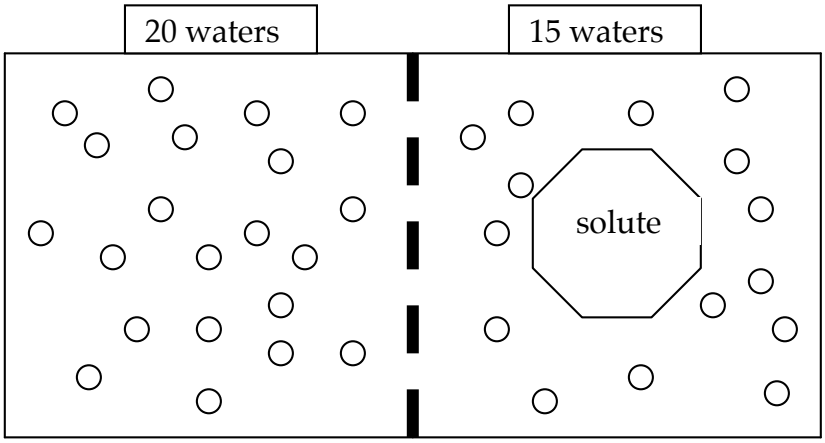

Figure 2. The "concentration of water" explanation for osmosis. The circles are water molecules and the dotted line is a semipermeable membrane.

If this is true, then the concentration of water should be able to predict the direction of osmosis when different solutes are used. For example, the *Handbook of Chemistry and Physics* tells us that a 0.2 molal sucrose solution has a water concentration of 958 g/L and a 0.2 molal NaCl solution has a much higher water concentration—995 g/L. The sucrose solution should gain water from the NaCl solution if the two were separated by a semipermeable membrane. The number of molecules of NaCl and sucrose shouldn't matter—only the water concentration should be important. The water concentrations used in this experiment are shown in Table 1. Note that higher molal concentrations of sucrose produce lower water concentrations than the same concentrations of NaCl because sucrose is a much larger molecule than NaCl and displaces more water.

Table 1. Water concentrations (g water/L of solution) in several solutions at 20° C (Wolf *et al.*, 1982, pages D227-D276).

Molality	**Solute**		
	NaCl	**Glucose**	**Sucrose**
0.0	998.2	998.2	998.2
0.1	996.5	987.3	981.2
0.2	994.6	976.2	958.5
0.3	992.5	966.5	940.9

0.4	990.6	956.1	923.4
0.5	988.7	946.2	907.2

Another osmosis theory that is often found in general biology books (e.g., Solomon, Berg and Martin, 2008, p. 117) is the **"bound** water**" explanation** (Figure 3). This theory says that any hydrophilic solute (like sucrose or NaCl) will bind up hydrating water and prevent it from moving freely. Therefore, the side of a semipermeable membrane with pure water has a higher "free" water concentration than the side with the solute molecules. In Figure 3, more free water molecules will hit the membrane on its left side, and there will be a net movement of free water from left to right.

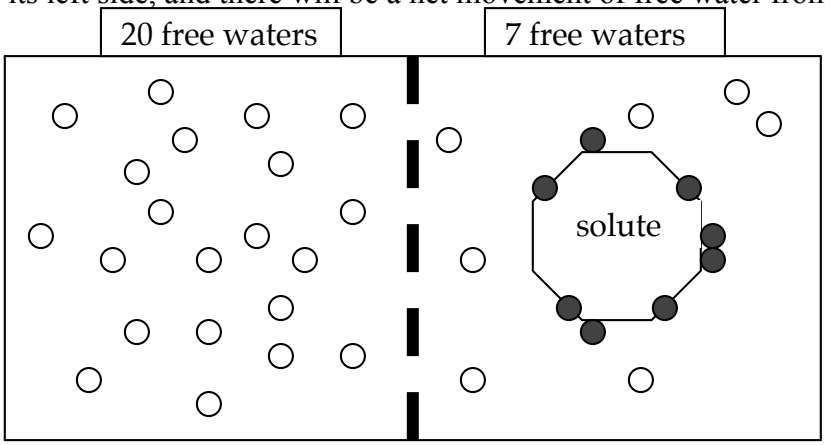

Figure 3. The "bound water" explanation for osmosis. The open circles are free water molecules, the darkened circles are water molecules bound to a hydrophilic solute, and the dotted line is a semipermeable membrane. In addition to displacing water molecules, solutes also bind them.

If this is true, we would expect that a greater mass of hydrophilic solute would bind more water. Whether a certain mass of solute is present in a few large molecules or in many small ones shouldn't matter. Also, if we were trying to predict osmosis, we would have to know how hydrophilic the solute is because we'd have to estimate how many water molecules each molecule of solute can bind.

The final explanation is not intuitively obvious but is supported by extensive data. This is the **"number of particles" explanation**. In the late 1800s, Jacobus H. van't Hoff estimated the osmotic potential of many kinds of solutions. He found that at a particular temperature and in relatively dilute solutions, the osmotic potential was proportional to the **concentration of solute particles**. The size or nature of the solute particles didn't matter. For example, a small sodium ion would have the same osmotic effect as a large sucrose molecule, and both of these would be equivalent to a very large starch molecule. This also means that ionizing substances like NaCl should have a greater osmotic effect than nonionizing substances like sucrose because when they ionize, they generate more particles. A measure of concentration called "**osmolality**" (the concentration of solute *particles* in moles/L) was invented to express this. A 1 molal solution of sucrose would have an osmolality of 1 osmol/L because each mole of sucrose produces one mole of solute particles. However, a 1 molal concentration of NaCl would have an osmolality of 2 osmol/L because each mole of NaCl ionizes

into *two* moles of particles. A 1 molal concentration of $CaCl_2$ would have an osmolality of 3 osmol/L because each mole of $CaCl_2$ ionizes into *three* moles of particles.

We will test the predictions of these theories with our data. We will determine whether water concentration, grams solute/L, or osmolality can best predict osmosis for these three different solutes. The way we will judge this is shown in Figure 4, below. Say we get the following results for osmolality in Fig. 4a:

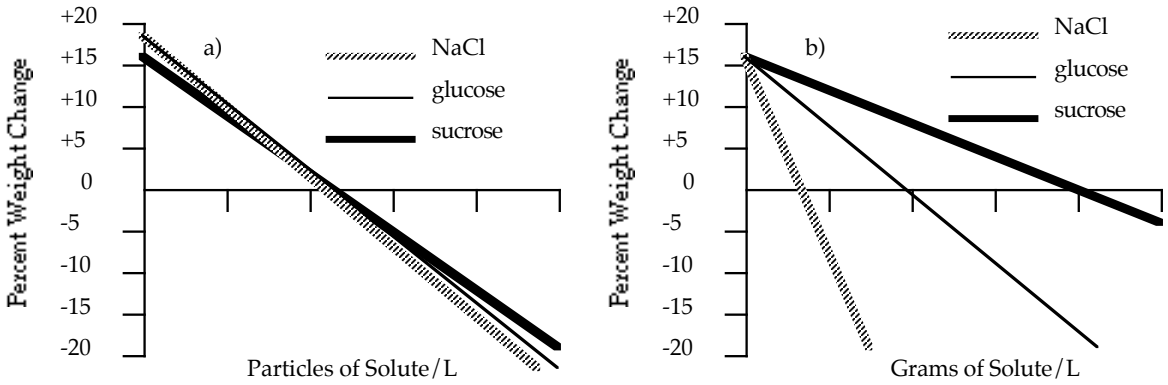

Figure 4. Hypothetical results: a) The number of particles/L (osmolality) has the same osmotic effect whether NaCl or glucose or sucrose is the solute, implying that number of particles is controlling osmosis. b) A gram of NaCl is far more osmotically active than a gram of glucose or of sucrose, implying that the mass of solute alone is *not* controlling osmosis.

In Figure 4a, both salt and the two kinds of sugar produce nearly the same effect on the potato cores, and they cross the x axis at a nearly identical value. Particles/L predicts osmosis consistently for three different solutes. This supports the idea that osmosis is controlled by particles/L. In Figure 4b, a gram of NaCl has a very different effect from a gram of sucrose or glucose. This casts doubt on the idea that it is the mass of solute that controls osmosis.

Procedure

1. Work in groups of four. Your lab instructor will tell you whether you will be working with NaCl, glucose, or sucrose. Two group members should start filling 6 beakers with 100 mL of each of the following 6 concentrations of your assigned solute: 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 molal. Label each beaker with its concentration.
2. This cutting step may be performed by your lab instructor. If not, the other two group members should obtain a white potato. Using a knife and the cutting board, cut a 3 cm thick segment of the potato:

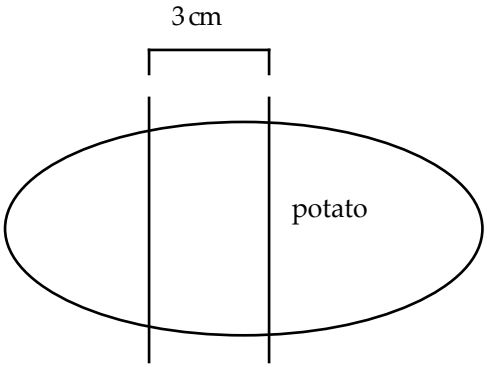

Figure 5. Cut a 3 cm segment out of the potato.

Cut 30 cores from this segment. Drive the corer into the cut ends of the segment. This allows you to avoid the skin:

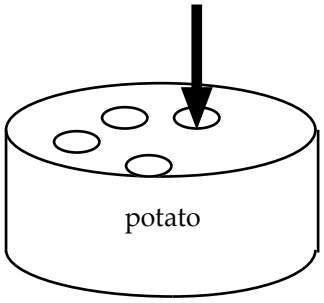

Figure 6. Drive the corer into the cut ends of the potato segment.

3. Push the cores out of the corer with a wooden dowel. Accumulate the cores in a covered Petri dish so they do not dry out.
4. When you have 30 cores, randomly select a group of 5 cores. Lightly roll each one in a paper towel to dry its surface, and then record an initial weight of all five. You do not need to record the weight of each core. Record this group weight in the "Initial Weight" column of Table 2, below:

Table 2. The initial and final weights of groups of five cores, plus their percentage changes in weight.

Molality	Initial Wt.	Final Wt	% Change
0			
0.1			
0.2			
0.3			
0.4			

0.5			

5. When you finish weighing a concentration, put the five cores in a covered Petri dish next to the beaker containing the solution. Finish recording the weights of five cores for all six concentrations, and *then* add the cores to their solutions *all at the same time*.
6. The cores must soak for about 1.5 hours. Swirl the beakers about every 10 minutes to keep shells of water from building up around the cores.
7. When your lab instructor tells you to harvest the cores, pull them out and **dry them exactly as you did before. Inconsistent drying is the most common error in this experiment.** Then weigh the cores and enter final weights for the cores from each solution in Table 2.
8. Compute the percent change in weight by the formula:

   % Change = 100*(Final Weight – Initial Weight)/Initial Weight (2)

   Some of your percent changes will probably be negative.
9. Find the "Potato Osmosis Data Sheet" at the end of this exercise. First, fill in the percent changes in the potato core mass in Table 3. Ignore the other columns for now. Use a piece of graph paper to plot the percent changes against molality. Your graph should run from 0-0.5 molal. Draw a best-fit line through the points and estimate the molality at which the line crosses the x axis. As you draw this line, emphasize the points closest the x axis. In other words, don't let your line be determined by an outlier point far from the x axis. Write this x-intercept on your "Potato Osmosis Data Sheet."
10. Use the van't Hoff equation and the molality at which the line crosses the x axis to estimate the osmotic potential of the solution at that molality. The equation to use is

    $$Y_s = -i*C*0.0831*T \quad (3)$$

    where Y_s is osmotic potential of the solution in bars, i is 1.0 for sucrose and glucose and 2.0 for NaCl, C is the molality where the line on your graph crosses the x axis, and T is the Kelvin temperature in the lab (the Celsius temperature plus 273°). If you're not told the Kelvin temperature, use 293° K (20° C). Y_s should come out between -1 and -15 bars. Write this water potential on your Potato Osmosis Data Sheet.
11. Now go back to Table 3 on the data sheet and fill in the water concentrations for each of your molalities. This will depend on what solute you used (see Table 1). Below the table, estimate the water concentration at the molality at which your line crosses the x axis.
12. Then we must include the g/L of solute data on this data page.
    a) *If you used sucrose*, a mole = 342.3 g. Multiply the six molalities on the data sheet by 342.3 g and put that in the column entitled "G Solute/L." Also, multiply the molality at which the line crosses the x axis by 342.3 and enter that in the "no-change g/L" line on the data sheet.
    b) *If you used glucose*, a mole = 180.2 g. Multiply the six molalities on the data sheet by 180.2 g and put that in the column entitled "G Solute/L." Also, multiply the molality at which the line crosses the x axis by 180.2 and enter that in the "no-change g/L" line on the data sheet.
    c) *If you used NaCl*, a mole = 58.4 g. Multiply all molalities on the data sheet by 58.4 and put them in the column entitled "G Solute/L." Also, multiply the molality at which the line crosses the x axis by 58.4 and enter that in the "no change g/L" line on the data sheet.

13. Finally, enter the moles of solute particles/L corresponding to each weight change. For glucose and sucrose, this will be equal to the molality. For NaCl, it will be the molality x 2. Below the table, estimate the moles of solute particles/L at which your line crosses the x axis.
14. Give your data sheet to your lab instructor. Your lab instructor might compile these data into a coursewide database that is published on a Web site. You also might only use the data from your lab section. You may be required to write a paper on your conclusions.
15. You will also need to keep your copy of Table 2 (with your group's data) because you will have to add a line for your group's data to your graph.

Writing Your Report (Clemson procedure described here)

You will write a report on this experiment. Refer to the Web site at

http://biology.clemson.edu/bpc/bp/Lab/110/osmosis.htm

This Web site will contain:

a) A literature review on the causes of osmosis and potato water potential;
b) Course-wide average changes produced by different molal concentrations of NaCl, glucose, and sucrose;
c) The average water concentration, g/L concentration, and moles of particles/L of NaCl, glucose, and sucrose that produce no weight change in the potato cores;
d) Chi-square median tests of the hypotheses that the water concentration that produced no weight change, the g/L of solute that produced no weight change, and the moles of particles/L that produced no weight change are the same for all three solutes.
e) The average estimated water potential of white potato tissue;

Using this information, you should write a report that contains the following:

Title and Abstract. Follow directions in the Writing Guide.

Introduction. Should explain what water potential is, and then review the literature both about a) the expected water potential of white potatoes, and b) whether osmolality (moles of solute particles/L), water concentration, or g/L of solute should be the more consistent predictor of osmosis. This literature review will allow you to develop your explanatory hypothesis. The null hypotheses are that the zero-weight-change osmolality, water concentration, and grams solute/L will be the same for NaCl, glucose, and sucrose.

Methods and Materials. Cite this handout for basic methods, and note any departures from the handout.

Results. You should have a Results text section that summarizes the major trends (see the *Writing Guide*). Then you should use data on the Web site in order to make three Excel graphs (Figures 1-3) in your report:

a) percent weight change (y axis) vs. water concentration of soak solution (x axis);
b) percent weight change (y axis) vs. grams/L of solute in soak solution (x axis);
c) percent weight change (y axis) vs. moles of solute particles/L in soak solution (x axis).

All of these x axis variables can be derived from the molality data above. Remember, moles of solute particles/L = molality for sucrose and glucose (because sugars do not ionize), and moles of particles/L = 2x molality for NaCl (because NaCl ionizes into two particles).

Each graph should have a line for the course-wide NaCl, glucose, and sucrose average weight changes above plus a fourth line for your lab group's data (either NaCl, glucose, or sucrose). The graphs should show points with an Excel "trend line" through the cloud of points. Each of the four trend lines should be distinctive (e.g., points with different shapes and dotted vs. solid lines). Use the techniques you practiced in the data presentation exercise the second week of lab.

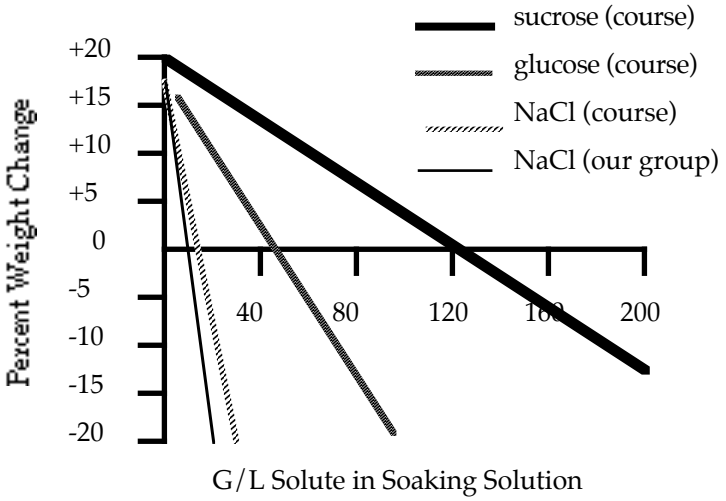

Figure 7. Illustration of student graph showing average course-wide results for NaCl, glucose, and sucrose, and your group's results for whichever solute you used. Your graph should show points in addition to the lines.

Then you will need a table. You will need to design this table, because the data you'll use come from four different tables on the Web site. This single table should summarize the chi-square results for the following four questions:

a) Are the potato water potentials estimated from the NaCl, glucose, and sucrose data the same?
b) Do potato cores have zero weight change in the same water concentrations when using NaCl, glucose, and sucrose as solute?
c) Do potato cores have zero weight change in the same grams/L of NaCl, glucose, and sucrose?
d) Do potato cores have zero weight change in the same osmolalities of NaCl, glucose, and sucrose?

You will need to summarize these data in a table that shows the means of each no-weight-change variable for NaCl, glucose, and sucrose, the chi-square for the test of equality of the three solutes for each variable, and the P-value for each variable's test. Note that you do not have to include the above- and below-median data in the tables below. It might be a good idea to devote one line of your table to each variable.

Discussion. The Discussion should have all the components mentioned in the Writing Guide (for example, the criticism of errors, ideas for future experiments, etc.) But primarily, it should address two questions:

First, how do the course-wide water potential estimates for white potatoes compare with values found in the literature?

Second, how successful is van't Hoff's Law as a predictor of osmosis? Van't Hoff's Law makes the predictions in Table 4, below. How many of them were borne out by the data? In all cases, "the same" and "different" below refer to differences between NaCl, sucrose, and glucose. Fill in the last column in Table 4 with "yes" or "no" when you get the course results, and use the comparison between prediction and results to organize your Discussion. It could be possible that all of these predictions, none of these predictions, or only some of these predictions were true. In other words, van't Hoff's Law might have a perfect "scorecard," might be partially successful, or might be wrong about everything.

Table 4. Predictions of van't Hoff's Law compared with the course-wide results. Comparisons are between potato cores in NaCl, sucrose, and glucose soaking solutions. VHL = van't Hoff's Law.

Variable	VHL Prediction	Prediction True?
Plot of weight change vs. water concentration	Solutes different	
Plot of weight change vs. g solute/L	Solutes different	
Plot of weight change vs. solute osmolality	Solutes the same	
Estimated water potential of cores	Solutes the same	
No-weight-change water concentration	Solutes different	
No-weight-change g solute/L	Solutes different	
No-weight-change solute osmolality	Solutes the same	

Literature Cited. See the Writing Guide. The citation of this handout is

Kosinski, R. 2008. Osmosis and the water potential of potato tissue. Class handout, Clemson University.

Potato Osmosis Data Sheet

Fill in this data sheet as directed and give it to your instructor.

Lab section meeting time _____

Last names of group members _____

_____

Solute _____ (NaCl, glucose, or sucrose)

Table 3. Percent changes in potato core weight.

Molality	Water (g/L)	G Solute/L	Moles Solute Particles/L	% Change
0.0	998.2	0	0	
0.1				
0.2				
0.3				
0.4				
0.5				

Estimated molality at which no weight change occurred = _____ molal

Water potential corresponding to this molality = _____ bars
Don't forget to include i = 2.0 in the equation if you're using NaCl.

Estimated water concentration at which no weight change occurred = _____ g/L

Estimated g of solute/L at which no weight change occurred = _____ g/L

Estimated moles of solute particles/L at which no weight change occurred = _____ moles of particles/L

Materials

Needed materials for a class of 24:
3-5 pounds of freshly purchased white potatoes. Large baking potatoes would be ideal.
36 beakers (250 mL) with volume gradations on their sides
36 petri dishes with lids
Solutions (for multiple sections, place each solution in a small carboy):

- 1000 mL of distilled water
- 300 mL of 0.1 molal NaCl, 0.1 molal glucose, and 0.1 molal sucrose
- 300 mL of 0.2 molal NaCl, 0.2 molal glucose, and 0.2 molal sucrose
- 300 mL of 0.3 molal NaCl, 0.3 molal glucose, and 0.3 molal sucrose
- 300 mL of 0.4 molal NaCl, 0.4 molal glucose, and 0.4 molal sucrose
- 300 mL of 0.5 molal NaCl, 0.5 molal glucose, and 0.5 molal sucrose

6 cutting boards and 6 knives for cutting the potatoes
6 balances that can weigh to the nearest 0.1 g
6 weigh boats
12 potato corers (metal cork corers with an internal diameter of approximately 0.5 cm)
12 dowels that will fit into the corers for pushing the cut cores out
6 sheets of graph paper
6 rulers with cm markings
A Celsius thermometer
A good supply of paper towels

Notes for the Instructor

At Clemson, we have used this laboratory twice in a large introductory biology course for majors (270 students in 2007 and 310 students in 2008). Results were very good. I will discuss the "wet lab" part, the data analysis, and the writing of the paper.

"Wet Lab" Component

At Clemson, we this include this exercise in an osmosis-diffusion lab with exercises on osmometers, dialysis bags, diffusion of ammonia in flask, diffusion of colored chemicals in agar, and even an exercise on cell fractionation that was displaced from the cell and microscopy lab. The potato cores must soak as long as possible, but need at least 90 minutes to reach a relatively stable weight. Therefore, when the students arrive, we immediately start half of them punching out potato cores while the other half gets beakers with solutions ready.

A third of the class will use NaCl as a solute, a third of the class will use glucose, and a third will use sucrose. We make up small carboys of each solution that will last the whole week rather than expecting the students to do the dilutions. A lab section of 24 would would have six groups of 4 and would need 600 mL of distilled water (because every group uses 100 mL of distilled water), but only 200 mL of each of 15 solutions: 0.1-0.5 molal NaCl, 0.1-0.5 molal glucose, and 0.1-0.5 molal sucrose. If simplification is desired, the impact is almost the same with NaCl and sucrose only. In this case, a section with six groups will use 300 mL of each of 10 solutions (0.1-0.5 molal NaCl and 0.1-0.5 molal sucrose). Keep in mind that these are molal solutions. To make 1 L of a 0.1 molal solution, 0.1 moles is simply added to a full liter of distilled water. It is not necessary to dissolve the

solute and then bring the solution up to volume. We use molal solutions rather than molar solutions because the concentrations in van't Hoff's equation (Eq. 3) are understood to be molal.

As the student outline explains, groups of five potato cores are placed in a covered Petri dish next to their beakers while the cores are being punched out. This way they can all be added to their beakers at the same time. Once the experiment has begun, the students can do other exercises for 90 minutes.

The most common error in the wet lab component is inconsistent drying of the potato cores. We tell the students to place a small group of potato cores on a paper towel, fold the towel over them, and roll the group of cores gently back and forth. This is done before weighing both when the potato cores have just been cut out and when they come out of the solution. The students must be warned to do this exactly the same way both at the beginning of the experiment and at its end. Also, while the potato cores are soaking, they must be swirled about every ten minutes.

At the end of the experiment, spot check to make sure the students are doing the percent weight change correctly (Eq. 2, p. 10). The computation of potato water potential is also troublesome (Eq. 3, p. 11). The zero-weight-change molality on their graph has the same water potential as the potato cores. Because the solution is at atmospheric pressure, it has zero pressure potential. Therefore, its water potential is equal to its osmotic potential. Osmotic potential can be computed from concentration and temperature. Eq. 3 computes it in bars; divide this result by 10 to find the water potential in megapascals.

Filling out the middle columns of the data sheet table (p. 15) may also cause difficulty. The water concentrations come from Table 1 (p. 7). Note that each concentration of each solute has a different water concentration. The student directions tell the students how to compute g solute/L and moles of solute particles/L.

Analysis of Results—Potato Water Potential

The first objective of the analysis is to compute the water potential of potato cores (using Eq. 3) and compare the values with the literature. Appendix A contains a literature review on potato core water potential. No matter what solute is used, the water potential should come out to be approximately -5 to -10 bars. At Clemson in 2008, the average of 27 water potentials determined using NaCl was -7.67 bars; the average of 27 determinations using sucrose was -6.99 bars; the average of 21 determinations using glucose was -6.84 bars. These estimates agree well with the literature.

At Clemson, we also perform a chi-square median test to test whether the NaCl, glucose, and sucrose estimates are the same. The principle of a median test is that if all the water potential estimates (irrespective of solute) are ordered from smallest to largest and there is no difference between solutes, the NaCl estimates should be half below the group median and half above it. The same should be true of the glucose and sucrose estimates. In other words, there should be no tendency for one solute's estimates to be below the group median and for another's to be above the group median. In 2008, the Clemson results were as follows:

Table 5. Number of student potato water potential determinations above and below the median of all determinations in 2008. The chi-square value here was 5.06 ($p = 0.08$). Therefore, the three solutes did not produce significantly different water potential estimates.

	NaCl	Sucrose	Glucose
Above Median	8.5	16	12
Below Median	18.5	11	9

A very handy spreadsheet for using this median test can be downloaded from
http://biology.clemson.edu/bpc/bp/Lab/110/mediantest.htm

Analysis of Results—Theories of Osmosis

The second objective of the exercise is to critically compare three explanations for osmosis—that it is caused by different concentrations of water, by different concentrations of *free* water, or that it depends on the concentration of solute *particles*, irrespective of their size. The student outline summarizes these theories; a more comprehensive literature review of the theories appears in the second half of Appendix A.

Ideally, the students should be able to take each of the theories, make predictions from them, and then determine if the predictions were realized. However, in 2008 we found that the students needed this task simplified. Our approach was to confine the analysis to van't Hoff's Law (osmosis is controlled by the number of solute particles/L) and the specific predictions it would make. The predictions are listed on pp. 3-4 in the Introduction for the Instructor and in Table 4 in the Student Outline. After the students got the course results, they would fill in the last column of this table and use that table to organize their discussion. Clemson's 2008 graphical results appear below, and are summarized in Table 5 as well.

The basic idea behind the analysis of the graphs is that if a variable (such as water concentration) is controlling osmosis, then a certain water concentration should have the same effect on the potato cores regardless of what solute is being used. For example, the 2008 weight change of potato cores is plotted against water concentrations for NaCl, glucose, and sucrose in Fig. 8 below.

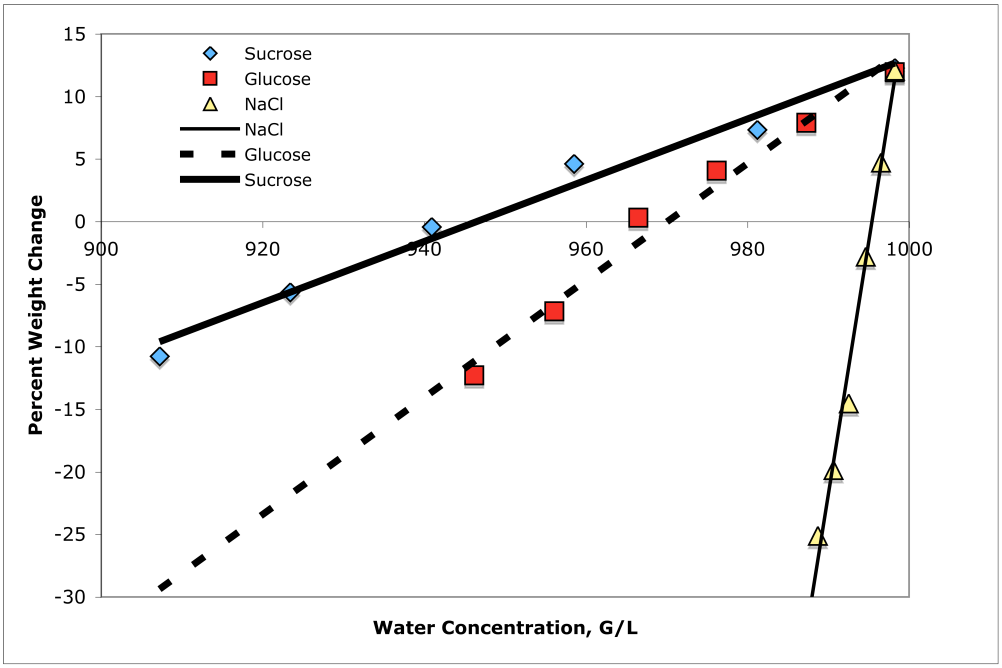

Figure 8. Percent weight change of potato cores after 90 minutes as a function of concentration of water in the soaking solution.

Consider a water concentration of 980 g/L. If water concentration is controlling osmosis, why does a potato core take up water from a 980 g/L solution when sucrose and glucose are the solutes, but suffer serious water loss when NaCl is the solute? The different solutes may affect water concentration in different ways, but the same water concentration should have the same effect on the potato cores. Since this is clearly not true, water concentration can be rejected as the major factor that is controlling osmosis.

It is difficult to estimate the concentration of *free* water, so we used the mass of dissolved solute as a proxy for it. The assumption is that larger masses of solute will bind more water. One could argue that the water-binding capacity *per gram* of NaCl and glucose are different, but it is harder to argue that a gram of glucose and a gram of sucrose would bind markedly different amounts of water. The weight change of the cores plotted against the mass of dissolved solute in our 2008 experiment appears below:

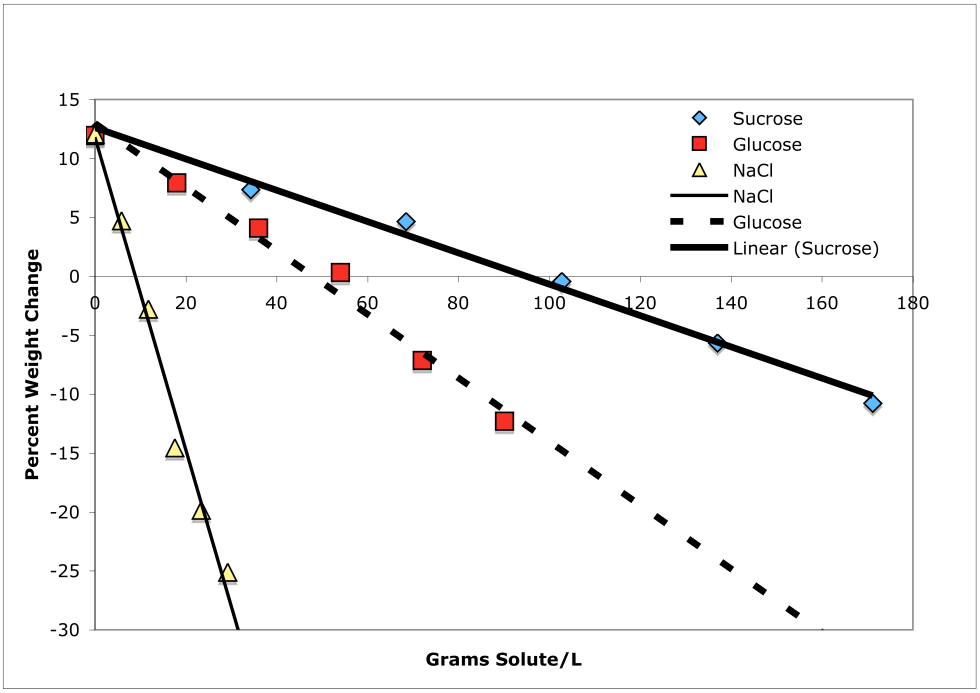

Figure 9. Percent weight change of potato cores after 90 minutes as a function of g solute/L in the soaking solution.

The argument is the same. Consider 80 g of solute/L. If g of solute/L controls osmosis, why should a potato core take up water from a solution containing 80 g/L of sucrose, but lose water to a solution containing 80 g/L of glucose? Why should potato cores be able to absorb water from a solution containing 30 g glucose and 30 g of sucrose/L, and rapidly lose water to a solution containing 30 g/L of NaCl? Grams of solute/L does not control osmosis, and this argues that bound water does not control osmosis either.

The final hypothesis is van't Hoff's Law. This states that osmosis is controlled by the moles of particles/L, irrespective of their size or properties. Moles of solute particles/L is called osmolality. The Clemson results were:

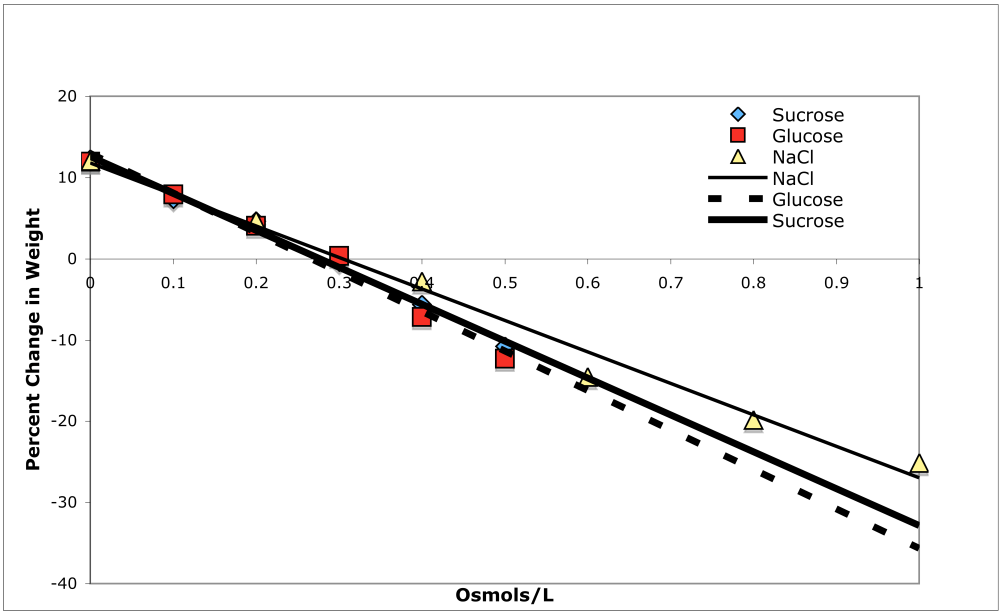

Figure 10. Weight change of potato cores after 90 minutes as a function of the osmolality of the soak solution.

After Figures 8 and 9 in which the three regression lines diverged sharply, the consistency of the potato response here is striking. No matter what the solute is, potato cores can gain water from a solution with 0.1 moles of solute particles/L, will be at equilibrium in a solution with 0.3 moles of solute particles/L, and will lose water to a solution containing 0.5 moles of solute particles/L. The results are consistent with van't Hoff's theory that the number of solute particles controls osmosis. As an example of this theory in action, it is a well-known piece of laboratory lore that either a 0.85% NaCl solution or a 5% glucose solution is isotonic with human red blood cells. A 0.85% NaCl solution and a 5% glucose solution have very different masses of dissolved material (8.5 g/L vs. 50 g/L), and very different water concentrations (995 g water/L vs. 968 g water/L). However, they have almost the same osmolality (0.291 osmol/L for NaCl vs. 0.278 osmol/L for glucose).

In addition to this casual examination of regression lines, we also did a chi-square median test comparison across solutes of water concentrations, grams of solute/L and solute osmolality that caused zero weight change in potato cores. The results in 2008 (plus the results for the lines above) are shown in Table 5 along with the predictions of van't Hoff's Law. Table 5 is the filled-in version of Table 4 for 2008.

Table 5. Predictions of van't Hoff's Law compared with the 2008 Clemson results. Comparisons are between potato cores in NaCl, sucrose, and glucose soaking solutions. VHL = van't Hoff's Law.

Variable	VHL Prediction	Prediction True?
Plot of weight change vs. water concentration	Solutes different	yes
Plot of weight change vs. g solute/L	Solutes different	yes
Plot of weight change vs. solute osmolality	Solutes the same	yes
Estimated water potential of cores	Solutes the same	yes
No-weight-change water concentration	Solutes different	yes
No-weight-change g solute/L	Solutes different	yes
No-weight-change solute osmolality	Solutes the same	no

Van't Hoff's Law made 6 out of 7 predictions successfully in 2008, only being wrong in the case of osmolality. While no-weight-change osmolality should have been the same for all solutes, we have always observed a tendency for NaCl to be slightly "weaker" per particle than glucose and sucrose, and this year there was a significant difference. It may be that van't Hoff's Law accounts for a high percentage of the weight change, but some other factor (perhaps bound water) also has a small effect.

The Paper

At Clemson, the students write an extended lab report in the format of a scientific paper on the results. In the student outline, we simply gave the Clemson directions because paper preferences of instructors at other institutions may be very different. To give some background for our paper directions, at Clemson we have a *Writing Guide* that gives the students the characteristics we desire in the Abstract, the Introduction, etc. The *Writing Guide* also assigns point values to each of these sections. We introduce the chi-square median test early and use it repeatedly through our two-semester sequence, but it is amazing how the students resist understanding even simple statistics. The lecture course introduces water potential and does many problems with it, so that part of the exercise is not new to the students. Finally, we do a "Data Presentation" exercise in the second laboratory in which the students learn how to do tables in Word 2007 and graphs of several types in Excel 2007.

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Appendix A
Clemson's Osmosis Literature Review Web Site
(October 2008)

The Water Potential of White Potatoes

White potatoes are important food crops in the cooler regions of the world. The tuber, the edible part of the white potato, is a very short and thick, starchy stem, with the "eyes" being the buds on the stem (Burton, 1989, Chapter 2). White potatoes have firm tissue and convenient size, so they are favorite subjects for the teaching laboratory determination of the water potential of plant tissue.

The "change in weight" method we use in our lab was published in 1935 by Meyer and Anderson as a modification of a method published by Ursprung (1923). Meyer and Wallace (1941) employed this method and found that white potato tuber water potential was between -7.7 and -8.3 bars, with the variance due to the length of time the sample was tested. Ashby and Wolf (1947) later confirmed these estimates. Epstein and Grant (1973) used gravimetric methods using sucrose solutions to test two varieties of white potatoes and found water potentials ranging from -2 to -8 bars. Ehlenfeldt (1992) used a technique very similar to ours (except that he used sorbitol as a solute rather than sucrose) and found that the solution with the same water potential as white potatoes ranged from 0.24 to 0.31 M, corresponding to about -7 bars. Instead of using a wide range of solutions, as we did, he focused on the point where the line crosses the x axis by using solutions that ranged from only 0.20 to 0.35 M. Using an entirely different technique, Gandar and Tanner (1976) used a pressure chamber to determine water potential in potato tuber cores. Here pressure was applied to the test specimen until sap just began to wet of the xylem traces at the ends of the cut sample. They calculated water potential of white potato tubers to be equal to -6 to -7 bars. Again, extreme variance (up to 5 bars) resulted, due to the moisture of the sample. Ros Barcelo and Calderon (1994) cited a value of -6.7 bars for white potato tuber tissue.

Despite the strong agreement in potato water potentials determined by these various methods, lengthy discussion has centered around the potential sources of error associated with each method. Meyer and Wallace (1941) suggest that weight change may not be due to the osmotic movement of water but rather due to the gain or loss of solutes to or from the soak medium. Bland and Tanner (1985) did a critical comparison of three methods of determining water potential and found that while two methods might agree in a certain range of water potentials, in another range they might diverge widely. Boyer (1969) reviewed numerous methods of water potential determination.

Growth conditions (especially soil water) cause significant differences in tuber water potential. As one would expect, drier soils produced drier white potatoes with more negative water potentials. For example, Burton (1944) found that the water content of some white potatoes in England increased from 73% to 77% as the rain increased from 30 mm/month to 70 mm/month. Win *et al.* (1991) found that white potato tubers in dry soil in New Jersey increased from a water potential of -4 bars before a rainstorm to -0.8 bars after the storm. Bland and Tanner (1985) measured the water potential of white potatoes and found a very wide range, from -0.1 to -0.9 MPa (-1 to -9 bars), although some stored white potatoes went as low as -15 bars. Bland and Tanner (1986) traced the drying of stored white potatoes, and found that their water potentials declined from -3 bars to -5 or -6 bars over the first 5 to 7 weeks of storage. After 25 weeks of storage, they had gotten down to -7 bars. We have no idea how long our potatoes were stored. Also, different parts of a tuber might have very different water potentials. Meissner (1997) found that beet storage organ

tissue had a water potential 5.6 bars lower than the tissue close to the water-conducting vessels (the xylem). Shibairo et al. (2002) found that weight loss in stored carrots was usually associated with changes in water potential or osmotic potential under common storage conditions.

White potatoes can acclimate to very dry conditions. Leone et al. (1996) found that the growth of white potato cells in tissue culture would be completely inhibited by sudden transfer to a solution with an osmotic potential of -23 bars, but the cells could continue to grow in this solution if they were gradually acclimated to it. Part of the acclimation was changing the fatty acid composition of the potato cell membranes, mediated by activation of different genes than in the unstressed cells (Leone et al., 1996 and 1994). Liu et al. (2006) found that white potato leaf water potentials declined from -5.3 bars to - 8.5 bars during an experiment testing the effect of water stress.

The same general conclusions also apply to sweet potatoes, although all the sweet potato water potentials tend to be lower. Sung (1985) found that sweet potato leaves became wilted when the leaf water potential dropped to about -16 bars. Ghuman and Lal (1983) found that sweet potato leaves in Nigeria had an average water potential of -9.6 bars. Despite the tough appearance of the tuber, sweet potato is sensitive to water stress, and the tubers increase their dry matter as water stress gets worse (Ekanayake and Collins, 2004).

Theories on the Factors Controlling Osmosis

Osmosis has been noticed by biologists since the middle 1700s, and by the 1870s, careful quantitative observations were being made of it (Baumgarten and Feher, 1998). However, while we can predict it exactly, the cause of osmosis is still in dispute (Baumgarten and Feher, 1998; Weiss, 1996, p. 216).

A simple and appealing explanation for osmosis is the concentration of water explanation--water in pure water is simply more concentrated than water in solutions because the solute has to take up some room in the solution. According to this idea, water diffuses into a hypertonic solution because it is diffusing down its concentration gradient. As Weiss (1996) points out, this predicts water movement in the right direction, but not of the right magnitude. Water movement in osmosis is faster than diffusion, and seems to be more like mass water movement caused by a pressure difference (Weiss, 1996, p. 218). Also, as Salisbury and Ross (1992, p. 39) point out, adding solutes to a solution decreases the concentration of water in most cases, but in some cases solutions have a higher concentration of water . The *Handbook of Chemistry and Physics* has a large section on solutions of common solutes, and it discloses that a 0.2 M solution of NaCl has a markedly higher water concentration (995 g/L) than a 0.2 M solution of sucrose (958 g/L) (Wolf et al., 1982, pp. D261 and D270). Yet our experiments will disclose that a potato cores loses water to a 0.2 M solution of NaCl but it gains water from a 0.2 M solution of sucrose. Also, if we compare 0.2 M solutions of sucrose and glucose, 0.2 M glucose has a higher water concentration than 0.2 M sucrose (976 g/L vs. 958 g/L) (Wolf et al, 1982, p. D239) . This makes sense because the smaller glucose molecules take up less room in the solution. However, these two 0.2 molal solutions have exactly the same osmotic potential.

A slightly more complex theory that is often found in general biology books is the “bound water” explanation. This says that any hydrophilic solute (like sucrose or NaCl) will bind up hydrating water and prevent it from moving freely. Therefore, the side of a semipermeable membrane with pure water has a higher “free” water concentration than the side with the solute molecules. Although it is popular in introductory texts, this theory is not even mentioned in several

reviews (Baumgarten and Feher, 1998; Weiss, 1996, pp. 216-222). If the bound water explanation were true, we would expect that a greater mass of hydrophilic solute would bind more water. Whether a certain mass of solute is present in a few large molecules or in many small ones shouldn't matter. Also, when predicting osmosis, we would have to carefully consider how hydrophilic the solute is (that is, how many water molecules it binds per molecule). In fact, the number of molecules present has a dominant effect on osmosis, and we can predict osmosis without considering how hydrophilic the solute molecules are.

Another explanation is van't Hoff's Law, or the "number of particles" explanation. Jacobus H. van't Hoff (1887) gathered much data on the osmotic potential of many kinds of solutions. As long as the solution was relatively dilute and the temperature was constant, he found that the osmotic potential was proportional to the concentration of solute particles. The size or nature of the solute particles didn't matter. So, for example, a small sodium ion would have the same osmotic effect as a large sucrose molecule, and both of these would be equivalent to a very large starch molecule (Baumgarten and Feher, 1998). This also means that ionizing substances like NaCl should have a greater osmotic effect than non-ionizing substances like sucrose because when they ionize, they generate more particles.

Van't Hoff's Law is an empirical relationship, not a physical description of why osmosis occurs. Why the number of particles should matter remains as unclear in 2008 as it was in 1887. Van't Hoff himself was only interested in predicting osmosis, and he expressed frustration with those who wanted him to explain why his law worked (van't Hoff, 1892; quoted in Weiss, 1996, p. 185).

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