1. Abstract

This is for faculty, not students.

a. Biological Question

How does the volume of a cell, as measured by weight, change when placed in environments of varying tonicity?

b. Statistical Content

- Organizing and summarizing data (cell weights vs. % change in cell weight)
- Descriptive statistics (median, mean ± SD/SEM)
- Inferential statistics (paired and unpaired T tests, ANOVA)
- Graphical representations of data

c. What students do

- Design hypotheses
- Make predictions
- Construct dialysis tubing cells
- Use a balance
- Organize, analyze and interpret data

d. Skills (those developed and practiced in the exercise, including things like writing, computer skills, etc.)

- Experimental design (formulate hypothesis, make predictions, identify controls, relate findings back to predictions and hypothesis)
- Practice compiling and organizing data sets
- Computer skills
- Use an analytical balance
- Learn to use data analysis programs (Excel, SPSS, SAS, etc.)
- Determine the best way to analyze the raw data (% change, descriptive statistics)
- Create various graphical representations of data
- Interpret data (graphical representations and the results of inferential statistics)

e. Student-active approaches (used in the exercise)

- Students design hypotheses and make predictions
- Students collect data
- Students organize, represent, analyze, and interpret their data
- Students compare graphical representations with each other and perform a peer review (constructive feedback)

●Students brainstorm about the appropriate components of good graph (these will be used to compile a rubric for assessment of future graphs)
●Students present their data analysis to the class for guided discussion

f. Assessable outcomes

Depends on the level and experience of the students.
After completing this module students will be able to:

1. Design an experiment (formulate a testable hypothesis, make predictions, incorporate experimental controls, design experimental groups, randomization)
2. Organize a data set
3. Perform descriptive statistics (mean, median, SD, SEM, each as appropriate)
4. Create appropriate graphical representations of their data (tables vs. scatter plots vs. bar graphs vs. line graphs)
5. Explain the advantages/disadvantages of different graphical representations for this data set
6.
7. Draw conclusions from their data and use the appropriate scope of inference

inferential statistics
graphical representations (
correlation vs. causation
scope of inference when drawing conclusions

2. Background
This section is written for faculty who can modify the background material as appropriate for their students.

Exploring the physical process of osmosis.

In order to understand how dissolved substances in the internal and external aqueous environments of cells influence their structure and function, we need to develop some vocabulary to describe the absolute and relative composition of these two solutions. Cells contain an aqueous internal environment, the cytosol, in which many substances are dissolved. The immediate external environment of living cells is also aqueous, but can have a very different composition. The concentration of dissolved substances (solutes) in a liter of liquid (solvent) is known as the **osmolarity** of the solution with units of osmoles solute/ liter solvent. Another measure of the amount of solute dissolved in a solution is **osmolality** which is osmoles solute/ kilogram of solvent. This measure is similar to osmolarity in

general concept, but is more precise in that it takes away any temperature-dependent changes in the volume of solvent.

When a cell is placed into a solution, it will lose or gain water dependent on a quantity called the **water potential (Ψ).** Water potential is the potential that water has to move across a semi-permeable membrane, such as that of a cell in solution, based on *difference across the membrane*. The two primary quantities that determine the water potential across a membrane are pressure potential and solute potential on each side of the membrane. Water potential is calculated as the total sum of pressure potential (Ψ_p) and solute potential (Ψ_π).

(*Equation*1)

Pressure potential is the potential determined by the physical pressure of enclosed fluid inside a cell pushing out and fluid outside a cell pushing in on the enclosed solution (Figure 1). An important function of this pressure can be observed in plants where the cell must be turgid in order to support the plant and the cell wall is structurally rigid. As water enters the cell, pressure potential goes up, since there is more force exerted by the greater volume onto the cell wall. When a plant cell is filled with a greater quantity of water, the pressure potential is positive, since pressure inside the cell is greater than that outside of it. If water is given a path across the cell wall, it would tend to move out down the water pressure gradient in this example. The plasma membrane in mammalian cells, for example, does not have a cell wall and thus these cells are more vulnerable to damage or changes in function with changes in cell volume.

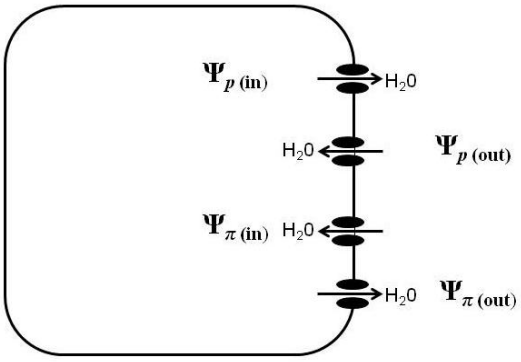


Figure 1. Schematic illustrating water and solute pressures inside and outside of cells and the direction of water movement they favor.

Solute potential is the potential for water to move into or out of the cell as based on dissolved *impermeable* solutes found in the cytosol and extracellular fluid. The differences in the composition of these two fluids across the membrane impact the direction of water movement (Figure 1). The solute potential of a solution is determined not only by the molarity of solute (M), but also by the number of ions into which it dissociates (i), and

the temperature in Kelvin (T) at which the measurements are being taken. It also incorporates the ideal gas constant R=8.314 joules per degree Kelvin per mole.

(*Equation* 2)

Tonicity refers to the relative abundance of *impermeable* solutes between two solutions separated by a barrier that underlie Ψ_π. The barrier for cells is the semi-permeable plasma membrane and the two aqueous solutions are the intracellular and extracellular solutions. The external solution is said to be **isotonic** with respect to the intracellular solution of the cell when $\Psi_{\pi(cell)} = \Psi_{\pi(extracellular)}$. In contrast, and remembering the negative sign on Ψ_π , the extracellular solution is said to be **hypotonic** with respect to the intracellular solution of the cells when $\Psi_{\pi(cell)} < \Psi_{\pi\ (extracellular)}$. Finally, the extracellular solution is said to be **hypertonic** with respect to the intracellular solution when $\Psi_{\pi(cell)} > \Psi_{\pi\ (extracellular)}$.

Predictions regarding the direction of water movements across a water permeable membrane, such as the plasma membrane, can be made by comparing the water potential on both sides of the plasma membrane. It is the balance of all of the potentials that will determine the net movement, or flux, across the membrane into or out of a cell.

Today we will be exploring the determinants of water movements across semi-permeable membranes by constructing a 'cell' using dialysis membrane. Dialysis membrane is a useful model for the plasma membrane in that it is semi-permeable; it selectively allows substances to cross it primarily according to their size relative to the size of the pores it contains. The membrane we will use today comes in a tube format and has pores of a size that will allow water to pass through, but not larger solutes in the solution (sucrose).

3. Student Instructions

These are detailed step-by-step instructions for the students to do the exercise.

***Pre-laboratory activities* (assumes the students have read the background information before coming to class and that they have learned about hypothesis-driven inquiry)**

You will be using the osmosis experiment to practice the process of scientific inquiry and to explore osmosis. To this end, you will work with your group to work through the scientific method. In your laboratory notebook write out the following questions/topics and your answers or responses:

a. ***What do you know about the determinants of water movements across semipermeable membranes?***

b. ***Design hypotheses for water movements across a semipermeable membrane for a cell places in isotonic vs. hypotonic vs. hypertonic solutions).***
c. ***Predict changes in cell volume and weight in the three tonicity scenarios.***

Experimental procedure:

Please obtain from the front desk:

i. a pair of gloves
ii. Three (3) pieces of dialysis tubing (**it is critical that you wear gloves any time you handle the dialysis tubing as the oil on your fingers can clog the pores in the membrane)**
iii. Six (6) dialysis closures (2 each of closures labeled A, B, and C)
iv. One (1) small beaker labeled 'X' (intracellular fluid)

2. Close one end of a length of tubing near an end with one of the A closures making sure that the clip snaps shut and that some tubing is sticking out from the clip.
3. Open the end of the tubing by gently rubbing it together between your fingers.
4. Fill the tubing ¾ full with solution from the small beaker (solution X). To get the filling started, it helps to put the tip of your finger in the opening and slowly pour the fluid along that finger into the tubing.
5. Remove any large air bubbles from the tubing.
6. Close the other end of the tubing with the other A closure close to the end with some tubing sticking out of the clip.
7. If constructed properly, your 'cell' should have some space in it, but not air. You can test this by gently pinching the 'cell' between two of your fingers. You should be able to touch your fingers together across the cell.
8. Put that 'cell' on a paper towel on the bench and repeat steps 2-7 for 'cells' B and C.
9. Gently dry off your three 'cells' and take them to the balance to weigh. Remember to tare the balance between each cell.
10. Weigh each 'cell' and record this baseline weight in your lab notebook
11. Get three (3) solution-filled beakers (A, B, and C, ~ 200mL of solution in each) from the front table.
12. Place each 'cell' in their appropriate beaker (Ex. 'cell' with A clips in the A beaker) and note the time.
13. After ~60 minutes have elapsed mark the time in your lab book, take your 'cells' out of their beakers, dry them, and record their post experiment weight in your lab notebook.

14. Compare the trends in your experimental data with what you and your group predicted would happen to the cell volume (as measured by weight) when placed in isotonic, hypertonic, or hypotonic extracellular solutions.
15. Gather the data from all of the lab groups to analyze.

Post-laboratory analysis and results

Today each group obtained data regarding the movement of water across a semi-permeable membrane in different osmotic conditions. You have now gathered the results from this experiment from each lab group. How do you go about organizing the data and making claims about it and your hypotheses?

» Draw *qualitative* conclusions about the data set and record them in your lab notebook (3-4 sentences)
» Compare the trend in your group's data set to that of the class data set and summarize the comparison in your lab notebook (3-4 sentences)
» Graphically represent the results from the whole group by hand in your lab notebook in at least two different formats

4. Faculty Notes

a. Objectives and audience

As originally designed, the target audience is freshmen biology majors taking a once-a-week introductory laboratory course with a research focus. The instructions for the post-laboratory analysis and results are intentionally left open in order for the instructor to gauge what the students know and don't know about the organization of data and its representation in graphical formats.

At the end of this module the students will be able to:

1. Design a testable hypothesis and create predictions based on the hypothesis
2. Properly use a digital balance
3. Organize a data set
4. Perform first-pass data analysis (descriptive statistics)
5. Choose the most appropriate graphical representation of their data
6. Perform the appropriate inferential statistic (paired or unpaired t test)
7. Peer review graphs

b. Materials and reagents

- Sucrose

- Deionized water
- Solution X (intracellular solution = 6.25% sucrose solution in dIH_20)
- Solution A (isotonic solution = 6.25% sucrose solution)
- Solution B (hypotonic solution = dIH_20)
- Solution C (hypertonic solution = 25% sucrose solution)
- Gloves
- Scissors
- Dialysis tubing (4 inch lengths; Dialysis Tubing, 22mm Dia. x 34mm W, 100' Roll (Cat. # 14 V 4512; Wards Natural Science)
- Dialysis tubing closures (6/group)
- 3, 500 mL beakers/group
- 1, 100 mL beaker/group
- Analytical balance
- Weigh boat (to place 'cells' when weighing)
- Paper towels
- Labeling tape
- Sharpies
- 3, 4L erlenmyer flasks to hold prepped solution

c. Suggestions for using the exercise

This includes logistical suggestions, such as "assign part 1 as homework before class" or "use the questions in part 2 for small group discussion". Also includes suggestions to shorten or expand the exercise as well as any potential misunderstandings or difficulties the students may have for the instructor to anticipate when teaching the exercise.

I assign the 'post-laboratory analysis and results' as homework for the next week of class (this was first designed and used in a once a week laboratory class)

On the second day of class I lead a guided discussion with instruction within Excel on how to manipulate data and plot them. This discussion covers the following questions: what data or data derivatives are the best to plot? what is the best type of graph to use? what are the components of a well-designed graph?

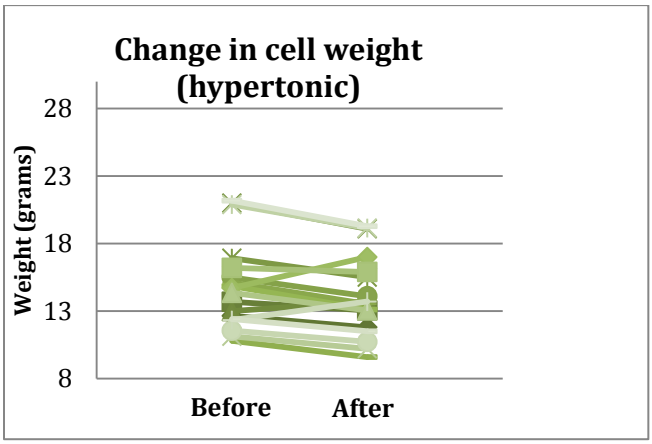
At the end of the class each student constructs a complete and appropriate graph of their data that they hand in with their lab notebook.

The guided discussion involves the following:

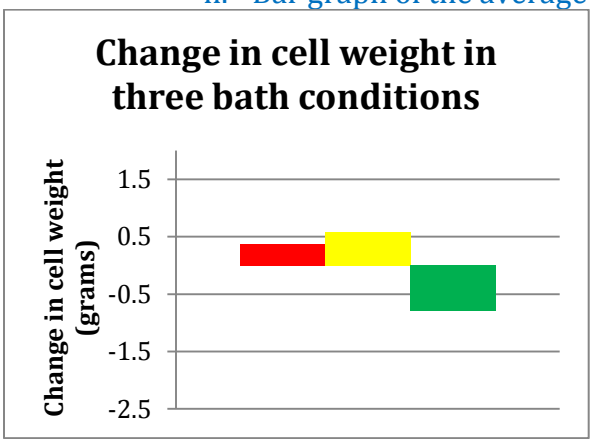
1. **What is best to plot?**
    a. Raw data
    b. Average cell weights before incubation and mean cell weights after incubation for the class data
    c. Average change in cell weight for the class data

d. Average % change in cell weight following incubation or relative cell weight for the class data (minimizes confounding variability of different starting cell weights)

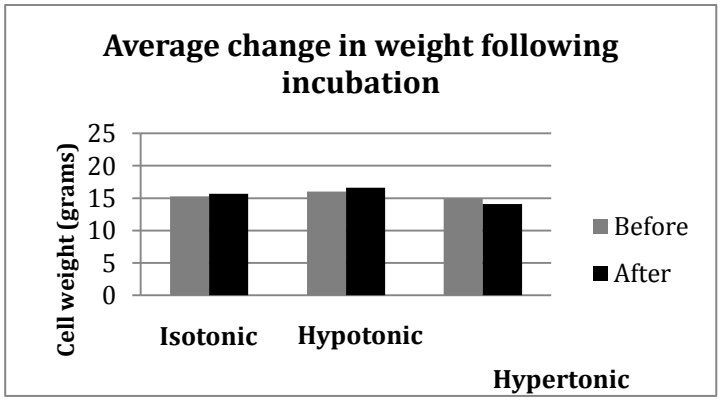
2. **What is the best type of graph to use with the data set? Depends on what you want to emphasize!**
   a. Emphasis on before and after
      i. Scatter plot of raw data cell weights before and after incubation



      ii. Bar graph of the average change in cell weight

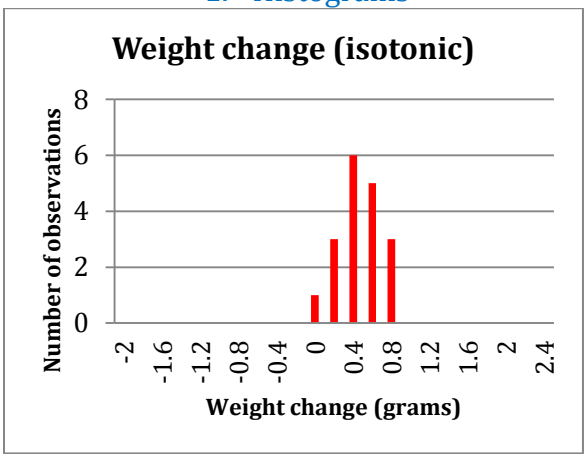


      iii. Bar graph of mean cell weights before and after incubation for the different tonicity conditions

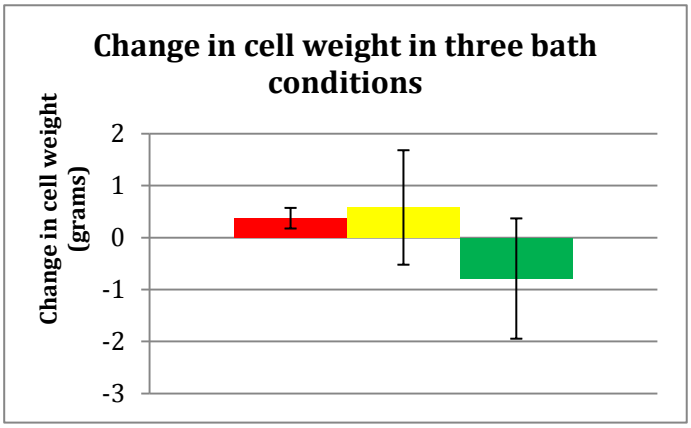


b. Emphasis on comparing different tonicity groups
    i. Bar graph of the average % change/relative cell weight in cell weight for the different tonicity groups in the class

c. Variability in the data set
    i. Visual representations
        1. Histograms

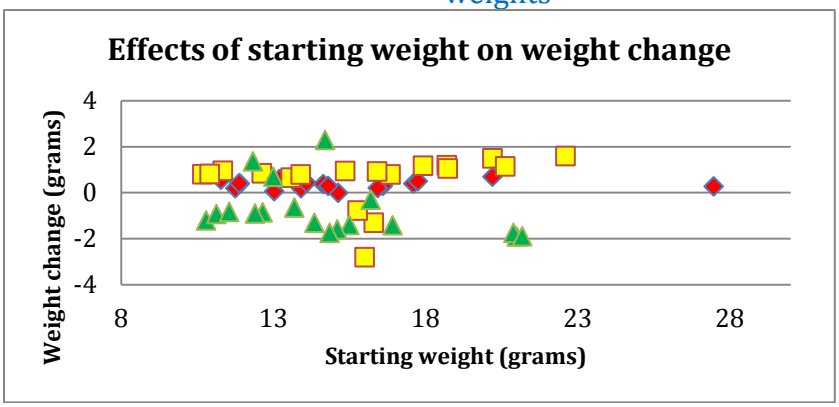


        2. Error bars (SD) ** ***this is something that nearly all freshmen and most upper level biology students omit/don't realize should be included.***



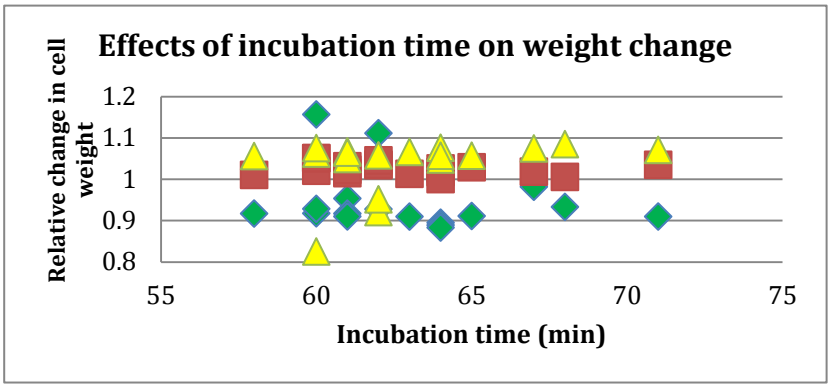
    ii. Discussion
        1. Expected variables

a. Different cells have different starting weights



yellow symbols are hypotonic, red are isotonic and green are hypertonic

2. Unexpected variables
   a. Dialysis tubing clips hold water so even isotonic cells gain some weight
   b. Different incubation times



yellow symbols are hypotonic, red are isotonic and green are hypertonic

c. Human error
   i. Using balance
   ii. Confusing cells

3. What are the components of a well-designed graph?

*This can be instructor-delivered criteria or a brainstorming session/peer review of graphs by the students to help them discover what is necessary (they develop the rubric that will be used to assess them!)

a. Title
b. Labeled axes
c. Concise, informative axis labels (units)
d. Legible symbols or color schemes for different data
e. A key defining the symbols or color schemes used
f. A figure legend describing what it plotted (average ± SD, for example)

Potential ways to expand this exercise are to:

1. Allow for more student-directed experiments (range of tonicities, measure pressure potentials, calculate pressure potentials, compare different osmolytes, etc)
2. incorporate a discussion on choosing the appropriate inferential statistical test to determine if differences are significant.

g. Assessment and evaluation

The students are assessed and evaluated based on:

i. Hypothesis
   1. Formulating a testable hypothesis

ii. Graphs
   1. Completing the 'homework' assignment in the post-laboratory analysis and results
      a. Graphs can serve as a 'pre-test'
   2. Construction of an appropriate and complete graph at the end of the class ('post-test')

iii. Conclusions
   1. Drawing appropriate conclusions based on the findings they have in hand within the appropriate scope of inference (eg. Findings apply to dialysis tubing cells).

h. Additional resources