BIOL 347L
Laboratory Three
Osmosis in potato and carrot samples

Introduction

Osmosis is the movement of water molecules through a selectively permeable membrane into a region of higher solute concentration, until there is an an equal solute concentration on the two sides.

Osmosis is essential in biological systems, as biological membranes are semipermeable. In general, these membranes are impermeable to large and polar molecules, such as ions, proteins, and polysaccharides, while being permeable to non-polar and/or hydrophobic molecules like lipids as well as to small molecules like oxygen, carbon dioxide, nitrogen, nitric oxide. (Haynie et al., 2001)

Tonicity is the osmolarity of a solution--the amount of ***solute*** in a solution. A Solute is any dissolved substance in a solution, such as sugars and salts. The term Tonicity is commonly used when describing the response of cells immersed in an external solution. Like osmotic pressure, tonicity is influenced only by solutes that cannot cross the membrane, as only these exert an osmotic pressure. Solutes able to freely cross the membrane do not affect tonicity because they will always be in equal concentrations on both sides of the membrane.

There are two things to ***always*** remember about osmoses and tonicity:

- Tonicity is always in comparison to a cell.
- The cell has a specific amount of sugar and salt

Remember the three key terms:

A ***Hypertonic*** solution has more solute (**so *LESS* water**) than the cell. A cell placed in this solution will give up water (osmosis) and shrink.

A ***Hypotonic*** solution has less solute (**so *MORE* water**) than the cell. A cell placed in this solution will take up water (osmosis) and expand.

An **Isotonic** solution has just the right amount of solute for the cell. A cell placed in this solution will stay the same.

An example of the effects of each of these osmotic environments on a typical plant cell is shown in Figure 1.

In ***plant cells***, the effect is more dramatic. The flexible cell membrane pulls away from the rigid cell wall, but remains joined to the cell wall at points called plasmodesmata. The cell takes on the appearance of a pincushion, and the plasmodesmata almost cease to function because they become constricted — a condition known as plasmolysis.

<u>In plant cells the terms isotonic, hypotonic and hypertonic cannot strictly be used accurately because the pressure exerted by the cell wall significantly affects the osmotic equilibrium point</u>.

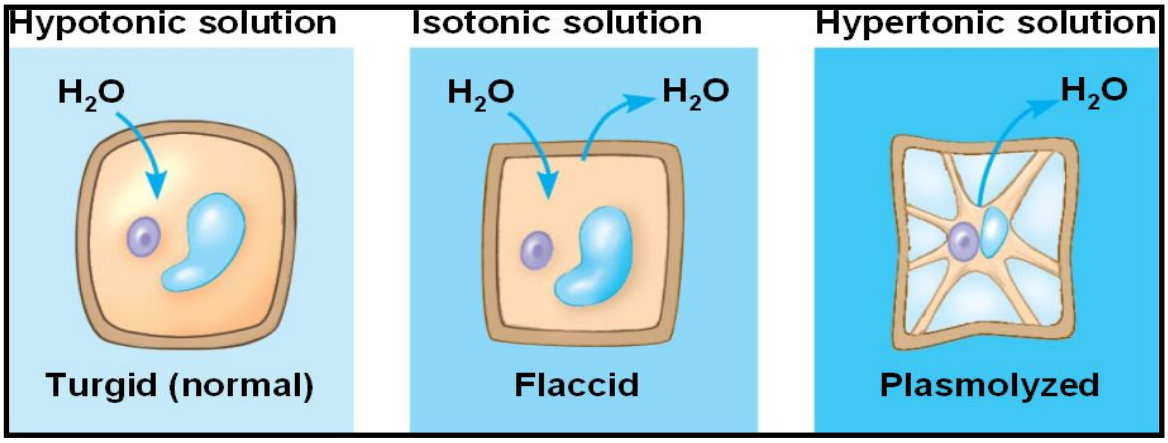

Figure 1 The effect on plant cells under different osmotic environments.

In a hypotonic environment, animal cells will swell until they burst, a process known as cytolysis. Fresh water fish urinate constantly to prevent cytolysis. Plant cells tend to resist bursting, due to the reinforcement of their cell wall, which provides effective osmolarity or osmolality (Kramer *et al.*, 2012).

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<u>OBJECTIVES for week one:</u>

During this lab, you should be able to:

1. Determine the effect of molecular mass on the diffusion rate of particles through a media.
2. Measure the osmotic pressure in cells of a potato using a gradient of solutions.

Solution preparations:

You will be provided with a 0.2 M stock solution of sodium chloride (NaCl) and firstly you will learn how to calculate dilutions for varying molar solutions

Calculating and making 10 ml of varying molar solutions from 0.2 M NaCl:

- First, calculate amount of stock solution (0.2M NaCl needed) to reach desired dilution

Molar Solution needed in dilution X 10 ml = **ml of 0.2 M NaCl**
0.2 M

e.g. to dilute a 0.2M NaCl (stock solution) to a 0.15 M NaCl (desired dilution)

(0.15M X 10 ml)/0.2M = 7.5 ml of 0.2M NaCl

Second, calculate the amount of dH_20 is needed to bring it up to 10 ml
Put these amounts in table below and calculate amounts of stock solution and dH20 needed to dilute to 0.1M and 0.05M solutions.

	0.2 M NaCl	**dH_20**
dH_20	0 ml	10 ml
0.2 M	10 ml	0 ml
0.15 M	7.5 ml	2.5 ml
0.10 M		
0.05 M		
0.025 M		

e.g. for a 0.15 Molar of NaCl in 10 ml, we need 7.5 ml of 0.2M NaCl and 2.5 ml of dH_20

- **Make above solutions and put into test tubes**

a. Using a cork borer cut cylinders from a single potato and carrot (the cuts are made parallel).

b. A razor blade is used to cut the ends of the potato cylinders square (all cylinders are equal in length). A length of about 30 mm gives good data.

c: All cylinders of samples must be equal in length, width, and appearance.

d. Measure and record the length and weight of each sample. All measurements must be similar.

e. Place a sample into a beaker.

f. Each of the test tubes is labeled and filled about 2/3 full with a different one of the salt solutions.

g. Weigh the potato and carrot cylinders after 10 mins and every 10 mins up to about 1 ½ hours. Then remove the cylinders from the test tubes.

dH20	0.2M	0.15M	0.10M	0.05M	0.025M
1					
2					
3					
4					
5					
6					
Average					

- Plot the average values in Excel. Plot change in cell size over time

Keep your samples and dry them at 60 °C and weigh them the following week to determine total water uptake

Week two:

a. Determine the weights of all of your sample cylinders, collect all class data.

b. Weight out 1/4th of your sample cylinders and place them in total protein extraction buffer (100 mM Tris, pH 7.4, 10% sucrose, 5 mM EDTA, pH 8.0, 0.19% EGTA, and 0.28% β-mercaptoethanol for the rest of the lab (I will place the at -20 °C the following morning). This will be used the following week.

c. **NOTE THE ACTURAL WEIGHT OF THE SAMPLE CYLINDER SECTION USED FOR TOTAL PROTEIN EXTRACTION.**

d. Return the remaining sample cylinders to the oven at 60 °C until the following week

Biochemical assays to be used in this Laboratory to make standard curves

Benedict's reagent assay procedure for reducing sugars:

This reagent contains 100 g sodium carbonate and 173 g sodium citrate dihydrate in a final volume of 850 mL water. Slowly, with stirring, add a solution of 17.3 g copper sulfate pentahydrate in 100 mL of water. Bring the final volume to one liter.

The blue copper (II) ions from copper (II) sulfate are reduced to red copper (I) ions by the *aldehyde groups in the reducing sugars*. This accounts for the color changes observed. The red copper (I) oxide formed is insoluble in water and is precipitated out of solution. This accounts for the precipitate formed. As the concentration of reducing sugar increases, the nearer the final color is to brick-red and the greater the precipitate formed.

- Sodium carbonate provides the alkaline conditions which are required for the redox reaction above.
- Sodium citrate complexes with the copper (II) ions so that they do not deteriorate to copper (I) ions during storage.

Procedure:

- Clearly label clean test tubes for your standards. ***Use a 2% glucose solution and distilled water to prepare a series of standard glucose solutions of different concentrations: 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, 1.2% 1.4%, 1.6%. 1.8%, and 2%***

Add 5ml of each standard and 1 ml of Benedict's solution together.

- Mix the contents of each tube by gently shaking the test tubes back and forth.

- Place the tubes in a test tube rack and set the rack in the BOILING water bath. CAUTION! The water is very hot.

- Incubate the tubes for 20 minutes.

- Remove your test tubes and allow them to cool.

- Transfer the contents to 1.5 mL centrifuge tubes and spin for two minutes. If there are still suspended debris in any of the tubes, centrifuge for two

more minutes. *****It is important that the solution be clear for the absorbance measurements. If there is solid matter suspended in the solution, the light being sent through the sample will be scattered and will cause error in the measurements.***

- Decant the supernatants into clean, labeled test tubes. Be careful that any sediment remains in the pellet at the bottom of the centifuge.

- Determine the O.D. of each of the samples using a spectrophotometer set to read at 735 nm. **Use distilled water to set the 100%T**.

- Plot the average values in Excel.

Bradford assay Procedure:

The Bradford assay is a very popular protein assay method because it is simple, rapid, inexpensive and sensitive. The Bradford assay works by the action of Coomassie brilliant blue G-250 dye, which specifically binds to proteins at arginine, tryptophan, tyrosine, histidine and phenylalanine residues.

Advantages

- Fast and inexpensive
- Highly specific for protein
- Very sensitive
- Compatible with a wide range of substances
- Extinction co-efficient for the dye-protein complex is stable over 10 orders of magnitude (assessed in albumin)
- Dye reagent is complex is stable for approximately one hour.

Disadvantages

- Non-linear standard curve over wide ranges
- Response to different proteins can vary widely, choice of standard is very important

Procedure:

- Add 1.4 ml of 1X Bradford reagent in each microfuge tube.
- Into the standard tubes place the following amounts of BSA protein standard (1 mg/ml).

Tube	µl BSA	µl H_2O	µg protein added
1	0.0	30.0	0.0
2	0.0	30.0	0.0
3	5.0	25.0	5.0
4	5.0	25.0	5.0
5	10.0	20.0	10.0
6	10.0	20.0	10.0
7	15.0	15.0	15.0
8	15.0	15.0	15.0
9	20.0	10.0	20.0
10	20.0	10.0	20.0
11	25.0	5.0	25.0
12	25.0	5.0	25.0
13	30.0	0.0	30.0
14	30.0	0.0	30.0

- Determine the absorbance at 595nm
- Plot the graph and find the linear regression line for the standard curve.

Carry out all of the assays and collect data from the whole class.

Week three:

a. Determine the final dry weight of your sample cylinder. Take into account the weight of the sections of cylinders you removed for protein extraction.

b. From all class data, determine the % weight change for each sample cylinder:

Incubation weight at each [NaCl] – initial weight X 100= % change

Total dry weight

c. Weigh out a portion of your cylinders (start with 0.002g) and carry out the Bradford assay to determine any potential change in total sugars in each sample during aa change in osmotic conditions. (This may need to be repeated if the % T is not in the central region of the standard curve).

d. Do the same with aliquots of the supernatant of the total protein extraction

SO, is there a correlation between sugar content and / or protein content of the sample cylinders during a change in the osmotic environment of each sample cylinder?

Relate this to the material we have covered in class, identify scientific papers to support (or refute) your findings

For the following week : *Individual* 9-10 page write up of the lab section (**1 inch margin, 12 pt font, 1 ½ spacing**). ***You know what to include!*** *Just in case:*

- Intro with background info and hypothesis: at least three references
- Methods in detail
- Written description of data, including standard curves. Legends and titles, and paste graphs into word!
- Detailed conclusion, explain results, include info from introduction. Did the experiment work? Possible errors and/or improvements.
- All references listed. No required format for this just be consistent!

References

Haynie, Donald T. (2001). Biological Thermodynamics. *Cambridge University Press*, pp. 130–136

Kramer, Eric M., and David R. Myers. (2012). Five Popular Misconceptions about Osmosis. *American Journal of Physics* **80**, **no**. 8 698