Lab 3: Osmosis in Model & Living Cells

<u>Objectives:</u> *To simulate the osmotic behavior of a model cell.*

We will begin our study of the workings of plant cells by looking at water movement across semi-permeable membranes—osmosis—in model cells. In the second part of the lab, we will look at real plant cells.

The movement of substances into and out of cells is accomplished largely by ***diffusion***. In addition, much of the movement of substances within organisms, and specifically <u>within</u> cells is accomplished by diffusion. In fact, few, if any of the physiological processes occurring in cells are not directly or indirectly involved with the phenomenon of diffusion. Substances tend to diffuse from areas of higher concentration to areas of lower concentration of their kind. Another way to say that is they move from areas of higher chemical potential to areas of lower chemical potential.

Chemical potential is a measure of the free energy available to do the work of moving a mole (6.02×10^{23}) of molecules from one location to another and, in some cases, through a barrier such as a plasma membrane. In a solution, the greater the concentration of molecules of a dissolved substance (***solute***), the higher the chemical potential (free energy per mole) of that substance. Therefore, we say that molecules tend to move from areas of higher concentration (higher chemical potential) to areas of lower concentration (lower chemical potential).

As you might remember, cells are bounded by a plasma membrane. All internal organelles of a plant cell (such as the nucleus, mitochondria, chloroplasts) are bounded by similar membranes. Water and dissolved solutes, such as sugars and ions, must traverse these membranes, but do so at vastly differing rates. Water can pass through the membrane easily, whereas other solutes cross the membrane only slowly, if at all; the membranes are ***semi-permeable***. All substances that enter the cell are in solution in water and diffuse through the water.

KEY TERMS

Diffusion: the movement of molecules from a region of higher concentration to a region of lower concentration.

Chemical potential: a measure of the free energy available to do the work of moving a mole of molecules (i.e., a certain number of atoms) from one location to another, sometimes through a membrane.

Solute: the substance that is dissolved (e.g., sugar).

Solvent: the liquid in which substances dissolve (e.g., water).

Semi-permeable membrane: a membrane through which different substances diffuse at different rates.

Osmosis: the diffusion of **water** molecules across a semi-permeable membrane from an area of greater concentration of water to an area of lesser concentration of water.

Water potential: a measure of the chemical potential of water molecules. A combination of osmotic and pressure potentials (see p. 3).

Diffusion of water molecules across a semi-permeable membrane is known as ***osmosis***. Osmosis is, therefore, a special case of diffusion. Because of the differentially permeable properties of the cell membranes there is a tendency for water to move across these membranes toward the solution with the higher solute concentration; or in terms of the water molecules, for the water molecules to move from a region of higher water potential to that of lower water potential. ***Water potential*** is a measure of the chemical potential, or free energy, of water molecules. Water potential is affected by the amount of solutes dissolved in the water. The more solute dissolved in the water, the lower the water potential. Pure water has the highest possible water potential.

Water potential (P_w) results from the combined actions of ***osmotic potential*** (or solute potential, Ψ_s), which is dependent on solute concentration in a solution, and ***pressure potential*** (Ψ_p), which results from the exertion of positive pressure or negative pressure (tension) on a solution. We can express this relationship as:

$$P_w = \Psi_s + \Psi_p$$

water potential	osmotic potential	pressure potential

(Your book uses the Greek symbol **Ψ** ["Psi," pronounced "sigh"] instead of P when talking about water potential).

- **Movement of water always goes "downhill" from areas of high water potential to areas of lower water potential.**

KEY TERMS

Osmotic potential: the pressure required to prevent osmosis.

Pressure potential: the result of pressure (positive or negative) on a solution.

Hypotonic: when the external solution has a lower solute concentration than the cell.

Isotonic: when the external solution has a solute concentration equal to the cell.

Hypertonic: when the external solution has a higher solute concentration than the cell, the solution is hypertonic relative to the cell.

Differences in water potential are a measure of the tendency of water to leave one area in favor of another. **The addition of solute to water *lowers* the osmotic potential** of a solution (makes Ψ_s more negative) and, therefore lowers the water potential of that solution. Cell membranes are permeable to water but often not to solutes, because solute molecules (e.g., sucrose or table sugar) are too big to pass through the pores of the permeable membrane. If there are more free water molecules on one side of the membrane than the other, there will be a net movement from high to low concentration (**Figure 1**). Thus, in the absence of other factors (such as pressure, $\Psi_p = 0$), osmosis results in the net movement of water from an area of high water potential (low solute concentration) to an area of lower water potential (high solute concentration). Water potential on two sides of a differentially permeable membrane will eventually become equal if there are no limits to expansion.

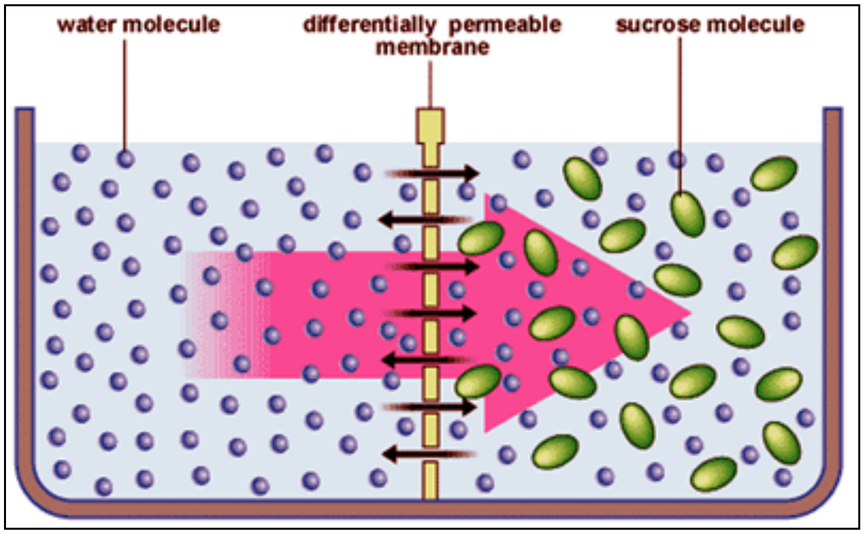

Figure 1. Osmosis – the movement of water molecules from a region of high water potential to a region of low water potential

When the external solution has a lower solute concentration than the cell, that solution is said to be ***hypotonic*** relative to the cell ("hypo" = "less than"). If the solute concentrations are equal, then the solution and the cell are ***isotonic*** ("iso" = "same"). Finally when the external solution has a higher solute concentration, then it is ***hypertonic*** ("hyper" = "more") relative to the cell (**Figure 2**).

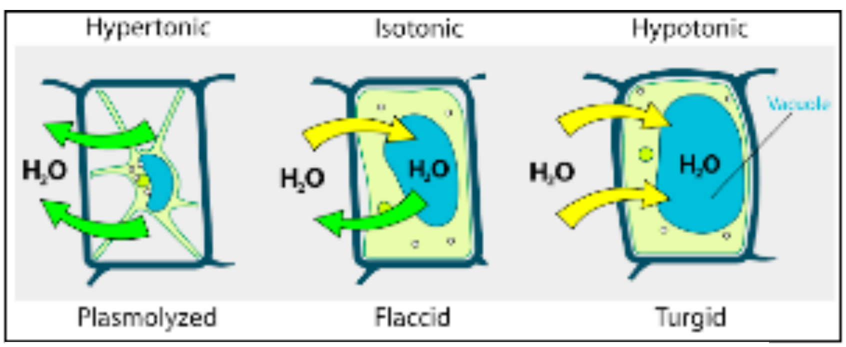

Figure 2.

THOUGHT QUESTION

If a cell is placed in a hypotonic solution, which way will water move and why?

If a barrier (such as a cell wall) prevents expansion, then pressure builds up inside the compartment (cell) into which the water is moving. An increase in positive pressure raises the pressure potential (**Ψ_p**) and therefore the water potential inside the compartment; this decreases the water potential gradient (difference) between the solution outside the compartment and the solution inside the compartment. Water continues to flow into the cell until the difference is offset by increased pressure and a dynamic equilibrium is reached. At this point, the ***net*** flow of water molecules from outside to inside the cell stops. Water molecules continue to travel randomly back and forth but the number is equal in both directions.

THOUGHT QUESTION

Osmosis can be either beneficial or detrimental to a plant cell, depending on the circumstances. Under what circumstances is osmosis detrimental to the plant cell? Use Figure 2 to help answer this question.

<u>Experiment: Osmosis in a model cell</u>

In this experiment, we will investigate the relationship between osmotic potential and solute concentration and between osmotic potential and the rate of movement of water through a semipermeable membrane by the process of osmosis. You will place dialysis tubes containing solutions of different sucrose (table sugar) concentrations into beakers containing distilled water (**Figure 3**). The rate and direction of water movement can be determined by weighing the tubes before and after placing them in distilled water; the dialysis tubes are permeable to water, but not to sucrose (as in **Figure 1**), and can gain or lose water.

At atmospheric pressure, the water potential of pure water in the beakers can be assumed to be zero ($P_w = 0$); no solute is present, thus osmotic potential is zero ($P_s = 0$), and pressure potential is also zero (**Ψ_p**). The water potential of the sucrose solutions in the dialysis tubes will be negative (recall that the addition of solute to pure water *decreases* osmotic potential), thus, the water potentials of the solutions inside the tubes will be lower (P_w is negative) than the water potential of the water outside the tubes, where P_w is zero.

Predict which way water will move under scenarios A and B:

A. **B.**

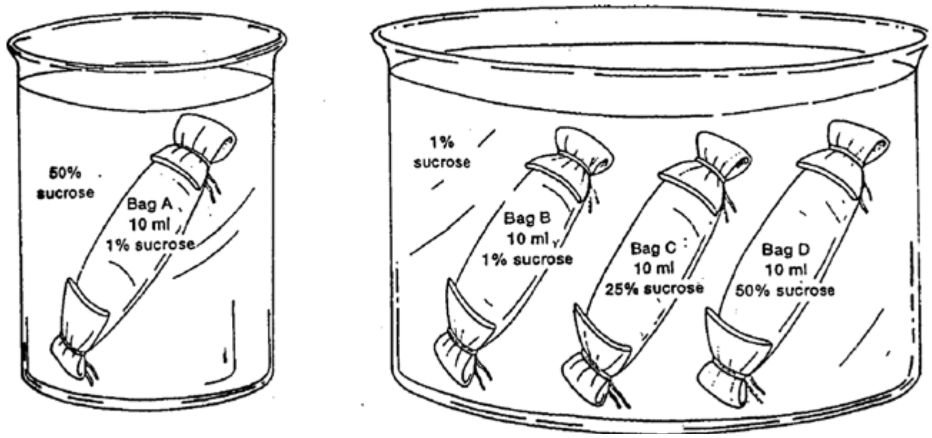

Figure 3. We will be placing tubes made out of semipermeable membrane into two different solutions. See table (p. 8) for the sucrose concentrations that we will use.

Experimental Procedure

SEE TABLE on p. 8 for details. Work in a group of five people.

1) **Soak six pieces of 15 cm long dialysis tubing** in distilled water for a few minutes.
2) While the tubing soaks, **label 6 clamps with masking tape: 5, 10, 20, 40, DI, DI.**
3) **Fold over one end of the tube 2 cm and clamp** 1 cm from that end of the tube. Do not let dialysis tubing dry out.
4) **Open the unclamped end of the tube and add 5 mL of the solution** of your experimental treatment. Then fold over the end, forcing out air, and **clamp it**. Make sure that there is space between the water and the clamp (but no air in the tube).
5) Thoroughly **rinse the tube with distilled water**.
6) **Weigh the tube on a scale**. Record weight in the table on p. 8 under 0 min and your tube number.
7) **Repeat steps 1-5 with the other 5 treatments**.
8) **Place your tube in the correct solution and start the timer** (tubes 1-5 go in distilled water in a beaker; tube 6 goes into a 40% sucrose solution). Make sure you place all the tubes into their solution and start the timer all at the same time.
9) **Weigh your tubes every 15 minutes for 1 hour** and record the results in the table on p. 8.
10) **Graph your results** on the last page.

Answer the questions on p. 6-8. Work in groups initially to answer the questions on pg. 6-8, and then come together as a whole lab and report your answers to the whole lab – your TA will record these on the white board.

Questions: Osmosis in model cells

1. Using the table below, you will first predict the results for the experiment you are going to do today. For each experimental tube listed, predict which way water and/or sucrose will move in the table below

Tube #	Tube contents	Beaker contents	Prediction
1	5% sucrose	Distilled water	
2	10% sucrose	Distilled water	
3	20% sucrose	Distilled water	
4	40% sucrose	Distilled water	
5	Distilled water	Distilled water	
6	Distilled water	40% sucrose	

2. What is the purpose of tubes # 5 and #6, in terms of our experiment?

3. What is happening to the concentration of water molecules and sugar molecules in the tubes and beakers? Use the terms **water potential** and **osmotic potential**.

4. What would happen to each tube if all bags were placed in 40% sucrose instead of distilled water? (**Use the table below to record your prediction**)

Tube #	Tube contents	Beaker contents	Prediction
1	5% sucrose	40% sucrose	
2	10% sucrose	40% sucrose	
3	20% sucrose	40% sucrose	
4	40% sucrose	40% sucrose	
5	Distilled water	40% sucrose	

5. To put this experiment in context, think about the literal tons of salt spread on the roads each winter the state of Vermont in the road crew's quest to keep roads free of ice. Plants near a heavily traveled highway sometimes show signs of salt damage by the end of winter. Sometimes you can see the strip of brown plants that grow right alongside the road. This happens because passing cars spray some of the salt onto the banks and this can affect the growth of trees several meters from the road. The highest concentrations of salt are usually found in the snow banks along the side of the road. Researchers in Canada measured salt concentrations in snow banks as high as 2%. Let's consider the molarity of sodium in snow that contains 2% salt. The molecular weight of sodium chloride (NaCl) is 58.44. A 2% solution of salt would therefore be 0.34 Molar. The concentration of salt in an isotonic solution is 0.9% or 0.15 Molar. **Based on your observations, are salt concentrations in the range of 2% likely to affect plant leaf cells? If so, how?**

Record your data here.

Tube	Tube Contents	Beaker Contents	Weight of Bag (Grams)				
			0 min	15 min	30 min	45 min	60 min
1	5% Sucrose	Distilled Water					
2	10% Sucrose	Distilled Water					
3	20% Sucrose	Distilled Water					
4	40% Sucrose	Distilled Water					
5	Distilled Water	Distilled Water					
6	Distilled Water	40% Sucrose					

Before leaving lab...
Turn in your drawings and complete lab assignment (all questions answered) to your TA. Keep your data on page 8 for your lab report. Points will be docked if you do not turn in your assignment before leaving lab.

TOTAL: /10

Please cite this lab manual in APA format:

Hill, L.M. and A. Clark. 2011. Osmosis in Model and Living Cells [lab manual]. PBIO 004, Introduction to Botany, University of Vermont, Burlington, VT. pp. 9.

PBIO 4 Osmosis Lab Report Rubric (for use in the coming weeks)

GENERAL REQUIREMENTS (1 pt)
- Complete (title, abstract, introduction, methods, results, discussion)
- Clarity of writing (how easily you can understand the ideas presented)

TITLE & ABSTRACT (3 pts)
- Title describes the specific topic analyzed
- Abstract is written concisely
- Rationale for why the analysis was performed is given, methods are stated briefly, key results and main conclusions are described

INTRODUCTION (4 pts)
- Includes a description of the broader topic with literature cited.
- Explains how the concepts of the broader question apply to the system studied
- Hypothesis is clearly stated
- Background information is relevant and complete

METHODS (3 pts)
- Complete description of materials and methods used to conduct Osmosis lab (put this in your own words, do not plagiarize and simply copy the lab manual)
- Enough detail given that another research could repeat the analyses
- Does not include irrelevant information

RESULTS (5 pts)
- Results are presented in a clear, organized fashion
- Complete presentation of results of experiments involving dialysis tubing (model cell) in terms of diffusion, osmosis and water potential.
- Data is presented clearly and figures are properly labeled
- Figure legends are descriptive enough to understand the data presented
- Results are described, but not interpreted

DISCUSSION (3 pts)
- States whether the data support/do not support the hypothesis with sufficient explanation given for why the data do/do not support the hypothesis
- Alternative interpretation of the data provided
- Clearly describes how the results fit into the broader question being addressed with literature cited.

REFERENCES (1 pt)
- At least 2 pertinent, peer-reviewed, APA cited references

OVERALL ASSESSMENT OF REPORT **Score:________ / 20**