

BIOLOGY 1230: BIOLOGY I LABORATORY

FALL SEMESTER 2000

OSMOSIS

September 25, 2000

Instructor Version

OSMOSIS: THE BIOLOGY OF WATER MOVEMENT

Introduction

The drawing below is a cartoon of the results of osmosis. Initially (Figure 1, left), the bag contains a high concentration of the solute (e.g., sugar, black dots) and a low concentration of water (open triangles). At some point later (Figure 1, right), the water has flowed into the bag while the sugar has remained inside. This increases the internal concentration of water and decreases the concentration of sugar. The number of sugar molecules remains the same.

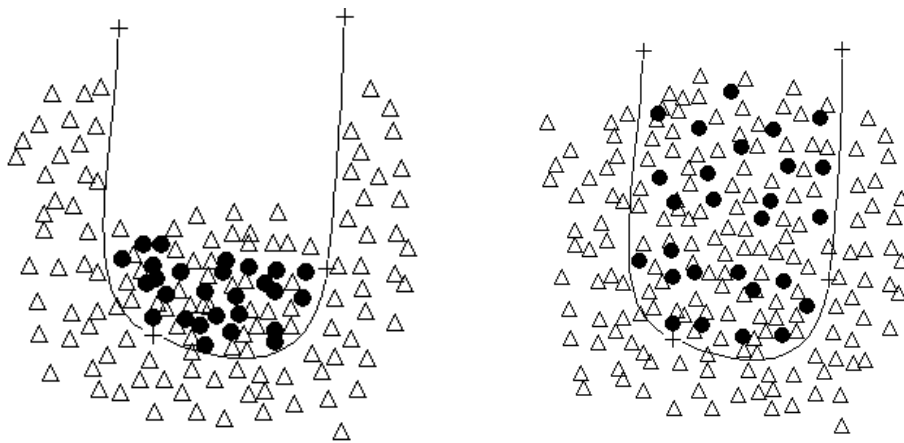


Figure 1: Left: Early stage of osmosis. Right: late stage of osmosis.

The purpose of this lab exercise is to describe how fast the bag will fill with water molecules.

Remember Cooling

A central idea underpinning the use of mathematics in biology is **analogy**: the identification of similarities between two apparently unrelated systems or processes. The similarities are made precise by our use of the same mathematical equations to describe the two systems. Now we are going to explore another analogy between the flow of heat from a beaker to the outside (cooling) and the flow of water to a bag enclosed by a semi-permeable membrane (osmosis).

First, we recall the rate of temperature change:

$$R(T) = -D(T - T_a)$$

To make the analogy between cooling and osmosis, we want an equation for the rate of water change in the bag similar to the one we used for temperature change. It is your task to develop this equation.

The Osmosis Process

To help you with stating the analogy, here, in brief, is the experiment we will do today.

1. Make a small bag from a section of dialysis tubing (thin plastic tubing with small holes that permit small molecules to pass through, but not large ones).
2. Inject a small amount of concentrated sugar solution into the bag.
3. Weigh the bag.
4. Place the bag in a beaker of distilled water.
5. At regular intervals, remove the bag, weigh it, and return the bag to the beaker.

Go to the next page to define the analogy between osmosis and cooling.

Step 1: The Analogy

What is the analogy between the osmosis experiment described on the previous page and the cooling experiment we did earlier? In your group, answer these questions:

1. What measured variables are analogous in the two situations?
2. What environment variables are analogous?
3. Is there a rate variable in both situations? If yes, what are they?
4. Is the rate of change of bag weight similar to the rate of change of temperature?
5. What is an equation for the change in bag weight?
6. Caution: the dialysis tubing is not perfectly elastic, do you think the concentration of water inside the bag will ever equal the concentration outside the bag (suppose outside is pure water). What does your equation predict?

When the instructor re-convenes the class, present your analogy.
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Step 2: The Model

Based on the discussion with the instructor and the whole class, here is the model that you developed after Step 1.

The Variables Involved

First, we need some definitions.

Box 1: Table of Symbols and Definitions

W_m	measured total weight of bag (liquid, clips, dialysis tube)
tare	weight of bag + clips without liquid
B	water concentration in bag as percent concentration (gm H ₂ O/gm liquid in bag)
O	water concentration outside bag (gm H ₂ O/gm H ₂ O) = 1.0
D	a water diffusion or conductivity constant
W	absolute weight of bag contents = gm water + gm solute = $W_m - \text{tare}$

The equation for water concentration (B) is:

Box 2: Water Concentration

$$B = \frac{W - \text{gm solute}}{W}$$

Simple Osmosis Model

The class discussion revealed how it will be impossible for the water concentration inside the bag to be exactly equal to 1.0: the tubing is not perfectly elastic. A back pressure exerts a force against the water flowing into the bag against the osmotic gradient. As a result, we use the empirical idea that there exists an "effective" outside water concentration. This is the inside water concentration that would be achieved if we waited a very, very long time for the system to equilibrate. We have the following models:

1. Perfectly elastic membrane (no internal pressure on walls limiting inflow):

Box 3: Rate of Concentration Change

$$\text{Rate}_{\text{elastic}} = -D(B - O)$$

2. Inelastic membrane:

Box 4: Rate of Concentration Change

$$\text{Rate}_{\text{inelastic}} = -D(B - O')$$

where O' is an empirical estimate of an *effective* outside water concentration (possibly not equal to 1.0).

Mini-quiz:

1. Why is there a minus sign in front of D ? Isn't the rate positive?
2. If the tare weight of the bag is 5 grams, the total weight W_m is 28 g, and the sucrose solution is 15%, what is B (the concentration of water inside the bag)?

When the instructor is ready, go on to perform the experiment on the next page.

Step 3: Experimental Protocol

This page describes the experimental protocol. After reading it and setting up your experiment, go to the next page and plot your data as you collect it.

Materials

Each lab team of 4 will need:

dialysis tubing (15 cm long)	2 dialysis bag clips	triple beam balance
stopwatch	400 ml beaker	weighing pan
distilled water	30% sucrose (wt/wt)	10 ml graduated cylinder
paper and pencil	graph paper (2 sheets)	
squeeze bottle of distilled water	paper towels	

Procedure

1. Obtain your tare weight: the weight of the bag and 2 clips without liquid. When weighing, estimate the weight to .01 gram. Weigh the materials on the triple beam balance (or the electronic balance) at your lab station. Put this amount in the box “tare” on your data sheet.
2. Prepare the dialysis bag:
 - (a) Obtain a 15 cm piece of dialysis tubing that has been soaking in distilled water for at least 5 min.
 - (b) Fold over the end of the bag about 3 mm twice. See Figure 2.

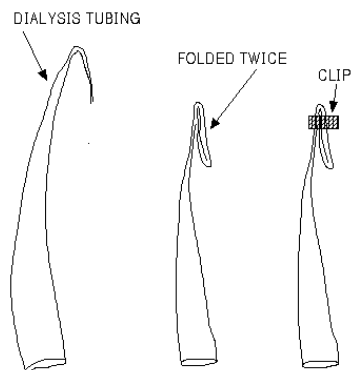


Figure 2: Folding the Dialysis Tube.

- (c) Place an orange clamp over the top of the folds and snap it shut. It is important that the bag not leak.
- (d) Rub the unclamped end between your fingertips until the bag opens. You have all had practice at this rubbing the top of a grocery store produce bag to open it at the top.
- (e) Pour 10 ml of 30% sucrose carefully into the bag. Squeeze the bag to bring the liquid up to the level of the open end, making sure **all air is out** of the bag. See Figure 3.

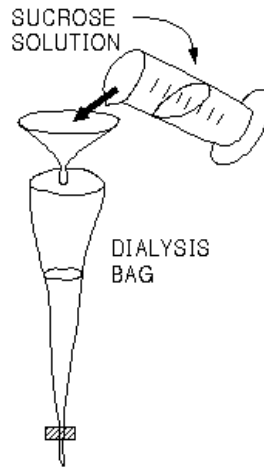


Figure 3: Filling the Dialysis Tube.

- (f) Fold the open end over 3mm twice, as you did the first end and secure with an orange clamp. It should be somewhat loose and floppy, allowing for some increase in volume due to osmosis. It should have no air bubbles and it should not leak. Squeeze it gently. If the sucrose solution comes out, reattach the clamps. If it leaks, your experiment will fail.
3. Initial weight: Rinse your bag gently with a squirt of distilled water over the sink, and blot dry by laying it on a paper towel. Record the W_m , the total weight of the bag, clips, and sucrose solution.
 4. Set your timer to the “count down” mode by pressing “Stop Reset” to bring zeros to the display. Then enter one “5” and “zero” and “zero” so that the display reads 5 minutes and zero (00) seconds.
 5. Fill the 500 ml beaker with distilled water from the jug on the prep bench.
 6. Place the dialysis bag in the beaker and press the “start” button on your timer.
 7. Get ready for weighing. Zero your triple beam balance with the weighing pan in place.
 8. When your beeper goes off, remove the dialysis bag, blot in on a paper towel and place in the weighing pan and record the weight. Team members not involved in weighing set the timer for 5 minutes again. See Fig. 4.

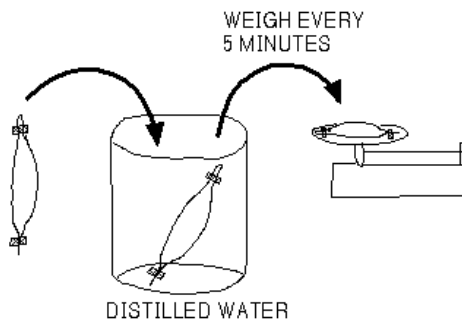
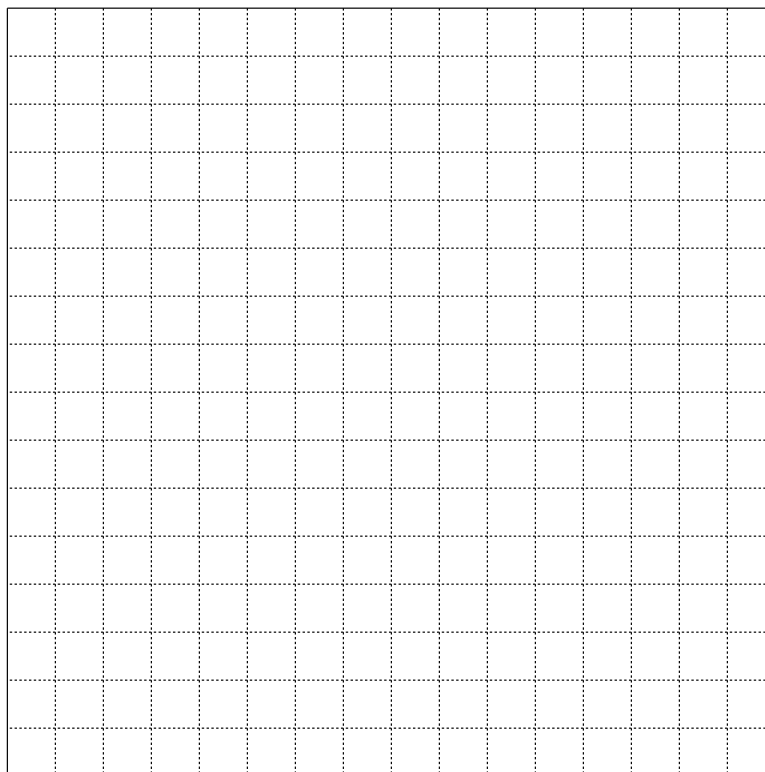


Figure 4: Weighing the Dialysis Tube.

9. Repeat this procedure for 30 minutes (6 weighings). Use the data sheet supplied in the lab.
10. Between readings, calculate the increased weight of the bag, the new water concentration of the bag, and the rate of change in the 5 minute interval. Determine with your team members what the weight of the sucrose is in your bag, and write it on your data sheet.
11. Using the graph paper below, plot
 - (a) Total weight vs time
 - (b) Water concentration vs time



12. When 30 minutes worth of data have been collected, calculate D the diffusion coefficient of your model cell.

Go to the next page.

Step 4: Estimating Diffusion Coefficients

Based on our previous discussion, the model of the rate of water uptake is:

Box 5: Equation for Rate

$$R = -D(B - O')$$

and this can be re-written in the standard form for a straight line: $y = mx + b$ as:

Box 6: Straight-line Version

$$R = -D \cdot B + D \cdot O'$$

which is the equation for a straight line.

Mini-quiz:

1. In Box 6, what is the independent variable and what is the dependent variable? What is the slope and what is the intercept?
2. Can you convert the **cooling** model into the same form as Box 6? Mathematically, does it matter which form we use?

We can use this equation to estimate D , plotting the observed rate against B . If the model is correct, the rate will decrease as B increases (as the bag gets heavier, see Figure 5). To check this, use your calculator to verify the numbers in the following table. There are only a few numbers, but calculate D from this table. Plot the rate against the average of the sequential weight measurements (e.g., the average of W_0 and W_5). Remember to divide by the time interval (5 min, below).

Time	Weight Measured	Weight - Tare	Water Concentration	Rate
0	17.6	10.0	0.8	0.0028
5	18.35	10.75	0.814	0.0016
10	18.81	11.21	0.822	—

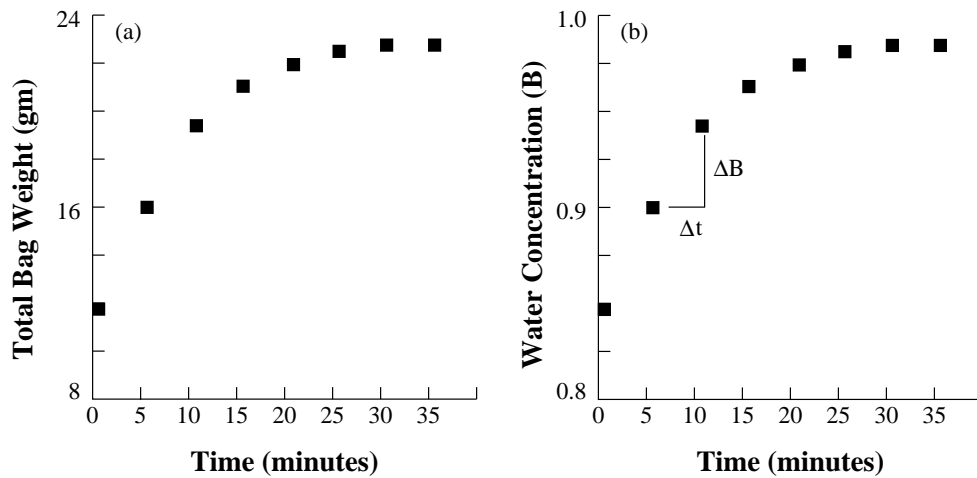


Figure 5: Hypothetical Results of Osmosis Across a Dialysis Tube.

Using the equation in Box 6, we have to estimate both D and O' . D is negative of the slope of the plot of rate versus concentration. O' is the x-axis intercept (where rate = 0). Guess what the unknown curve will look like in the graph below. Think about our analogy with cooling.

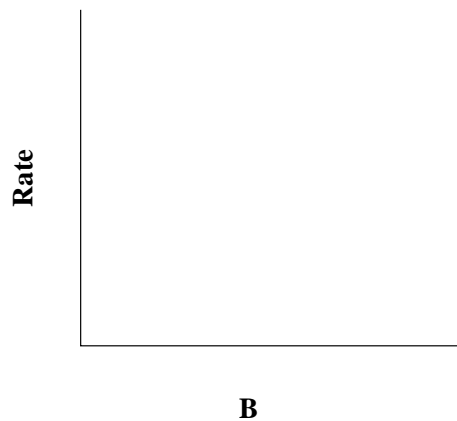
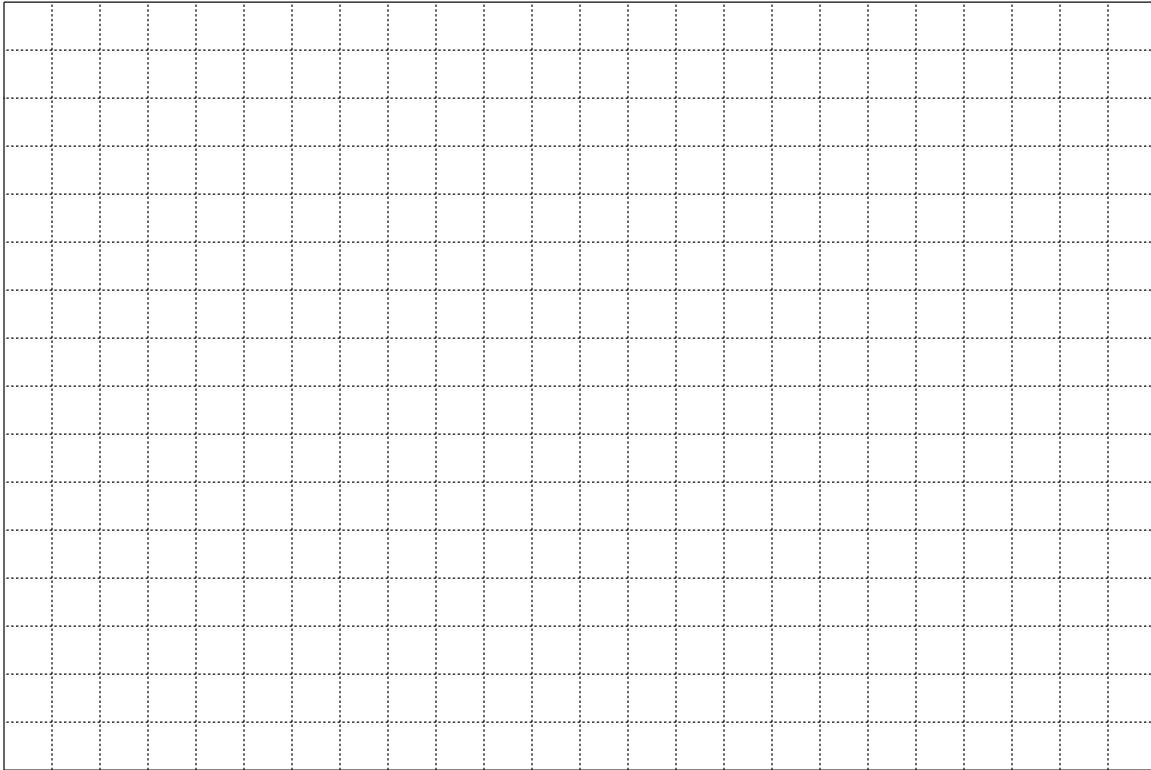


Figure 6: Unknown relation between rate of concentration change and the current bag concentration (B).

When everyone in your group is confident that they understand this and can do it on their own, go to the next page.

Step 5: Analyzing Your Data

After all your data are collected, plot the weight gain versus weight using graph paper. Be sure to label the axes completely, including the units. Use this plot to estimate the diffusion coefficient in the model.



The instructor will lead you through the next step.

Step 6: Iterating the Equation

The instructor will lead the class through this part.

We are now ready to predict the values of weight gain using our model. The process is analogous to the method we used to predict cougar cooling, except now our variable (weight) is increasing, not decreasing.

Since we calculated rates based on 1 minute time intervals, we iterate every minute (not 5 minutes).

Box 7: (Equations for concentration at minutes 1, 2, and 3)

$$\begin{aligned}B_1 &= B_0 - D(B_0 - O') \\B_2 &= B_1 - D(B_1 - O') \\B_3 &= B_2 - D(B_2 - O')\end{aligned}$$

and so on. B increases because $B < O'$.

For example, from the data in the above table and using $B_0 = 0.8$

Box 8: Example calculation

$$\begin{aligned}B_1 &= 0.8 - 0.035(0.8 - 0.88) = 0.8028 \\B_2 &= 0.8028 - 0.035(0.8028 - 0.88) = 0.8055 \\B_3 &= 0.8055 - 0.035(0.8055 - 0.88) = 0.8081\end{aligned}$$

Use your estimates and data and iterate the equation using your calculator. Plot the model predictions and the observed values on the same graph.

Mini-quiz:

Did the model predict your data exactly? If not, how did it fail? Why?

Step 7: Solved Problems

Below are some solved problems. Since these types of problems will be given to you in quizzes and exams, you should be sure that you understand how to solve them.

1. At time $t = 0$ (time in minutes), a bag and its liquid contents weighs 20 gm. The tare weight of the bag is 6 gm and the effective outside concentration of water is 0.9. The bag contains 40% sucrose by weight. If the bag weighs 20.8 gms at time $t = 1$ minute, what is the value of D ?

ANSWER:

We know that $B_1 = B_0 - D(B_0 - O')$. We are given O' . We are also given data that will allow us to calculate B_1 and B_0 . We want to know D . Our strategy will be to plug the given data into the equation and solve for D .

Liquid weight = bag - tare = 14 g. Weight of sucrose = $(14.0)(0.4) = 5.6$ g.

Weight of water in bag = $14.0 - 5.6 = 8.4$ g at $t = 0$

$B_0 = 8.4/14.0 = 0.60 =$ water concentration at $t = 0$.

Weight of water in bag at $t = 1$ is $20.8 - 6 - 5.6 = 9.2$ g.

$B_1 = 9.2/14.8 = 0.62 =$ water concentration at $t = 1$.

Therefore, $0.62 = 0.60 - D(0.60 - 0.9)$.

Solving,

$$D = -\frac{0.62 - 0.60}{0.60 - 0.90} = 0.006/\text{minute}$$

2. A bag starts with 5 gm of sucrose and is measured at 2 minute intervals. After subtracting the weight of the bag materials, the following measurements were made.

Time	Wt.
0	20.0
2	22.22
4	24.69
6	27.43
8	30.48
10	33.87

What is the diffusion coefficient and effective water concentration outside the bag?

ANSWER:

The question asks us to estimate D and O' . We have done this before by plotting the **change** in water concentration against water concentration. Here is how to do it:

Liquid contents = 20 g. Sucrose contents = 5 g.

Weight of water at $t = 0$ is 15 g.

$B_0 = 15/20 = 0.75$

Using 2 minutes as the basic time unit, the data are analyzed in terms of concentration and rates:

TIME	0	2	4	6	8	10
B_i	0.7500	0.7750	0.7975	0.8178	0.8360	0.8524
RATE	.025	.0225	.0203	.0182	.0164	.0148

Plot RATE vs B_i and draw a straight line through the points. The slope is D , and the x-axis intercept is the effective external concentration. When you do this, you should get $D = 0.1$ and $O' = 1.0$.

3. If D is 0.04, the effective outside water concentration is 0.8, and a bag whose total liquid contents initially weighs 15 gm including 20% sucrose (by weight), what is weight of the bag in 5 minutes?

ANSWER:

We wish to know B_5 . Do we have an equation for B_5 ? Yes, we use the iterative equation to get B_5 from B_4 .

$$B_5 = B_4 - D(B_4 - O')$$

But we don't know B_4 , so we get B_4 by iterating using B_3 , and so on until we can compute B_1 from B_0 (which we do know):

$$B_1 = B_0 - D(B_0 - O')$$

Which we can compute because we know how to calculate B_0 and the other variables are given to us in the problem

So the first problem is to find B_0 . Do we have an equation for B_0 ? Yes,

$$B_0 = \frac{W - \text{sucrose}}{W}$$

where W is the initial weight of the bag's contents (water plus sucrose).

The amount of sucrose is 20% concentration of the bag's contents, or $0.20(15) = 3.0$ g. Therefore

$$B_0 = \frac{15 - 3}{15} = 12/15 = 0.8$$

Now we compute B_1 :

$$B_1 = B_0 - 0.04(0.8 - 0.8) = B_0 - 0.0$$

or

$$B_1 = B_0$$

This means the bag is not changing weight. It is at **equilibrium** or **steady state**. There is no net change of water in the bag. After 5 minutes, this will still be true so the weight of the bag will be 15 g. We started the bag with the same water concentration inside as outside, so there is no net change in the bag.