

LAB 4: Biological Membranes

I. INTRODUCTION

Membranes are another molecule that make life possible. Most importantly, they provide a compartment for cells, separating the cytoplasm (the material within a cell), with its multitude of molecules and structures, from the outside environment. Most intracellular processes, especially enzyme-catalyzed reactions, depend on a stable internal environment (in terms of both molecular concentrations as well as pH). Beyond being a wall, membranes both regulate and are the site of a whole host of important biochemical reactions. The cell's membrane helps regulate the intracellular environment by regulating what types of molecules go in and out of the cell. Furthermore, many life processes are mediated by membrane-bound proteins. In this lab we will examine several characteristics of biological membranes, and physical forces acting upon a cell's membrane.

II. MEMBRANE THERMODYNAMICS

In one sense membranes are very simple – they are a double layer of phospholipids. As you learned in lecture, phospholipids are amphipathic molecules composed of a polar phosphate head group and two non-polar hydrocarbon tails (see Figure 4.1 at right).

When amphipathic molecules are added to water they can self-assemble into aggregates. This self-assembly occurs mainly due to hydrophobic interactions (what are these interactions?). The polar water molecules repel the hydrophobic tails, with the tails tending to become closely packed with one another.

The simplest self-assembling aggregate is a micelle, a small droplet with the hydrophilic heads on the outside and the hydrophobic tails on the inside. This most commonly forms with amphipathic molecules with a small hydrophobic region, such as ionized fatty acids with a single hydrocarbon tail. Phospholipids, however, do not often form micelles because the two hydrocarbon tails are too bulky to fit in the interior of a micelle. Instead, phospholipids usually form a thermodynamically more stable lipid bilayer. The resulting aggregate is a liquid-filled balloon called a liposome, with the wall of the liposome composed of the lipid bilayer (see Figure 4.2). Both sides of the

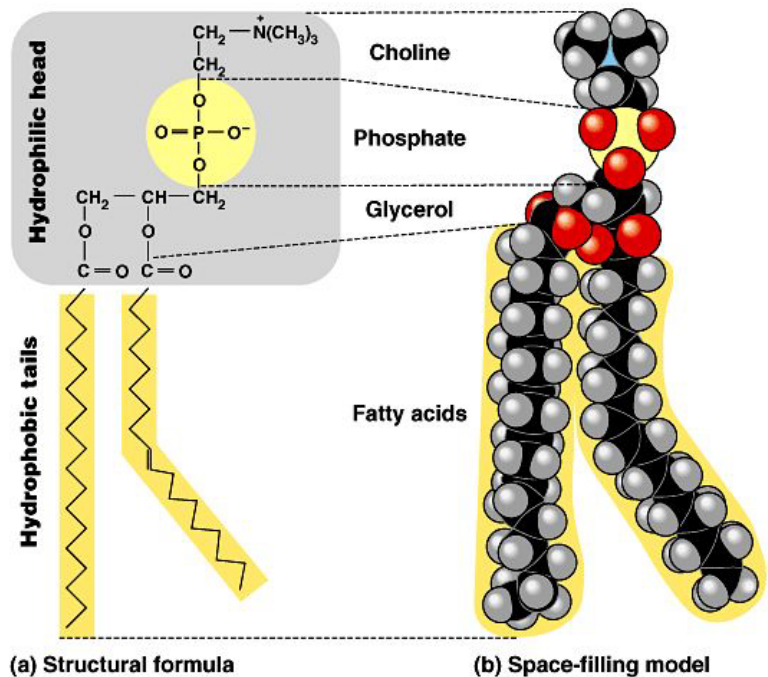


Figure 4.1. Structure of phospholipids. (a). Structural formula and (b) space-filling model. Phospholipids can vary in the identity of the head group (in this case a choline) and differences in the two hydrophobic tails, each having a carbon backbone and hydrogens attached. The kink in one of the tails is due to a double bond. Figure adapted from Campbell and Reece (2002) "Biology", 2nd ed.

lipid bilayer are bounded by water, with the

non-polar tails in the center of the bilayer. The stability of the bilayer is the result of two factors: first, water molecules are released from interacting with the non-polar tails, which are in the center of the bilayer; and second, van der Waals forces between the tails favor the closely-packed bilayer arrangement.

The complexity of membranes is due to other molecules present in the lipid bilayer, such as cholesterol, glycolipids (lipids with a sugar group attached to the hydrocarbon chain), and proteins. In this lab we will be examining the characteristics of the **lipids** in the bilayer and the behavior of a transmembrane protein channel called **aquaporin**.

III. MEMBRANE PERMEABILITY

Biological membranes are lipid bilayers that compose the boundaries of cells. These barriers prevent molecules generated in the cell from leaving the cell and keep unwanted molecules out. Lipid components of the membrane determine the permeability of the membrane itself. In particular, membranes have very low permeability to ions and large polar molecules. Water, though a polar molecule, can move across membranes due to its small size, high concentration and lack of a complete charge. In general, the more nonpolar a molecule, the more readily it crosses a membrane. Note also that there are a variety of membrane proteins that transport molecules (such as ions) that would not otherwise be able to cross the membrane, though we won't be looking at these in lab today.

What we will do is use red blood cells to investigate the permeability of cell membranes. Red blood cells are a good model because they are easy to obtain, they are uniform in size and it is relatively easy for us to determine when they undergo

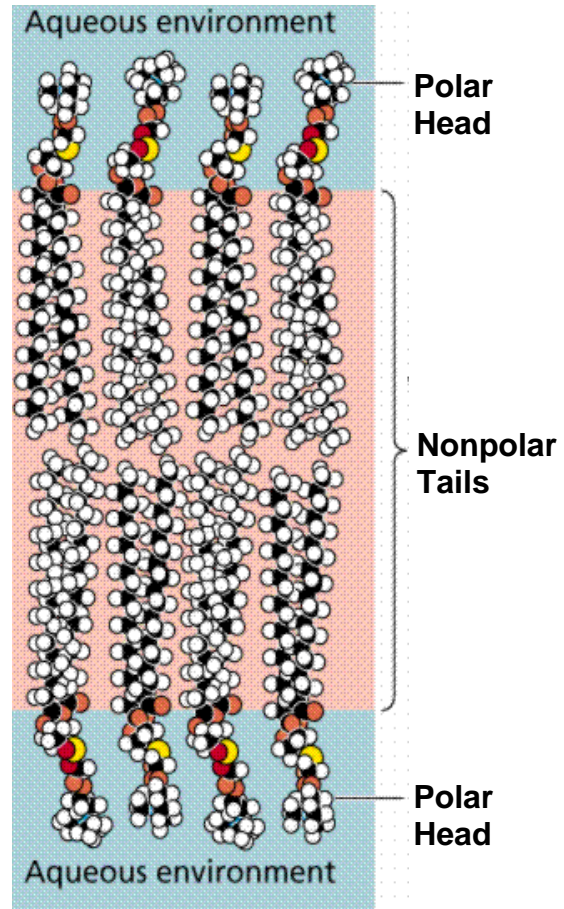


Figure 4.2. A lipid bilayer.

hemolysis (cell rupture). You will first look at red blood cells under the microscope to observe the changes in cell shape which take place in hypertonic and hypotonic solutions. Then you will perform a second experiment comparing the *rate* of hemolysis with different compounds in solution.

As mentioned above, water can move across membranes; however its movement through a lipid bilayer is relatively slow. In many tissues, water moves across the membranes very quickly due to the presence of pore-forming proteins called aquaporins. (Aquaporins were discovered in 1991 by Peter Agre, and he won the Nobel Prize in

Chemistry for his research in 2003 – see Preston et al. 1992, which is available on the lab web page.). Each aquaporin (AQP) is a

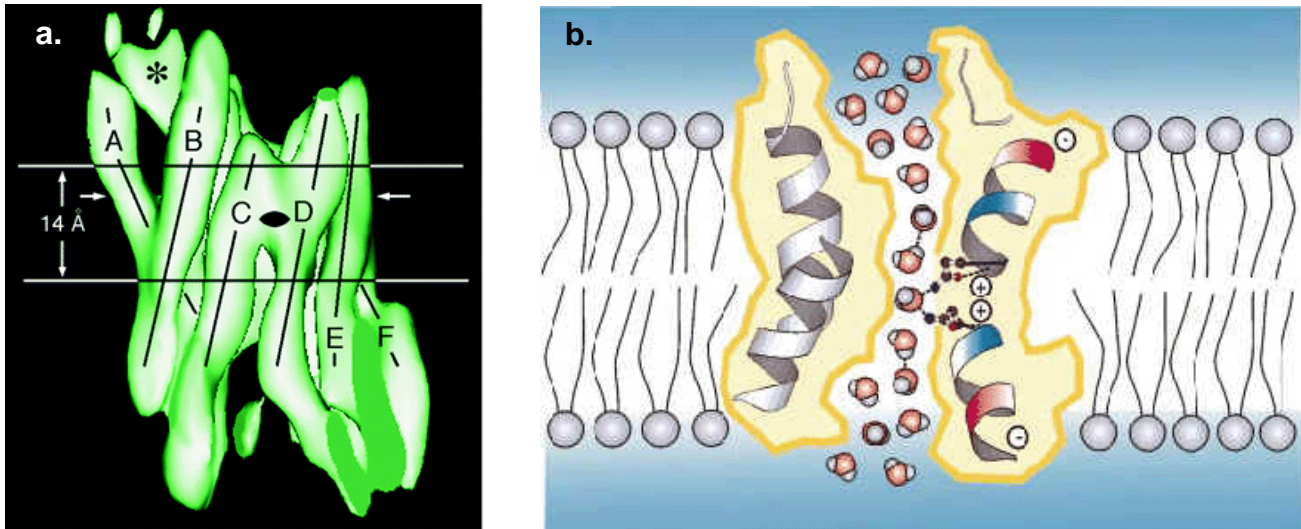


Figure 4-3. Structure of Aquaporin-1 in human red blood cells. Aquaporin is composed of six helices that span the membrane. **(a)** A representation of the six-helix barrel viewed parallel to the bilayer with the lines indicating the approximate axes for the six helices (labeled A-F). The arrows identify the center of the lipid bilayer. **(b)** Cross-section of an aquaporin molecule that shows the passage for water in the center. Images adapted from: (a) Cheng et al. 1997 *Nature* 387:627-630 and (b) Murata et al. 2000 *Nature* 407:599-605.

transmembrane protein with a passage in the center that allows water to pass through (see Figure 4.3). Each molecule is a monomer with six α -helices that form a barrel-shaped passage through the membrane.

Aquaporins are present in the cell membranes of red blood cells, as well as other water permeable membranes found in the epithelial lining of intestines and kidneys. When we observe the effect of ionic concentrations on red blood cells, we are indirectly observing the activity of the transmembrane aquaporins.

IV. OSMOLARITY

Organisms and their component cells often find themselves aqueous environments with differing solute concentrations. For example, the protozoan that we saw in lab earlier commonly occur in freshwater environments with low solute concentrations, much lower than that found within the organisms. This

difference in solute concentration produces a concentration gradient across the organism's membrane. This gradient is a kind of order, in the sense that it is not random. And if you

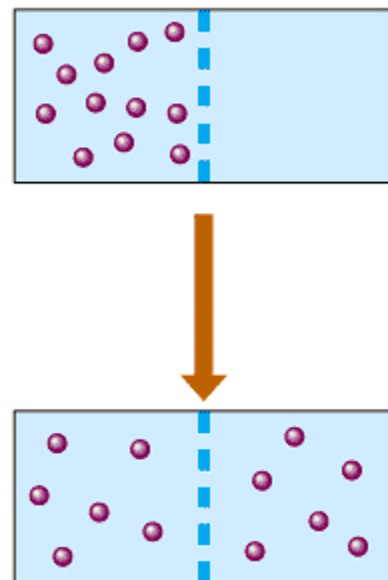


Figure 4-4. Solute particles can diffuse across a semi-permeable membrane from an area of high concentration to an area of low concentration. This diffusion will occur until solute concentration is equal on both sides of the membrane.

recall the second law of thermodynamics, all process processes occur so as to increase randomness (entropy). So there will be pressure, in this case called **osmotic pressure**, that will tend to act in such a way to equalize the solute concentrations on each side of the membrane. There are two ways to equalize the solute gradient. First, the solutes can move across the membrane – this process is called **diffusion** and is shown in Figure 4.4. Second, water can move across the membrane in a process called **osmosis** – this process is important when water can move across the membrane, but the solutes cannot. Note that in both diffusion and osmosis, particles move in order to equalize the solute concentration across the membrane.

Osmolarity is a measure of the amount of solute present in solution. This concept does not take into account differences between molecules which ionize in solution, such as NaCl, and those which do not, such as sucrose. A 1 M solution of NaCl (each molecule of which dissociates into two particles, Na^+ and Cl^-) has a concentration of 2 Osm (osmolar = osmoles/L). Note that a 30 mM solution of CaCl_2 has an osmolarity of 90 mOsm because each molecule of CaCl_2 dissociates into three particles in solution. For a substance like sucrose, which does not ionize, the osmolarity of a solution is the same as the molarity.

There are three terms used to compare solute concentrations across a membrane. **Isotonic** (or isoosmotic) refers to a solution having the same solute concentration as

another solution. If a cell is placed in an isotonic solution, there will be no net movement of water across the membrane. In medicine, intravenous fluids must be given at a concentration that is isotonic with blood (normally 296 ± 5 mOsm). A solution with a lower osmolarity than another solution is said to be **hypotonic** (or hypoosmotic), whereas a solution with a higher osmolarity is **hypertonic** (or hyperosmotic). Note that all of these terms are relative terms.

V. MICROSCOPIC OBSERVATIONS OF RED BLOOD CELLS

In isotonic solutions, red blood cells (RBC's) are biconcave disks with a remarkably uniform diameter ($7\mu\text{m}$ for human RBCs). This shape allows them to fold up slightly as they squeeze in single file through capillaries. The cell membrane has a relatively fixed surface area. As the cell volume decreases in a *hypertonic* solution, the cell membrane wrinkles, and the RBC takes on a *crenated* appearance. Conversely, as the cell volume increases in a *hypotonic* solution, the RBC will first swell and lose its biconcave shape, then as the membrane integrity becomes compromised, the intracellular contents spill out of the cell (called **hemolysis**).

In this experiment you will observe microscopically the effect of solutions of various osmotic strengths on the gross appearance of RBCs (we are using sheep blood). These qualitative observations of RBC behavior should lend further support to the hypothesis that biological membranes are semipermeable. Please wear purple nitrile gloves when working with blood.

Experimental Procedure:

Repeat the following procedure with 3 different concentrations of sucrose: First 300 mM (this is isotonic with the RBC cytoplasm), then 600 mM (hypertonic), then 100 mM (hypotonic). If you have three people in your lab group, have each person in your group can do one of the solutions, and then look at each other's slides. Use the Nikon Alphaphot microscopes for this exercise.

1. Place a small drop of 2% RBC suspension on a microscope slide.
2. Add 1 medium drop of the Sucrose solution directly on top of the RBC suspension.
3. Add a cover slip and examine the slide immediately through the compound microscope. Focus first at low power, then 10x, then 40x.

If the RBCs are crowded too closely together to see individual cells clearly, repeat the procedure with a smaller volume of RBC suspension. You should be able to describe the appearance of the RBCs under each condition and discuss what happened in each case.

Why do RBCs change shape when the different solutions are added? What is happening to water in these three cases?

VI. RBC PERMEABILITY IN DIFFERENT SUBSTANCES

As you observed in the exercise above, the red blood cell (RBC) is normally a flattened, biconcave disk. When the RBC's are placed in a hypotonic solution, water moves into the cells through aquaporin channels. The cells will initially swell and become spherical. As more water moves into the cells, membranes will stretch and hemoglobin will begin to leak out. Eventually, the cell membrane will burst, leaving behind the empty cells, or "ghosts".

If you look at the 2% blood solution from the previous exercise, you will notice that dilute blood is opaque (i.e., it is not transparent). However, when the cells burst or "hemolyze", the blood mixture will become a transparent red color. The membranes no longer block light and the hemoglobin goes into solution, tinting the liquid red.

In this experiment, you will observe the time of hemolysis (i.e., how long it takes for red blood cells to lyse or burst) in different solutions. These substances (shown below) include a series of salt (NaCl) solutions at different concentrations and alcohols with different lipid solubilities.

Test Solutions used in Experiment:

- | | | | |
|----|-------------------------------------|----|--|
| A. | Distilled water (DH ₂ O) | E. | 0.3 M ethanol (EtOH) in DH ₂ O |
| B. | 0.06 M NaCl | F. | 0.8 M ethanol (EtOH) in DH ₂ O |
| C. | 0.145 M NaCl | G. | 0.8 M ethylene glycol in DH ₂ O |
| D. | 0.350 M NaCl | H. | 0.8 M glycerol in DH ₂ O |

Solutions **A** through **D** are NaCl salt solutions of different osmolarity. Distilled water (DH₂O) is used as a control for all treatments and is, by definition, hypotonic. Solutions **E** through **H** contain a series of alcohols that differ in lipid solubility (i.e., how readily the alcohol passes through the membrane). Ethanol is the most lipid-

soluble (meaning that it can easily diffuse through the membrane) and glycerol is the least lipid-soluble.

If the normal osmolarity of the blood body is about 0.290 Osm (Osm refers to the concentration of particles, so a 1 Osm solution has a particle concentration of 1 M), what concentration of salt is isotonic to blood? (Remember that NaCl breaks up into

2 ions, Na^+ and Cl^- , when dissolved). You should understand why the hemolysis rates differ as they do for these different substances, and predict from a series of similar molecules which will have the highest hemolysis rates.

Experimental Procedure:

1. Using a 10-ml glass pipette, transfer 10 ml of each of eight test solutions into separate test tubes. You should label the tubes (with tape and/or a marker). A separate pipette should be used for each solution – you can do this by placing a pipette in each of the solution tubes on your bench. All solutions should be at room temperature.
2. Measure the **time of hemolysis** for one solution at a time, as follows. You will need a clock/watch and one small square of Parafilm to seal each test tube. The measurement is based on the time that it takes for hemolysis to progress to the point where a black and white image can be seen through an RBC suspension in the test solution. Each run should be conducted quickly and efficiently. If you have three people in your group, one person can watch the clock, one person can hold the test tube and image, and the third person can pipet the blood.
3. While holding the test tube with parafilm at ready, use a P-200 pipettor to transfer 50 μl (0.05 ml) of heparinized **whole blood** to the test tube. When pipetting blood, push and release the plunger on the pipettor slowly so that the RBC's are not ruptured by shear forces as they pass through the pipet tip. Start timing when you add the blood - this is time-zero.
4. Immediately seal the tube with Parafilm. Mix it quickly by tilting it upside down once.
5. Immediately hold the tube in front of the black and white image provided at your lab bench. Looking at the image through the diluted blood in your test tube, watch for the point when the image on the film can first be clearly resolved. This is the stop time. Record elapsed time, in seconds, on your datasheet. If the image cannot be discerned within ten minutes, then record the hemolysis time as >600 sec. You should design a table to record these values before coming to lab.
6. Repeat steps 4-6 for each test solution.
7. When you are done with the measurements for all 8 solutions, discard the blood and rinse out all test tubes.
8. Copy your data onto the computer datasheet opened by your TA. You will use data for your entire lab section for your analysis.

You recorded the time, in seconds, that it took for the image to resolve through the RBC suspension. When you analyze the data (section IX below), you will take the reciprocal of time of hemolysis and record that as the **relative rate of penetration** in sec^{-1} .

VII. EXPRESSION AND INHIBITION OF RBC AQUAPORIN

Aquaporins were only recently discovered (Preston et al. 1992), first in red blood cells, and later in water-permeable membranes of other tissues such as in kidneys and intestines. A common model for observing the behavior of aquaporins is the oocyte (that is, an unfertilized egg) of the African Clawed Frog, *Xenopus laevis*. If mRNA for the aquaporin protein from human RBC's is injected into a *Xenopus* oocyte, the oocyte will translate this mRNA into the aquaporin protein. Then by an unknown mechanism the aquaporin proteins insert themselves into the oocyte membrane. In this experiment, we will attempt to observe the effect of human aquaporin expression in *Xenopus* oocytes.

Aquaporins can be inhibited by certain compounds, such as mercury salts and Tetraethylammonium Chloride (TEA). If aquaporins are important in the movement water across an oocyte membrane, how can we use TEA as a control in experiment?

For this experiment, you will observe the rate at which the oocytes change diameter, using an ocular micrometer. Since the oocytes are large (>1mm in diameter), we will be using **dissecting microscopes**. You will need two microscopes per group. When adding the TEA inhibitor, you will incubate the eggs for at least 5 minutes prior to testing to make sure that TEA has bound to the aquaporins **before** the oocytes are added to the low-osmolarity test solution. This experiment will be discussed further during your lab session. Below is a summary of the experimental design.

You will receive oocytes in a buffer called ND96, which is isoosmotic with oocytes and has an osmolarity of 200 mOsm. For the experiment, you will first choose an oocyte from the appropriate treatment and measure its diameter. Then using a pipet, transfer the oocyte to a small Petri dish containing a 1:3 dilution of ND96 (2 parts water to 1 part ND96) – what is the osmolarity of this test solution? After transferring the oocyte to the test solution, measure two types of data: (1) oocyte diameter at 30 second intervals (until 5 minutes or oocyte rupture, whichever comes first), and (2) the time at which the oocyte ruptures.

| Oocyte Experimental Design: | | |
|------------------------------------|-----------------------------|------------------------|
| Treatment #: | Type of Oocyte used: | TEA Incubation? |
| 1 | Injected with 50µl water | No |
| 2 | Injected with 50µl RNA | No |
| 3 | Injected with 50µl RNA | Yes |

BIO 126: ENERGY FLOW IN BIOLOGICAL SYSTEMS

VIII. DATA ANALYSIS - COMPARISON OF MEANS USING STANDARD ERROR

When you have recorded your data, the class data will be pooled (i.e. the data set will include data collected by all students in your lab). You should first convert each data point to **relative rate of penetration** in sec^{-1} , by taking the reciprocal of time to resolve the image on the slide. In order to present and interpret these results, you will plot the rate of penetration for each of the solutions used and add standard error bars for each mean.

Standard Error Bars

To help understand the meaning and use of standard error bars, pretend that you conducted an experiment on red blood cell hemolysis with three solutions that we'll designate 'X' and 'Y.' Assume that you conducted several trials (replicates) for each solution using the same methods as in our hemolysis experiment, measuring time to lysis and calculating relative rate of penetration for each trial. The next step is to find the **mean** (or **average**) rate of penetration for each solution – these means will be compared to see if the solutions differed in terms of rate of penetration. A statistical problem immediately presents


itself, however. There may very well be

differences in the means, but **how** different must the means be before we are confident that one solution (or treatment) is different from another. (Note that there are two treatments in this imaginary experiment – solution 'X' and solution 'Y'.) Scientists answer this question by comparing the differences among replicates within treatments to the differences between treatments. If the differences between the treatment means are no greater than the difference among replicates in the same treatment, then the treatments themselves (i.e. the type of solution to which red blood cells were added) had little or no effect blood cell hemolysis. To illustrate this, suppose that our hypothetical experiment had five replicates per treatment (i.e., there were three tests for each solution). Suppose that the mean rate of

penetration for solution X is 0.65 sec^{-1} , and the mean rate of penetration for solution Y is 0.46 sec^{-1} . Does this difference mean anything? The only way to tell is to look at the variation within the treatments. We will examine two possible cases, which we'll call Case I and Case II, each having the same treatment means. The experimental data for the two cases are shown in Table 4.1.

Table 4.1. Hypothetical data for hemolysis experiments, denoted Case I and Case II.

| | Case I | | Case II | |
|-------|----------------------------|----------------------------|----------------------------|----------------------------|
| | Treatment 1: Solution X | Treatment 2: Solution Y | Treatment 1: Solution X | Treatment 2: Solution Y |
| | 0.84 | 0.74 | 0.69 | 0.57 |
| | 0.35 | 0.24 | 0.51 | 0.37 |
| | 0.68 | 0.59 | 0.70 | 0.50 |
| | 0.44 | 0.34 | 0.55 | 0.42 |
| | 0.94 | 0.39 | 0.80 | 0.44 |
| Mean: | 0.65 | 0.46 | 0.65 | 0.46 |


 Note that treatment means are the same for both cases

In Case I there is a lot of variation within the treatments (i.e., the individual amounts eaten from the three disks in each treatment differ greatly from each other), whereas in Case II there is very little variation within treatments. These relative differences between the two cases are only evident after studying the data closely – what we really want is a convenient way to graphically represent these differences. This is where the standard error comes in: the **standard error of the mean** is a statistical measure that is commonly used to graphically show variation within a treatment. (In fact, the **standard error** is only one of several statistics that measures the variation within a group of numbers, but it is the one most

commonly used on graphs.) We will discuss this statistic below – the going may be tough, but please stick it out and read through the entire discussion.

The standard error of the mean (**SE**) is defined by the following complicated-looking formula:

$$SE = \sqrt{\frac{\sum_{i=1}^n (\bar{x} - x_i)^2}{n(n-1)}} \quad (2)$$

$$= \sqrt{\frac{\sum_{i=1}^n (x_i)^2 - \frac{1}{n} \left(\sum_{i=1}^n x_i \right)^2}{n(n-1)}} \quad (2)$$

In the equation, “n” is the number of replicates in the sample (3 in our hypothetical example), and “x” represents the value for the parameter being measured (e.g. penetration rate). Many electronic calculators have “statistics functions” and will calculate the SE for you if you type in the individual observations; this method is highly recommended.

Note that though many calculators with statistics functions do not calculate SE, they most likely do calculate standard deviation, or ‘s.’ A simple conversion from standard deviation to standard error is:

$$SE = \sqrt{\frac{\sum_{i=1}^n (\bar{x} - x_i)^2}{n(n-1)}}$$

$$= \frac{\sqrt{\frac{\sum_{i=1}^n (\bar{x} - x_i)^2}{n-1}}}{\sqrt{n}}$$

$$= \frac{s}{\sqrt{n}} \quad (3)$$

where “s” = standard deviation calculated from the sample data.

To calculate the standard error by hand, it is easiest to use the right half of equation (2) above. It is convenient to set up a table with values for x and x² for each replicate. Table 4.2 is such a table for case I of the hypothetical experiment on the previous page.

Table 4.2 Calculation of standard error for Case I

| Treatment 1: Solution X | | Treatment 2: Solution Y | |
|----------------------------|----------------|----------------------------|----------------|
| x | x ² | x | x ² |
| 0.84 | 0.7056 | 0.74 | 0.5476 |
| 0.35 | 0.1225 | 0.24 | 0.0576 |
| 0.68 | 0.4624 | 0.59 | 0.3481 |
| 0.44 | 0.1936 | 0.34 | 0.1156 |
| 0.94 | 0.8836 | 0.39 | 0.1521 |
| Sum: | 3.25 | 2.30 | 1.2210 |

For treatment 1 in Table 4.2,

$$\sum_{i=1}^n (x_i)^2 = 2.3677$$

and

$$\sum_{i=1}^n x_i = 3.25$$

Therefore,

$$SE_{\text{treatment 1}} = \sqrt{\frac{\sum_{i=1}^n (x_i)^2 - \frac{1}{n} \left(\sum_{i=1}^n x_i \right)^2}{n(n-1)}}$$

$$= \sqrt{\frac{2.3667 - \frac{1}{5} (3.25)^2}{5(5-1)}}$$

$$\approx 0.113$$

A large SE indicates much variation, a small SE little variation. In our hypothetical examples the SE's were:

Table 4.3. Standard Errors for Hypothetical Hemolysis Experiment

| | Treatment 1: Solution X | Treatment 2: Solution Y |
|----------|----------------------------|----------------------------|
| Case I: | 0.113 | 0.090 |
| Case II: | 0.053 | 0.034 |

(To be certain that you can do so, use the data in Table 4.1 to calculate the SE's for Case II.)

There are formal statistical techniques for using SE's to determine whether differences among treatment means are significant. For the purposes of this lab, we will simply point out that if the difference between means is small relative to the SE, then the difference is not meaningful. If the difference between the means is large relative to the SE, then the difference is likely to be significant. For both hypothetical cases above, the difference between means is 0.19; this is small relative to the SE's for case I, but it is large relative to the SE's in case II. Only in case II would the experiment have shown a potentially significant result.

Now we have the tools to create the graphical comparison that we've been working towards. The comparison between means relative to SE's can be seen at a glance when data are presented graphically, as shown in Figure 4.4. In each case, the top of the large bar or box represents the mean for the treatment. The SE is represented by an error bar above and below the mean – the length of each error bar is the value of the SE. Thus for treatment 1 of case I, the top of the large bar is positioned at 0.65; one error bar extends from the mean to 0.663 (i.e., mean + SE = 0.65 + 0.113), and the other error bar extends from the mean down to 0.537 (i.e., mean - SE = 0.65 - 0.113). The visual comparison of sample means is as follows: two means are significantly different if their corresponding standard error bars do not overlap. It is easy to see from the graph of case I that the error bars for the two treatments overlap, and that the difference between means is minor compared to the SE – we would conclude that in case I, the treatment means are not significantly different. For case II, the error bars do not overlap and the SE's are small compared to the difference between treatment means – in this case the treatment means are most likely significantly different..

Warning!! The Standard Error Bar Method is a crude visual test

One thing we need to stress is that the standard error bar method described above is a **crude visual test**. The visual comparison of means does not take the place of a statistical test. You can use this visual test if you are in the audience at scientific seminar or reading someone else's paper - in these cases, you personally do not have the data and cannot do a statistical test. However, if you are presenting your own data to the scientific community, you should perform the appropriate statistical test. The bottom line here: when you have the data, always use a statistical test to determine the significance of differences between treatments.

In this lab we are using this 'quick and dirty' method to avoid using a set of complex statistical tests (in the hemolysis experiment, they would be Analysis of Variance and a set of post-hoc comparisons of means), which are actually the more valid method of comparing means. When we compare two means using standard error bars and find that the error bars do not overlap, our conclusion should be that the two means are **probably** significantly different – to have a definitive answer we must compute the appropriate statistical tests. For the purposes of this lab, though, the visual test will suffice.

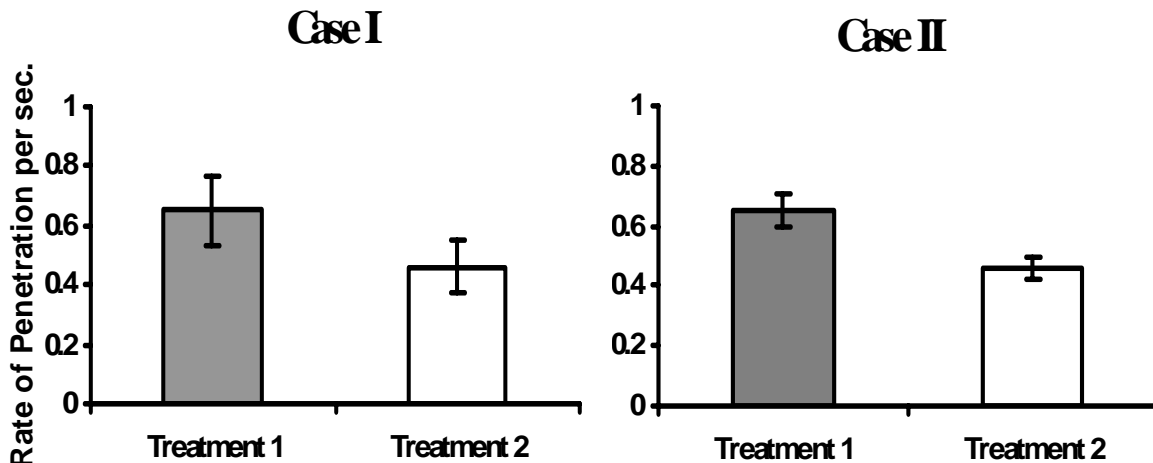


Figure 4.4 Means and standard error bars for the example data.

VIII. WRITTEN ASSIGNMENT

You will use the whole class dataset for your lab assignment, which will be in the Collab folder for your lab section. You are welcome to discuss the class data with your lab partners. However, you should work alone when making the final graphs and writing answers to the questions listed below.

ASSIGNMENT CONTENTS

A. Two graphs: RBC hemolysis and Oocyte data.

You should use the datasheet in Collab folder for your lab section. Open the data spreadsheet with Microsoft Excel and save it in your HOME folder – you might want to give it a different name.

- RBC graph:**
- 1). Convert all values for Rate of hemolysis to Relative Rate of Penetration – i.e., take the reciprocal of rate of hemolysis.
 - 2). Calculate averages and standard errors for each of the test solutions
 - 3). Create a bar graph with the different solutions on the x-axis and rate of penetration on the y- axis. You should have Excel draw error bars for each bar on the graphs, with the size of each error bar equal to the standard error. See the link on the lab web page for help with plotting error bars. See also pages 175-205 in Knisely's "A Student Handbook for Writing in Biology, 2nd ed." for help on using Excel to graph data. The graph should have a complete caption.

- Oocyte graph:**
- 1). Calculate averages and standard errors for each of the three treatments.
 - 2). Create a bar graph with the oocyte treatments on the x-axis and rate of oocyte expansion on the y- axis. You should have Excel draw error bars for each bar on the graphs, with the size of each error bar equal to the standard error. The graph should have a complete caption.

B. Interpret the data.

The first step in interpreting the data is deciding which bars on your bar graph seem to be significantly different. Then, answer the questions below. **Please type all answers.**

- 1.) For the distilled water and NaCl solutions, which seemed to be significantly different?
- 2). How do you explain the apparently significant differences between the water and salt solutions?
- 3). Were 0.145M NaCl and 0.350M NaCl different – why or why not?
- 4). How do you explain differences between the different alcohols? (Hint: Remember that alcohols vary in their ability to cross the membranes, and water **and** alcohol can move across the membrane simultaneously.)
- 5). Were aquaporins expressed in the *Xenopus* oocytes? Support you answer.
- 6). Did TEA seem to inhibit transport of water by aquaporin? Support you answer.