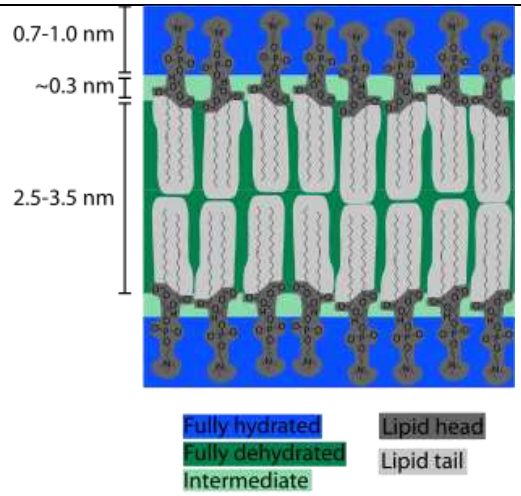
Cell Membrane Permeability

Names:_____

_____

Date:_____

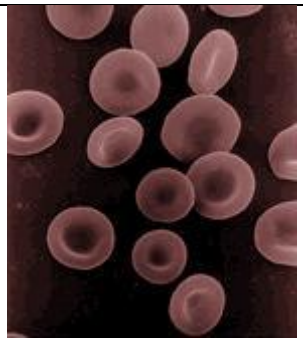
Introduction: The membrane of a cell is only two molecules thick, but it is of incredible importance in terms of the evolution of life on the planet as we know it. The plasma membrane is composed of two layers of **phospholipids**, special molecules that are kind of confused, with a hydrophilic head group (the phosphate end) and a hydrophobic tail end (made of fatty acid chains). These molecules organize into a bilayer, with the head groups facing toward the outside and the inside of the cell, and the lipid tails pointing towards each other (Figure 1). The **amphipathic** nature of these molecules define the role of the membrane, to allow for the separation of "self" from "non-self" and the specialization of organelles inside the plasma membrane of the cell to perform functions that could otherwise be toxic to the cell. Also, membranes are important components of how the cell makes energy (remember cellular respiration and the proton gradient that makes ATP) from glucose, and glucose from the light of the sun during photosynthesis in plants.



http://en.wikipedia.org/wiki/Lipid_bilayer

Figure 1: Lipid bilayer, with chemical structures indicated. Figure from Wikipedia, accessed on June 14, 2012.

Because of the hydrophobic interior of the cell membrane (Figure 1), materials that are hydrophobic themselves can cross the membrane ("like dissolves like"). Polar or charged molecules have a difficult time crossing the membrane because of the hydrophobic core – water molecules "hydrate" the surface of polar molecules, and the hydrophobic core of the membrane strongly resists water. Functional groups like hydroxyl groups (OH) increase the polarity of molecules, and the more hydroxyls a molecule has the more polar it will be. The more polar the molecule, the less likely it will be to cross. This information will be useful when you work through your hypotheses below.



http://en.wikipedia.org/wiki/Red_blood_cell

Figure 2: Human red blood cells, 6-8 microns. Photo from Wikipedia, date accessed June 15, 2012.

However, there are other factors like the size of molecules that can affect whether it will cross, with large molecules having a more difficult time crossing than smaller molecules. To investigate this, comparing molecules that have CH_2O in that rough ratio (the sugars, from methanol to sucrose) could be useful. Then there are molecules that have a shape like

the phospholipids that work better than they do and work to dissolve the membrane itself and surround the pieces of membrane with chemical to wash the membranes away.

Your job today is to work with a list of chemicals, many of them available in your household, to determine which of them will cross a biological membrane and which of them won't. The membranes you will use in the lab are the membranes of red blood cells (RBC's), simple cells that basically consist of membrane and hemoglobin proteins inside. In mammals, these cells don't have a nucleus or any membrane bound organelles inside, and so don't use the oxygen that they are so important for transmitting around the body. The energy inside these red blood cells is made completely from glycolysis to make ATP with NADH recycled to NAD+ by creating lactic acid from pyruvate during the lactic acid fermentation process. We recommend that to prepare for this lab, that you read up on red blood cells in your textbook and other sources (such as http://en.wikipedia.org/wiki/Red_blood_cell). The internal concentration of RBC's is approximately 300 mOsm, or about 0.3M. Most of the solutions we have prepared for you are at 0.3M, and as a result, these solutions will be iso-osmotic with the internal environment of the cell. If a material can cross the membrane of the red blood cell, then it will cross and will cause the cell to lyse, dumping its contents. If the chemical cannot cross, then the cell will not lyse because the internal and external concentration of solutes is the same across the membrane. Some of the materials we have given you to test ionize in the watery (aqueous) environment of the cell, and as a result, may have a higher than 0.3M concentration and you might observe a different response. Use this information to think through the development of your hypotheses below.

Hypothesis formation: For the first part of this laboratory, you are to look at the page of chemical structures provided as a handout, think about what those chemicals would do inside the watery (aqueous) environment of the cell. Would they break apart and ionize? What effect would water have interacting with these chemicals? For each compound, you need to think about and write down what the material would do in water, and if the compound or its components would be able to cross the red blood cell membrane. If you determine that a material can't cross the membrane, then what would the water inside the cell do in the environment of that solution (if you add the solution to the outside of the cell membrane)? In thinking about your hypotheses, you must consider the

1. Hydrophobicity of the molecule (is it charged, polar or nonpolar in the aqueous environment of the cell?). **List here what you remember from the section of the course on chemistry, regarding what functional groups/elements you should look for to determine if a molecule is polar, nonpolar, or charged.**

2. Size of the molecule (is it large or small? Are the atoms similar between molecules, just higher numbers of atoms in the larger molecules?). **What do you look for to determine the size of a molecule?**

3. Shape of the molecule (does the molecule look like a phospholipid?) **Describe and draw what a phospholipid looks like here:**

Before you create your hypotheses, first put the molecules on your chemical structure sheet into as many different groupings as possible, and describe the criteria that organizes each of your groups (for example, will behave like a salt and ionize in a normal cell). Develop at least 4 different groupings, below:

Group 1: _____ Group 2:_____

Group 3: _____ Group 4:_____

Next, form your hypotheses as to whether these materials will cross the membrane, and provide explanations for your hypotheses. In the explanation column, you can/should also draw what you think the cells will do when provided with the chemical.

Compound	Mol. Wt.	M/L	Hypothesis	Explanation
Distilled Water (H_2O)	18.015			
Methanol (CH_3OH	32.04	0.3		
Sodium Laureth Sulfate (dishwashing liquid)	420.0	0.3		
Ammonium Sulfate $(NH_4)_2SO_4$	132.14	0.3		
Acetic Acid (vinegar) (CH_3COOH)	60.05	0.3		
NaCl (table salt)	58.44	0.15		

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Ethylene Glycol (antifreeze) (HOC_2H_4OH)	62.07	0.3		
Isopropanol (rubbing alcohol; C_3H_8O)	60.1	0.3		
Glycerol ($C_3H_8O_3$)	92.09	0.3		
Dextrose ($C_6H_{12}O_6$)	180.16	0.3		
Sucrose ($C_{12}H_{22}O_{11}$)	342.3	0.3		
Butane (C_4H_{10})	58.12	0.3		

Procedure for testing your hypotheses:

1. **Materials Needed**:
    a. Compound microscopes for students
    b. Slides
    c. Cover slips
    d. Stopwatch
    e. Red blood cells
    f. Transfer pipets
    g. Very small containers for solutions; eyedropper bottles to be passed around the lab would work too, and would reduce the need for transfer pipets
    h. Solutions below, plus **distilled water**
        i. Methanol
        ii. Sodium Laureth Sulfate
        iii. Ammonium Sulfate

iv. Acetic Acid
v. NaCl 0.15 M
vi. NaCl 0.3 M
vii. Ethylene Glycol
viii. Dextrose
ix. Sucrose

2. **To test your hypotheses, you will need to:**
    i. Obtain a set of microscope slides. Place a drop of blood on the center of a slide, and gently place a coverslip on it. Gently treat the RBC's because the membranes will rupture if they get shaken too violently, heated up to far, etc.
    ii. Place the slide on the microscope, and focus at the lowest magnification, then and the next level, and then at the highest magnification. RBC's are very small, so this is best done at the highest magnification.
    iii. Describe in detail the shape of your cells before you add any solutions to them
    iv. While focused at the highest magnification, with one lab partner watching, the other one should place a drop of the solution to be tested on the edge of the coverslip. **The material should move under the coverslip and mix with the blood. If it doesn't, then a kimwipe or a paper towel can be used on the opposite side of the coverslip to wick the fluid under it.**
    v. You should then describe your observations. RBC's are plate shaped, with a clear indentation on one side in their normal state, so if they look very round (like a ball) or shriveled, then water or solute has either moved into them or out of them. If you see clear cell membranes in pieces, you know that the cells have broken open (lysed)or been dissolved (in this last case, the membranes tend to congregate all together in clumps surrounded by the material that dissolved them).
    vi. Students should run each solution at least twice, with an opportunity for each student in the group to watch the RBC's respond to the chemical solution.

Evaluating your hypotheses:

What was the shape of your red blood cells before you added anything to them? Describe them in detail and draw a picture of them.

Compound	Mol. Wt.	M/L	Detailed description of your observations on the effect of solution on your cells – draw them as well	How long did it take for most cells to lyse?	Explanation – why do you think this happened?
Distilled Water (H_2O)	18.015				
Methanol (CH_3OH)	32.04	0.3			
Sodium Laureth Sulfate (dishwashing liquid)	420.0				
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Did your hypotheses match your expectations? Why or why not? If they didn't then explain how this affected your thinking about the material and its interaction with the membrane.

i. Distilled water:

ii. Methanol

iii. Sodium Laureth Sulfate

iv. Ammonium Sulfate

v. Acetic Acid

vi. NaCl 0.15 M

vii. NaCl 0.3 M

viii. Ethylene Glycol

ix. Isopropanol

x. Glycerol

xi. Dextrose

xii. Sucrose

Overall, what was the effect of the hydrophobicity of the molecule? Give some specific examples in your answer.

What was the effect of polarity? What happened to the solutions that had more or less –OH groups?

Why are some of the solutions at different concentrations? Is every isoosmotic solution isotonic? Is every isotonic solution isoosmotic? Explain.

How do isopropyl alcohol and glycerol differ from each other? How do you think that affected their ability to cross the membrane?

The effect of molecule size? Give some specific examples in your answer. What was the effect of different CH_2O units? Make a rough graph of the effect of molecule size and number of CH_2O units. What if we had different sized molecules with different polarities?

The effect of the shape of the molecule? Give some specific examples in your answer.

Resources used to create lab:

Amherst College instructors, 2001. Bio 19: Permeability of the Plasma Membrane, BIO 19 Laboratory Manual.

Wikipedia, accessed June 2012.