

# MEMBRANE FUNCTION

## CELLS AND OSMOSIS

Consider placing a cell in a beaker of pure water (Fig. 1). The cell contains a water solution with many different kinds of dissolved molecules and ions so that it is about 94% water. Thus, the concentration of water is greater outside the cell than inside (there is a concentration gradient for water). Since the plasma membrane does not prevent its diffusion, water will flow into the cell by osmosis. The cell will swell and eventually burst. The bursting of a cell is called lysis, but since red blood cells are often the subjects of study, it's commonly called hemolysis.

On the other hand, if the cell is placed in a 10% sucrose (sucrose is not freely permeable) solution, the solute concentration outside the cell is greater than inside, and thus, the concentration gradient for water would be in the reverse direction. Water would leave the cell causing it to shrink and appear crenate (Fig. 2).

This demonstration makes it clear that the fluids which bathe the cells must be kept at the same osmotic strength as the intracellular fluids.

Giving the concentration of solutions in percent by weight can lead to problems when studying osmosis. For example, in comparing a 10% solution of glucose and a 10% solution of sucrose (Fig. 3), each has the same weight of sugar (10g per 90g H<sub>2</sub>O), but because glucose is about half the size of sucrose, the glucose solution has about twice the concentration of dissolved solute molecules. This means that the 10% glucose solution has almost twice the osmotic strength of the 10% sucrose solution.

To get around this problem, solutions are often described by their solute concentration using the Molar designations. To make a 1 Molar solution, water is added to the molecular weight in grams to make exactly 1 liter of solution (Fig. 4). In comparing Molar solutions, the number of solute

molecules per unit of volume (liter or milliliter) is always the known. Thus, a liter of 1M glucose solution has exactly the same number of dissolved molecules as a liter of 1M sucrose solution (Fig. 4) even though the weight of sucrose used was twice that of glucose.

Biological solutions often contain a mixture of solute molecules. The molar designation doesn't work for these solutions because it is defined in terms of the molecular weight of one

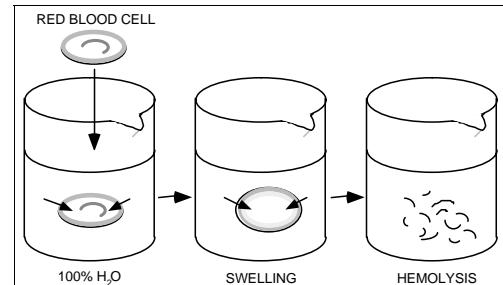


FIGURE 1. A cell is placed in a beaker of pure water. Water enters the cell and hemolysis occurs.

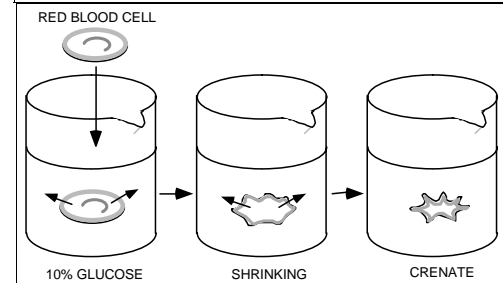


FIGURE 2. A cell is placed in a 10% Sucrose Solution. Water leaves the cell, it shrinks and is finally crenate in appearance.

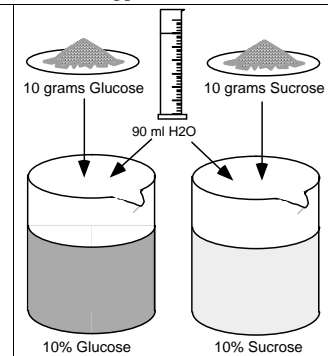


Figure 3. Comparison of the composition of solutions by weight

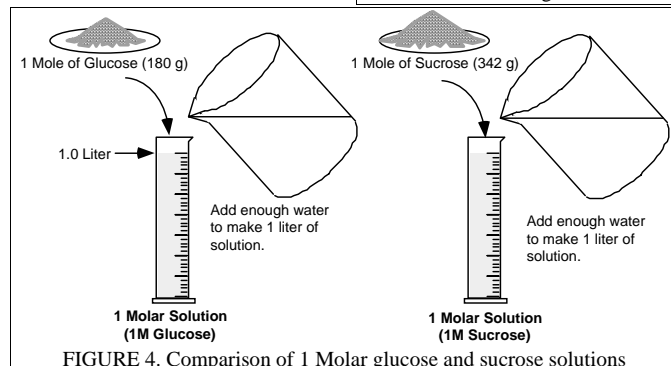
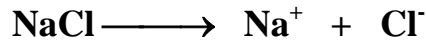


FIGURE 4. Comparison of 1 Molar glucose and sucrose solutions

type of solute molecule per solution. When there is a mixture of solute molecules in a solution, concentrations of solute (and water) are designated by the Osmole (Osm.). A 1 Osm solution has Avogadro's number (1 mole) of solute molecules in 1 liter of solution. If water is added to 0.5 mole of sucrose and 0.5 mole of glucose to make 1.0 liter, the resulting solution is 1.0 Osm and has the same solute concentration as any other 1 Osm or 1M solution.

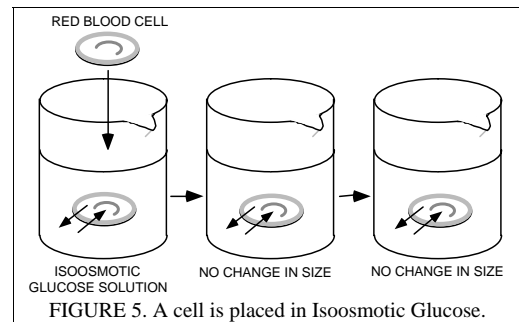
Ions contribute to osmolarity in the same way as glucose or other solutes do. This changes the relationship between Molarity and Osmolarity. Sodium Chloride for example, completely ionizes when it dissolves in water:



Since each molecule of NaCl dissociates into two ions, each Mole of NaCl yields 2 Moles of ions. Since both ions are osmotically active, a 1 M NaCl solution has an osmolarity of 2 Osm. A NaCl solution will be used in the next exercise, so keep in mind that a 0.5 M NaCl solution has the same osmolarity as a 1 M Glucose solution.

Solutions which have the same solute concentration as intracellular fluid are called Isoosmotic. Hypoosmotic solutions have solute concentrations less than intracellular fluid, and Hyperosmotic solutions are more concentrated than intracellular fluid.

If a cell is placed in an isoosmotic solution (Fig. 5), the concentration of solute and water on either side of the membrane is the same, and one would not expect osmosis to occur. The cell would neither swell or shrink, but rather remain the same size (this assumption is true only as long as the solute is not permeable across the plasma membrane... more on this later). Cells placed in hypoosmotic solutions tend to swell and hemolyze (Fig. 1), while cells placed in hyperosmotic solutions tend to shrink and become crenate (Fig. 2).



This strategy is used to investigate the osmolarity of the intracellular fluid the demonstration that follows.

## MATERIALS

1. Five test tubes and rack
2. 1.0 M NaCl solution
3. Blood - diluted with saline. Mix well before taking sample!
4. Plastic Squeeze Pipettes
5. Exam gloves
6. Compound Microscope, clean slides and cover slips

## PROCEDURE

7. Set up five clean test tubes in the rack and label: 1.0M; 0.3M; 0.15M; 0.075M; H<sub>2</sub>O
8. Make 10 ml of each of the dilutions using the 1.0 M NaCl stock and place in the appropriately labeled tubes.
9. Add four drops of blood to each tube, and mix by inversion. Make sure the person handling the horse blood is wearing gloves.
10. Note the appearance of each tube and record in Table 1. Set the tubes aside for 30 minutes, but mix the tubes well every five minutes.

11. After 30 minutes, mix all the tubes well. Compare their appearance by holding test tube rack up to a white piece of paper. A transparent solution indicates that hemolysis has occurred. If the solution is cloudy, there hasn't been any hemolysis. Note your observations in Table 1.
12. Prepare a slide from each of the solutions that haven't hemolyzed, using one drop of well- mixed solution and cover with a cover slip.
13. Examine the red blood cells under the 1,000X objective (oil immersion) and note their size and appearance. Since no measuring device is available, make sure you compare solutions carefully. Record your observations in Table 1.

Table 1. Osmolarity of Intracellular Fluid Data			
NaCl Test Solution	Osmolarity	Appearance of Tube (clear, opaque, etc.)	Size and Appearance of Cells
H <sub>2</sub> O			
0.07 M			
0.15 M			
0.30 M			
1.00 M			

## **ANALYSIS OF CELLS AND OSMOSIS**

---

You should now have enough information to determine which of the test solutions are hypoosmotic, hyperosmotic, and isoosmotic. Keep in mind that the red cells will swell and eventually hemolyze in hypoosmotic solutions. They will shrink and appear crenate in hyperosmotic solutions; and will remain the same size indefinitely in isoosmotic solutions.

You should also note that once you have identified the isoosmotic solution, you then know the osmolarity (solute concentration) of the intracellular fluid of red blood cells. Since these cells don't swell or shrink while in the blood, then you can assume that the plasma osmolarity is the same.

## **MEMBRANE PERMEABILITY: THE EFFECT OF MOLECULAR SIZE AND POLARITY**

---

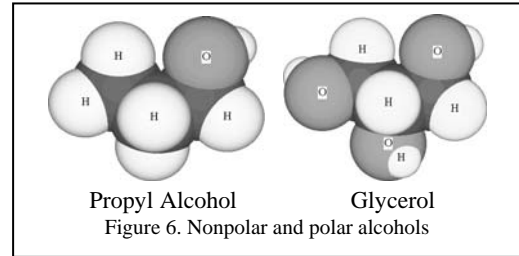
### INTRODUCTION

In order to study the effect of molecular polarity and size on membrane permeability, you will place red blood cells into isoosmotic solutions of various alcohol solutions. If the alcohol is not permeable across the membrane, the cells will not gain or lose water due to osmosis (because the solution is isoosmotic). If however, the alcohol is permeable, the alcohol molecules will diffuse through the membrane (due to a concentration gradient) and the cell will expand and eventually hemolyze.

In the first part of this experiment, you put the cells into two isoosmotic alcohol solutions: propyl alcohol and glycerol. Both of these molecules are about the same size (Fig. 5), but greatly differ in their polarity,

with glycerol being much more polar than propyl alcohol. Thus, any difference in observed hemolysis time must be due to differences in polarity, not size.

In the second part, you add the cells to three isoosmotic solutions: ethylene glycol; glycerol; and ribose (Figure 6). All of these molecules have one -OH group for each carbon in their structure, and therefore have about the same polarity. They do differ in size however, having 2,3 and 5 carbon atoms (and -OH groups) respectively. So in this case, any difference in observed hemolysis time must be due to differences in size, not polarity.



### MATERIALS AND EQUIPMENT

1. 5 - 3 ml plastic squeeze pipettes
2. 5 small test tubes in rack
3. Isoosmotic Alcohol solutions (0.3M): Propyl Alcohol; Glycerol; Ethylene Glycol; and Ribose.
4. Horse blood diluted with saline. Mix well before taking sample!
5. Parfilm squares to cover test tubes.
6. Disposable exam gloves
7. Label your squeeze pipettes: "blood;" "Propyl Alcohol;" "Glycerol;" "Ethylene Glycol;" and "Ribose." Make sure you use only the appropriately labeled pipette for each solution.

### THE EFFECT OF MOLECULAR POLARITY

8. Label 2 small test tubes: one Propyl Alcohol and the other Glycerol.
9. Add 3 ml of appropriate solution to each tube with a squeeze pipette.
10. To each tube add two drops of horse blood and immediately cover with parafilm square and mix by inversion. Record the time the blood is added in Table 2
11. The tubes should be cloudy immediately after the addition of blood. As hemolysis occurs, the solutions will become transparent.
12. Record the time when the tubes become transparent in Table 2.
13. Calculate the "Hemolysis Time" by subtracting the time the blood was added from the time the tube became transparent and record the number of minutes and seconds in Table 2.

Table 2. Effect of Molecular Polarity Data					
Molecule	Time blood added	Time solution became transparent	Hemolysis Time	Number of:	
				# Carbon Atoms (size)	Polar OH Groups
Propyl Alcohol				3	1
Glycerol				3	3

### THE EFFECT OF MOLECULAR SIZE

14. Label three tubes: Ethylene Glycol; Glycerol; and Ribose.
15. Add 3 ml of appropriate solution to each tube with a squeeze pipette.
16. To each tube add two drops of horse blood and immediately cover with parafilm square and mix by inversion. Record the time the blood is added in Table 3

17. The tubes should be cloudy immediately after the addition of blood. As hemolysis occurs, the solutions will become transparent.
18. Record the time when the tubes become transparent in Table 3.
19. Calculate the "Hemolysis Time" by subtracting the time the blood was added from the time the tube became transparent and record the number of minutes and seconds in Table 3.

Table 3. Effect of Molecular Size Data					
				Number of:	
Molecule	Time blood added	Time solution became transparent	Hemolysis Time	# Carbon Atoms (size)	Polar OH Groups
Ethylene Glycol				2	2
Glycerol				3	3
Ribose				5	5

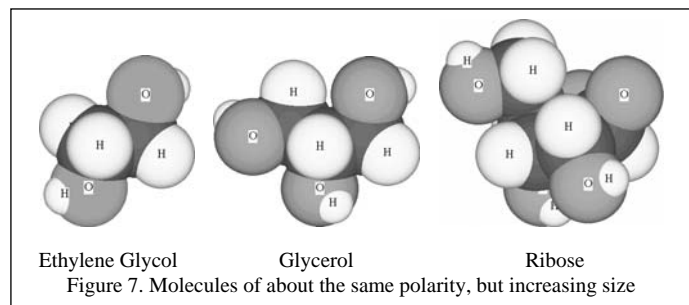
## ANALYSIS OF MEMBRANE PERMEABILITY

### MOLECULAR POLARITY

1. Examine the structures of Propyl Alcohol and Glycerol in Figure 6 and in the molecular models provided in the lab.
2. Note that both have a backbone of three Carbon atoms and about the same overall size.
3. The main difference between them is the number of polar -OH groups: Propyl Alcohol has only one, while Glycerol has three, one for each Carbon atom.
4. This makes Glycerol a much more polar molecule than Propyl Alcohol.
5. Thus differences that you observe in permeability must result more from the polar nature of the molecules rather than from their dimensions(size).
6. Keep in mind that the more nonpolar a molecule is, the more easily it will dissolve in lipid.
7. If molecules are to cross the plasma membrane and enter a cell, they must dissolve in the middle lipid layer of the plasma membrane.
8. One would predict then, the more lipid soluble the molecule (the more easily it dissolves in lipid), the more permeable it will be and the shorter its hemolysis time.

### MOLECULAR SIZE

9. Examine the structures of Ethylene Glycol, Glycerol and Ribose in Figure 7 and in the molecular models provided in the lab.
10. All of the molecules in this series have one polar -OH group associated with each Carbon atom (Fig. 7).
11. This makes the polarity of these three molecules roughly the same.
12. Thus, any differences observed in permeability must be due to their molecular size rather than polarity.



## QUESTIONS FOR YOUR REPORT

---

1. (2 pts) Present an abstract of your work. Your abstract should include a brief description (1/2 page) of 1) what you set out to learn, 2) what procedures you performed, and 3) a summary of your data or discoveries.
2. Explain why a 1.0 M solution of NaCl has an osmolarity of 2.0 Osm.
3. Predict the osmolarity of red blood cell cytoplasm. Support your prediction using your observations from the demonstration. Include Table 1 in your report.
4. Explain why osmotic homeostasis is an important consideration for the all the body fluid compartments.
5. Use your data to support the argument that a molecule's polarity affects its membrane permeability. Include Table 2 in your report.
6. Use your data to support the argument that molecular size affects a molecule's membrane permeability. Include Table 3 in your report.
7. Calculate the osmolarity of a solution containing 100 grams of KCl in 250 ml of water. (Assume the molecular weight of K is 39 and Cl is 35)
8. (2 pts)  $\text{MgCl}_2$  can break down into  $\text{Mg}^{2+}$  and  $\text{Cl}^-$  ions in water. Calculate the *percent solution* of  $\text{MgCl}_2$  necessary to give you a 2 Osm solution. (Mg = 24 g/l. Assume that  $\text{MgCl}_2$  has 0 density)