Lab 1: Cell transport Mechanisms, Permeability and Osmosis

Exercise 1: Interpreting and understanding Osmolarity and Tonicity

Each cell in your body is surrounded by a plasma membrane that separates the cell from interstitial fluid. The major function of the plasma membrane is to selectively permit the exchange of molecules between the cell and the interstitial fluid, so that the cell can take in the substances it needs while expelling the ones it does not. These substances include gases, such as oxygen and carbon dioxide; ions; and larger molecules such as glucose, amino acids, fatty acids, and vitamins.

Molecules move across the plasma membrane either passively or actively. In active transport, molecules move across the plasma membrane with the expenditure of cellular energy (ATP). In passive transport, molecules pass through the plasma membrane without the expenditure of any cellular energy. Examples of passive transport are simple diffusion, osmosis, and facilitated diffusion. Simple diffusion is the spontaneous movement of molecules across a biological membrane's lipid bilayer from an area of higher concentration to an area of lower concentration. Osmosis is the movement of water across a semipermeable membrane. Facilitated diffusion is the movement of molecules across a selectively permeable membrane with the aid of specialized transport proteins embedded within the membrane.

Diffusion

Diffusion is defined as the movement of molecules from one location to another because of their random motion. All molecules, including solids, liquids and gases are in continuous motion. This motion causes collisions between neighboring molecules, thus altering directions and creating a state of "random" motion. This random motion can be further altered by temperature, with increases in temperature stimulating a more rapid random movement.

Simple Diffusion

Simple Diffusion is the spontaneous movement of molecules across a biological membrane and is driven by concentration gradients. More specifically, molecules will move across a biological membrane from an area of higher concentration to an area of lower concentration. This movement will eventually lead to a state in which the concentration of the diffusing solutes will be constant in space and time. The rate at which a molecule moves across a membrane depends in part on the mass, or molecular weight, of the molecule. The higher the mass, the slower the molecule will diffuse (rate proportional to $1/MW^{1/2}$). Another key factor affecting the rate of diffusion across the membrane is the solubility of the substance. Solutes that are capable of simple diffusion must be able to pass through the hydrophobic core of the membrane. Nonpolar substances, such as oxygen, carbon dioxide, steroids, and fatty acids, will diffuse rapidly while polar substances, having a much lower solubility in the membrane phospholipids move through more slowly, or not at all. Diffusion across cellular membranes tends to equalize the concentrations on the two sides of the membrane. In addition to lipid solubility and size, diffusion rate is proportional to the cross-sectional area of the membrane and to the difference in the concentration of the diffusing solute on the two sides of the membrane. These relationships are expressed by Fick's first law of diffusion, below:

$J = -DA(\Delta C/\Delta X)$

J	=	net rate of diffusion in moles or grams per unit time
D	=	diffusion coefficient of the diffusing solute in the membrane (this coefficient considers the size of the substance as well as its solubility in the membrane)
A	=	area of the membrane
ΔC	=	concentration difference across the membrane
ΔX	=	thickness of the membrane

Facilitated Diffusion

Facilitated diffusion permits the movement of substances across the membrane that are too big and/or too polar to pass through the membrane. The name, facilitated diffusion, implies that these substances require assistance to cross the membrane. Indeed, there are integral membrane proteins that assist in the transport. Ions, such as Na^+, K^+, Ca^{2+} and Cl^-, move through membrane via channel proteins. Picture a fluid filled tube passing through the membrane that the ions can diffuse through. These channels can be specific for a single ion or can allow multiple ions to pass through. Additionally, the channels are often gated and only open in the presence of certain signals. We will discuss this gating in more detail in lecture. In addition to ion channels, other integral membrane proteins called transport proteins mediate the movement of larger, polar molecules, like glucose, across the membrane. Transport proteins require binding of the solute to specific receptors and then a conformational change to move the solute across the membrane. Unlike simple diffusion, facilitated diffusion exhibits saturation and its rate is directly related to the concentration of specific transport proteins within the membrane. More importantly, this type of transport, like simple diffusion, does not require an input of energy.

Osmosis

Osmosis is the process by which a fluid (**solvent**) tends to move through a membrane from a solution of lower **solute** (particles) concentration to a solution of higher solute concentration. (*Note: An important condition for osmosis is that the membrane is impermeable to the solutes). Thus, water moves down its concentration gradient, across the membrane, toward a region of higher solute (lower water) concentration.

In biological systems, the universal **solvent** is water, whereas a large variety of organic and inorganic particles comprise the **solute** component of the solution. As more solute is added to a solution, the water concentration decreases in direct proportion to the increasing solute concentration. As the concentration of solutes increases on one side of a membrane, the tendency for water to diffuse, by osmosis, toward the region of greater solute concentration concomitantly increases. Thus, the concentration of solutes is an important determinant for the rate of osmosis.

One convenient way to express solute concentrations is in terms of molarity (M, moles/L). For example, glucose has a molecular weight of 180, therefore a 1 M solution of glucose contains 180 g of glucose dissolved in 1 liter of solution. Because glucose does not dissociate (come apart) in solution, its molarity is a good measure of its particle concentration, and therefore a good indicator of its effect on osmosis. However, compounds such as NaCl, when placed in water, dissociate into separate ions (Na+ and Cl^-). Thus, although the molecular weight of NaCl is only 58.5 grams, if we were to dissolve 58.5 grams of NaCl in 1 liter of fluid, we would have 2 particles for every NaCl added. Hence, the number of particles in a solution for 58.5 grams of NaCl would be greater than the number of particles for 180 grams of glucose and the overall osmotic effect of NaCl twice that of glucose.

The osmotic effect is determined by the number of particles in solution, not by the substance's molecular weight. Knowing a solution's molarity will not necessarily determine the osmotic effect of that solution. To express the osmotic effect of a substance we use **osmolarity**, which is defined as the number of osmotically active particles in one liter of pure water.

To illustrate, a 1 molar solution of glucose (180 g glucose in one liter) is also a 1 osmolar solution. However, in contrast, a 1 molar solution of NaCl (58.5 g NaCl in one liter) is a 2 osmolar solution. Thus, a 1 M NaCl solution will have twice the osmotic effect as a 1 M glucose solution. In biological solutions we deal with very small concentrations of solutes, so instead of osmolar solutions we often refer to milliosmolar solutions. The normal osmolarity of human body fluids (intracellular and extracellular fluids and plasma) is about 300 milliosmolar (normal range is from 280 to 296 mOsm).

<u>Osmosis and Physiological Systems</u>

To compare the relative concentrations of particle in two solutions we use the terms isosmotic, **hypoosmotic** and **hyperosmotic**. For example, if solution A has more particles per liter than solution B we say that solution A is hyperosmotic compared to B. If both have the same number of particles per liter, we say they are isosmotic, and if solution A has fewer particles per liter than B we say it is hypoosmotic to B. We can use these terms to compare solutions regardless of the nature of the particles.

In physiological systems some particles can freely cross the cell membrane (**penetrating solutes**) and others cannot (**non-penetrating solutes**). In terms of osmosis, it is only the not-penetrating ions that are important. When a cell is placed in a solution, water and penetrating solutes will move until the intracellular and extracellular osmolarites are equal. Therefore, in physiological systems solutions are described based on the effect they have on the cell. If a cell is placed in a solution that contains a lower concentration of non-penetrating solutes than are found within the cell, it will swell and may even burst of lyse and we say that the solution is **hypotonic**. On the other hand, if the cell is placed in a solution that has a higher concentration of non-penetrating solutes than the cell it will shrink or crenate and we say that the solution is **hypertonic**. If there is no change in cell size, we say that the solution is **isotonic**. Hence, depending on the nature of the solutes a hyperosomotic solution could be either isotonic or hypotonic to the cell, while a hypoosmotic solution will always be hypotonic to the cell. As you can imagine, in a clinical setting it is vital to know the tonicity of a solution before infusing it into a patient.

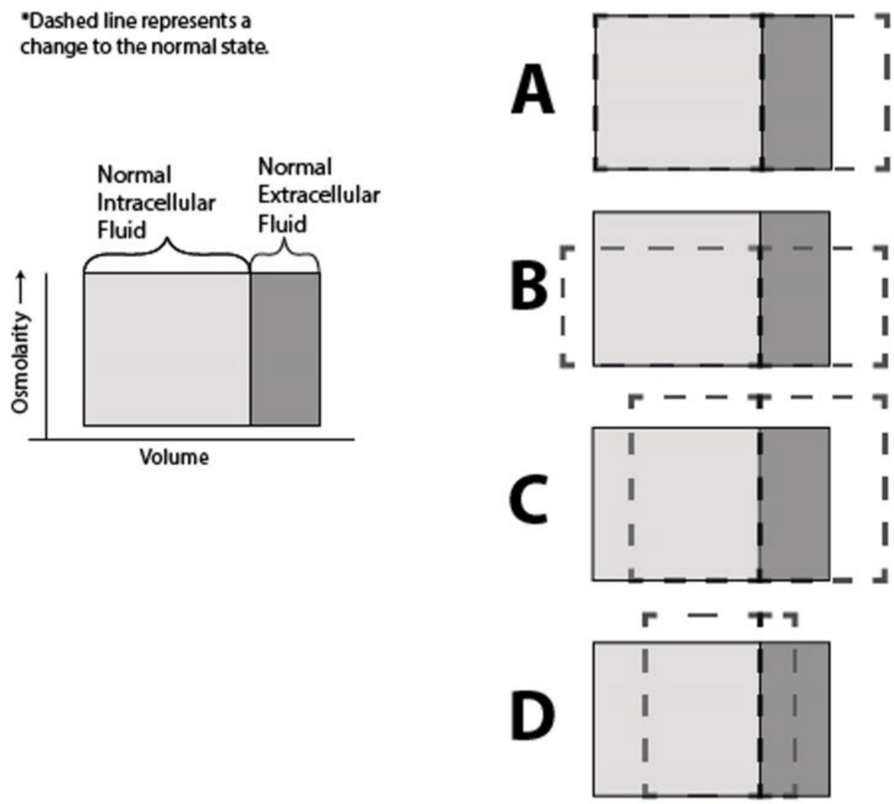
Questions (Exercise 1)

1. Complete the following table by correctly identifying the osmolarity and tonicity of the listed solutions. Use the following prefixes: iso = same, Hyper = more, Hypo = less

SOLUTION	ALSO KNOWN AS	OSMOLARITY	TONICITY
0.9% Saline	Normal saline		
5% Dextrose in 0.9% Saline	D5-normal saline		
5% Dextrose in water	D5W		
0.45% saline	Half-normal saline		
5% dextrose in 0.45% saline	D5-half normal saline		

Osmosis problems.

2. The diagram represents various states of abnormal hydration. In each diagram, the normal state (dark grey and light grey) is superimposed with the abnormal state (dashed lines) to illustrate the shifts in the volume (width of rectangles) and total osmolarity (height of rectangles) of the extracellular and intracellular fluid compartments. In other words, if the dotted lines increase in height there was a change in osmolarity, if the dotted lines increase in width there was a change in volume. Look at example A. Something was added to the ECF that caused the volume to increase (ie., the dotted line moved to the right) but didn't change the osmolarity (ie, the dotted line didn't move height) and the dotted line in the ICF didn't move. Thus, an isotonic solution was added to the ECF of A increasing the volume of the ECF but did not affect the ICF volume nor osmolarity.



Which of the following CORRECTLY identifies a cause for the lettered outcomes above? In other words, this is essentially a true/false question, does the description of A (below) match the diagram labeled A (above in the picture). You may also want to try and match a description below with its correct diagram above, but note that not all descriptions will work. Hopefully, you will be able to say True or False on each one below

(A matches A above T/F) A case of severe dehydration that has caused the loss of an excessive amount of salt and water
(B matches B above T/F) Adding a couple of liters of an Isosmotic IV solution called lactate ringers solution to the ECF. Lactate ringers solution has "non-permeable" solutes.
(C matches C above T/F) Adding a couple of liters of an IV solution that is labeled as 2.5% dextrose to the ECF.
(D matches D above T/F) A condition called diabetes insipidus that causes kidneys to excrete excessive amounts of free water (water without electrolytes) as urine (out of the body).

3. A 70 kg subject has a total body water of 40 liters, with 25 liters in the intracellular compartment (ICF) and 15 liters in the extracellular compartment (ECF). The osmolarity of both the ECF and ICF is 290 mOsm/liter, initially. Subsequently, this person drinks 200 ml of 0.333 M $CaCl_2$ which is completely absorbed from the gastrointestinal tract into ECF. (MW of $CaCl_2$ = 111.7, i=2.1)

   What will the osmolarity of the ECF compartment be prior to osmosis occurring? Assume that the $CaCl_2$ solution is evenly distributed throughout the ECF compartment and that the cell membranes are completely impermeable to Ca^{++} and Cl^-. What will the ECF and ICF volumes be after equilibrium is established?

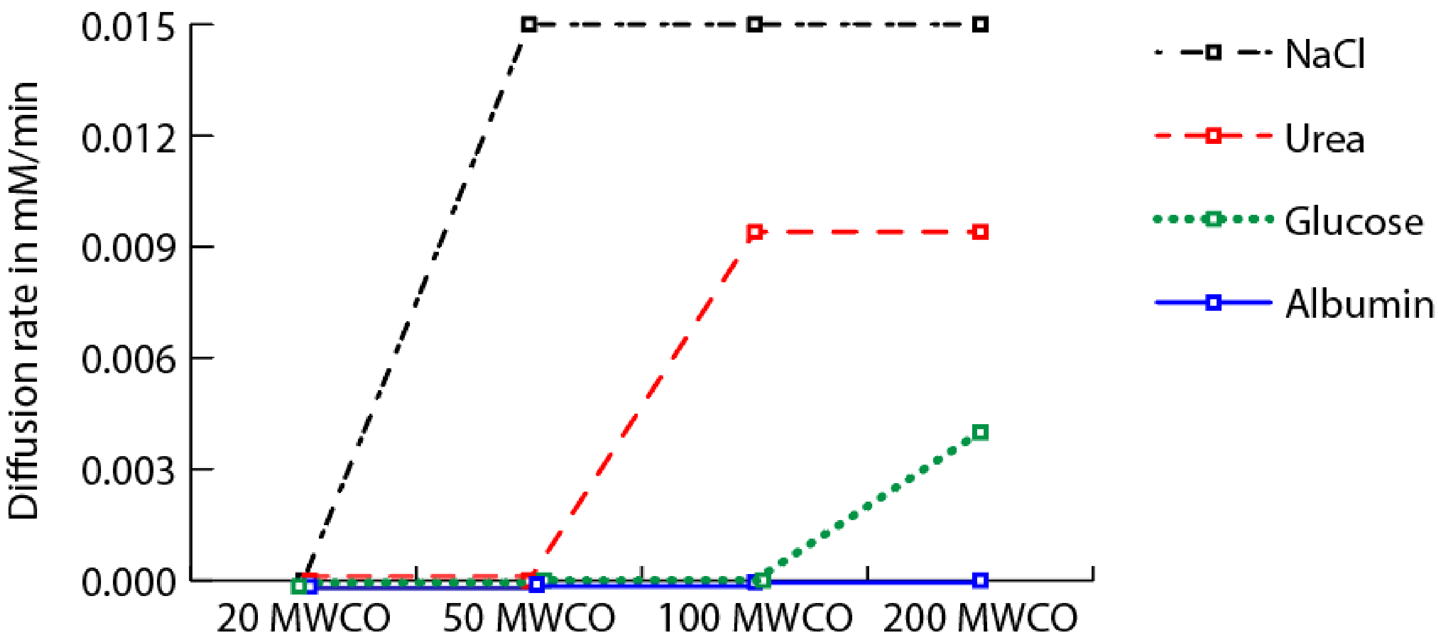
4. You are a third-year medical student, alone in the hospital emergency room. It is a quiet night, and the residents are getting some needed sleep. A patient, Ms M.B., is brought in showing serious signs of dehydration. You try to give her water, but she vomits it up. Feeling that you must try something else and not wanting to wake the residents, you administer 1 liter of sterile distilled water intravenously. For simplicity assume the RBC's have an osmolarity of 300 mOsm/Liter and that all the particles in the RBC's are non-penetrating. Also assume that the infused water does not move into the extracellular compartment but stays in the circulatory system. Prior to the IV Ms M.B.s plasma volume was 3 L and her RBC volume was 2L.

   a. Calculate Ms M.B.'s plasma osmolarity after the infusion has mixed with her plasma but before any diffusion across RBC membranes.
   b. Calculate the plasma and RBC osmolarity after the infused water equilibrated between her plasma and RBC's.
   c. What would the final plasma volume and RBC volume be after the water had equilibrated?

If you want even more practice you may [CLICK HERE] to see yet another practice problem

Exercise 2: Interpreting and Understanding Simple Diffusion

Consider data obtained on the diffusion rates of 9 mM solutions of the following solutes: NaCl, urea, albumin, or glucose. The data was obtained using the following system. An aquarium was filled with water and then divided into two fluid compartments by the addition of a semipermeable membrane. The semipermeable membrane design allowed for the easy exchange among membranes of varying molecular weight cut offs (MWCO) ranging in pore size of 20, 50, 100 and 200. The diffusion rates for each solution, subjected to the different MWCO membranes, was then determined by adding a solution to one side and allowing the solute to move to equilibrium (assuming the solute was able to diffuse). Once equilibrium was reached, the concentration of the solute was compared between the two sides and an average diffusion rate (mM/min) was calculated. The tank was then rinsed and prepared for the addition of another solution or different membrane until all solutions were compared against all membranes. The data obtained can be found in the following graph.



Questions (Exercise 2)

1. Rank the relative size of each molecule.
2. Rank the diffusion rates of the molecules and explain the observed differences. How does this correlate with Fick's 1st Law of Diffusion?
3. Why does the diffusion rate not change for NaCl with an increase in MWCO from 50 to 100 to 200?

Exercise 3: Diffusion through membranes

The following experiment will utilize dialysis tubing as a representation of the cell membrane.

Procedure:

a. Cut five 10-inch pieces of dialysis tubing and soak them in 100 ml distilled water for about 5 minutes.
b. While the dialysis tubing is soaking, set out five paper cups (label them A-E) and add 6ml of "stock" solution (20%) to cups B-E (the cup labeled A will get distilled water).
c. Add water to dilute accordingly: A = 6ml distilled water (DW), B = add 4.5ml DW to 6ml stock (5% solution), C = add 3ml DW to 6ml stock (10% solution), D = add 1.5ml DW to 6ml stock (15% solution), E = don't add water, already at 20%.
d. Remove the dialysis tubing from soaking and tie one end of each tubing in a knot
e. Add 6ml of each dilution to a corresponding bag, be careful to not mix up the bags!
f. Push out most of the air and then tie the other end of the dialysis tubing. When all is said and done, you should have 5 solution filled dialysis bags (cells) that range in concentrations from 0% to 20% labeled (A-E).
g. Carefully blot each bag dry and then weigh each bag on the scale. (Do not mix up the bags!) Record the weight of each bag at time zero in the chart below.
h. Pour 30 ml of "bath" solution into five additional cups. Add one dialysis bag (A-E) to each cup (Do not

mix up the bags!).

i. Allow the bags to soak for 10 minutes and then remove them, blot each bag dry, weight each bag, and replace back into the bath solution for another 10 minutes. Record the weight.

j. Repeat this procedure for 50 minutes, record the weight of each bag in the chart below.

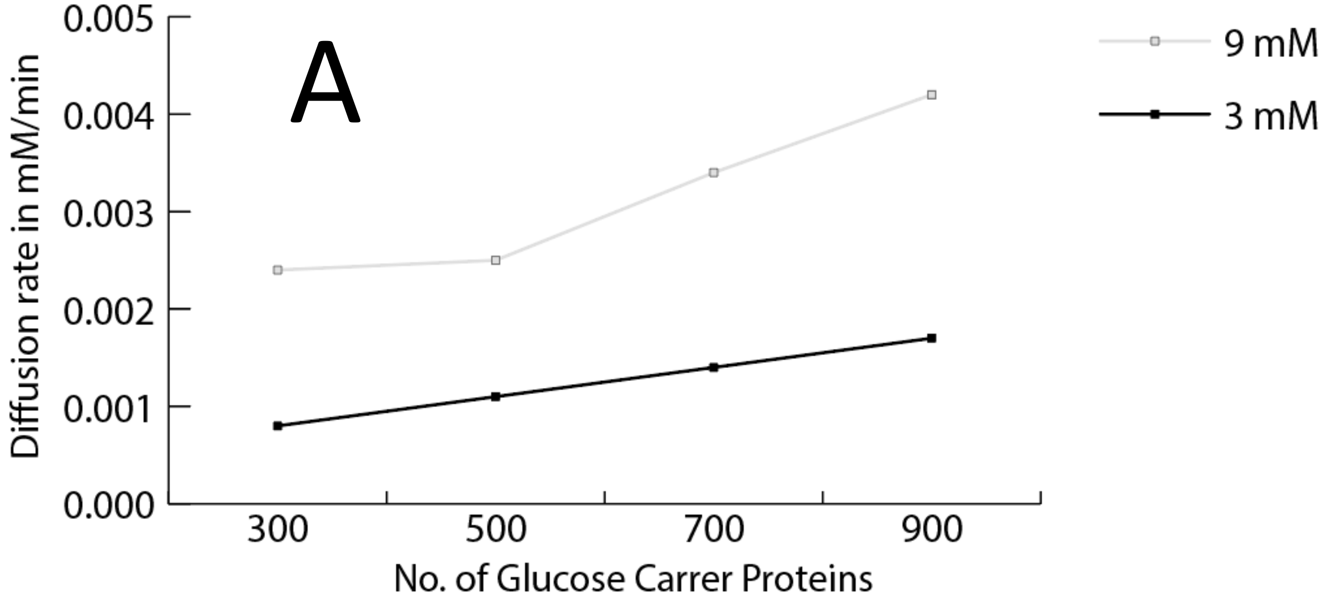
Materials	Time zero	10 minutes	20 minutes	30 minutes	40 minutes	50 minutes
Bag “Cell” A Weight (grams)						
Bag “Cell” B Weight (grams)						
Bag “Cell” C Weight (grams)						
Bag “Cell” D Weight (grams)						
Bag “Cell” E Weight (grams)						

Questions (Exercise 3)

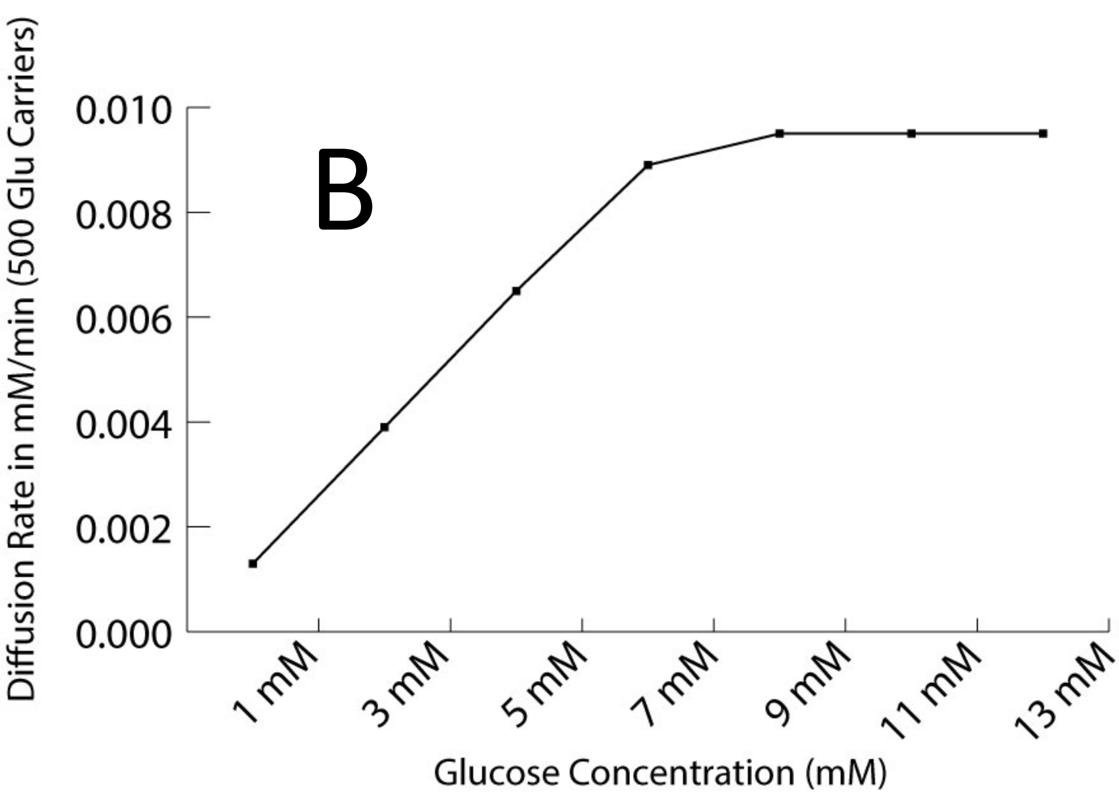
1. Is the dialysis membrane selectively permeable? What evidence supports your answer?
2. Graph the results and then based on your data, what percent solution is the stock solution?
3. Which bags contain hypotonic solutions at 30 minutes?
4. Which bags contain isotonic solutions at 30 minutes?
5. Which bags contained hypertonic solutions initially?

Exercise 4: Interpreting and Understanding Facilitated Diffusion

In **graph A**, data was collected from a two-chamber system separated by a membrane but instead of using MWCO as the barrier, membranes were added that contained carrier proteins for glucose. The amount of carrier proteins could be modified from 300, 500, 700 to 900 carriers. Two different solutions of glucose were used, either 3mM or 9mM. Diffusion rates were measured in mM/min.



In **graph B**, data was collected from the same system but with a fixed number of glucose carriers (500) but with varying mM concentrations of glucose (1,3,5,7,9,11 and 13). Diffusion rates were measured in mM/min.



Questions (Exercise 4)

1. At a given glucose concentration (either 3 or 9mM), how does the amount of time it takes to reach equilibrium change with the number of carriers used? (Graph A)
2. What happens to the rate of diffusion as the concentration gradient is increased? (Graph B)

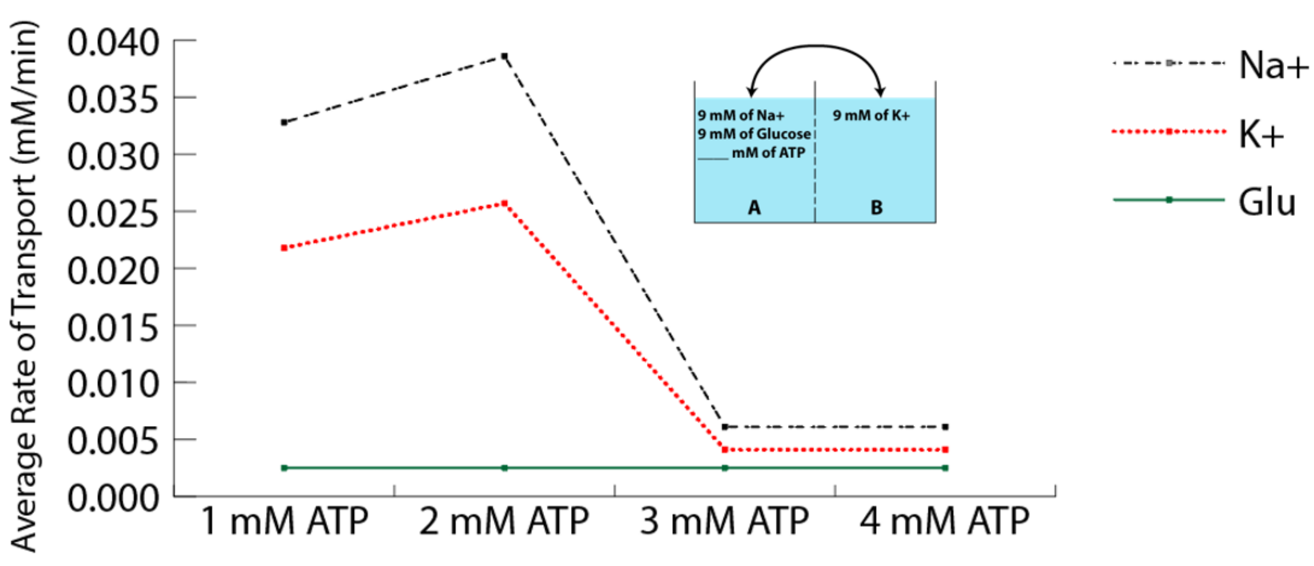
Exercise 5: Interpreting and Understanding Active Transport

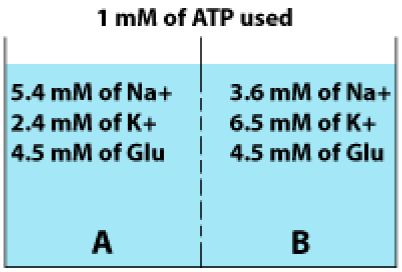
The graph represents data obtained from a two-chamber system separated by a membrane containing Na/K ATPase pumps (primary active transport proteins). Each side is filled with either a 9mM NaCl solution or a 9mM KCl solution. The membrane can be altered to contain various numbers of Na/K ATPase pumps and the amount of ATP can be altered from 1mM up to 10mM. The data is displayed as rate of transport.

The graph below represents the average rate of Na+, K+, and Glucose transport over a period of time that it takes for all transport to come to completion.

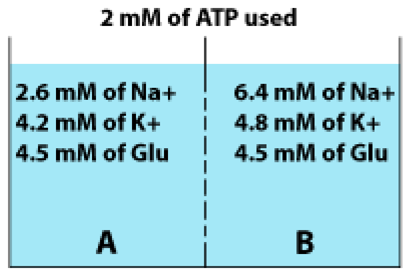
4 experiments were performed where side "A" started with 9 mM of Na+ and Glucose and side "B" started with 9 mM of K+. Each experiment used a different amount of ATP introduced to side A. The experiment was allowed to run until all transport of all solutes stopped.

The images below the graph shows the concentrations of solutes after transport has completed and the time it takes to complete all transport is shown below the images.

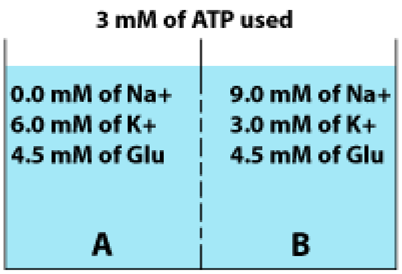




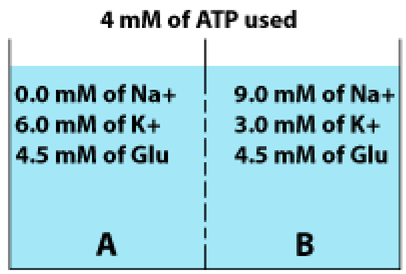
Na+ transport finished in 2 min
K+ transport finished in 2 min
Glu transport finished in 60 min



Na+ transport finished in 5 min
K+ transport finished in 5 min
Glu transport finished in 60 min



Na+ transport finished in 50 min
K+ transport finished in 50 min
Glu transport finished in 60 min



Na+ transport finished in 50 min
K+ transport finished in 50 min
Glu transport finished in 60 min

Questions (Exercise 5)

1. As each run progressed with varying amounts of ATP (mM), the concentrations of the solutes changed in between side A and Side B. The rate also slowed down markedly even stopping before completion. Why?
2. Did the amount of ATP added make any difference in the rate of transport? Why?
3. Why is the transport rate slower when you add 3 and 4 mM ATP compared to when you add only 1 to 2 mM ATP?