pH, CELL STRUCTURE, DIFFUSION & OSMOSIS

Links with Useful Information & Animations

pH, Acids & Bases

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http://www.bgfl.org/bgfl/custom/resources_ftp/client_ftp/ks3/science/acids/neutralisation.htm

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Molecules that fall apart. http://www.chemheritage.org/EducationalServices/pharm/asp/acid01.htm
The Weak & the Strong. http://www.chemheritage.org/EducationalServices/pharm/asp/acid02.htm
Acids, Bases, Salts. http://www.chemheritage.org/EducationalServices/pharm/asp/acid03.htm
Water: Acid & Base. http://www.chemheritage.org/EducationalServices/pharm/asp/acid04.htm

Kyrk, J. 2007. pH Scale. (animated) http://www.johnkyrk.com/pH.html

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Cellular Transport (Diffusion & Osmosis)

Bowen, R. 2000. Hypertext for Biomedical Sciences. Colorado State Univ.
Osmosis. http://arbl.cvmbs.colostate.edu/hbooks/cmb/cells/pmemb/osmosis.html
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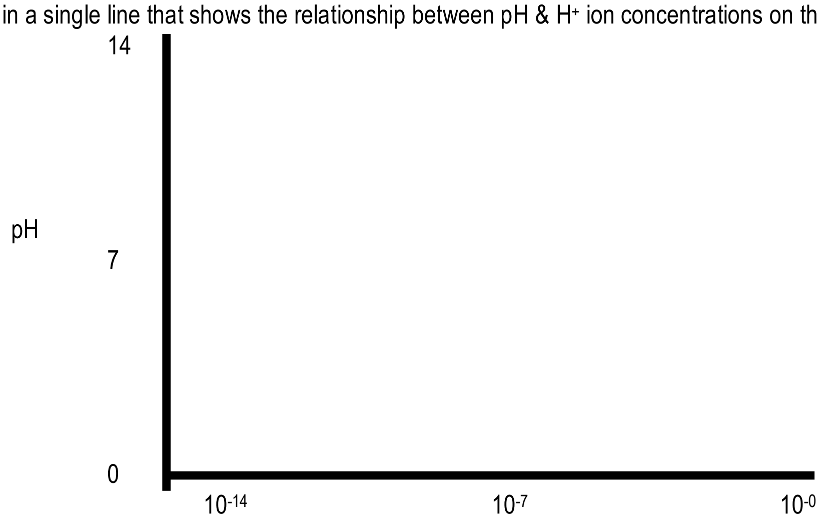
Simple Diffusion. http://physioweb.med.uvm.edu/diffusion/SDiffPages.htm
Diffusion of Many Particles. http://physioweb.med.uvm.edu/diffusion/MultiNoMembPages.htm
Measuring Diffusion. http://physioweb.med.uvm.edu/diffusion/MultiExtraPages.htm
Diffusion Across a Selectively Permeable Membrane.
http://physioweb.med.uvm.edu/diffusion/MultiWithMembPages.htm
Introduction: solute, solvent and solution. http://physioweb.med.uvm.edu/diffusion/OsmPages1.htm
A solution. http://physioweb.med.uvm.edu/diffusion/OsmPages2.htm
In solution, solute reduces solvent activity. http://physioweb.med.uvm.edu/diffusion/OsmPages3.htm
Solvent Movement Between Two Compartments. http://physioweb.med.uvm.edu/diffusion/OsmPages4.htm

pH , CELL STRUCTURE, DIFFUSION & OSMOSIS

Biol. 119 **Name** ______________________________________

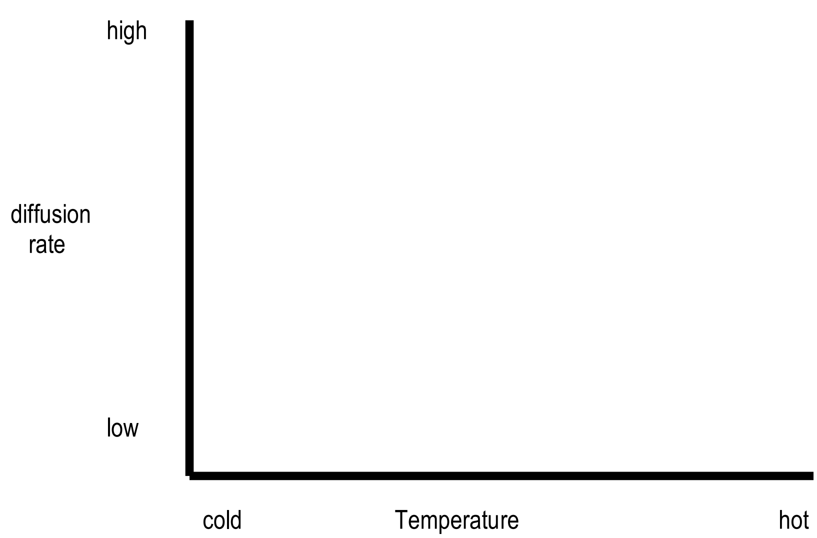
Pre-Lab Questions **Lab Section** _______

1. Draw in a single line that shows the relationship between pH & H^+ ion concentrations on this graph:

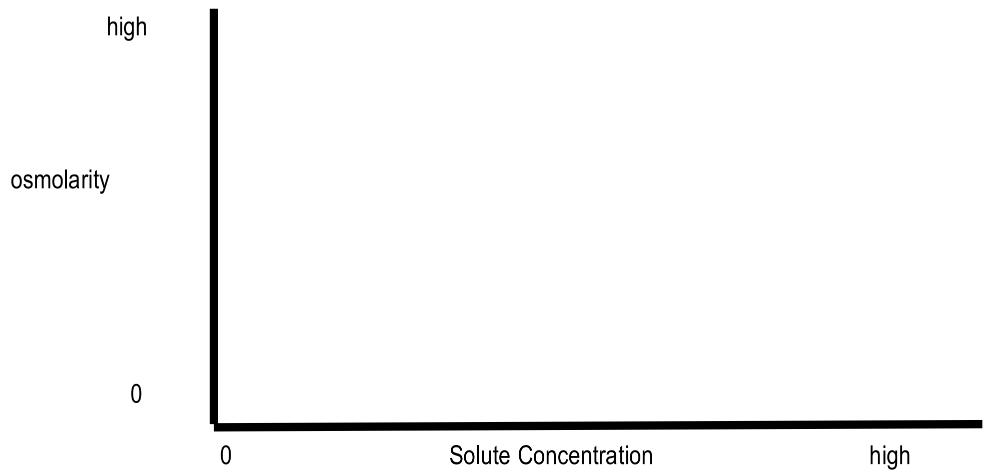


2. Circle the neutral point on your line in question 1 & then explain why water has a neutral pH.

3. Draw a line to explain the general relationship between temperature & diffusion rate using this graph:



4. Draw a line to explain the relationship between osmolarity of a solution & solute concentration using this graph:



5. Calculate the molecular weight of potassium chloride (KCl) if their atomic masses are: K = 39.1 and Cl = 35.5.

6. Molecule X ionizes into 3 particles when it is placed in water, while substance Y does not ionize. If you are given solutions of molecule X & of molecule Y, each with a 0.2 molarity, what is the osmolarity of solution X & the osmolarity of Y? Show your work.

pH Effects in the Body

Acids increase the concentration of H^+. They typically release > 1 H^+. Some examples are:

- Carbonic acid H_2CO_3 - ----> $H^+ + HCO_3^-$ (bicarbonate ion)
- Acetic acid CH_3CHO_2 ----> $H^+ + CH_3CO_2^-$

Bases (alkaline substances) decrease the concentration of H^+ by binding to free H^+ they remove H^+ from solutions.

- (Hydroxyl ion) $OH^- + H^+$ -----> H_2O
- Sodium hydroxide readily ionizes in water to release a hydroxyl ion, so it is a base:
- NaOH ----> Na^+ (sodium ion) + OH^- (hydroxyl ion)

Water is both an acid and a base, if it ionizes. However, water ionizes rarely.

- H_2O -----> $OH^- + H^+$

The **pH scale** is a measure of the number of H^+ present in a solution. The symbol for H+ concentration is $[H^+]$. A pH value is proportional to the inverse of the concentration of $H^+ \sim (1 / [H^+])$. The pH scale is log transformed, $pH = - \log[H^+]$. This means 1 unit difference in value equals a 10X difference in the amount of hydrogen ions. A pH of 3 is 10X more acidic than a pH of 4. A pH of 12 is 100X more alkaline than a pH = 10 because there is a 2 unit difference in the pH values, (10 X 10 = 100) ,

- Normal plasma has a pH = 7.35 - 7.45 so it is slightly alkaline.
- Neutral pH = 7 of pure water releases an equal number of H^+ & OH^- ions
- Acidic pH < 7 means more H^+ are present or fewer OH^- ions are present
- Basic/Alkaline pH > 7 means fewer H^+ are released or more OH^- are present

A variety of homeostasis imbalance problems can lead to pH imbalances in the body's extracellular fluids (e.g. blood plasma pH). We will discuss more during the quarter, but digestive tract imbalances are relatively common & easily explained as follows: Diarrhea or chronic use of laxatives causes the loss of alkaline fluids from the intestines. If this is severe or chronic, the blood pH becomes more acidic. An acidic shift (below the normal range) in plasma pH is called acidosis. Acidosis can inhibit activity of the brain & muscle tissues, which can lead to muscle weakness, fatigue, and finally a coma & death.

Vomiting caused by an illness or bulimia leads to loss of extremely acidic stomach fluids. Loss of acidic stomach fluid shifts your blood pH to a more alkaline range. Extreme alkaline shifts of blood plasma are referred to as alkalosis. This has the opposite effect on the brain& muscle tissue to acidosis. Severe alkalosis may trigger excessive muscle tension, a faster heart rate & ultimately convulsions & death. Enzymes & other cellular proteins may begin to denature (unwind or lose normal shape) as a result of extreme shifts in extracellular pH (either acidosis or alkalosis). These changes result in the malfunction of many metabolic processes.

Introduction to the Cell Membrane

Cells are the basic building blocks of living systems. All living things are made up of cells and all cells come from preexisting cells. A watery environment that is called extracellular fluid surrounds cells. Examples of extracellular fluids include: **plasma** (the fluid portion of blood) and **interstitial fluid** (fluid that is in the small spaces or interstices that surround most cells).

Most animal cells are very similar in design. The outer surface of a cell is called the cell or plasma membrane. The cell or plasma membrane is made up primarily of phospholipids so that it is selectively permeable or semi-permeable. Most lipids or **non-polar** (uncharged) substances move easily through a cell membrane. An exception is water. Water is an extremely small molecule, in very high concentrations in all body fluids. There are almost always small water channels in the membrane that allow water to move freely into or out of a cell. Most **polar** (charged, or ionic) substances move more slowly than water if they move at all across a cell membrane, in part because they are not as numerous as water molecules. Some polar molecules require active transport (which includes the expenditure of cellular ATP & the presence of special carrier proteins) to enter or leave a cell. Substances that move via active transport move more slowly than water across a cell membrane, because carriers have "rate limits" or maximum speeds at which they function. Some polar molecules may not move at all if the necessary carriers are missing from that cell membrane. The internal environment of a cell is called **cytoplasm**. Cytoplasm contains **intracellular fluid** & organelles such as the nucleus and mitochondria. Intracellular fluid is highly viscous (sticky).

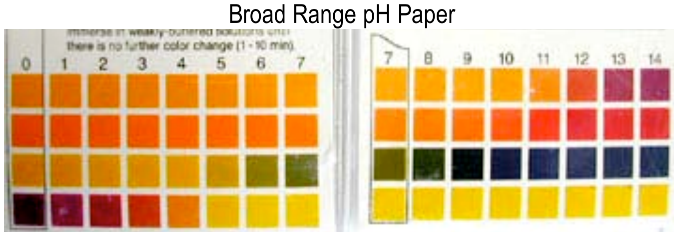
Experiments: pH Testing

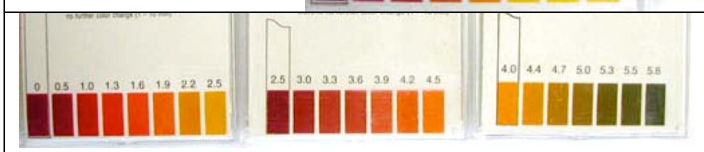
1. One or two groups of students will measure the pH of some common substances.
   a. First test each substance with a broad scale pH paper. These strips have a series of color bars that can be matched to standards on the box. The paper will indicate integer values from 0-14.
   b. Second, test each substance with an appropriate more narrowly defined pH paper scaled to 1 decimal place.
   c. For example, if a substance had a pH = 6 on the broad scale paper, use the narrow range paper for values between 5 & 7. Record this more accurate value. For example the solution may have a pH = 6.4.
   d. Was this substance acidic, basic or "relatively" close to neutral (>6.5 but < 7.5)?
   e. Check the results with the TA's data. Be sure your values are in the correct range.

2. One group will mix a small amount of baking soda with vinegar in a beaker. Can you explain the reaction? What is the pH of the mixture you created?

3. Your group(s) will answer the review questions on pH for the entire class.

Broad Range pH Paper





Solution	Narrow Range pH	Acidic, Basic or Neutral
Tap water		
Pepsi or Coca Cola		
Milk		
Coffee		
Bleach (1:10 dilution)		
Apple juice		
Baking soda		
Milk of Magnesia		
Vinegar		
Liquid soap		
What was the pH of the solution you created by mixing some baking soda with vinegar?		

Diffusion Experiments

All molecules are in constant motion. As molecules bump into each other, directions are changed, causing random dispersal of the molecules. The random movement of molecules results in diffusion. Diffusion occurs when molecules of substance X move away from an area or source of higher concentration towards an area of lower concentration of substance X. Molecules of substance X move away from the area of higher concentration because the molecules encounter fewer obstructing molecules in the area of lower concentration. The rate of diffusion is variable and depends on temperature, molecular weight, distance to travel, solvent density, and other factors.

In your experiment, we are using an agar or gelatin gel. The agar plates were made using 1.5g of agar in 100 ml of water, so this is a 1.5% agar solution. Agar is a gel extracted from a type of red seaweed found along the Pacific coast. The methylene blue dye has a Molecular Wt. = 320, and thus is a very heavy molecule. Your experiments & information from the web links should help you understand the effect of molecular weight, concentration and temperature on diffusion.

WARNINGS

- The dye should not come in contact with your skin or clothes!
- Wear latex or vinyl gloves while handling the dye solutions.
- Place paper towels on the table beneath your agar plates while they are filled.

- Obtain 2 petri dishes prepared with agar/gelatin.
- Use a 1.0 cm cork borer to remove 4 disks of agar/gelatin from each plate.
- Keep your holes away from the edge of the plate & at least 2 cm apart from each other.
- The holes should be cleanly cut, no nicks or cuts. If not, cut a new hole.
- Check afterwards to be sure the agar remains firmly stuck to the bottom of the dish.

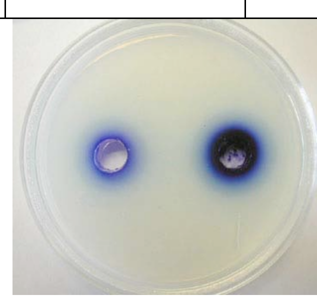
- Fill 1 well on each of your plates with each of these solutions: a. 0.01M methylene blue dye, 0.001M methylene blue dye, c. red food coloring, and d. green food coloring.
- Use the glass pipettes to fill each well without spilling dye on the surface of the agar/gelatin.
- Be sure ALL wells are filled to the SAME height (nearly to the top of the agar/gelatin).

- When both plates are ready, place 1 plate on a heating pad at low heat & 1 plate on ice.
- Leave the plates in position for 20-30 min., but measure all plates after the same time interval.
- Measure the maximum spread of dye from each well by measuring the outermost diameter of each dyed circle in mm, by placing a sheet of white paper under the petri dish & then placing the ruler underneath the dish.
- After you have measured the dye wells, save your agar plates so the rest of the class can see your samples.
- Your group will answer the review questions on diffusion for the entire class.

	Max. Diameter (mm)	**Max. Diameter (mm)**	**Max. Diameter (mm)**	**Max. Diameter (mm)**
Dye Conc.	Methylene blue 0.010M	Methylene blue 0.001M	Red food dye Unknown conc.	Green food dye Unknown conc.
Warm Plate				
Cold Plate				

Sample with just methylene blue:

Which circle on this agar plate was filled with the higher concentration of dye?



Osmosis Experiments

Water is a charged or polar molecule (H^+ - O^- - H^+) that is always moving across cell membranes. Scientists theorize that this is possible because it is such a small molecule or because there are special gap or pores that allow water movement through the cell membrane. The predominant direction of water flow is determined by the concentration of the solutes (non-water molecules) inside and outside of the cell. Water molecules will show a new movement from an area of higher water concentration (& lower in solutes) to an area of lower water concentration (& higher in solutes). In other words the net water flow tends to dilute an area of higher solute concentration. When water moves by diffusion through a semi-permeable membrane it is called osmosis. This is a type of passive transport because no cellular energy (ATP) is involved in the movement of water.

For convenience we will use tonicity & osmolarity as interchangeable terms. In fact, there are exceptions when these terms do not have identical meaning. An extracellular solution is isotonic ["iso" = same, tonicity = tone or tension] or iso-osmotic to a cell if the cell has no net gain or loss of water. This is a dynamic equilibrium. The cell & the extracellular solution have the same concentration of water & the same concentration of solutes. Our extracellular fluids need to stay isotonic in order for cells to survive. Iso-osmotic solutions can be used as intravenous solutions or during kidney dialysis because they maintain the osmotic balance of the body's extracellular fluids.

If cells are placed in a solution that contains a higher concentration of solutes than the cell, cells suffer a net loss of water and appear crenated ["cren" = notched] or wrinkled. These cells are in a hypertonic or hyperosmotic solution. Cells in a highly hypertonic solution may die from this **dehydration**.

A solution that has a lower solute concentration than is present in cells is said to be a hypotonic or hypo-osmotic solution. In this case, excess water flows into the cells and the cells swell. Neurons begin to malfunction when **overhydrated**. Blood cells & other cells may eventually rupture or burst open in a process called **lysis**.

Although we simplify osmolarity problems by using the % of a solute to represent its concentration, two solutions with the same % of solutes may NOT have the same number of solutes. Accurate osmolarity calculations must use a more complex calculation. All molar solutions contain the same number of molecules:

- 1 mole unit of any molecule, has 6.022×10^{23} molecules in 1 liter of solution.
- 1 mole of a substance equals its molecular wt.

Osmolarity is calculated as n * moles, where n = the number of dissociated particles that are present when a substance is placed in water. 1 mole of glucose has an osmolarity = 1 Osmole because glucose doesn't ionize in water. 1 mole of sodium chloride has an osmolarity = 2 Osmoles because it ionizes freely into two ions: Na^+ and Cl^- when placed in water.

0.30 Osmoles of any solute is isotonic with a normal plant or animal cell.

- Sodium chloride (NaCl): molecular weight = 58.5 g
- 1 mole of sodium chloride (NaCl) = 58.5 g NaCl / 1000 ml water
- Sodium chloride readily ionizes into Na^+ and Cl^- so its osmolarity is 2 X its molarity.
- Isotonic NaCl = 0.30 osmoles of NaCl / 2 particles = 0.15 moles of NaCl
- 0.15 moles of NaCl = 58.5g NaCl/1000 ml water (1 mole NaCl) * 0.15 = 8.8 g NaCl / 1000 ml
- 8.8 g NaCl / 1000 ml water = 0.88 g NaCl/100 ml water = 0.88% NaCl solution
- **An isotonic NaCl solution has 0.88% NaCl**

B. Facilitated Diffusion

1. Open the PhysioEx: 01 Cell Transport Exercise
2. Go to the Facilitated Diffusion Experiment
3. Run a series of simulations of using this range of variables described in the table.
4. Record the time to equilibrium when its shown, it will not be in the simulation's data table.
5. Graph your results on an overhead & explain the results to the class.

Glucose Conc. in R & L Beakers at Start	250 glucose carriers in "membrane" you build		500 glucose carriers in membrane you build		1000 glucose carriers in membrane you build	
Record these values	Time to Equilibrium	Rate of diffusion	Time to Equilibrium	Rate of diffusion	Time to Equilibrium	Rate of diffusion
Left – 5 mM Right – 0 mM						
Left – 10 mM Right – 0 mM						
Left – 20 mM Right – 0 mM						

C. Experimental Procedure - Using Potato Sticks

- You will be given 5 potato sticks that are 4-6 cm long with a 1 cm diameter.
- You will be provided with 5 vials each with a different salt or sugar solution.
- Determine the initial weight (in g) of each potato stick. **Be sure the balance is set for gram units!!**
- Record this initial potato weight to 2 decimal places, for example: 3.15 g.
- Immediately after each potato stick is weighed, place it in one of the solution vials.
- Re-zero the balance after recording the weight for each potato slice.

- Predict which solutions SHOULD BE iso-osmotic, hyper-osmotic or hypo-osmotic to potato cells.
  — Clue: We identified which solutions should be isotonic earlier in your notes.
- Predict which solutions will cause the potato stick to gain or lose water & which solutions won't change.
- THEN rank them by relative gain or loss:
  0 = no change, +1 minimal gain, +2 moderate gain, +3 maximum gain
  -1 minimal loss, -2 moderate loss, -3 maximum loss
- Predict (relatively) how much water potatoes will gain or lose in those solutions.

- After 20 minutes weigh each potato stick again & record the data.
- Calculate the change in weight: final wt - initial wt = + or - change in weight.
- Calculate the + or - %change as [change in weight (g) / initial weight (g)] X 100
- Your group will answer the osmosis questions for the entire class.

Salt Conc.	**INITIAL WT.** **of Potato**	**FINAL WT.** **of Potato**	**CHANGE IN WEIGHT (g) (show + or -)**	**CHANGE IN WEIGHT % (show + or -)**	**PREDICTED TONICITY RELATIVE TO LIVING CELL**	**RANK RELATIVE WATER LOSS OR GAIN**
10% NaCl						
3.5% NaCl						
0.88% NaCl						
water						

REVIEW OF EXPERIMENTS - GROUP DISCUSSION

pH

1. Explain the relative acidity of a pH = 5 vs. a pH = 7.

2. Describe 2 reasons why the water sample you tested did NOT have a pH = 7.

3. Describe & explain the chemical reaction between vinegar & baking soda.

4. Describe 2 events that may cause your extracellular fluid to become too acidic.
   a.

   b.

5. What physical symptoms do you suffer from when your body becomes too acidic (i.e. you suffer acidosis)?

6. Describe 2 events that may cause your extracellular fluid to become more alkaline.
   a.

   b.

7. What physical symptoms do you suffer from when your body becomes too alkaline (i.e. when you suffer alkalosis)?

8. How can you correct these pH imbalances?
   (We'll discuss the homeostatic regulation of pH later, so answer here what you might eat or drink to fix the problem).
   a. acidosis

   b. alkalosis

Diffusion

9. Describe 2 practical problems that can lead to measurement errors in the diffusion experiments.

10. Explain why methylene blue should travel farther in the agar/gelatin when its the dye concentration is higher.

11. Explain why the dyes should travel farther under hot conditions.

12. Look up the molecular weights of red & green food dye. Would you expect these food colors to diffuse more quickly or more slowly than methylene blue? If these food colorings didn't follow your expectations, what could cause that result?

Facilitated Diffusion Simulation

13. Define facilitated diffusion & show the graph of your results to the class.

14. Explain the effect of the number of carrier proteins & the effect of the glucose concentration gradient on the diffusion rates & time to reach equilibrium.

Osmosis

15. Go to this link on a computer: http://arbl.cvmbs.colostate.edu/hbooks/cmb/cells/pmemb/osmosis_eg.html
Study the osmosis simulations 1, 3 & 6, until you understand what factor(s) affect the osmolarity of a solution.
Be able to diagram & describe examples 1, 3 & 6 to the class.

16. Explain or diagram the online osmosis simulations & discuss 2 factors that affect the osmolarity of a solution.

    a.

    b.

17. Explain 2 practical measurement problems in the potato experiment that could cause your results to differ from our expectations based on normal cellular osmolarity of potato cells.

18. Define the terms hypertonic & hypertonic relative to a cell. Why do cells placed in hypertonic solutions lose water? Why do cells placed in hypotonic solutions gain water?

19. Did the potato cells placed in more extremely hyper- or hypo-tonic solutions gain/lose more water than less extreme solutions? Explain why this did or did not happen.

20. Define what it means to be dehydrated, physiologically. Describe some ways that you can become dehydrated.

21. What are the noticeable symptoms of dehydration? (Look this up on the computer or use your textbook!)

22. Define what it means to be overhydrated, physiologically. Describe how you can become overhydrated.

23. What are the physical symptoms of overhydration? (Look this up on the computer or use your textbook!)