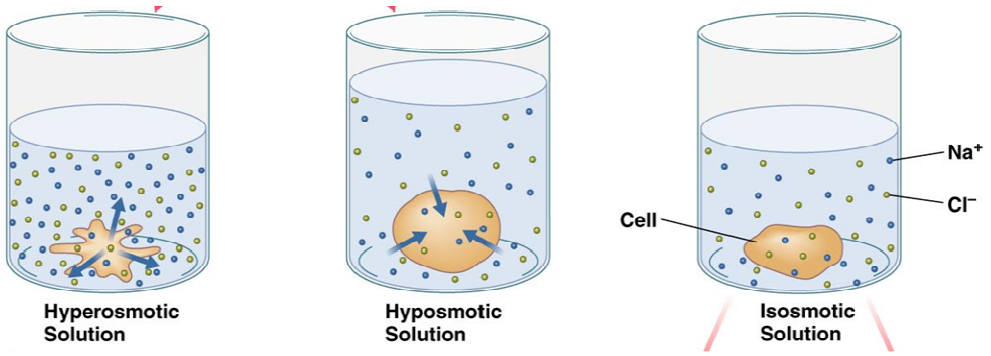
WATER AND SOLUTE MOVEMENT THROUGH RED BLOOD CELLS

Purpose

This exercise is designed to demonstrate the properties of cellular membranes and the movement of water and solutes across them. In this lab, you will review important concepts of membrane physiology described in lecture and perform basic calculations relevant to understanding and predicting the movements of water across cell membranes. In addition, you will gain experience in developing and testing scientific hypothesis and in properly presenting and discussing your data.

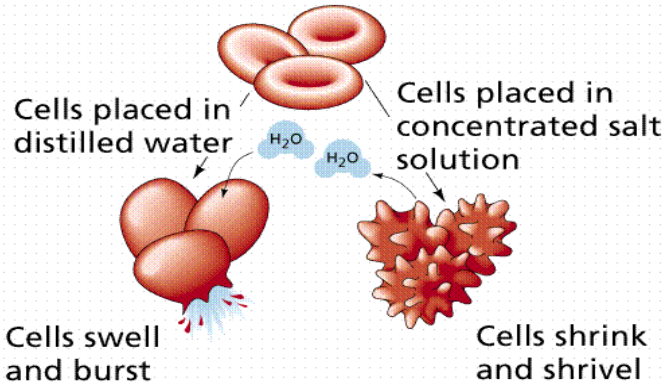
Background

Mammalian **ERYTHROCYTES** (red blood cells) are ideal for the study of water and solute movement across plasma membranes. Due to their flattened and concave shape, erythrocytes have a large surface area and therefore a large membrane area. Erythrocytes are also very active in transmembrane transport and are tolerant of relatively large changes in cell volume: they can both shrink and swell by more than 100% of normal size.



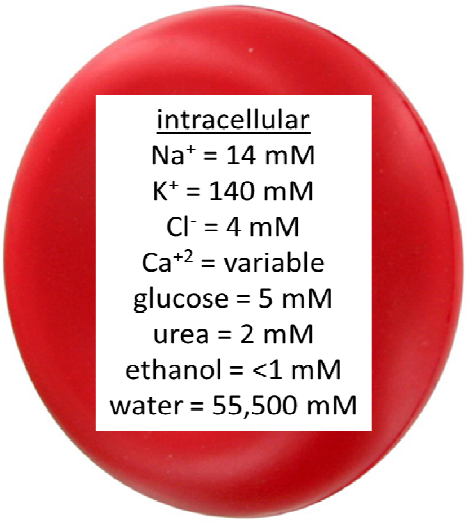
textbook Fig 2.9 pg 31

However, even erythrocytes with their enhanced ability to tolerate changes in cell volume have points where the diffusive movements of water induce changes in cell volume that cannot be compensated. **CRENATION** is the shrinkage of red blood cells (as in a hyperosmotic solution) that results in folding of the edges of cell membranes. **HEMOLYSIS** is the rupture of red blood cells due to rapid increases in cell volume that cannot be compensated for (as in from swelling in a hyposmotic solution).



Turn on your spectrophotometer now. The spec needs to warm up for 20 min prior to use.

The normal osmotic concentration inside mammalian red blood cells is approximately 288 mOsm (mOsm = number of millimoles of solute particle in one liter of fluid) and the extracellular fluid surrounding the cells is isosmotic to the interior of red blood cells (i.e. also approximately 288 mOsm).



extracellular	membrane permeability
Na^+ = 142 mM	Na^+ = very low
K^+ = 6 mM	K^+ = low
Cl^- = 116 mM	Cl^- = low
Ca^{+2} = variable	Ca^{+2} = low
glucose = ---	glucose = moderate
urea = ---	urea = high
ethanol = <1 mM	ethanol = high
water = 55,500 mM	water = very high

concentrations are given in millimoles per liter

As you can see from the figure above, cell membranes are generally impermeable to solutes, but extremely permeable to water. Thus, the changes in cell volume driven by differences in solute concentration on either side of the cell membrane are due to the passive diffusion of water down its concentration gradient. Red blood cells hemolyze when the extracellular solution becomes too hyposmotic relative to intracellular concentrations. Under physiological conditions, erythrocytes can regulate cell volume to some extent by actively transporting solutes across the membrane. However, there are limits to the ability of erythrocytes to regulate cell volume (i.e. water moves in or out of cells faster than solute concentration can be adjusted). In general, hemolysis occurs at extracellular concentrations less than 180 mOsm and crenation occurs at extracellular concentrations greater than 500 mOsm.

Review of osmolarity

OSMOLARITY is the concentration of molecules in a solution that can influence the movement of water. Thus, the osmolarity of solutions can be used to determine the net movement of water. Water will move from compartments with low osmolarity (fewer dissolved particles and a higher concentration of water) to compartments with high osmolarity (more dissolved particles and a lower concentration of water). Osmolarity is closely related to molarity, which you should recall from introductory chemistry. If 1 mole (6.022×10^{23} molecules) of sucrose is dissolved in 1 liter (L) of solution, the osmolarity would be 1.0 osmolar (Osm) or 1000 milliosmolar (mOsm). However, this relationship changes for molecules that dissociate or **IONIZE** in solution. NaCl ionizes to Na^+ and Cl^- when in solution, therefore if 1 mole of NaCl were dissolved in 1 liter of solution, the resulting osmolarity would be 2 Osm, because both Na^+ and Cl^- influence the movement of water. For example, the osmolarity of seawater is about 1000-1200 mOsm and results from many different dissolved solutes, including Na^+ and Cl^-.

Importantly, not all solutes are osmotically active. If a molecule does not dissolve into the solution it does not contribute to osmolarity. For example, lipids, uric acid, and proteins are considered osmotically inactive.

Review of passive diffusion

Red blood cell volume will depend not only on the initial osmolarity of the extracellular fluid, but also on the movement of any solutes. Water will move almost instantaneously in response to any difference in osmolarity between intracellular and extracellular fluids. However, some solutes may also be able to cross cell

membranes down their own concentration gradient (see figure at top of page). Remember that each solute moves independently according to its own gradient, not according to the overall concentration indicated by osmolarity. In considering your hypotheses and results, think about both the movement of water (nearly instantaneous) and the movement of solutes (which may be delayed, and will secondarily affect water movement). Also keep in mind that hemolysis is irreversible.

Measuring the state of red blood cells using spectrophotometry

A **SPECTROPHOMETER** is an instrument that can both emit light at a given wavelength and measure the intensity of incoming light through a solution. The amount of light that is transmitted through a solution, and therefore not absorbed by any molecules within that solution, is termed **PERCENT TRANSMITTANCE** (or %T). Percent transmittance is expressed in relative terms as the amount of light transmitted in a particular sample relative to a "blank" solution assumed to be 100% transmittance.

Percent transmittance at 510 nm will be used as an indirect measure of red blood cell volume and changes in cell volume will be used as an indicator of the osmotic response of red blood cells to solutions with differing osmolarity. As a cell shrinks, the membrane folds and scatters the light so that less light is detected by the spectrophotometer. Thus a solution containing crenated red blood cells will absorb more light and will have a reduced percent transmittance. Conversely, as a cell swells, the plasma membrane scatters less light and more light is detected by the spectrophotometer. Thus a solution containing swollen or hemolyzed red blood cells will have an increased percent transmittance. To summarize:

95-100% transmittance = hemolyzed red blood cell
0-5 % transmittance = severely crenated red blood cell

Small changes in transmittance (1-2%) are not of interest in this experiment and likely result from variations in the opacity of the glass and solution (i.e. experimental error) so consider this when performing your measurements and interpreting your data.

Protocol: Spectrophometric analysis of cell volume change in red blood cells

1. Form groups of three students. Introduce yourself and exchange email addresses.

2. Check your equipment and supplies. Verify that you have the required items and are familiar with their use. Please ask instructor for clarification if you have any uncertainty and the instructor will review operation of the spectrophotometer and the pipettors.

3. Make a stock solution of defibrinated sheep blood for sampling
   a. From the instructor, obtain 1.4 ml of "Teacher's Stock Blood Solution" in a 15-ml plastic vial with cap.
   b. Add 12.6 ml of physiological saline to make the "Student Stock Blood Solution"
   c. Mix the solution by gently inverting the vial. Keep the vial closed when not in use. This is the blood solution you will use throughout the experiment.
      - ***Remember to invert the vial gently 2-3 times before taking each sample.***

4. Obtain 25 ml of each of the following stock experimental solutions:
   a. 250 mM NaCl (equal to 500 mOsm) MW = 58.44 g
   b. 500 mM urea (equal to 500 mOsm) MW = 60.06 g
   c. 500 mM glucose (equal to 500 mOsm) MW = 180.16 g

Calculate the mass of each dry solute that was needed to make 500 ml of each of the above stock solutions. Remember that a 1M solution contains MW (g) of solute/L solution. Be prepared to show and explain your calculations in your notebook at the beginning of lab next week.

5. Make a 5.0 ml blank solution in a clean test tube marked "BLANK" with a marker. The blank should ideally contain all of the elements of your test solution except the substance of interest (in this case, the intact plasma membranes). Thus, for these experiments the blank should contain hemolyzed red blood cells that will control for substances in the samples that are not of interest.

How do you ensure that all of the cells are hemolyzed in the blank?
By adding 0.5 ml of student stock blood solution to 4.5 ml distilled water (a very hyposmotic solution). Record this in your lab notebook.

6. Adjust the spectrophotometer according to the directions specified by the instructor (read the sign attached to the front of the spec). The display should read 510 nm (i.e. the wavelength of light to be detected) and 100%T (i.e. transmittance mode). If the spectrophotometer says "A", press the A/T/C button until it reads "%T" on the top of the display. Insert the blank tube and set the maximum value (100% transmittance).

Calculate and record the volumes of water and stock solution required for each 4.5 ml test solution as indicated in 7a. **Outline your predictions for the effect of each solute (experiments 7-10) in your lab notebook**. Hypothesize whether the cells will crenate or hemolyze in when added to each solution. Specify why you made each hypothesis?

7. **The effect of NaCl concentration on the volume of red blood cell**
   What do you hypothesize will happen?
   a. Take a 25-ml aliquot of the stock solution of 500 mOsm **NaCl** to your lab bench. Using the stock solution and distilled water, make 6 dilutions in the test tubes, each containing 4.5 ml test solution:

4.5 ml of 500 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O
4.5 ml of 300 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O
4.5 ml of 250 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O
4.5 ml of 200 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O
4.5 ml of 100 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O
4.5 ml of 0 mOsm	=	_____	ml stock 500 mOsm	+ _____	ml dH2O

   Warning: add the blood only after you have mixed each test solution.
   What would happen if you added blood first, then water, then the stock solution?

   b. Add 0.5 ml "Student Stock Blood Solution" to the first solution. Cover the test tube with a small piece of parafilm and immediately mix by inverting the test tube. **Measure the %T on the spectrophotometer within 5 seconds of mixing**. Repeat this process for each of the remaining NaCl solutions, remembering to measure %T immediately after mixing.

   c. After 30 minutes, gently remix and resample your same six tubes.
   Are the readings any different? Why might they change over time? See table on permeability rates.

   d. For your report (due week 5), prepare a two graphs of % T (y-axis) vs. osmolarity (x-axis).
      - plot percent transmittance at 5-sec for each osmolarity using your group's data. On the same graph include the class average at 5-sec for each osmolarity. Add trendlines to each dataset and include error bars on the class average data.

- plot percent transmittance at 30-min for each osmolarity using your group's data. On the same graph include the class average at 30-min for each osmolarity. Add trendlines to each dataset and include error bars on the class average data.

8. The effect of glucose concentration on the volume of red blood cells
What do you hypothesize will happen?

a. Take a 25-ml aliquot of the stock solution of 500 mOsm **GLUCOSE** to your lab bench. Using the stock solution and distilled water, make 6 dilutions in separate test tubes, each containing 4.5 ml test solution:

4.5 ml of 500 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 300 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 250 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 200 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 100 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 0 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O

b. Add 0.5 ml "Student Stock Blood Solution" to the first solution. Cover the test tube with a small piece of parafilm and immediately mix by inverting the test tube. Measure the %T on the spectrophotometer within 5 seconds of mixing. Repeat this process for each of the remaining glucose solutions, remembering to measure %T immediately after mixing.

c. For your report (due week 5), prepare a graph of % T (y-axis) vs. osmolarity (x-axis).
- plot percent transmittance at 5-sec for each osmolarity using your group's data. On the same graph include the class average at 5-sec for each osmolarity. Add trendlines to each dataset and include error bars on the class average data.

9. The effect of urea concentration on the volume of red blood cells
What do you hypothesize will happen?

a. Take a 25-ml aliquot the stock solution of 500 mOsm **UREA** to your lab bench. Using the stock solutionand distilled water, make 6 dilutions in separate test tubes, each containing 4.5 ml test solution:

4.5 ml of 300 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 100 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 50 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 10 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 5 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O
4.5 ml of 0 mOsm = _____ ml stock 500 mOsm + _____ ml dH2O

b. Add 0.5 ml "Student Stock Blood Solution" to the first solution. Cover the test tube with a small piece of parafilm and immediately mix by inverting the test tube. Measure the %T on the spectrophotometer **as quickly as possible**. Note that the readings will not be steady, so take the first reading from the spectrophotometer after inserting the sample. Repeat this process for each of the remaining urea solutions.

c. For your report (due week 5), prepare a graph of % T (y-axis) vs. osmolarity (x-axis).
- plot percent transmittance for each osmolarity using your group's data. On the same graph include the class average for each osmolarity. Add trendlines to each dataset and include error bars on the class average data.

Why are the results so different from data in experiments 7 & 8?

To finish today's lab:

1. Save ALL of your data into the Documents folder in a folder labeled with your group name. Make a personal copy of these data in case someone deletes them.
2. Record your raw data into the common spreadsheet. Add your group data to the common data table.
3. Rinse with tapwater (no soap!) and turn upside down to dry all of your glassware, including tubes.
4. Clean up your work area.
5. Turn off your spectrophotometer
6. Have the instructor approve that all these tasks are completed before you leave.

Next week: Discussion-Membrane Physiology

We will be discussing your data in our "Discussion" lab next week. You should come prepared to discuss the class's data. Additional instructions will be posted on Blackboard prior to meeting. Each group will generate a lab report complete with Title Page, Results with figures and Discussion sections.

- complete figure captions are required.
- if data are represented in a figure, do not repeat the presentation of these numbers in a table.
- Results should include a paragraph summarizing the major trends in your data.
- in the Discussion, provide a physiological explanation for your observations
- provide references for any sources cited (e.g. articles posted on Blackboard or your textbook).
- Further details can be found in the "Writing a Lab Report" file to be posted on Blackboard

Table: Solute Permeability Rates

Solute	*Membrane Permeability Rate*
Sodium (Na^+)	0.00000001 μm s^{-1}
Potassium (K^+)	0.0000001 μm s^{-1}
Chloride (Cl^-)	0.000001 μm s^{-1}
Calcium (Ca^{+2})	0.000001 μm s^{-1}
Glucose	0.001 μm s^{-1}
Urea	0.1 μm s^{-1}
Ethanol	0.5 μm s^{-1}
Water (H_2O)	30 μm s^{-1}