

BIOLOGY 163 LABORATORY

RBC PERMEABILITY TO DIFFERENT SOLUTES

(Revised Fall 2011)

Plasma Membranes

Cells continually exchange materials with their external environments. The exchange of such materials is controlled by a plasma membrane composed of a phospholipid bilayer, proteins, and associated carbohydrates. Some substances are able to cross the plasma membrane through simple diffusion driven by a concentration gradient. Others utilize specialized membrane proteins that facilitate diffusion or actively transport materials using energy from ATP. The distinction here is important—this exercise will investigate the ability of different substances to cross the plasma membrane through simple diffusion.

Although the phospholipid bilayer is fluid in nature, not all substances are able to freely diffuse across it—the size, charge, solubility characteristics, and other chemical properties of a substance all influence how it will interact with the phospholipids in the membrane. These interactions will in turn determine if the substance is able to cross (or *permeate*) the membrane. Because the chemical nature of membranes allows them to “control” which substances pass through them, they are said to be *selectively permeable*.

Diffusion and Osmosis

Investigating the ability of different solutes to permeate a plasma membrane is easy if you understand the basic principles of *diffusion* and *osmosis*. Diffusion is the movement of a particular substance from an area of higher concentration of that substance to an area of lower concentration of that substance. Osmosis refers specifically to the diffusion of water across a selectively permeable membrane. Again, this is driven by a concentration gradient—water will move from an area where it is more concentrated to an area where it is less concentrated.

In an aqueous environment, the concentration of water is linked to the concentration of solutes. In simple terms, where there are more solutes, there is less water, and vice-versa. For example, one liter of a 1.0 M solution of NaCl has a lower concentration of water than does one liter of a 0.5 M solution of NaCl. If these two solutions were separated with a membrane permeable only to water, in which direction would the water move?

It is important to understand that while the diffusion of a given solute depends upon the concentration gradient of *that solute*, the diffusion of water (osmosis) is determined by the *total solute concentration* on either side of the membrane. In other words, it is the total *number of particles* in the solution that affects the concentration of water, not the *kind* of particle!

Red Blood Cells (Erythrocytes)

Mammalian red blood cells (RBCs) function primarily to carry oxygen to the many tissues of the body and to carry carbon dioxide to the lungs. A mature mammalian RBC is essentially a bag of hemoglobin (an oxygen-carrying protein). It is an extremely simple cell, with no nucleus or other internal membranes, and therefore is ideal for the study of membrane permeability. The RBC is normally shaped like a biconcave disc, but it is very flexible and often squeezes through very thin capillaries. The biconcave shape is determined primarily by the underlying cytoskeleton attached to membrane proteins.

Red blood cells are ordinarily found in plasma of approximately the same solute concentration as the interior of the RBCs. (Such a solution is said to be *isotonic* to the cell.) Given your knowledge of osmosis and diffusion, you should realize that this would result in NO NET MOVEMENT of water into or out of the cell, and the normal shape of the cell would be maintained.

In contrast, an RBC placed in a solution that is more concentrated than the inside of the cell (i.e., *hypertonic* to the cell) will find itself with a higher concentration of *water* on the inside of the cell. Since water can freely cross plasma membranes, it will diffuse out of the cell. This causes the RBC to shrink and take on an obvious “spiky” (or *crenated*) appearance. Crenation usually does not damage the cell—returning it to an isotonic solution will typically allow it to return to its normal biconcave shape.

Also in contrast, an RBC placed in a solution that is *less* concentrated than the inside of the cell (i.e., *hypotonic* to the cell) will find itself with a higher concentration of water on the *outside* of the cell. This will result in water diffusing *into* the cell, causing them to swell. The cell can tolerate only a limited amount of swelling before it eventually ruptures and releases its hemoglobin into the surrounding medium. This process, called *hemolysis*, will equilibrate the osmotic conditions, and the now hemoglobin-free cells will often revert to their original shape. These “empty” cells are called ghosts—although they have the size and shape of normal RBC’s, they appear much more faint and can usually only be viewed using phase-contrast microscopy.

Investigating Membrane Permeability

The ability of different substances to permeate the plasma membrane can be explored by placing RBC’s in relatively concentrated solutions of those substances and observing the response of the cells. If you have followed the discussion so far, this is simpler than it may seem at first.

If an RBC is placed in a relatively concentrated solution of a solute that is *UNABLE to cross the plasma membrane*, HYPERTONIC conditions will be maintained. Water will leave the cell by osmosis and the cell will crenate.

If an RBC is placed in a relatively concentrated solution of a solute that is *ABLE to cross the plasma membrane*, the response is a bit more complicated. In this situation the solute would permeate the membrane and diffuse into the cell until that solute reaches equilibrium (the point at which the concentration of that solute is equal on both sides of the membrane). With the solute in question at equilibrium, the TOTAL solute concentration is now higher on the INSIDE of the cell. (This is because the inside of the cell already contains a large number of “trapped” proteins and other substances.) The cell now finds itself in a HYPOTONIC environment—water enters by osmosis (essentially “following” the solute) and the cell hemolyzes.

Keep in mind that *time* can be a confounding factor in these observations. If an RBC is exposed to a concentrated solute that is able to permeate the membrane, but does so very SLOWLY, the cell may initially crenate, later return to a normal appearance, and then eventually hemolyze. If you truly understand diffusion, osmosis, and selective permeability, you should be able to explain why this occurs!

PROCEDURE

This exercise has two parts. In Part A, you will practice working with RBCs, and become proficient in identifying “normal” vs. crenated vs “ghost” (hemolyzed) cells. In Part B, you will investigate the ability of various solutes to permeate the RBC membrane.

Work carefully and follow these general procedures to avoid frustrating complications:

- **Label** accurately and carefully **all** tubes and microscope slides you use.
- Use only clean, dry test tubes and microscope slides. **Clean and dry them yourself if needed!**
- You will work with very small volumes of blood (usually 2 drops) to which you will add particular solutions. **Do not allow the blood to dry at all** before adding the appropriate solution.
- Always prepare a **fresh slide** when making observations.

A. Microscopic Observations of Red Blood Cells in Different Solutions

Red blood cells in an isotonic solution:

Use a transfer pipette to add two drops of blood to a small test tube. Immediately add 4 ml of mammalian Ringer's solution (or isotonic saline) to the tube. Cover the tube with a small piece of Parafilm and invert to mix. Note the appearance of the suspension in the tube--is opaque or transparent? Remove a drop of this suspension, prepare a wet mount, and observe the cells using both standard and phase-contrast microscopy. Make a quick sketch of the cells.

Red blood cells in a hypertonic solution:

Use a transfer pipette to add two drops of blood to a small test tube. Immediately add 4 ml of 0.5M NaCl (a strong salt) to the tube, cover with Parafilm, and mix. Note the appearance of the suspension. Prepare a wet mount, and observe the cells with both normal and phase-contrast microscopy. Make a quick sketch of the cells and describe the process that has occurred.

Red blood cells in a hypotonic solution:

Use a transfer pipette to add two drops of blood to a small test tube. Immediately add 4 ml of distilled water (which has very few solutes in it) to the tube, cover with Parafilm, and mix. Note the appearance of the suspension. Prepare a wet mount, and observe the cells with both normal and phase-contrast microscopy. (Why are the cells not as easily visible with the standard compound microscope?) Make a quick sketch of the cells and describe the process that has occurred.

Before continuing, review your macroscopic (naked eye) and microscopic observations. Does the appearance of the solution in the test tube provide any clue as to the condition of the cells?

B. Permeability of RBC membranes to different solutes

Using the techniques practiced in Part A, design an experiment to determine whether each of the substances listed in Table 1 is able to permeate the RBC membrane. Note that all of these 0.5M solutions are *more concentrated* than the inside of the cell. For reasons discussed previously, the particular response of the cells depends on whether or not the solute is able to pass freely across the phospholipid bilayer of the plasma membrane.

Design your experiments carefully and keep in mind that **certain effects may not happen immediately**. A good experimental design will take this into account!

Before you begin, it is recommended that you set up a data table to record your observations (both microscopic and macroscopic) over the course of the experiment.

Table 1. Characteristics of solutions available for RBC permeability testing.

CHEMICAL	CONCENTRATION	MOLECULAR WEIGHT	OTHER FEATURES
Urea	0.5 M	60	polar
Ethylene glycol	0.5 M	62	polar
Glycerol	0.5 M	92	polar
Ribose	0.5 M	150	polar
Glucose	0.5 M	180	polar
NaCl	0.5 M	58	ionic
KCl	0.5 M	75	ionic
MgCl ₂	0.5 M	95	ionic
Ethanol	0.5 M	47	slightly lipid soluble
Propanol	0.5 M	61	slightly more lipid soluble
Butanol	0.5 M	74	even more lipid soluble
Pentanol	0.5 M	88	highest lipid solubility

Clean Up

Clean the glassware you have used and leave all items as you found them. Wash all test tubes and leave them upside-down to drain in the rack at your station. Dispose of used coverslips in the waste container or glass box (NOT the trash!) Thoroughly wash and DRY all slides and return them to your lab bench. If you have used immersion oil, be sure to clean it COMPLETELY off the microscope objective using lens paper.

Assignment*Results*

Concisely summarize the observations you made in Part B of the procedure. Be sure to note any trends or patterns evident with regard to solute type or size.

Conclusions

What do your results suggest about the ability of polar, ionic, and lipid soluble molecules to permeate the RBC membrane? Is size a factor? Do your conclusions support the notion that plasma membranes are selectively permeable?

This exercise is adapted in part from the following sources:

Giese, Arthur C. 1975. Laboratory Manual in Cell Physiology. Boxwood Press, Pacific Grove, CA. 319 pp.

Von Blum, Ruth. 1981. Experimental studies of permeability in red blood cells. *In* Tested Studies for Laboratory Teaching (Glase, Jon C., ed.). Kendall/Hunt Pub. Co. Dubuque, IA. pp. 63-119.