# **Lab 3: Cell transport**

## **Pre-lab Reading**

## **Vocabulary**

|  |  |
| --- | --- |
| **Lysis:** bursting open of cell in response to too much fluid entering cell (term **hemolyze** is used for RBCs) | **ICF**: intracellular fluid (inside cell) |
| **Crenate**: shrinking of cell in response to fluid exiting the cell | **ECF**: extracellular fluid (outside cell) |
| **Mole (mol)**: equal to the molecular weight of a substance | **Osmoles: (**# of mol) **X** (# of particles substance breaks into when put into solution) |
| |  | | --- | | # of moles of solute | | 1 liter of solution |   **Molarity:** | |  | | --- | | # of osmoles of solute | | 1 Liter of solution |   **Osmolarity**: total vol of solutes +solvent=1 L; measurement of *all* solutes (as opposed to tonicity) |
| **Permeant solutes**: solutes are able to cross plasma membrane (can reach equilibrium between **ICF** & **ECF**,so they have NO effect on the eventual vol of the cell and no relevance for measuring **tonicity**) | |  | | --- | | # of osmoles of solute | | 1 Kg water |   **\*Osmolality**: total vol of solvent only= 1 L |
| **Impermeant** **solute**: solutes are NOT able to cross plasma membrane (cannot reach equilibrium between **ICF** & **ECF**, so they WILL have an effect on the eventual vol of cell) | **Tonicity**: the ability of solutions to pull water across membrane; determined by the concentration of **impermeant solutes** only |
| **Semi-permeable**: permeability is based on size of particles (ex: dialysis tubing) | **Hypotonic solution**: solution has a lower concentration of impermeant solutes than the **ICF**; causes water to move from **ECF** to **ICF** |
| **Selectively permeable**: uses active biochemical transport, diffusion, and osmosis to choose which particles will cross (ex: cell membrane) | **Isotonic solution:** solution has the same concentration of impermeant solutes as the **ICF**; causes no net movement of water between **ECF** and **ICF** |
| **Diffusion**: movement of particles from high to low concentrations | **Hyertonic solution**: solution has a higher concentration of impermeant solutes than the **ICF**; causes water to move from the **ICF** to the **ECF** |
| **Dialysis**: diffusion of solutes across a semi-permeable membrane | **Osmosis**: diffusion of water |

\*In osmolality, when water is the solvent, then 1Kg=1L, so Kg can be replaced with L in the denominator of the equation

## **BACKGROUND & REFERENCES**

Many of the terms and concepts in this lab will be a review from your general biology course. Please read through this information in your lecture textbook if you need a reminder.

**Making solutions**: To make a (w/v) solution, you measure the same # of grams as the desired % solute solution (ex: if you want a 2% solution, you measure 2 g of the solute). You place this in a container and then add water until the final vol is 100mL. This means you will be adding less than 100 mL of water

Example: What is the molarity of a 2% w/v solution of NaCl?

Answer: Remember, the solution will contain 2 g of NaCl in 100 mL final vol, molarity is moles/ 1 L, you use molecular weight (NaCl=58g) to convert grams to moles, So…

|  |
| --- |
| 2 g X 1000 ml = 20 g X 1 mol = 0.34 mol |
| 100 ml 1 l 1l 58 g 1 L |

To find the osmolarity of the same solution, you first need to determine if the solute breaks apart when placed in water. In this case, NaCl breaks up (ionizes) into 2 particles, Na+ and Cl-. So you take the molarity (0.34 mol/L) and multiply it by 2, which gives us an osmolarity of 0.68 Osm/L.

**Note on osmolarity vs. osmolality**: For our purposes, the difference between osmolarity and osmolality does not become significant; however, you should be aware of the fact that there is a difference between the two terms and that the word osmolality is not a "typo" error. These two terms are often used interchangeably; however, when dealing with osmosis, osmolality is actually the correct term to use.

**Tonicity vs Osmolarity**

Because osmolarity and tonicity are measuring different things (all solutes, as opposed to

impermeant solutes only) it is possible for a solution to be *hyperosmotic* to the ICF of cells, but still *isotonic*, as long as the concentration of impermeant solutes of the solution matches that of cells. For example, a solution with 320 mOsm NaCl (impermeant) and 100 mOsm urea (permeant) would have a total osmolarity of 420 mOsm, which is hyperosmotic to ICF (320 mOsm). But this solution would be isotonic. Cells placed in such a solution would INITIALLY shrink, but eventually their final volume would be the same as their initial volume, as the permeant solutes and water equilibrated. Overall, the solution does not cause shrinking or swelling of the cell (it is isotonic).

In contrast, a solution can be *iso-osmotic* to the ICF of cells but be *hypotonic* if the concentration of impermeant solutes is lower than that of the ICF. For example, a solution with 200 mOsm NaCl and 120 mOsm urea has a total osmolarity of 320 mOsm, which is equal to the osmolarity of ICF. In this case, water will flow into the cell (as it follows the permeant solute urea). That water flow occurs because the high concentration of impermeant solutes in the ICF (320 mOsm) can NOT be balanced by the low concentration of impermeant solute (200 mOsm NaCl) outside the cell. Overall, the solution causes water to flow into the cell, and the cell will swell (therefore, the solution is hypotonic). After equilibration of the urea, the concentration of the ICF is 380 mOsm (i.e. 320 + 60) while that of the solution is 260 mOsm (i.e. 200 +60). The solution is hypotonic to the cell.

Since osmolarity measures ALL solutes and tonicity only measures impermeant solutes, it is possible for a solution to be *hyperosmotic* to **ICF**, but still be **isotonic**. BUT… the solution will not be hyperosmotic to the ICF for long, as the permeant solutes will cross into the cell and reach equilibrium:

**Red**=impermeant solutes; **Black**=permeant solutes

Isotonic solution (same red in ICF & ECF) Black solutes cross and reach equilibrium

Hyperosmotic solution (more black in ECF) Red stay b/c the cannot cross

It is also possible for a solution to be iso-osmotic to the ICF but be hypotonic. In this scenario, the impermeant solutes need to be “balanced”. Since they cannot cross, water will move into the cell to balance the concentrations (ECF to ICF):

Iso-osmotic solution (same black in ICF & ECF) Water moves from ECF to ICF, making cell larger

Hypotonic solution (fewer red in ECF than ICF)

**Activity 1: Selective Permeability & Osmosis:**

The rate at which osmosis occurs is determined by such factors as:

1. osmolality of the two solutions separated by the membrane (the higher the difference in osmolality, the faster osmosis occurs)
2. permeability of the membrane to the solutes involved (the more permeable the membrane is to the solute, the faster that solute will cross)

In part A of this Activity, you will observe the interaction of solutions with different osmolalities that are separated by a **semi-permeable** membrane (dialysis tubing). The direction and rate of **osmosis** will allow you to determine if the solution in the thistle tube is **hypotonic**, **isotonic**, or **hypertonic** to the solution in the beaker. The dialysis tubing represents a cell membrane, which separates the intracellular and extracellular fluids; however, the tubing is **semi-permeable** while the cell membrane is **selectively permeable**. Thus, the dialysis tubing is a good, but not perfect approximation of how a cell membrane behaves.

In part B of this Activity, you will observe dialysis: the movement of a solute across the semipermeable membrane of a dialysis bag. One of the solutes will be able to cross the dialysis tubing while the other solute will not be able to cross the tubing.

In part C of this Activity, you will observe what happens to red blood cells when placed in solutions with different tonicities. Red blood cells will **hemolyze** if they are placed in a hypotonic solution, and they will **crenate** if they are placed in a **hypertonic solution**. A 0.32 osmolar saline solution is both iso-osmotic and isotonic to red blood cells. Red blood cells act as osmometers; the hemolysis or crenation of the red blood cells will be used to determine whether different solutions are **hypotonic**, **isotonic**, or **hypertonic** to the cells.

**Activity 2: Effect of Molecular Weight and Lipid Solubility on Membrane Permeability:**

In this Activity you will observe the rate at which **hemolysis** of red blood cells occurs when placed in two different **iso-osmotic**, **hypotonic** alcohol solutions. The difference in hemolysis rates will be used to compare how the molecular weights and lipid solubilities of these two alcohols affects their membrane permeability and rate of diffusion. Remember that molecular weight (size) and lipid solubility are two factors that affect the rate of diffusion of a substance across a membrane.

As you know, the cell membrane is made mainly of phospholipids and proteins. A particle that is highly soluble in lipids (i.e. oxygen) will be able to cross the cell membrane more easily and faster than one that is not soluble in lipids (i.e. an ion). Some particles that are not soluble in lipids can still cross the membrane because of special proteins that form channels and pumps in the cell membrane.

Alcohols do not use carriers or pumps because they are lipid soluble and are able to diffuse directly across the plasma membrane. Generally speaking, the larger an alcohol molecule is, the more lipid soluble it is. This experiment will allow you to determine whether size or lipid solubility is more important for movement of these alcohols across red blood cell membranes. At the beginning of this Activity, the red blood cells will be placed in a solution which contains 0.3 mol/L alcohol and 0.02 mol/L NaCl.