

Lab 2: Osmosis and Hemolysis

Cells are surrounded by a plasma membrane that separates the contents of the cells from the solution bathing them. The cell membrane is a **selective barrier** allowing some molecules to pass through it easily, some not so easily, and some not at all. We will use the red blood cell (RBC) to study the movement of water and other molecules across the cell membrane. RBCs have **aquaporins** (water channels) in their plasma membranes that permit water to diffuse across the membrane, a process known as **osmosis**.

Osmotic Flow

A difference in water concentration ($[H_2O]$) on two sides of a membrane provides a driving force for water diffuse from the region of high $[H_2O]$ to the region of low $[H_2O]$. For example, the $[H_2O]$ is higher in a solution of 0.1 M NaCl than it is in a solution of 10 M NaCl.

Rather than speaking in terms of $[H_2O]$ one usually speaks of osmolarity, which is a reflection of total solute particle concentration. The presence of solute in a solution reduces the water concentration of that solution. Body fluids (both intracellular and extracellular) generally have an osmolarity of about 300 mOsmolar (which is the same as 0.3 Osmolar).

Volume Changes in the RBC Caused by Osmotic Forces - Hemolysis

Consider a red blood cell placed in a bathing solution of a non-penetrating solute (i.e. a solute that can't cross the membrane) that has a lower osmolarity than the cell contents. The difference in water concentration causes water to move down its concentration gradient into the cell, and consequently the cell swells. An associated effect is the diluting of the cell contents by the entering water. Water will continue to diffuse into the cell until the osmolarity is the same inside and out, at which point there is no longer a driving force for water to diffuse. In this instance, we are considering a solution with only non-penetrating solute, so diffusion of water into the cell is the only way that the osmolarity of the intra- and extra-cellular solutions can be equalized. The extent of swelling depends on the osmolarity of the non-penetrating solute in the bathing solution. If the bath osmolarity is very low, the cell membrane will be stretched until the cells burst (i.e., hemolysis). By finding the lowest osmolarity at which the cells can swell but not burst, one can estimate the amount of swelling the cell can withstand, i.e. the limit of cell fragility. This test is performed clinically, and changes in cell fragility are observed in several pathological situations (e.g. increased fragility in hereditary spherocytosis).

Intact RBCs scatter light in all directions, making it difficult to see through the solution (cloudy). The bursting of RBCs results in the release of hemoglobin and other cell contents into the medium containing them. These molecules in solution, being much smaller, allow the light to pass undeflected. Therefore, when hemolysis occurs, a suspension of RBCs turns from cloudy to clear.

Observations with NON-PENETRATING Solutes

Red blood cells will be added to solutions of NaCl ranging from 40mM to 160mM. The NaCl molecule dissociates into separate Na^+ and Cl^- ions in solution. The membrane is effectively impermeable to Na^+ and Cl^- ions. **What is the osmolarity of each solution? In which solutions do you see hemolysis? Which way does water move across the cell membrane? Record your observations in the following table.**

	Distilled Water	NaCl 60mM	NaCl 70mM	NaCl 80mM	NaCl 100mM	NaCl 120mM	NaCl 140mM	NaCl 160mM
Osmolarity (mOsm)	0	120	140	160	200	240	280	320
Hemolysis?	Y	Y	Y	Y/N	N	N	N	N
Movement of Molecules?	H_2O in	H_2O in	H_2O in	H_2O in	H_2O in	H_2O in	H_2O in	H_2O out
Shape Changes?	Swell - burst	Swell - burst	Swell - burst	Swell - burst	Swell	Swell	Swell	Shrink

From the lowest NaCl concentration at which no hemolysis occurs, calculate the relative change in volume these red blood cells can tolerate before they lyse (burst).

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Laboratory Experiments in Microbiology Ted R. Johnson, Christine L. Case, 1989

Teaching Innovation in University Education: Case Studies and Main Practices Saura, Jose Ramon, 2022-06-17

In the last decade the development of new technologies has made innovation a fundamental pillar of education Teaching innovation includes the evolution of both teaching and learning models to drive improvements in educational methodologies

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Education Instructional Materials Ohio State University. Center for Vocational and Technical Education,1972

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