

## **Measurement of Erythrocyte Fragility (Osmotic Hemolysis)**

Cell membranes are semi permeable barriers, and osmotic gradients are established between intracellular and extracellular fluids which can cause water to flow into and out of the cells. The amount of osmotic pressure depends upon the difference between the concentration of non-diffusible ions (osmols/L) on each side of the membrane. One osmol is equal to one mol of dissolved, non-diffusible, non-ionizable substance if the substance completely ionizes into two ions, then one mol of the dissolved substance yields two osmols.

The intracellular fluid of erythrocytes is a solution of salts, glucose, protein, and hemoglobin. A 0.9% NaCl (MW 58.5) solution is said to be isotonic: when blood cells reside in such a medium, the intracellular and extra cellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water. When subjected to hypertonic media (e.g. 1.8% NaCl), the cells lose their normal biconcave disc shape, undergoing collapse (leading to crenation) due to the rapid osmotic efflux of water. On the other hand, in a hypotonic environment (e.g. 0.4% NaCl or distilled water), an influx of water occurs: the cells swell, the integrity of their membranes is disrupted, allowing the escape of their hemoglobin (hemolysis) which dissolves in the external medium.

In this experiment, we make use of the property that the osmotic fragility (or susceptibility to hemolysis) of erythrocytes is not uniform, and the number of cells undergoing hemolysis depends on the degree of hypotonicity of the extracellular medium. Thus, the percentage of erythrocytes which hemolyse when suspended in media of different hypotonicity (between isotonic saline and distilled water) will vary. The concentration of liberated hemoglobin in each test medium is an index of the extent of osmotic hemolysis. Your problem is to examine experimentally the relationship between extent of hemolytic and osmolarity of the medium in which the erythrocytes are suspended.

*See Instructor For Procedure:*