

Objectives

1. Describe which cell structures are present on a cheek sample.
2. Explain cell membrane transport, focusing on osmosis.
3. Explain the factors that might affect the enzymes normal functions.

Review of Microscope Care

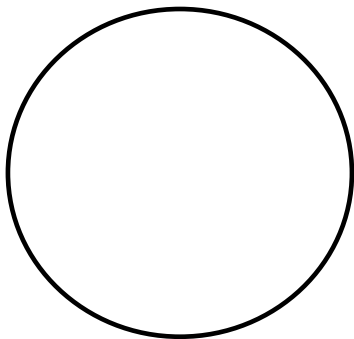
- Hold with two hands, one under the base and the other supporting the arm
- Always begin with the lowest objective magnification lens first

Activity 1: Cheek Cell Wet Mount

This activity will help you observe and understand the basic components of cells, namely the plasma membrane, cytoplasm, and nucleus. You will be obtaining a sample of cheek cells from one of your group members. The cells found in the lining of the oral cavity are known as squamous epithelial cells.

Procedures:

- A. Place a drop of 0.9% NaCl saline solution onto a microscope slide.
- B. Choose one member of your group and with a toothpick, carefully scrape the lining of your cheek to obtain a sample of epithelial cells.
- C. Transfer the sample to the saline solution on your microscope slide and mix using the toothpick.
- D. Add a drop of methylene blue stain to your microscope slide. Again, use the toothpick to stir.
- E. Apply a coverslip to your sample and gently blot excessive fluid with a Kimwipe (tissue).
- F. Examine the stained epithelial cells using a microscope

Sketch your observation of a cheek cell below

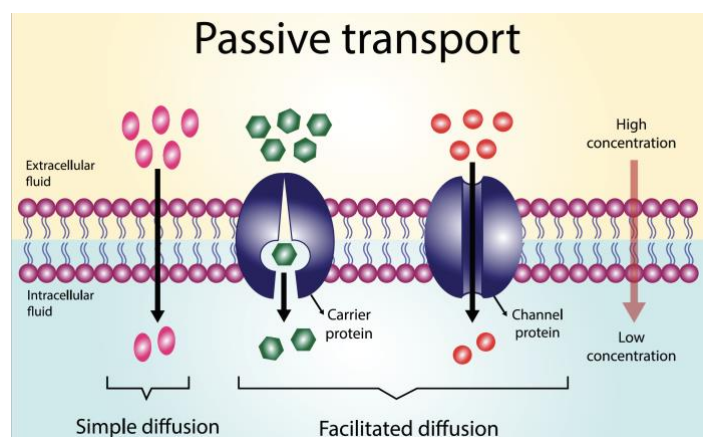
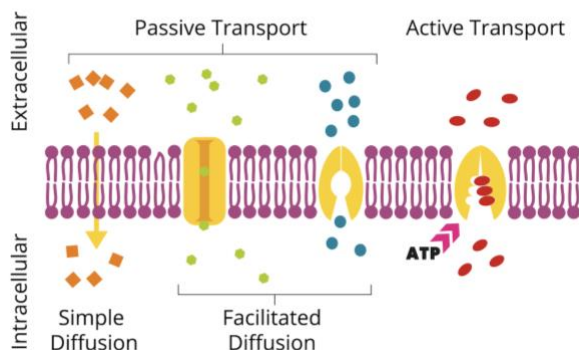
- a) What parts of the cells were visible under the microscope?
- b) List 2 organelles that were not visible but should have been in the cheek cell?
- c) Methylene blue is a cationic, basic stain. For this reason, methylene blue binds strongly to negatively charged cell components. How might this explain its role in staining the nucleus?
- d) One adaptive trait of viruses is the ability to envelope themselves in host-derived membrane after viral progeny budding from cells. How might this help viruses proliferate?

- e) A patient is deficient in the HMG-CoA reductase enzyme responsible for cholesterol synthesis. A cell biologist notices an odd morphology to this patient's cell. What is the connection?
- f) In some neurological disorders such as amyotrophic lateral sclerosis (ALS) where motor neurons in the brain and spinal cord die, the nuclear pores of neurons become clogged. In relation to the central dogma of molecular biology, what is one product that would be unable to exit the nucleus, and which step of gene expression would be inhibited?

Activity 2: Cell Membrane Transport

There are two types of transport, passive and active transport. When the transport requires energy, it is active transport, and this is due to the molecule moving against its concentration gradient. If it doesn't require energy, it is passive transport. In this laboratory, we are going to focus on passive transport. Simple diffusion is when molecules move into and out of the cell by their own thermal motion, down their concentration gradient. Osmosis is the movement of water molecules down their concentration gradient. Osmosis and diffusion can be a little confusing, but keep in mind that osmosis can only function in a liquid medium, but diffusion can occur in all three mediums (solid, liquid, and gas). Furthermore, osmosis requires a semi-permeable membrane, while diffusion does not. The intake of water in plants is an example of osmosis. This movement is caused by an unequal distribution of solutes on the two sides of the membrane. Water is "drawn" towards the side of the membrane with the higher solute concentration (as there is less water). No osmosis occurs when the solute concentrations on the two sides of the membrane are equal. Today we are going to study osmosis in erythrocytes across a semipermeable membrane.

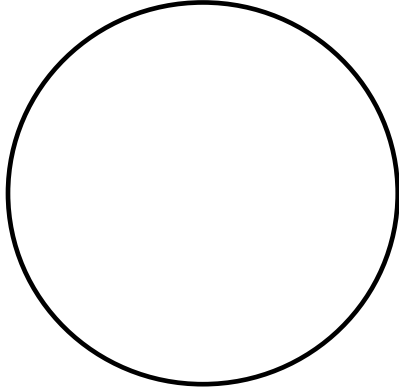
Cell Transport



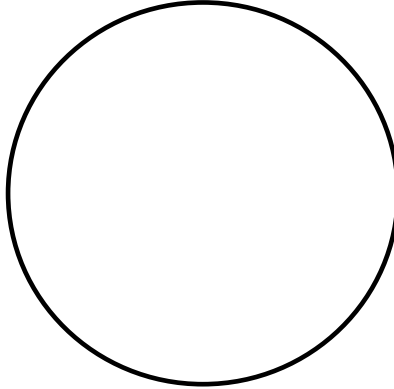
Procedures

- A. Obtain three test tubes and label them 1, 2 and 3.
- B. Add 1 mL of 0.9% NaCl to the test tube labeled 1, 1 mL of 10% NaCl to the test tube labeled 2, and 1 mL of deionized (DI) water to the test tube labeled 3.
- C. Using a pipet, place 3 drops of sheep's blood into each test tube
- D. After carefully stirring the solutions and waiting five minutes, add 1 drop from each of the test tubes onto microscope slides.
- E. Examine the slides at 40x and record your observations.

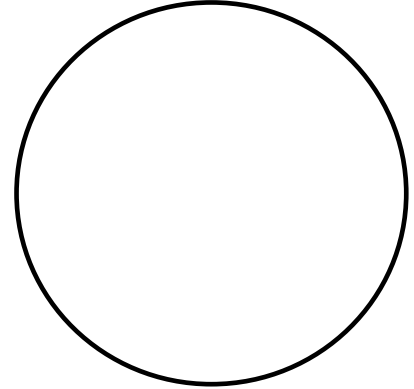
Hypertonic Solution



Hypotonic Solution



Isotonic Solution



Questions

- a) Which solution is hypertonic?
- b) Which concentration of NaCl lysed the cells?
- c) Which of the three solutions most closely resembled the solute concentration in a red blood cell? How do you know?
- d) How might these results relate to the effect of a salty diet on blood pressure?
- e) Why might the absence of chloride ion channels in cystic fibrosis patients result in the common symptom of excessively thick mucus secretions?

Activity 3: Metabolism and Enzymes

Metabolism is defined as the sum of all chemical reactions in a cell. In the human body, the energy is extracted from biomolecules ingested in food. Once this food is digested, it is processed and then used as work or for storage. Many metabolic pathways are dependent on the presence of enzymes. Enzymes are biomolecules that act as catalysts, meaning they increase the rate of chemical reactions by lowering the activation energy. Enzymes bind to a reactant molecule, which we call a substrate. The area of the enzyme that binds to the substrate is called the “active site”. The enzyme then acts on the substrate to make a product molecule. A general enzymatic reaction can be seen below:

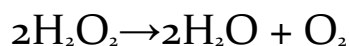


As it can be seen in the reaction above, enzymes are not used up in reactions. After the reaction occurs, the enzyme is free to bind to another substrate molecule and catalyze the reaction.

It is important to remember that enzymes bind to specific substrates. They can recognize one substrate from another. This is due to their complementary shapes. This is known as the “lock and key model”. A more accurate description of the specificity of enzymes is the “induced fit model”. This model states that the binding of the substrate alters the shape of the active site by inducing a conformational change in the enzyme.

Enzymes can be affected by many factors, including “external factors” such as temperature and pH. In this laboratory, we will not go into detail about other factors such as enzyme and substrate concentration, activators, and inhibitors. Just know that these factors are important. Changes in temperature or pH can alter the shape of the enzyme, denaturing it. This means the enzyme will not function properly. In the human body, changes in temperature and pH rarely occur, and this is because they are highly controlled.

Catalase catalyzes the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. The reaction can be seen below:



Hydrogen Peroxide is a byproduct of some metabolic processes, but it can be harmful. This makes catalase important for preventing oxidative stress. Live cells have a high concentration of Catalase.

Questions

- How might temperature affect the rate of reaction of certain enzymes? What if it is too hot or too cold?
- What can pH do to the rate of reaction of certain enzymes? What if this enzyme lives in the stomach? The small intestine? Blood?
- In cyanide poisoning, cyanide binds to and inhibits the cytochrome c oxidase enzyme of the electron transport chain. Which organelle would be affected and how would its function be impaired?

Making organelles from clay! Each group will be assigned an organelle and you must make it from clay. You will present it and explain the function in front of the class!

- Nucleus
- Mitochondria
- Rough ER + Ribosome
- Golgi Apparatus
- Lysosome
- Centrosome
- Cell Membrane
- Smooth ER