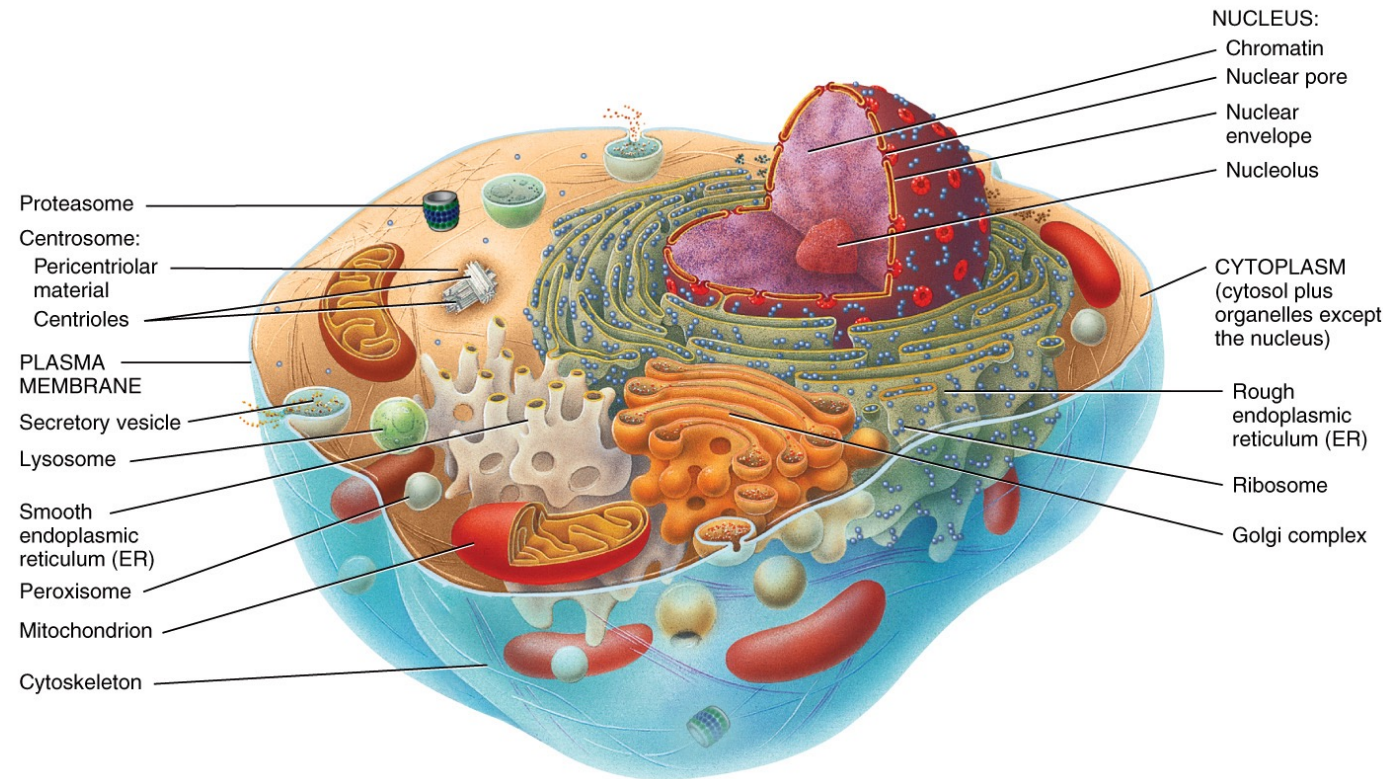


LAB 1: CELL BIOLOGY

Protocol Slides
PCB 3702L
FIU

LAB 1 PROTOCOL 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.



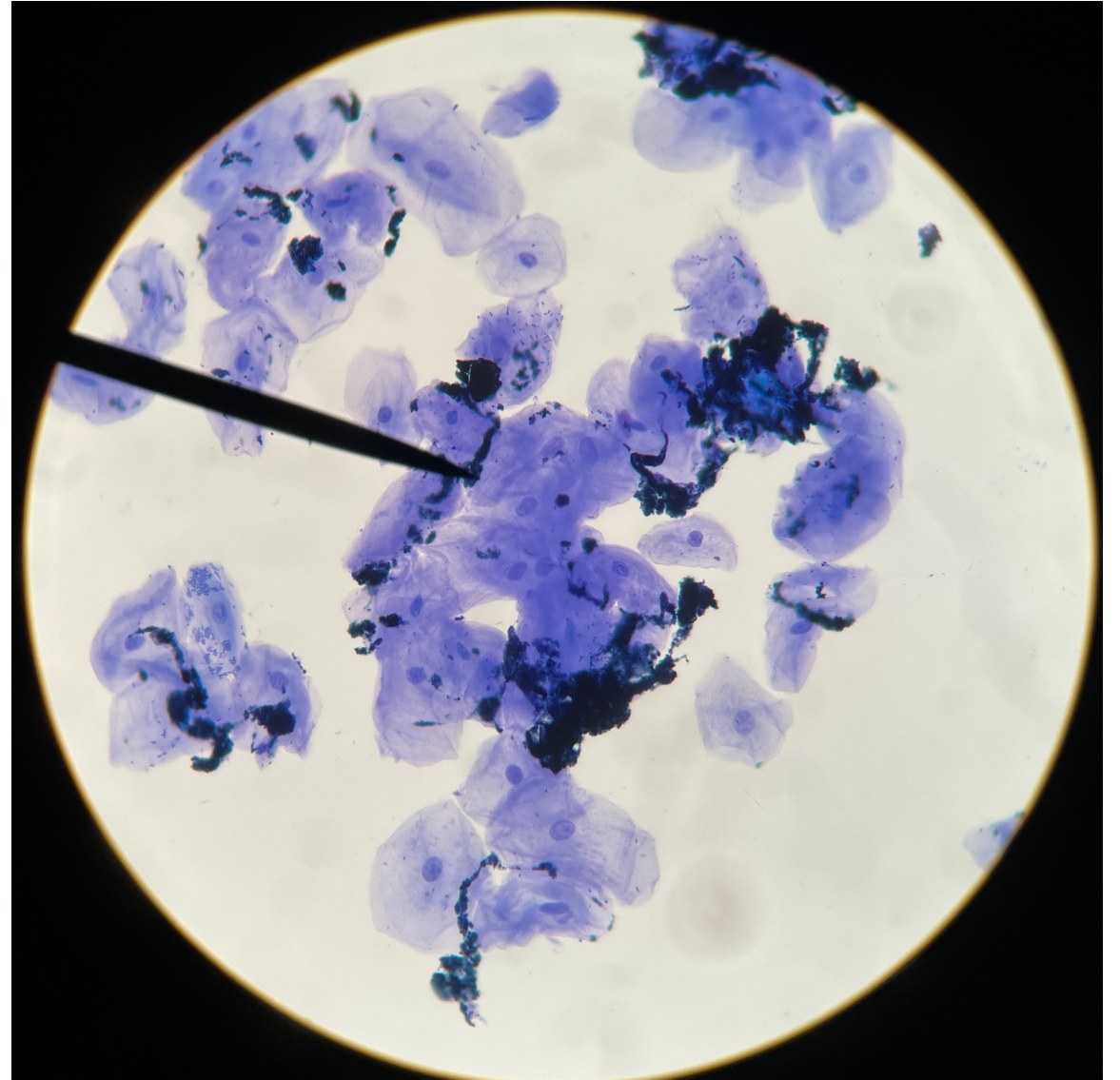
MICROSCOPE BASICS



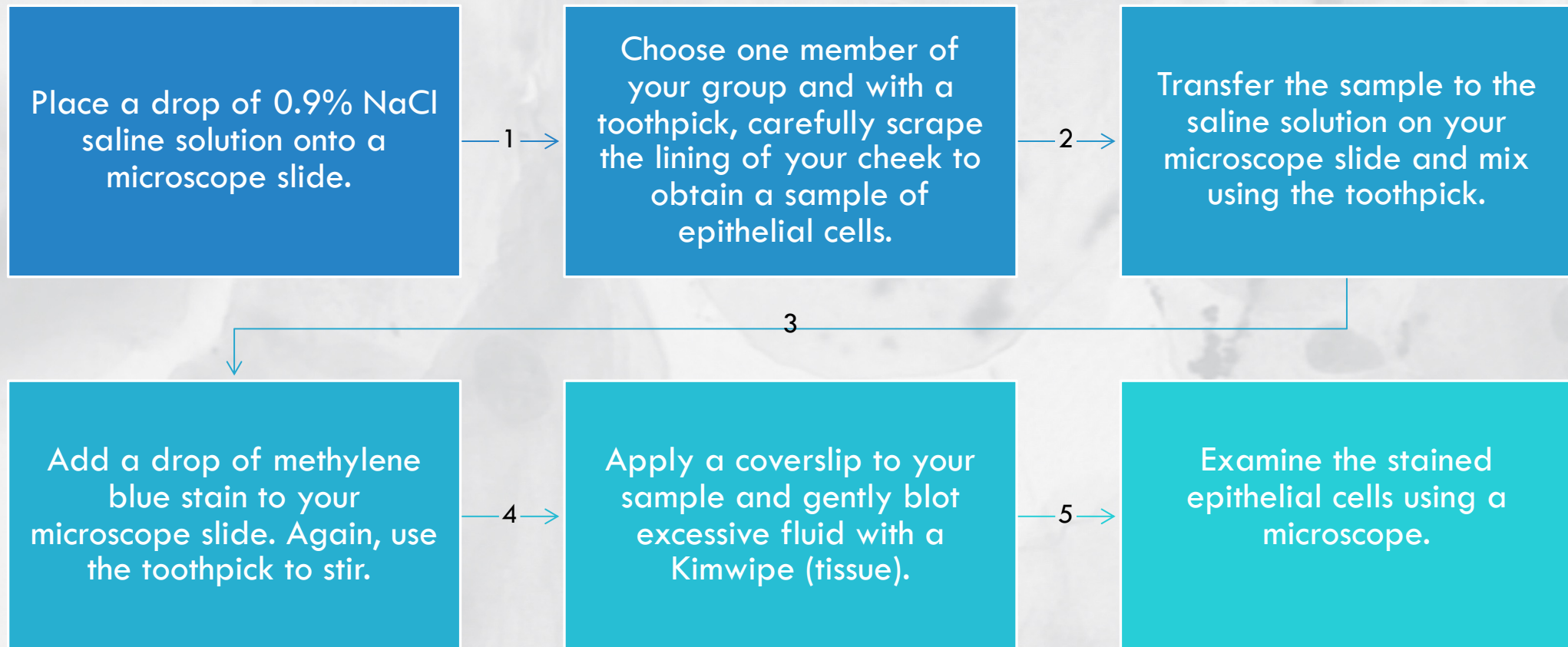
1. Turn ON the Microscope's **light switch**
2. Adjust the illumination
3. Place **objective lens** on the LOWEST magnification (x4)
4. Pull on the **stage clip** to load the specimen slide onto the stage
5. Adjust the stage using the **stage control knobs** in order to find the specimen on the slide
6. First, use the **COARSE adjustment knob** to bring the stage up until the specimen is in sight (should look like a clump)
7. Then, use the **FINE adjustment knob** to slowly bring the stage further up until the specimen becomes clearer (more features will become noticeable)
8. IF you need to see a more magnified version of the specimen, carefully move up to the next magnification lens and REPEAT steps 6 & 7 until you have a clear view of the specimen

Always grip the microscope by the arm and put your hand beneath its base. Always hold the scope upright. Do not bump it against anything.

ACTIVITY 1: CHEEK CELL WET MOUNT



CHEEK CELL WET MOUNT PROCEDURES



CHEEK CELL WET MOUNT QUESTIONS

What kind of cell are we staining?

- Diploid, Somatic Cell
- Stratified Squamous Epithelial Cell

What parts of the cell were visible under the microscope?

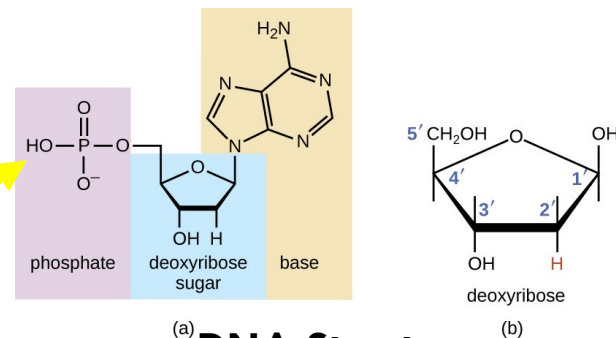
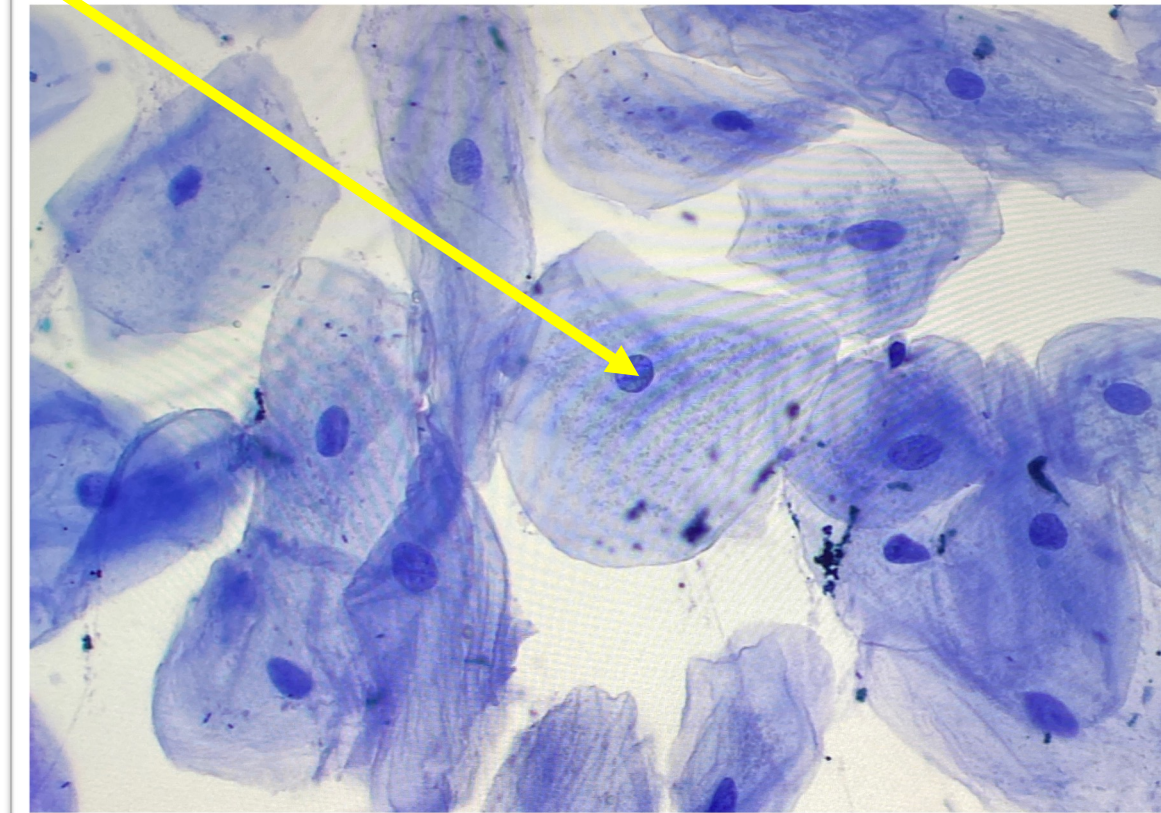
- Nucleus, cytoplasm, cell membrane

List 2 organelles that were not visible but should have been in the cheek cell.

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?

- Binds to the negatively charged molecules in the cell such as DNA/RNA

nucleus



(a) **DNA Structure**

(b)

APPLICATION QUESTIONS

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?

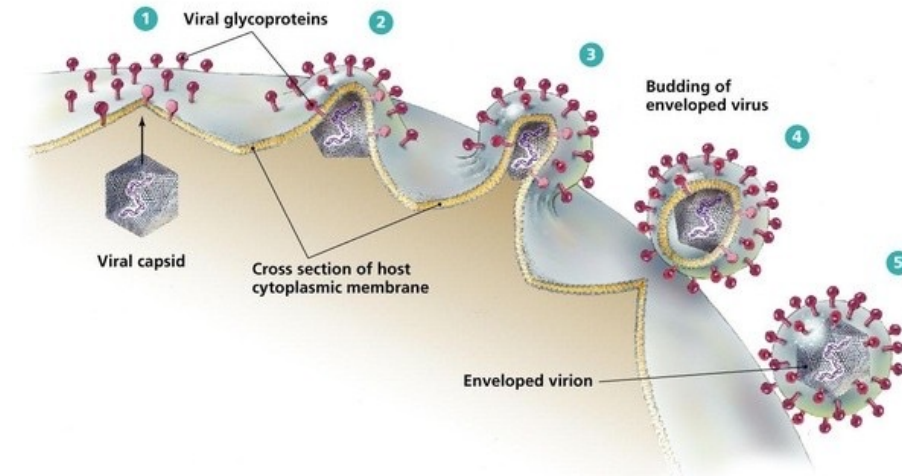
- The host membrane might help viruses avoid detection by the host's immune system

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?

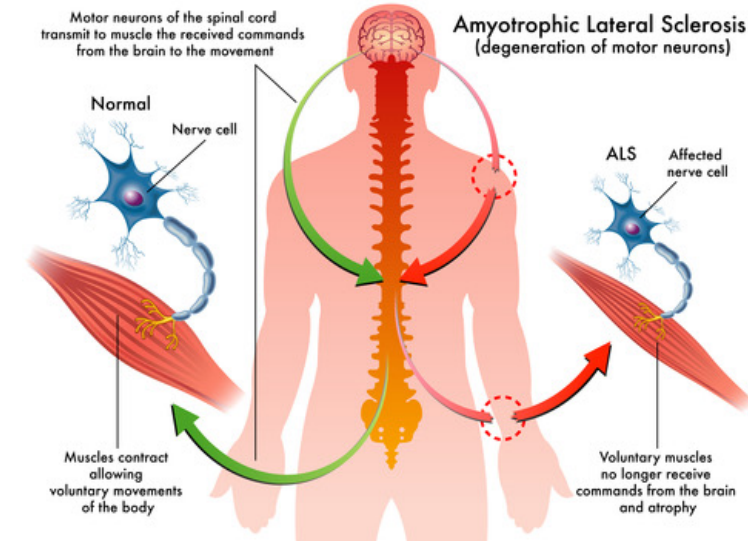
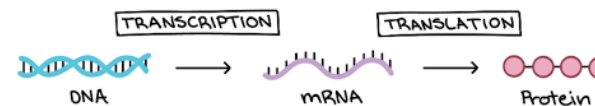
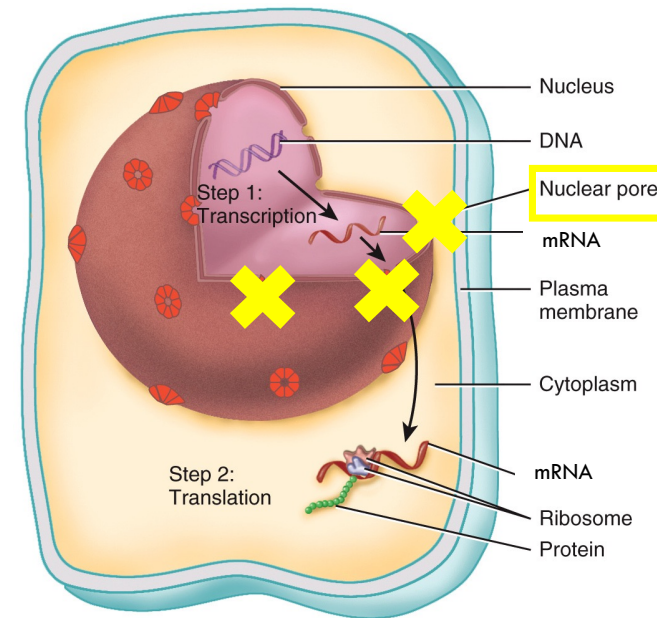
- Cholesterol regulates membrane fluidity, depending on environmental conditions

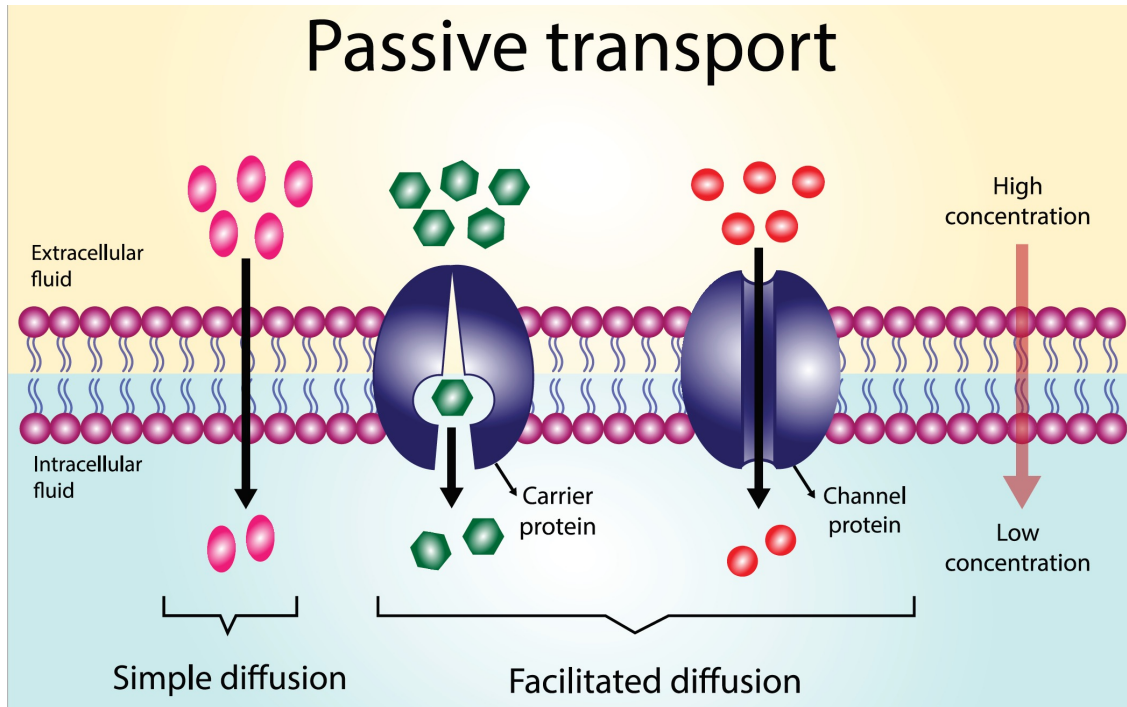
In some neurological disorders such as amyotrophic lateral sclerosis (ALS) where motor neurons in the brain and spinal cord die, the nuclear pores of neurons become clogged. In relation to the central dogma of molecular biology, what is one product that would be unable to exit the nucleus, and which step of gene expression would be inhibited?

- mRNA will be unable to exit the nucleus
- Translation would be inhibited

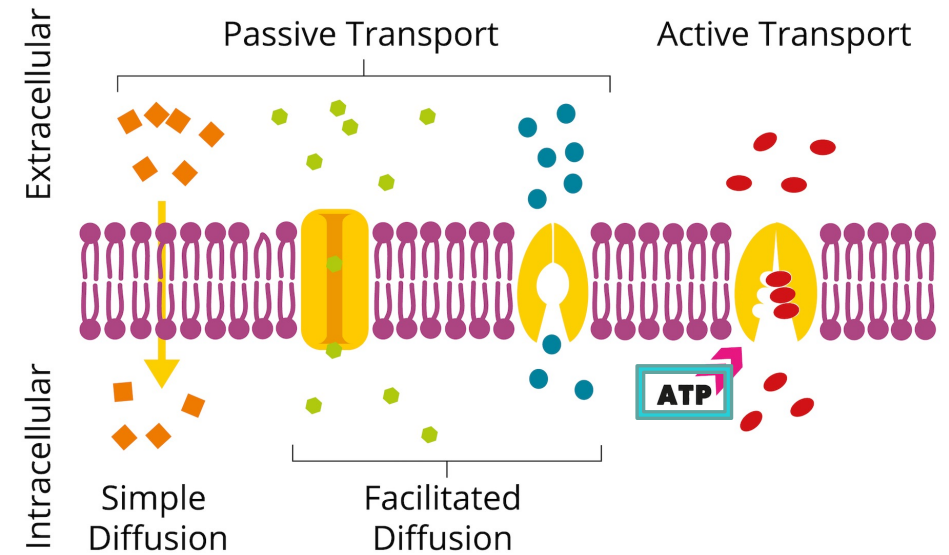


Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.





Cell Transport

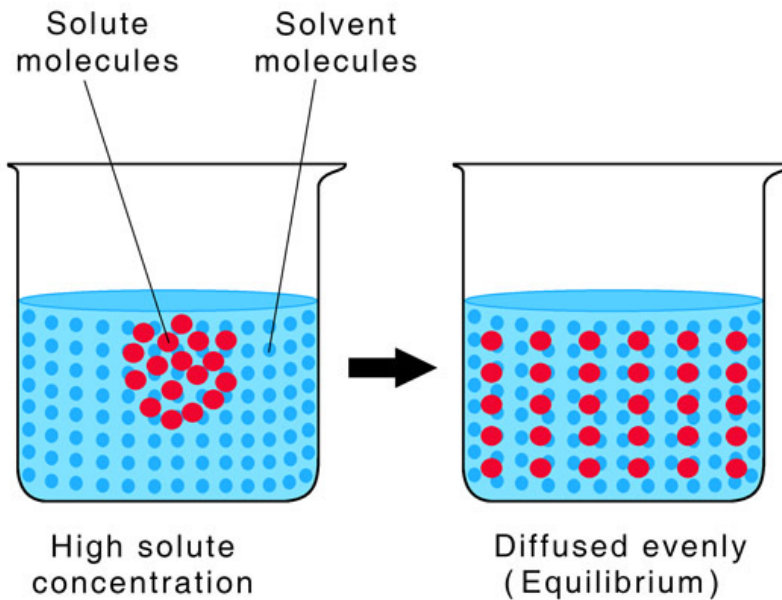


ACTIVITY 2: CELL MEMBRANE TRANSPORT

What is the difference between passive and active transport?

Diffusion

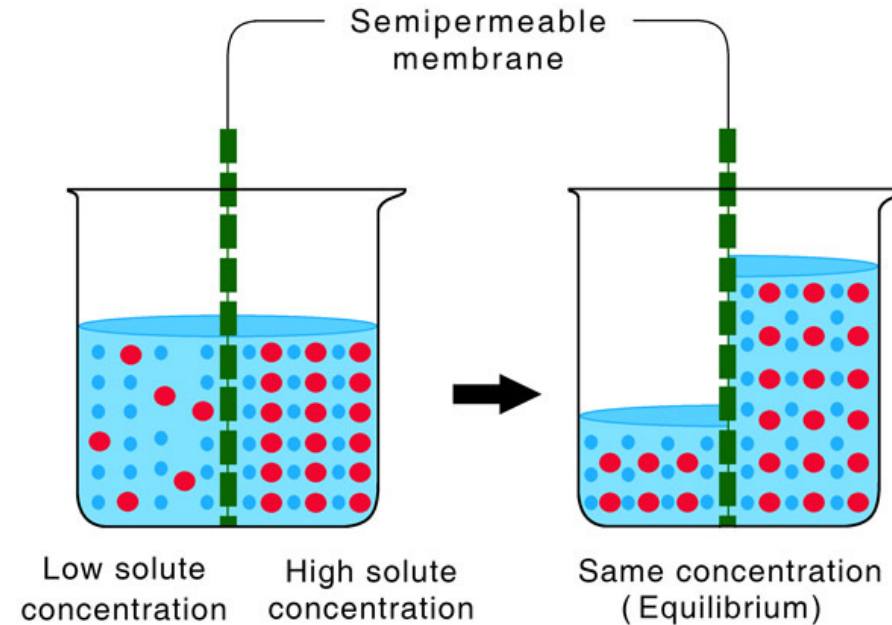
Solute molecules move from high to low concentration



vs

Osmosis

Solvent molecules move from low to high solute concentration

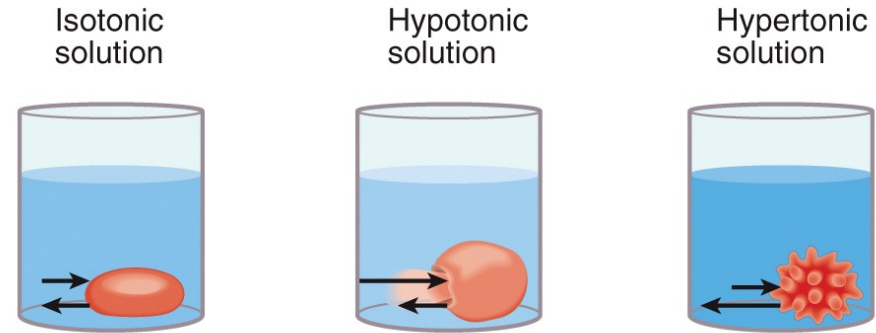


TONICITY OF SOLUTIONS

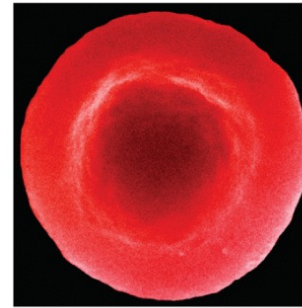
Describes the ability of a solution to change the volume of cells by altering their water content via osmosis. It depends not only on relative solute concentrations, but also on solute permeability

- Isotonic solutions cause no net movement of water

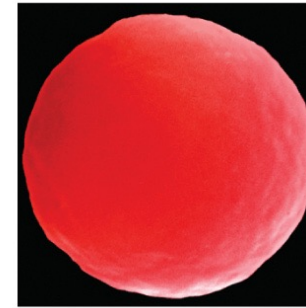
What is the effect of a hypertonic solution? A hypotonic solution?



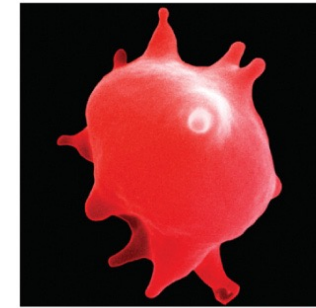
(a) Illustrations showing direction of water movement



Erythrocyte maintains normal shape in an isotonic solution



Erythrocyte undergoes hemolysis in a hypotonic solution



Erythrocyte undergoes crenation in a hypertonic solution

SEM

David M. Phillips/Science Source

(b) Scanning electron micrographs (all 15,000x)

SHEEP'S BLOOD ACTIVITY PROCEDURES



1. Obtain three test tubes and label them 1, 2 and 3.



2. Add 1 mL of 0.9% NaCl to the test tube labeled 1, 1 mL of 10% NaCl to test tube labeled 2, and 1 mL of deionized (DI) water to the test tube labeled 3.



3. Using a pipet, place 3 drops of sheep's blood into each test tube.

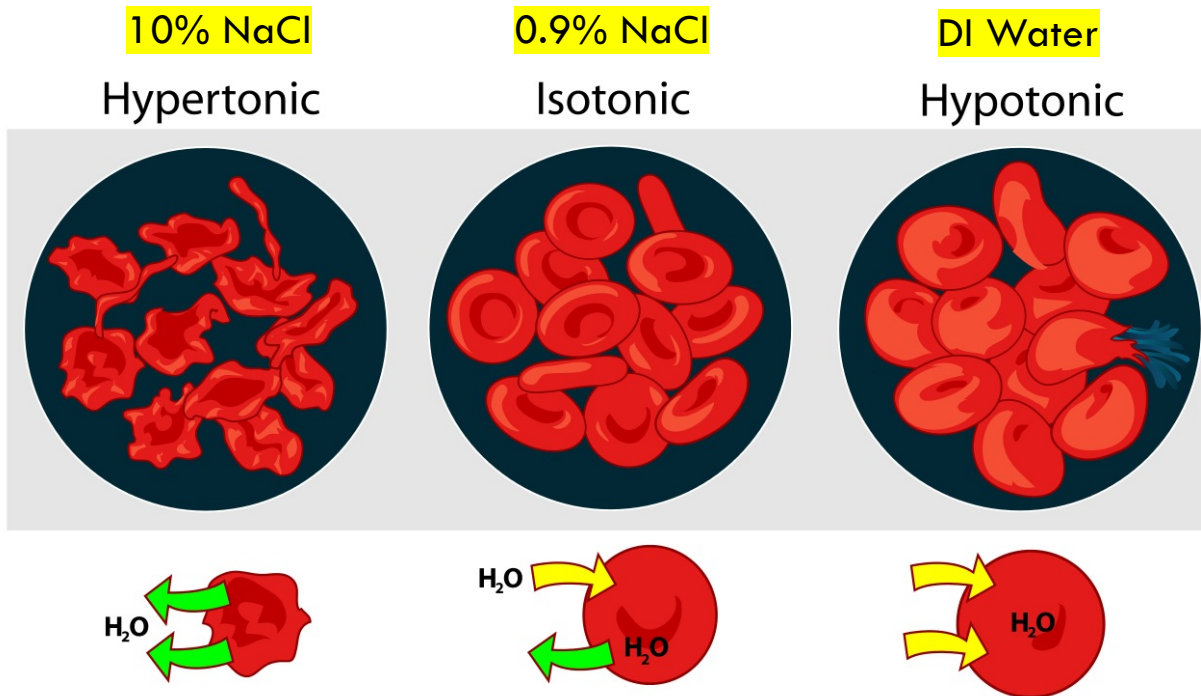


4. After carefully stirring the solutions and waiting five minutes, add 1 drop from each of the test tubes onto microscope slides.



5. Examine the slides at 40x and record your observations.

SHEEP'S BLOOD ACTIVITY - RESULTS



Which solution is hypotonic?

Which solution is hypertonic?

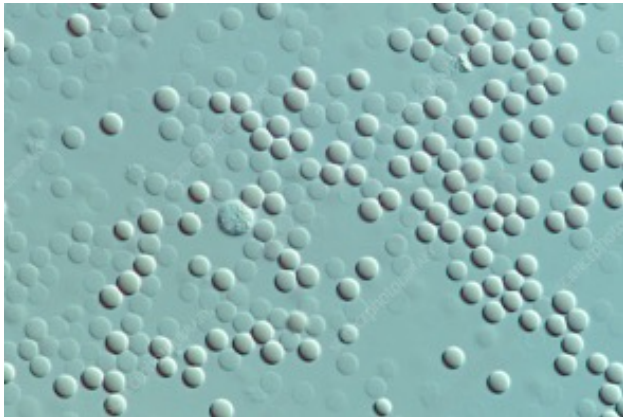
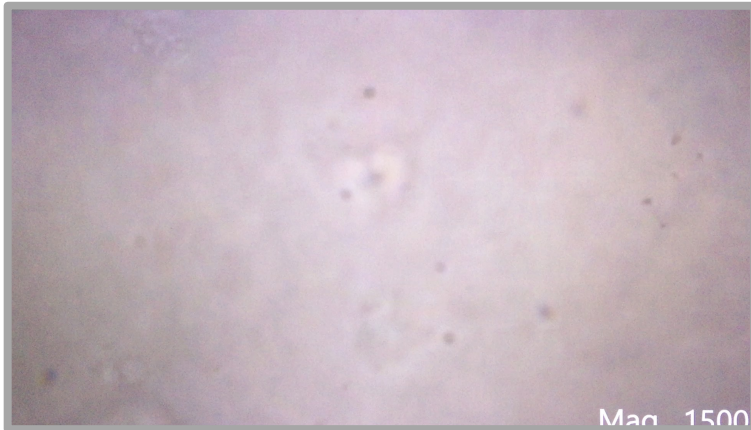
Which concentration of NaCl lysed the cells?

Which of the three solutions most closely approximated the solute concentration in a red blood cell? How do you know?

OSMOSIS IN ERYTHROCYTES

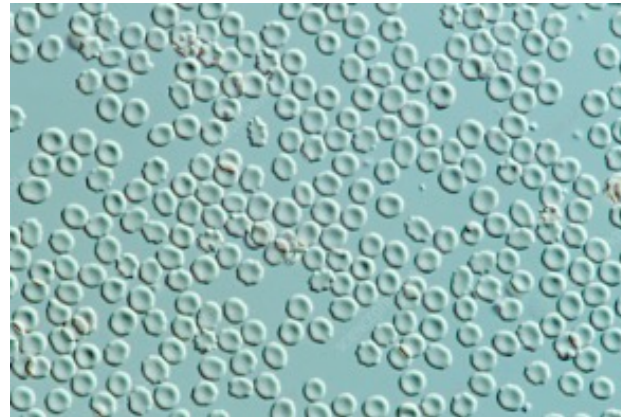
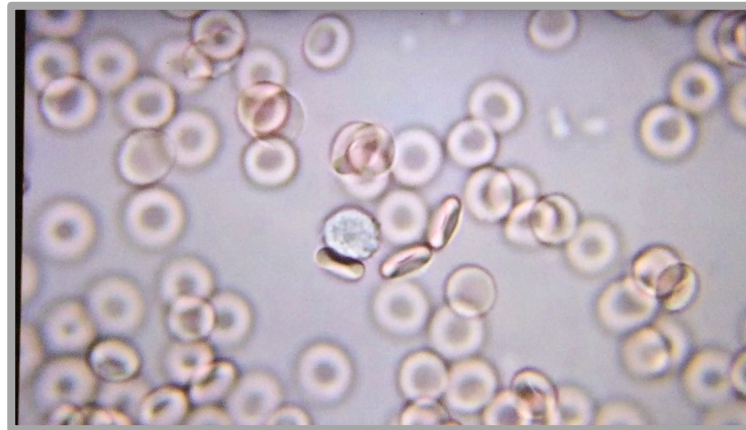
DI Water

Hypotonic



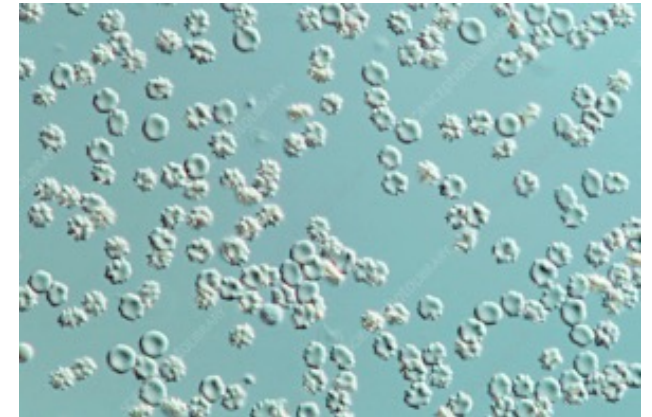
0.9 % NaCl

Isotonic



10 % NaCl

Hypertonic



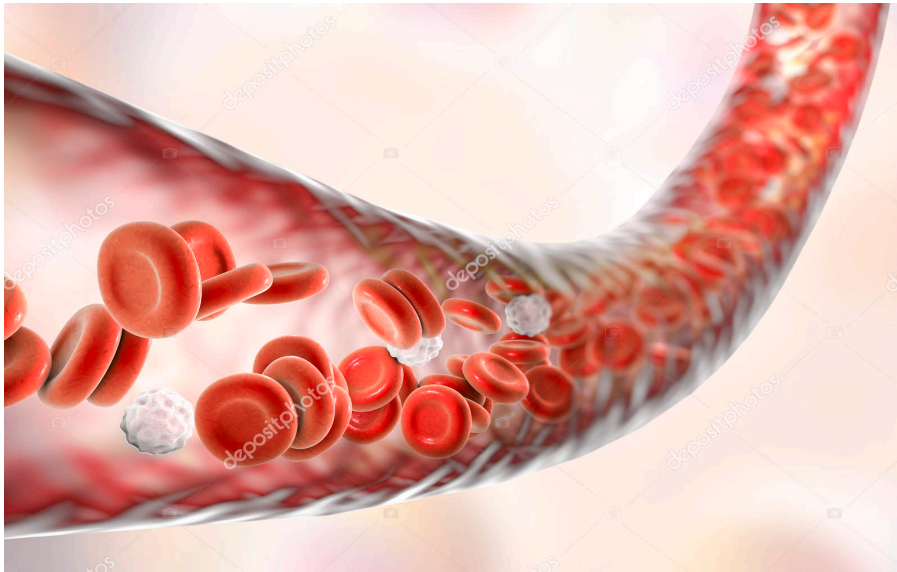
Full video can be found here:

<https://www.youtube.com/watch?v=A8cl6FkcG4c>

APPLICATION QUESTIONS

BLOOD PRESSURE

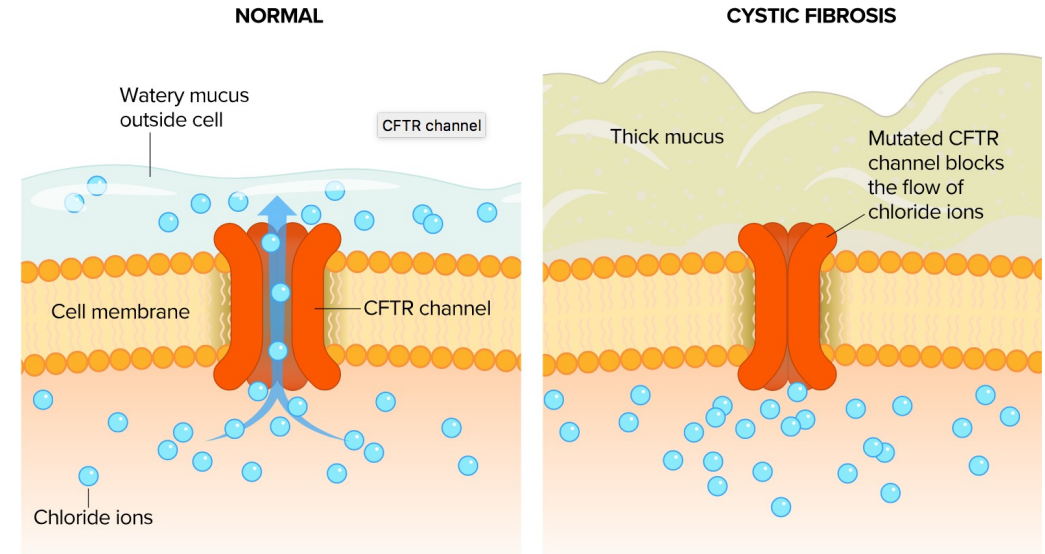
How might these results relate to the effect of a salty diet on blood pressure?



A high concentration of ions from dietary salt in blood plasma leads to increased water retention in the body, which increases blood volume and consequently, blood pressure.

CYSTIC FIBROSIS

Why might the absence of chloride ion channels in cystic fibrosis patients result in the common symptom of excessively thick mucous secretions?



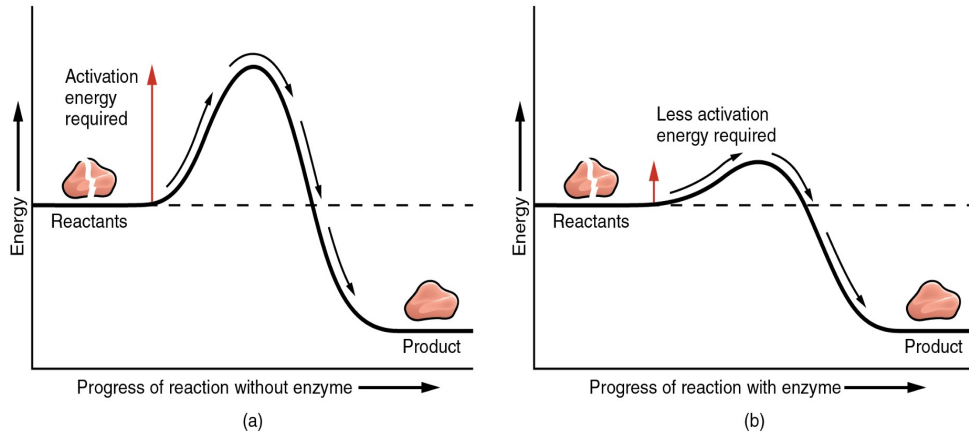
A mutation in the CFTR gene causes a dysfunction of the salt and water balance. This results in dehydration of mucous secretions and excessive loss of salt in sweat.



ACTIVITY 3: METABOLISM & ENZYMES

ENZYMES

Figure 2.13



- **Enzymes**

- Enzymes decrease the activation energy required for a given chemical reaction to occur.

(a) Without an enzyme, the energy input needed for a reaction to begin is high.

(b) With the help of an enzyme, less energy is needed for a reaction to begin.



Central Dogma of Biology

Serving as biological catalysts, enzymes catalyze chemical reactions by **accelerating** the conversion of reactants into products. This is done by **lowering the amount of activation energy required for a reaction to occur**

Enzymes are not used up or changed at the end of a given reaction

Enzymes are pH and temperature sensitive, with each enzyme having optimal activity within a specific pH and temperature range

Enzymes do not alter the equilibrium constant and do not affect the overall ΔG of a reaction

How are they produced?

- Enzymes are usually proteins but can be RNA (Ribozymes)
- Proteins are synthesized by ribosomes on the rough ER; they are then modified by the Golgi apparatus

Which factors can affect the performance of an enzyme?

- pH, temperature, salinity, inhibitors, activators, enzyme concentration, substrate concentration, cofactors, etc.

ACTIVITY 3: METABOLISM & ENZYMES



How might temperature affect the rate of reaction of certain enzymes? What if it is too hot or too cold?

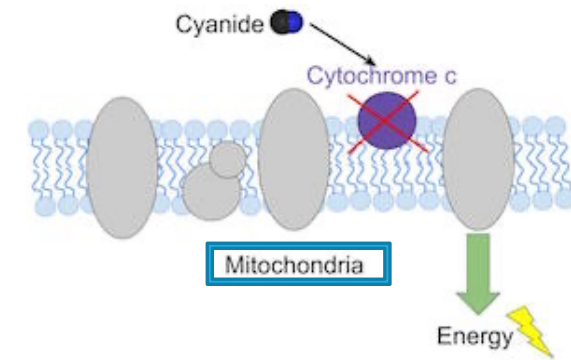
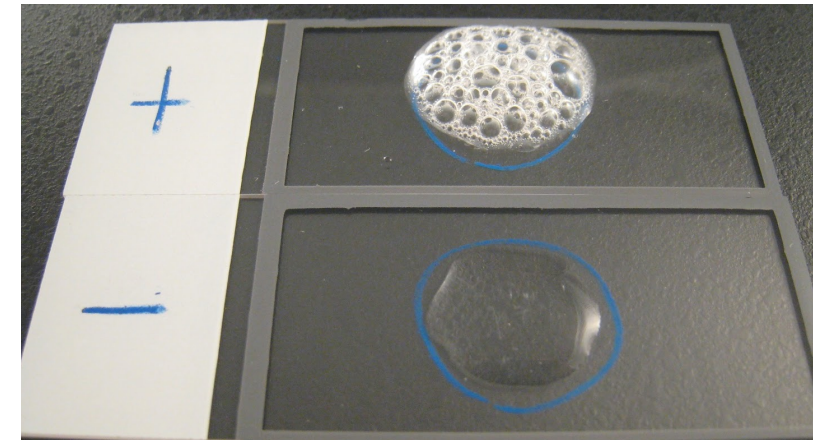
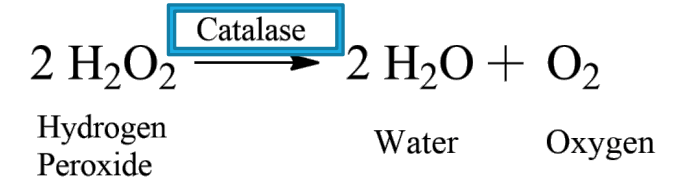


What can pH do to the rate of reaction of certain enzymes? What if this enzyme lives in the stomach? The small intestine? Blood?

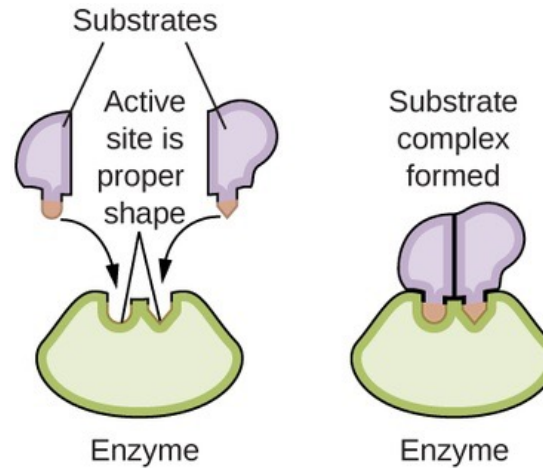


In cyanide poisoning, cyanide binds to and inhibits the cytochrome c oxidase enzyme of the electron transport chain. Which organelle would be affected and how would its function be impaired?

What enzyme is responsible for converting hydrogen peroxide into water and oxygen?

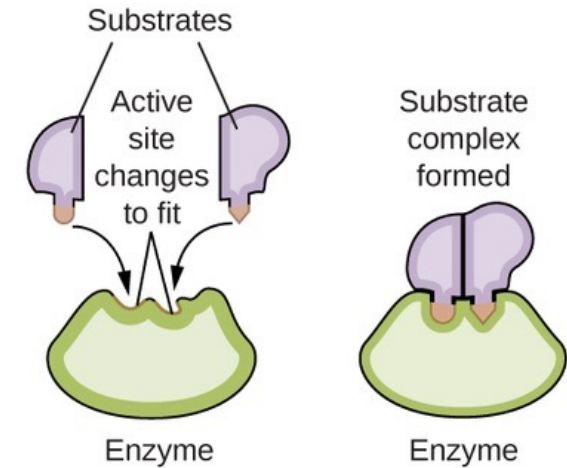


ENZYMES: LOCK-AND-KEY VS. INDUCED FIT MODELS



(a) Lock-and-key model

- Active site is the “lock” and the substrate fits like a “key”
- Both the substrate and enzyme active site should fit like lock and key to initiate a reaction



(b) Induced fit model

- Generally accepted model
- Binding of substrate induces a conformational change in the active site of the enzyme