

## **LAB 4 : ENZYMES**

Organisms can perform a variety of activities such as movement, chemical synthesis, growth, digestion and secretion, and respond to stimuli. They are able to do all of these things using the set of chemical machinery that guides and controls the multiple chemical processes occurring at the cellular level. The properties that are inherent to all living things are more or less related to these chemical processes.

A cell is the site of continuous chemical activity. Growth and reproduction depend on the biochemical synthesis needed for the production of new protoplasm. Secretion, accumulation, movement, and heat production require energy and result only from the chemical reactions within the living cells. Energy is constantly being extracted (as ATP) from the catabolism of large organic molecules and used in a wide variety of synthetic reactions. Even the processes of nerve conduction and other stimulus-response mechanisms depend upon the chemical reactions in nerve cells and the sense organs. In other words, ***ALL LIFE ACTIVITIES ARE BASED ON CHEMISTRY.***

Plant and animal cells contain thousands of substances that undergo chemical reactions. If these substances were taken away from the cell (separated from the living machinery) and simply mixed together, few reactions would occur. Within the chemical contents of cells, there is the potential for many kinds of chemical reactions, BUT only in the living cell does this potential manifest itself. Why? Because, the living machinery supplies an essential ingredient for each of these reactions. **ENZYMES** are large protein molecules that act as catalysts in biochemical reactions.

A **catalyst** speeds up a chemical reaction. For example, if iron is mixed with oxygen, nothing happens. However, in the presence of water, the iron and oxygen undergo a chemical reaction to form ferric oxide (rust). The water, which greatly speeds up the reaction, is the catalyst. Enzymes, which are produced by living cells, perform the same function in the chemical reactions on which all life depends. Without the help of a catalyst, most of the chemical reactions occurring in cells would proceed too slowly to be effective.

As catalysts, enzymes speed the reactions of hydrolysis, decomposition, oxidation, double displacement, polymerization, racemization, and other chemical processes. Each enzyme is a specific catalyst for one type of reaction. The chemical components whose reactions are catalyzed by an enzyme are called **substrates** or **reactants**.

Most enzymes are specific; i.e. they tend to react with one or only a few specific molecules (substrates). Whenever a reaction of a group of substrates is susceptible to catalysis by a particular enzyme, these substrates are always closely related chemically. This remarkable specificity is characteristic of

enzymes. The enzyme binds temporarily with its substrate or substrates to form an **enzyme-substrate complex**. In the reaction that follows, the substrate is changed to **products**. {Figure 1}

An enzyme works by lowering the energy of activation required for a reaction {Figure 2}. The enzyme is then regenerated at the end of the reaction and always appears among the reaction products. Therefore, each enzyme molecule reacts over and over again—so a little goes a long way! Each cell has room for possibly a few thousand different enzymes. Although each one is present only in very small amounts, the speed that enzyme molecules use to catalyze the reaction of one substrate molecule after another (up to 1,000,000/second!) compensates for the low concentrations of the individual enzymes that are found in each living cell.

Almost any reaction requires a catalyst, and each catalyst is somehow different. Therefore, there are several thousand different catalysts speeding along the same number of chemical reactions in each living cell.

Because enzymes are proteins, which are large molecules, they have the physical properties of other large molecules. Also, proteins (i.e. enzymes) are changed or destroyed by heating, and their properties (i.e. catalyzing ability) are sensitive to changes in pH (i.e. acidity or alkalinity) and to various chemical and physical agents.

All known enzyme-catalyzed reactions occur with the loss of some energy (heat). The reactions are either **1: driven reactions**: which must have energy supplied as heat, light, electrical energy, or other forms, or **2: spontaneous reactions**: which release {lose} energy from the substrate. Catalysts can only speed up spontaneous reactions. For example, digestive enzymes catalyze the breakdown by hydrolysis of the large molecules of protein, carbohydrates, and lipids into smaller fragments—substances usable by living organisms. The starting materials—i.e. starch and water, contain more chemical energy than the products—i.e. small sugar molecules. The excess energy is evolved as heat. Similarly, oxidation of food materials in respiration releases some energy as heat. The substrates of each oxidative reaction are more energy-rich than the products. Since enzymes cannot contribute the energy to drive reactions that cannot occur spontaneously, how can a living cell synthesize the energy-rich molecules from substrates containing less energy? The living machinery accomplishes this trick without violating any laws of nature by **coupling** each energy-requiring (driven) reaction with an energy-yielding (spontaneous) reaction. In a way, the latter reaction drives the former. In living organisms, the energy-yielding reactions are called catabolic processes, or **catabolism**. The synthetic reactions, which must be driven, are anabolic processes. The sum total of biological syntheses is called **anabolism**. Anabolic reactions are possible at the expense of the energy made available by catabolism.

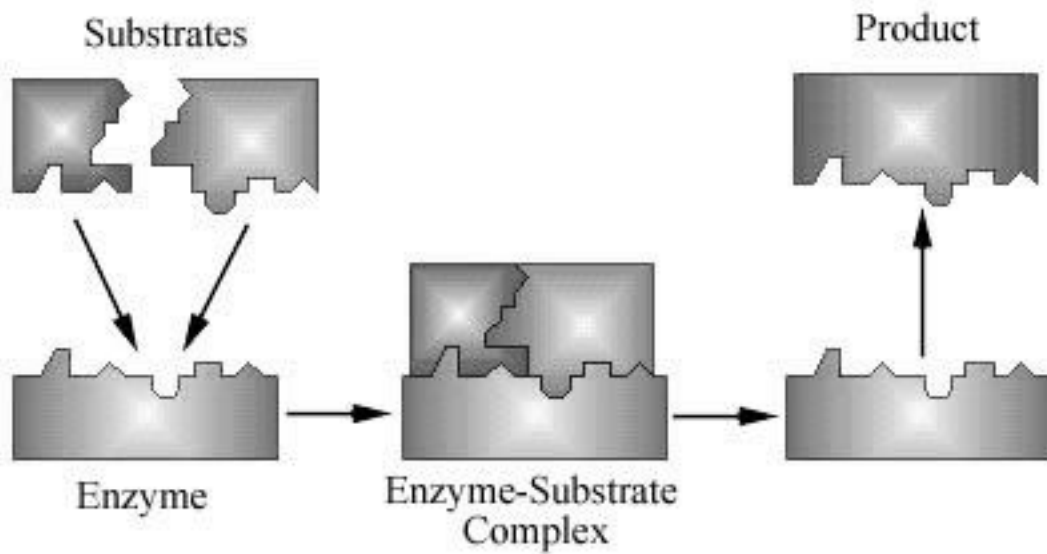


Figure 1. Formation of an enzyme-substrate complex.

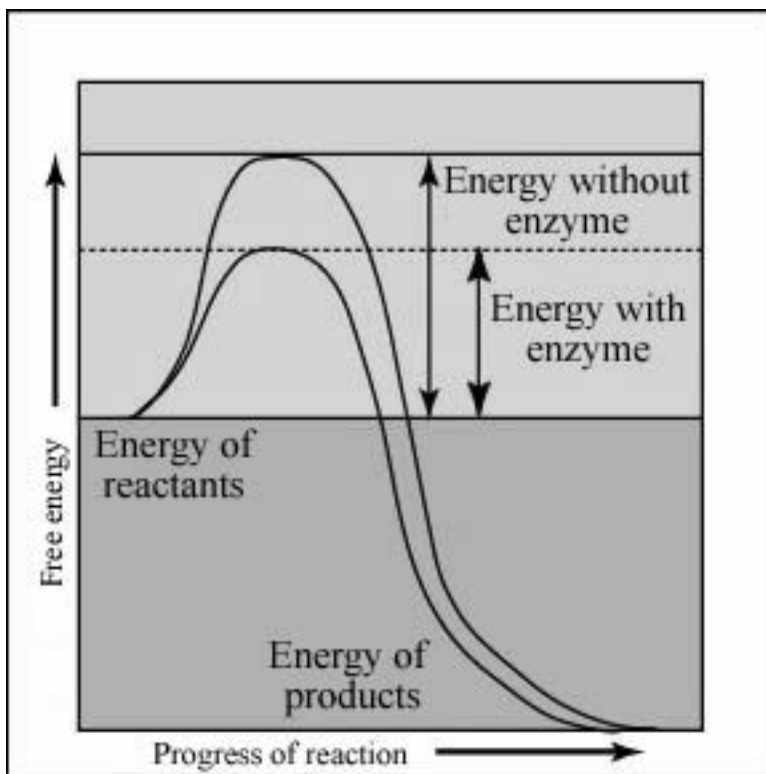


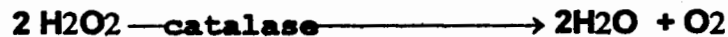
Figure 2. Free energy of a reaction with and without an enzyme.

It is important to realize that the energy released as heat cannot be used by the cell to drive synthetic (anabolic) reactions. The clever trick used by living cells to couple anabolic with catabolic reactions is to conserve, in one of the products of a catabolic reaction, some of the energy of the initial reactants. This product then becomes one of the substrates of the anabolic reaction. Therefore, the overall process can be thought of in two steps. Each step is a spontaneous reaction. Although some energy is lost in the reaction, high-energy products are made from low-energy substrates. Anabolism is accomplished by coupling, and coupling only happens one way--by making a product of one reaction the substrate of another.

### **PROCEDURE 1**

#### **ENZYME ACTIVITY AS A FUNCTION OF SUBSTRATE CONCENTRATION**

In the absence of a catalyst, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is a relatively stable substance. However, it will decompose slowly, forming oxygen (O<sub>2</sub>) and water (H<sub>2</sub>O). Various chemical agents can change this rate of decomposition. The most effective catalyst known for this reaction is the enzyme, catalase.



In this experiment, you will work with the enzyme catalase (from ground-up cow liver), which speeds up the breakdown of hydrogen peroxide.

Observe the crystals of purified catalase on the demonstration table. A dilute aqueous solution of the enzyme has been prepared for your use.

1. Put 6 test tubes in a test tube rack.
2. Using a small graduated cylinder, measure out 1ml of catalase and pour into one of the 6 test tubes. Repeat for each of the test tubes.
3. Using a dropping pipette, add 3, 6, 9, 12, 15, & 18 drops of the hydrogen eroxide solution--a different amount in each of the test tubes.
4. Do not shake test tubes.
5. Mark the outside of each test tube with a grease pencil at the top of the bubble columns.
6. Measure and record the height of the bubble column after 30 seconds. The tops of the bubble columns should be in a relatively straight line, increasing in height with the amount of enzyme.

7. Record the data as a graph on the sheet to hand in.



## **PROCEDURE 2**

### ***EFFECTS OF TEMPERATURE ON ENZYME ACTIVITY***

Temperature is a measure of the speed at which molecules are moving. As the temperature increases, the molecular movement also increases. Increasing temperature causes the enzyme and the substrate to come together at a faster rate, increasing the rate of enzymatic activity.

1. Place 1 ml of hydrogen peroxide (substrate) in a test tube. Place the test tube in the appropriate temperature water bath.
2. Place 1 drop of catalase (enzyme solution) in the bottom of a second test tube. Place this test tube in the same water bath as the first one.
3. These 2 test tubes are allowed to acclimate 15 minutes in the water bath so that the temperature inside the 2 test tubes is equal to the temperature of the water bath.
4. The substrate is added to the enzyme and the height of the bubble column is measured after 30 seconds (time is kept constant).
5. Record the data as a graph on the sheet to hand in.
6. Repeat steps 1-5, for 4 temperatures: 0°, room temperature ( $\approx 26^\circ$ ), 50°, 70°. The effects of temperature on enzyme activity are measured by increasing the temperature of the water and repeating the experiment until a decrease in enzyme activity occurs. \*\*Different (new) catalase and hydrogen peroxide are used at each temperature.

**NOTE:** The quantities of enzyme and substrate are kept constant so that the only variable is temperature.

**PROCEDURE 3**  
***ENZYME ACTIVITY AS A FUNCTION OF pH***

In this experiment, you will examine the affect of pH on enzyme activity.

1. Using a graduated cylinder, measure 1 ml of each of the buffer solutions (2, 4, 7, 10, 11) provided and pour each solution into a different test tube. Label the 6 test tubes with the pH of its solution.
2. Carefully add 1 drop of catalase to each of the test tubes.  
NOTE: Make sure the catalase does not touch the side of the test tube.
3. After 30 seconds, mark the outside of each test tube with a grease pencil at the top of the bubble column.
4. Measure the height of each bubble column and record the data in a graph on the sheet to hand in.

**PROCEDURE 4**  
***TESTING FOR ENZYME'S PRESENCE***

Catalase is found in almost all living cells. Test for its presence in yeast and in blood.

1. Place a drop of yeast suspension on a slide.
2. Add a drop of hydrogen peroxide solution.
3. Note the result.
4. Repeat steps 1-3, using a drop of blood in place of the yeast.

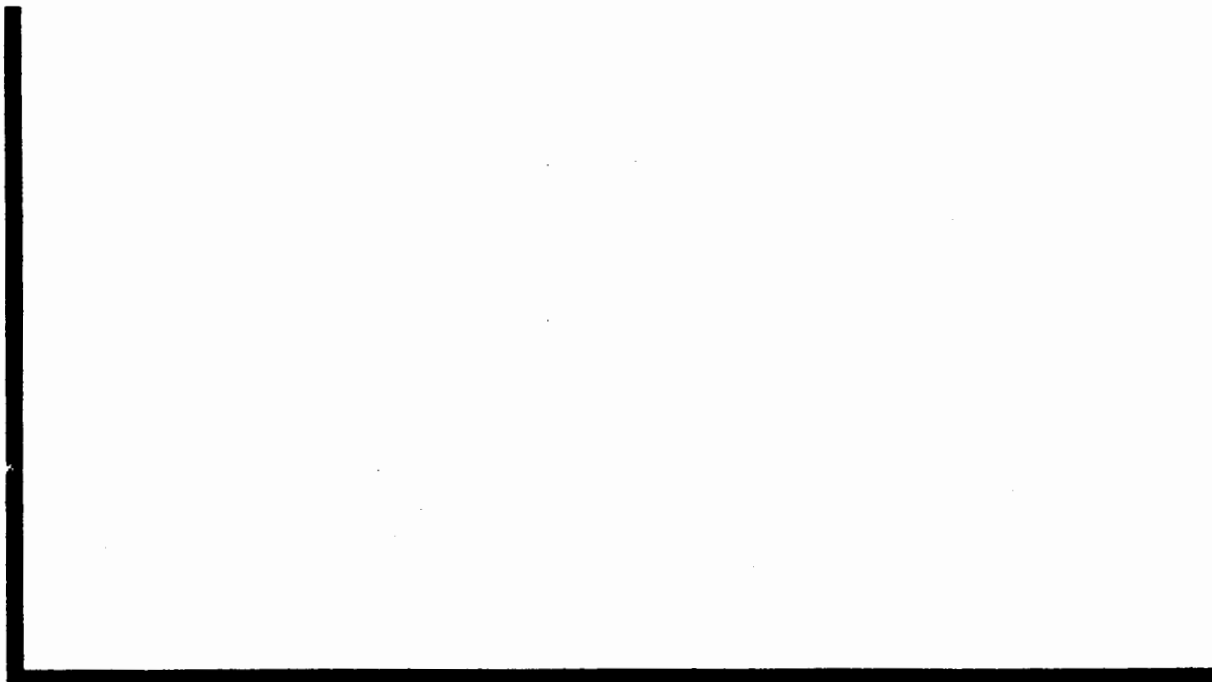
## LAB 4: ENZYMES

Student Name \_\_\_\_\_

1: Data obtained on enzyme activity.

Number of drops of enzyme	Height of bubble column (cm.)

Graph of data obtained. (Enzyme activity as a function of substrate concentration.)

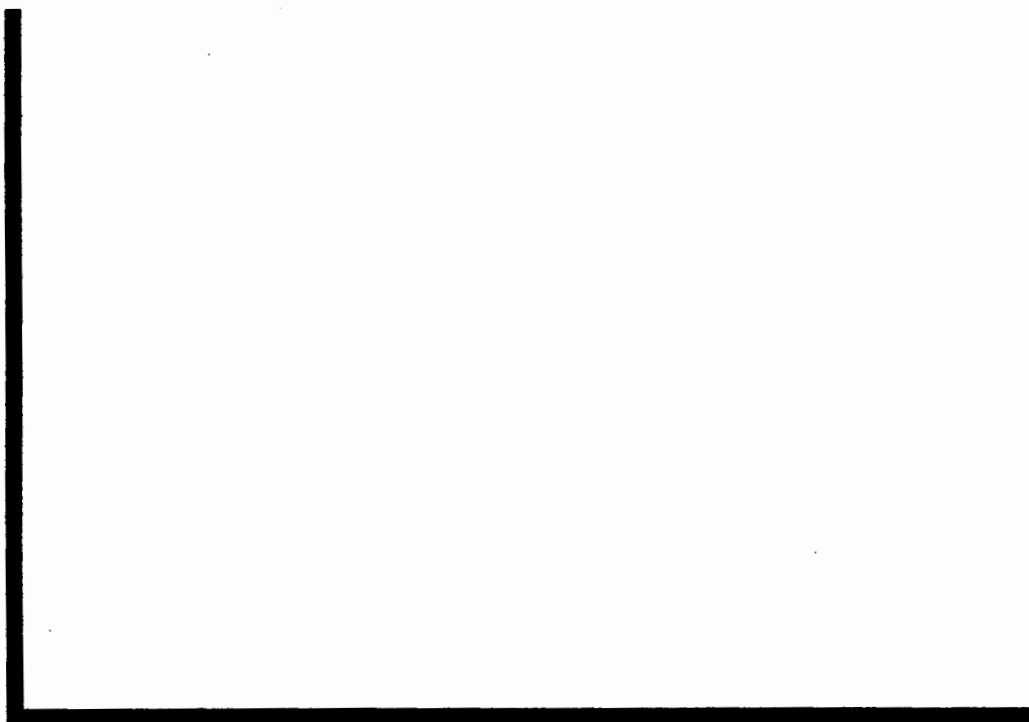


Make a general statement regarding the effect of substrate concentration on the rate of reaction.

2. Temperature vs. enzyme activity.

Temperature	Height of bubble column (cm.)

Graph of data obtained (Effects of temperature on enzyme activity)



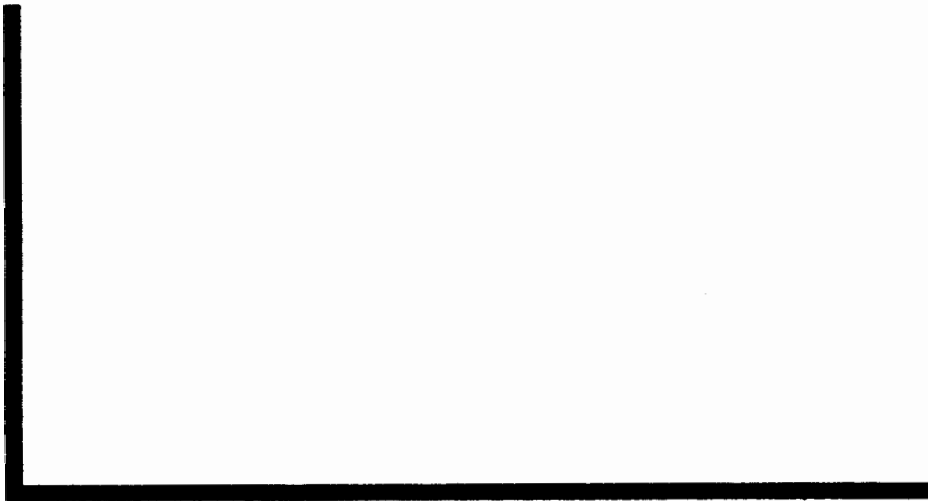
Make a general statement comparing the effect of temperature on the rate of enzyme activity.

**3. pH vs. enzyme activity**

pH	Height of bubble column (cm.)

**Graph of data obtained. (Enzyme activity as a function of pH)**

**The optimum pH for this enzyme is about \_\_\_\_\_.**



**4. List four factors that affect the activity of an enzyme, and describe how each effects enzyme activity.**

5. Enzymes are proteins with specific 3-dimensional shapes. Define each of the following:

**amino acid:**

**peptide bond:**

**polypeptide:**

**active site:**

**catalysis:**

**substrate:**

**enzyme-substrate complex:**

**anabolism:**

**driven reaction:**

6. In the hydrogen peroxide decomposition, you may have noticed that some of the reaction mixtures warmed up slightly. Explain the source of this heat.