

Enzyme Lab

Background information

Liver and other living tissues contain the enzyme catalase. This enzyme breaks down hydrogen peroxide, which is a harmful by-product of the process of cellular respiration if it builds up in concentration in the cells. If we use potato or other tissue containing this enzyme, we can use this to measure the relative influence of varying several different factors on the activity of enzymes in living tissue.

In order to obtain energy and building blocks from food, the digestive system must break down proteins, fats and carbohydrates. In this process, specific enzymes catalyze hydrolysis reactions in which food polymers are broken up into monomers. In this lab, you will perform reactions involved in digestion of carbohydrates, lipids, and proteins and observe the results of these reactions.

AP Objective met:

- * Measure the effects of changes in temperature, pH, and enzyme concentration on reaction rates of an enzyme catalyzed reaction in a controlled experiment.
- * Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

INTRODUCTION: What would happen to your cells if they made a poisonous chemical? You might think that they would die. In fact, your cells are always making poisonous chemicals. They do not die because your cells use enzymes to break down these poisonous chemicals into harmless substances. Enzymes are proteins that speed up the rate of reactions that would otherwise happen more slowly. The enzyme is not altered by the reaction. You have hundreds of different enzymes in each of your cells.

Each of these enzymes is responsible for one particular reaction that occurs in the cell. In this lab, you will study an enzyme that is found in the cells of many living tissues. The name of the enzyme is catalase (KAT-uh-LAYSS); it speeds up a reaction which breaks down hydrogen peroxide, a toxic chemical, into 2 harmless substances--water and oxygen.

The reaction is as follows: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ (fix)

This reaction is important to cells because hydrogen peroxide (H_2O_2) is produced as a byproduct of many normal cellular reactions. If the cells did not break down the hydrogen peroxide, they would be poisoned and die. In this lab, you will study the catalase found in liver cells. You will be using chicken or beef liver. It might seem strange to use dead cells to study the function of enzymes. This is possible because when a cell dies, the enzymes remain intact and active for several weeks, as long as the tissue is kept refrigerated.

MATERIALS:

- 1molar HCl solution (in dropper bottle)
- 1molar NaOH solution (in dropper bottle)
- 6 Test tubes and Test tube holder
- 10-ml Graduated cylinder

40 ml 3% Hydrogen peroxide solution (found in stores)
Straight-edged razor blade
Scissors and Forceps (tweezers)
Thermometer
Stirring rod
pH paper
Fresh liver, chicken meat, Apple, and Potato

PART A - Observe Normal Catalase Reaction

1. Place 2 ml of the 3% hydrogen peroxide solution into a clean test tube.
2. Using forceps and scissors, cut a small piece of liver and add it to the test tube. Push it into the hydrogen peroxide with a stirring rod. Observe the bubbles;

HINT: The arrows point out questions that should be answered on your data table.

What gas is being released? Throughout this investigation you will estimate the rate of the reaction (how rapidly the solution bubbles) on a scale of 0-5 (0=no reaction, 1=slow, 5=very fast). Assume that the reaction in step 2 proceeded at a rate of "4"

Recall that a reaction that absorbs heat is endothermic; a reaction that gives off heat is exothermic. Now, feel the temperature of the test tube with your hand. ► Has it gotten warmer or colder - Is the reaction endothermic or exothermic?

Is Catalase Reusable?

1. Place 2 ml of 3% hydrogen peroxide solution into a clean test tube and add a small piece of liver. What is happening in your test tube?
2. Pour off the liquid into a second test tube. Assuming the reaction is complete. What is this liquid composed of? What do you think would happen if you added more liver to this liquid? Test this and record the reaction rate. ► Explain your results (what is the liquid composed of?)
3. Add another 2 ml of hydrogen peroxide to the liver remaining in the first test tube. ► Is catalase reusable?

Part B - What Tissues Contain Catalase

You will now test for the presence of catalase in tissues other than liver. Place 2 ml of hydrogen peroxide in each of 3 clean test tubes and then add each of the three test substances to the tubes.

To the first tube, add a small piece of potato.
To the second tube, add a small piece of chicken.
To the last tube, add a small piece of apple.

As you add each test substance, record the reaction rate (0-5) for each tube.

► Which tissues contained catalase? Do some contain more catalase than others? How can you tell?

PART C - What is the Effect of Temperature on Catalase Activity?

1. Put a piece of liver into the bottom of a clean test tube and cover it with a small amount of distilled water. Place this test tube in a boiling water bath for 5 minutes. ► What will boiling do to an enzyme?
2. Remove the test tube from the hot water bath, allow it to air cool, then pour out the water. Add 2 ml of hydrogen peroxide. CAUTION: Use a test-tube holder when handling the hot test tubes. Record the reaction rate (0-5) in DATA TABLE
3. Put equal quantities of liver into 2 clean test tubes and 1 ml H₂O₂ into 2 other test tubes. Put one test tube of liver and one of H₂O₂ into each of the following water baths: Ice bath and Warm water bath (not boiling)
4. After 3 minutes, pour each tube of H₂O₂ into the corresponding tube of liver and observe the reaction. Record the reaction rates (0-5) in DATA TABLE

You recorded the reaction rate for room temperature earlier. ► What is the "optimum" temperature for catalase? (This is the temperature at which the reaction proceeds fastest.)
 ► Why did the reaction proceed slowly at 0 deg.C? ► Why did the reaction not proceed at all at 100 deg.C?

PART D - What is the Effect of pH on Catalase Activity

1. Add 2 ml hydrogen peroxide to each of 3 clean test tubes. Treat each tube as follows:

Tube 1--add a drop of HCl (acid) at a time until pH 3.

Tube 2--add a drop of NaOH (base) at a time until pH 10.

Tube 3--adjust the pH to 7 by adding single drops of either 1molar HCl or 1molar NaOH as needed.

CAUTION: Do not let acids or bases contact your skin or clothing. Swirl each test tube after adding each drop and measure the pH of each solution with pH paper. To do this, remove a drop or two of solution from a test tube using a clean glass stirring rod. Rinse your stirring rod and wipe dry before you dip it into each test tube. Place the drop on pH paper. Record the pH of each solution in DATA TABLE

► Does there appear to be a pH "optimum" - at what pH? ► What is the effect of low or high pH on enzyme activity?

DATA TABLE			
		Rate of Reaction (1-5)	Observations & Conclusions (answer section questions)

PART A	Normal Liver		▶
	Liver added to Used Peroxide		▶
	Reused Catalase		▶
PART B	Potato		
	Chicken		▶
	Apple		
PART C	Boiled Liver		▶
	Ice Bath Liver		▶
	Warm Liver		▶
PART D	Basic Solution (pH 10)		
	Acidic Solution (pH 3)		▶
	Neutral Solution (pH 7)		▶