Name:_____ Date:_____

Investigation: Enzymes

Objectives

- Measure the effects of changes in temperature, pH, and enzyme concentration on reaction rates of an enzyme
- 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; it speeds up a reaction which breaks down hydrogen peroxide, a toxic chemical, into two harmless substances → water and oxygen.

The reaction is: $2H_2O_2 \longrightarrow 2H_2O + O_2$

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:			
6 Test tubes Test tube holders 3% Hydrogen peroxide	Straight-edged razor blade Scissors and Forceps Measuring Pipettes Stirring rod	Fresh liver, Apple, and Potato, Yeast Vinegar / Baking Soda HCL and NaOH pH paper (optional)	Ice bath Warm water bath Boiling water bath

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.

What gas is being released? (Consider the equation.) _____

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"

3. 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? _____

4. Pour off the liquid into a second test tube. Assuming the reaction is complete.
What is this liquid composed of? _____

5. What do you think would happen if you added more liver to this liquid? _____

Test this and record the reaction rate. Reaction Rate _____ (1 – 5)

6. Add another 2 ml of hydrogen peroxide to the liver remaining in the first test tube.
What is the reaction rate? _____ (1 – 5)

Synthesis -- **Answer the question: Is catalase reusable?**

CLAIM:

EVIDENCE

REASONING.

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

Substance	Apple	Potato	Yeast	Raw chicken
Rate of Reaction (0-5)				

Synthesis -- **Do all living tissues contain catalase?**

Claim:

Evidence:

Reasoning:

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 water. Use a test tube clamp to place it in a boiling water bath for 5 minutes.

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.

What is the reaction rate for the boiled liver and peroxide? _____

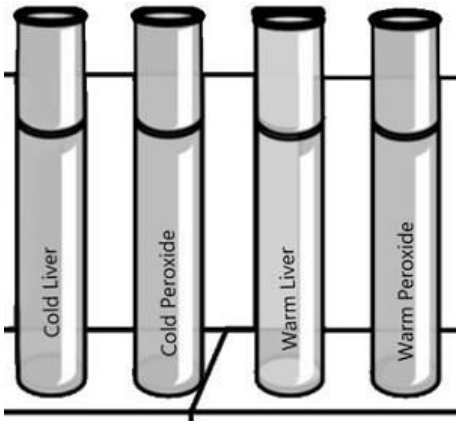
3. Put equal quantities of liver into 2 clean test tubes and 1 ml H_2O_2 into 2 other test tubes. Put one test tube of liver and one of H_2O_2 into an ice bath. Place the other set in a warm water bath (not boiling).

After 3 minutes, pour each tube of H_2O_2 into the corresponding tube of liver and observe the reaction

What is the reaction rate for the cold liver/peroxide? _____
What is the reaction rate for the warm liver/peroxide? _____

Synthesis -- **How does temperature affect the catalase enzyme?**

Claim:



Evidence:

Reasoning:

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

Obtain or create solutions with varying pH from the following chemicals. Caution HCl and NaOH are strong acids and bases and can burn the skin, safety goggles needed.

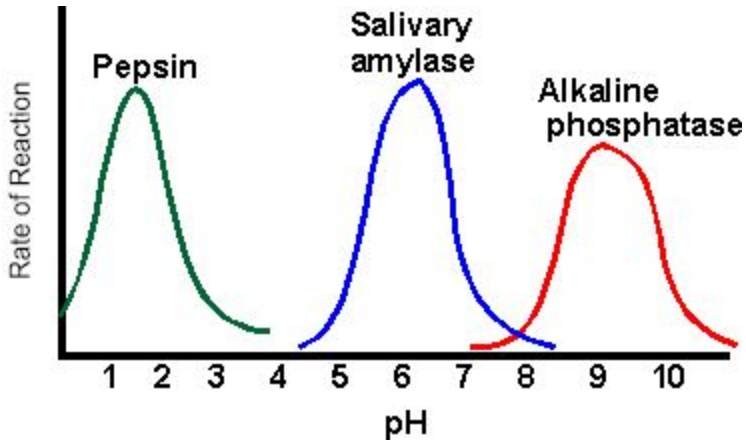
3M HCl 3M NaOH Vinegar Baking Soda Water

Use pH paper to determine the actual pH of each and determine the enzyme reaction rates for each using either yeast or liver.

	Solution 1	Solution 2	Solution 3	Solution 4	Solution 5
pH					
Reaction Rate					

1. How does pH affect the reaction rate of catalase? Propose a way to **refine** your experiment to find the **exact**, or OPTIMAL pH and temperature of catalase.

2. The following graph shows reaction rates of various enzymes in the body. Pepsin is found in the stomach, amylase in the saliva, and phosphatase in the liver.



Synthesis: How does pH affect the activity of enzymes?

Claim:

Evidence:

Reasoning:

Part E - Design an Experiment

Lactaid is a product designed to help people who cannot digest milk sugar (lactose) because they are missing the enzyme lactase. Many people are lactose-intolerant, a condition that is mainly genetic. Lactase breaks down lactose into two subunits: glucose and galactose.

To test for the presence of monosaccharides and reducing disaccharide sugars in food, the food sample is dissolved in water, and a small amount of Benedict's reagent is added. The solution should progress in the colors of blue (with no glucose present), green, yellow, orange, red, and then brick red or brown (with high glucose present).

Design an experiment where you would determine how quickly lactaid works to break down milk sugar. Be specific in your description, use drawings if necessary.