Name:_____ Section: _____ Lab#6

Objectives:

* 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.

Investigation: Enzymes

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. Light can also break down H_2O_2 which is why the chemical is sold in dark containers.

The reaction is: $2\ H_2O_2$ ----> $2\ H_2O + O_2$

This reaction is important to cells because hydrogen peroxide 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:	3% Hydrogen peroxide solution (found in stores)	Stirring rod Fresh liver, Apple, and
Vinegar (acid)	Straight-edged razor blade	Potato
Baking soda (base)	Scissors and Forceps (tweezers)	Test tube holders
6 Test tubes		
Measuring Pipette	pH paper (optional)	Ice bath Warm water bath Boiling water bath

PART A - Observe Normal Catalase Reaction

1. Place about 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 above) _____

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"
Assume that the normal reaction rate of catalase is a 3.

Recall that a reaction that absorbs heat is endothermic; a reaction that gives off heat is exothermic. Feel the temperature of the test tube with your hand.

Has it gotten warmer or colder? _____
Is the reaction endothermic or exothermic? _____

3. 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. Reaction Rate _____ (1 – 5)

4. Add more hydrogen peroxide to the liver remaining in the first test tube.
What is the reaction rate? _____

Is catalase reusable? Explain how you know.

Part B - What Tissues Contain Catalase?

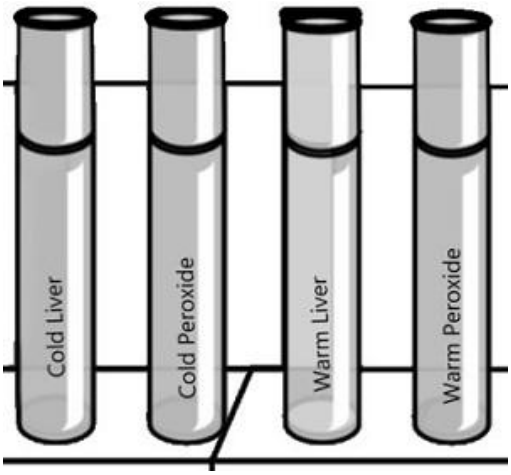
You will now test for the presence of catalase in tissues other than liver. Place 2 ml of hydrogen peroxide in each of 3 clean test tubes and then add each of the three test substances to the tubes. As you add each test substance, record the reaction rate (0-5) for each tube.

Substance	Rate of Reaction (0-5)
Potato	
Apple	
Chicken	

Based on your observations, 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 water. Place this test tube in a boiling water bath for 5 minutes.



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 for hot test tubes.

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? ____

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

1. Add 2 ml hydrogen peroxide to 3 clean test tubes, then add:

Tube 1--add acetic acid (vinegar) pH =_____

Tube 3 – add sodium bicarbonate solution (base) pH =_____

Tube 5 – add water (neutral) pH =_____

Now add liver to each of the test tubes (try to do it all at about the same time, so you can easily compare)

Rate of Reaction for: Acid _____ Neutral _____ Base_____

DATA ANALYSIS

1. Describe the relationship between catalase and hydrogen peroxide. Indicate which is the enzyme, which is the substrate and what occurs during the reaction. (2)

2. Is catalase reusable? Use your **data** to support your answer. (1)

3. How does temperature and 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. (3)

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 when there is a large amount of glucose present. (Google benedict's test to see the way this looks.)

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

- Problem:
- Hypothesis:
- Independent Variable
- Dependent Variable
- Control Group
- Experimental Groups
- 2 Constants
- Data to be collected
- Example of a conclusion