

Enzymes Lab
Pre-Lab Exercise

Name _____

1. For the reaction we are studying in this week's lab:
 - a. What is the name of the enzyme?
 - b. What is the substrate?
 - c. What are the products of the reaction?
2. What is the purpose of having this enzyme in your cells?
3. How will you rate enzyme activity? What will you use as a positive control?
4. What is the effect of high temperature on hydrogen bonding and three dimensional protein structure?
5. Write out a hypothesis for each experiment. What result for each experiment would support your hypothesis?

Experiment B:

Experiment C:

Experiment D:

Experiment E:

Enzymes Lab

Work in groups of four

Enzymes are responsible for catalyzing all of the biochemical reactions that run your cells and keep them alive. Without enzymes, your cells would not be able to respond to your environment and maintain all the characteristics of life you have already defined.

Enzymes work by speeding up the rate of a chemical reaction, but the enzyme itself is not part of the reaction. It is not used up during the reaction and can be recycled. Chemically, enzymes are usually **proteins**. Any physical or chemical condition that damages a protein will damage an enzyme as well. When an enzyme is damaged, the chemical reaction it catalyzes cannot occur at biological speeds.

Use a separate piece of paper to answer the questions as you do each exercise (not after you go home!). Use the Data Sheet to record your results. Include any observations you may make independent of what is directly asked of you.

Summary of Activities

1. Test normal catalase activity.
2. Test what effect the **chemical reaction** has on the enzyme itself.
3. Test what types of **tissues** contain the enzyme catalase.
4. Test the effect of **temperature** on enzyme activity.
5. Test the effect of **concentration** on enzyme activity.

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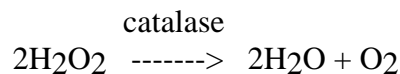
Your cells are constantly exposed to poisonous chemicals, many made by the cells themselves as metabolic byproducts. Some examples of these types of substances are alcohol, caffeine, or hydrogen peroxide. Your cells are not damaged by these chemicals because they contain **enzymes** that can breakdown toxins and turn them into harmless substances.

Enzymes are proteins that speed up the rate of chemical reactions that might otherwise occur so slowly, they may as well not be occurring at all. The enzyme is not part of the chemical reaction, so it is not changed when the reaction occurs. Your cells have hundreds, if not thousands of different enzymes. Each different enzyme catalyzes a different specific chemical reaction.

We will examine two factors that affect the rate of reaction: **temperature** and **concentration**. Temperature can destroy the three-dimensional shape of a protein. Can you predict what the loss of an enzyme's three-dimensional shape would have on its activity? What do you think increasing the concentration of an enzyme would do?

In this lab, we will study the action of the enzyme **catalase**. This enzyme helps your body get rid of hydrogen peroxide. Hydrogen peroxide (H₂O₂) is a byproduct of many normal cellular reactions. It acts as powerful oxidizing agent that can damage or kill your cells. Think about what it can do to hair! This enzyme is produced in the liver and can be obtained from beef liver that has been ground up. It turns peroxide into harmless water and oxygen gas.

The chemical reaction catalyzed by the enzyme catalase is:



Materials:

- 40 ml 3% hydrogen peroxide (H₂O₂)
- test tubes
- glass stirring rod
- razor blade

- forceps
- paper towel
- pea-sized pieces of liver
- small pieces of fresh potato, apple, and chicken muscle
- water baths: ice; 37° C; and boiling
- distilled water

Procedure:

A. Normal Catalase Activity at Room Temperature

1. Using the forceps and razor blade, cut your liver into at least 12 small pea-sized pieces. Try to make all of them as uniform in size as possible.
2. Add a piece of liver to the test tube, pushing it into the test tube with the stirring rod.

Note: Be sure to clean your stirring rod each time you use it.

3. Add 3 ml of the 3% hydrogen peroxide solution to the liver in the test tube and give the tube a brief shake. Measure the amount of time it takes for the bubbles to reach their peak. Measure the peak height of the bubbles (If the bubbles overflow, use the height of the tube and the time it took the bubbles to get there.) Record both the time and the height on your data sheet. Measure the height of all of your subsequent reactions after this amount of time.
4. Record your results and observations.

A chemical reaction that absorbs heat (gets cold) is endothermic; a reaction that gives off heat is exothermic. Feel the temperature of the test tube with your hand.

B. What Happens to an Enzyme After a Chemical Reaction?

1. Get a clean test tube and place a small piece of liver in it. Add 3 ml H₂O₂ and shake.

2. Measure the height of the bubbles using the appropriate time. Record your results.
3. When the bubbling stops, pour off the liquid into a second test tube.
4. Test your prediction by adding a small piece of fresh liver to this test tube. Measure the bubble height and record your results.
5. Add another 3 ml of H₂O₂ to the liver remaining in the first test tube. Measure the bubble height and record your results.

C. Occurrence of Catalase in Different Tissues

1. Label 4 clean test tubes with "liver", "potato", "muscle", and "apple".
2. Cut an equal sized cube of each specimen and place at the bottom of the appropriate tube.
3. Add 3 ml of H₂O₂ to each tube and shake.
4. Measure the bubbles using the appropriate time and record your results.

D. Effect of Temperature on Enzyme Activity

1. Put a small piece of liver into the bottom of a clean test tube and add enough distilled water to cover it. Place this tube in a boiling water bath for 5 minutes.
2. Remove the test tube from the boiling bath using a test tube holder and allow it to cool. Pour off the liquid. Add 3 ml of H₂O₂ and shake. Measure the bubbles and record your results.
3. Put equal amounts of liver in 3 clean test tubes labeled "ice", "RT", and "37° C". Record the actual room temperature on your Data Sheet. Put 3 ml H₂O₂ in 3 separate test tubes with the same labels.

4. Put the separate tubes of liver and H_2O_2 into each of the appropriate water baths. Leave the tubes labeled "RT" on your bench. After 10 minutes, the liver and H_2O_2 in separate tubes will reach the desired temperature. Pour the H_2O_2 into the tube of liver for each different temperature. Measure the reactions and record your results.
5. Make a graph of the estimated reaction rate as a function of temperature. You should have 4 points: 0°C (ice bath); 25°C (room temperature); 37°C (human body temperature); and 100°C (boiling).

E. Effect of Concentration on Enzyme Activity

1. In this case, you will be varying the amount of enzyme used. You will need four pieces of liver:
 - a. One piece the **same size** as the pieces used in the previous experiments.
 - b. One piece **twice the size** as the original.
 - c. One piece **half the size** as the original.
 - d. One piece **one quarter the size** of the original. You may have some difficulty getting this piece into the tube. Use a spatula if needed.
2. Place the pieces of liver in separate tubes that have been appropriately labeled. Add 3 ml H_2O_2 . Measure and record the results. Graph your results.

**Enzymes Lab
Data Sheet**

A. Normal catalase activity (4): Height of bubbles: _____ Number of seconds: _____

B. Catalase Activity After a Reaction

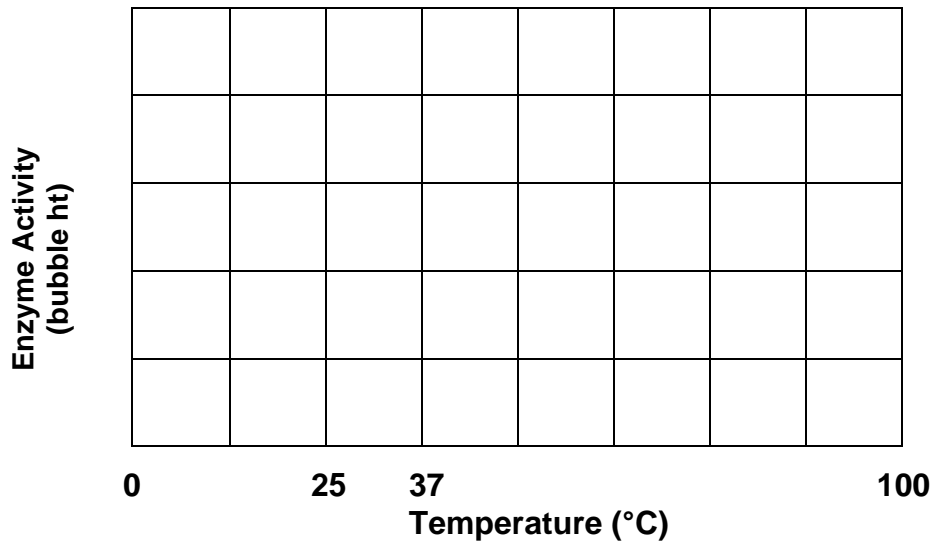
Sample:	Height of bubbles(cm)
Tube #1	
Tube #2: Liquid from Tube #1 + fresh liver	
Liver from Tube #1 + fresh H ₂ O ₂	

C. Catalase in Different Tissues

Sample	Height of bubbles(cm)
liver	
potato	
chicken muscle	
apple	
other	

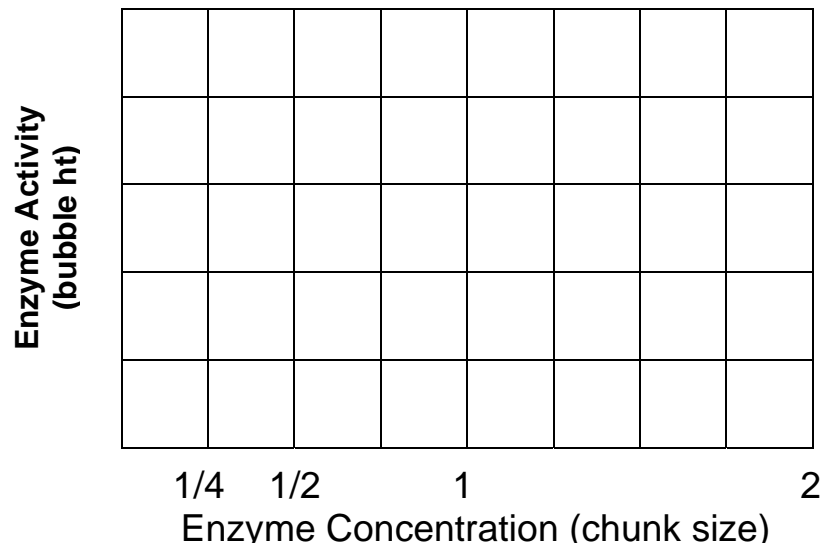
D. Effect of Temperature on Enzyme Activity

Temperature	Height of bubbles(cm)
Ice (0° C)	
Room Temperature (25° C)	
37° C	
Boiling (100° C)	



E. Effect of Concentration on Enzyme Activity

Size of Sample	Height of bubbles(cm)



Enzymes Lab

Lab Report

1. Turn in your data sheets.
2. Is the reaction catalyzed by catalase endothermic or exothermic? How do you know?
3. Is catalase reusable? How do you know?
4. What tissue had the most catalase? What tissue had the least?
5. What does the previous question tell you about the function of the liver?
6. Describe the shape of your graph of catalase activity vs. temperature. What is the optimum temperature for catalase activity?
7. Compare optimal temperature for catalase to physiological conditions in a cell. How do they compare?
8. What can you say about the effect of enzyme concentration on enzyme activity?
9. (2 pts) What condition today inhibited enzyme activity? Give a molecular explanation for how this condition inhibited enzyme activity.