Lab 3 Enzymes
Pre Lab 3 Test Questions

1. What type of chemical reaction gives off energy?
   a) endergonic
   b) exergonic
   c) catalytic
   d) all reactions are the same

2. Most chemical reactions in the human body occur without the assitance of enzymes. True or False

3. Llist 3 characteristics of an enzyme.

   a) _____

   b) _____

   c) _____

4. List 4 things that influence enzyme function.

   a) _____

   b) _____

   c) _____

   d) _____

Lab 3: ENZYMES

Objectives:

- Discuss the function and role of enzymes in living organisms.
    - Identify the substrate (reactant), enzyme and product of a chemical reaction.
    - Differentiate between exergonic and endergonic reactions.
    - List 5 things that influence enzyme activity and discuss some of the properties of enzymes.
    - Describe denaturation.
    - Describe the pH scale and test a variety of chemicals for their pH.

Metabolism refers to all the chemical reactions that occur in a cell. One way of illustrating a chemical reaction is:

$$A + B \Leftrightarrow C + D$$

- ❖ Where A and B are called the substrates (what you start with) and C and D are called the products (what you end up with).

- ❖ This chemical reaction can move in either direction as noted by the 2 way arrow.

- Some chemical reactions consume energy (endergonic) and therefore yield products that have more energy than the original substrates.

- Other chemical reactions give off energy (exergonic) and therefore yield products that have less energy than the original substrates.

All chemical reactions that occur in a cell have a unique enzyme that lowers the activation energy required to run the reaction and therefore speeds up the rate at which the reaction can occur.

So what are enzymes?

- Enzymes are large protein molecules (macromolecules)
- They catalyze or speed up chemical reactions
- But they are not altered in the reaction.

Proteins can be just about any size or shape, which is useful since it's the shape of an enzyme that determines the reactions it can catalyze.

- ❖ However, proteins are sensitive to changes in temperature and pH, which alter their shapes and can even destroy catalytic activity.
- ❖ An enzyme whose shape is changed and is no longer active is called "denatured".
- ❖ Proteins have evolved to work most efficiently at the temperature and pH found in the part of the body where they are needed.

Lab "Rules"

- It's very important you follow instructions during this lab. Assign someone in your group of four to read the instructions to the rest of the group *before* you do each part of the experiment.
- It's also important that everyone in your group participate. You won't learn much if you don't, and your grade may suffer!
- Clean up after yourself!!! Or bring your mom along to do it for you. That means—all spills wiped up, all glassware cleaned, all equipment returned to its place. No paper towels on the floor or bits of potato in the sink. THANKS!
- YOU WILL WORK IN GROUPS OF 4 -5.

Experiment 1: RENNIN and TEMPERATURE

Rennin is an enzyme found in the stomach lining of mammals. Rennin curdles (solidifies) milk in the stomach so that the milk remains in the stomach longer and can be digested more efficiently.

Instructions:

1. **Dissolve** the rennin (provided as a tablet). Open the rennin tablet's foil package and place the tablet in a porcelain mortar. Crush the tablet with a pestle. Using a graduated cylinder, measure 15 ml distilled water (in carboy) and pour it into a small beaker. Scrape the rennin powder into the beaker of water and swirl to mix. The solution will be cloudy.

2. **Label t**hree test tubes with your initials (everyone will use the same water baths) and the words "cold," "warm" or "boiled."

3. **Pour** the rennin solution out of the beaker into the three tubes, in approximately equal amounts (one-third to each tube). If they come out very uneven, use a clean squeeze bulb to adjust the amounts.

4. **Pre-treat the enzyme** (rennin) at different temperatures.
   Put the "cold" tube in the ice water bath, the "warm" tube in the 37°C water bath, and the "boiled" tube in boiling water for 5 minutes, then in the 37°C water bath.

5. **Pre-treat the substrate (milk)** at different temperatures.
   a. Label three more test tubes with your initials and the words "cold," "warm" or "boiled." Into each tube, place about 1 inch of milk *or* three squirts (1 squirt = the amount of liquid pulled into a plastic squeeze bulb with one good squeeze of the bulb—it will not fill completely).

   b. Put the "cold" tube of milk in the ice water bath, the "warm" *and* "boiled" tubes in 37°C water bath. Wait 5 min. (Don't boil the milk!)

6. **Mix the rennin and milk to start the enzyme reactions**.
   a. With a clean squeeze bulb, put **3 <u>drops</u>** of rennin solution from the "cold" tube of rennin into the "cold" milk. Leave *both* tubes in the ice water bath.

   b. With a clean squeeze bulb, put **3 <u>drops</u>** of the rennin solution from the 'warm" tube into the "warm" milk. Leave *both* tubes in 37°C water bath.

   c. With a clean squeeze bulb, put **3 <u>drops</u>** of the rennin solution from the "boiled" tube into the "boiled" tube of milk. Leave *both* tubes in the 37°C water bath. ***<u>Do not boil a second time</u>***!

7. Incubate each test tube for 30 minutes. Write down the time that you finished adding the rennin to all the tubes: Time = _____

8. After 30 minutes, record your observations in the table below.

Tube	Observations 30 min after adding rennin to milk (Has the milk curdled or not? Is the enzyme active?)
Cold	
Warm	
Boiled	

After this portion of the lab, be able to answer the following:

1. At which temperature was rennin most active?

2. What was the effect of boiling the rennin solution on enzyme activity?

During the 30 minute incubation period, do Experiment 2 (pH measurements) and Experiment 3 (catalase and pH).

Experiment 2: pH MEASUREMENTS

- pH is a measure of a solution's hydrogen ion concentration.
- The pH scale ranges from 0 to 14. The solutions are classified as having acidic, basic (alkaline) or neutral pH depending on the concentrations of hydrogen ions (H^+) and hydroxyl ions (OH^-) that are present in the solution.
- Just as temperature is measured in °F, acidity is measured in pH units. If you have a swimming pool or fish tank at home, you may have measured the pH of the water.

A buffer is a system of chemicals that takes up excess hydrogen ions or hydroxide ions causing a change in the pH.

- Our body possesses certain molecules that can assist in maintaining pH (homeostasis) by easily accepting H+ ions or releasing H+ ions. These molecules act as buffers.
- All living things are extremely sensitive to pH changes. In living organisms, pH plays an important role in the functioning of systems.
- Many body fluids have a pH near neutrality. For example, the pH of blood is normally between 7.35 and 7.45; lymph has a pH of 6.8, as does saliva. However, gastric juice produced in the stomach has a pH of about 2—highly acidic. (So how does the stomach avoid digesting itself?).

Like the Richter scale for earthquakes, pH uses a logarithmic scale where each number represents a 10-fold change in the concentration of hydrogen ions. For example, a solution with a pH of 3 is 10 times more acidic than a solution with a pH of 4, 100 times more acidic than a solution with a pH of 5, and 1000 times more acidic than a solution with a pH of 6.

A pH of 7 is considered neutral (distilled water is pH 7). Most tap water has a slightly acidic pH due to dissolved CO_2 from the air. Examine the scale below to see the pattern of pH with respect to acidic, neutral and basic solutions.

pH	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	strong acid			weak acid			neutral				weak base				strong base

Instructions:

- pH test paper is an easy way to get a quick estimate of the pH of a solution. Just dip the strip of paper in the solution to be tested, then compare the colors to the color charts printed on the dispenser box (note that the front of the box goes from pH 0 to 7, the back of the box from 7 to 14).

- Measure the pH values of the solutions provided in lab, and record your values in the chart below. Do this activity just *once* in your group, please, and share your results.

Solution	pH	Acid, Base, Neutral
.1 M HCL		
.01M HCL		
Lemon Juice		
Vinegar		
Water		
Alka Seltzer		
.1 M NaOH		
.01M NaOH		

Experiment 3: CATALASE and pH

Hydrogen peroxide (H_2O_2), a toxic chemical, breaks down slowly to water and oxygen.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2\ (gas)$$

This reaction can be speeded up in several ways. One way is to add an enzyme called **catalase**, which is found in many living cells. Our source of catalase will be potatoes.

Step 1

First, you will observe the effect of adding several different substances to hydrogen peroxide. You should record your observations using the following scale:

No bubbling:	**-**
Moderate bubbling:	**+**
Strong bubbling	**++**
Very strong bubbling	**+++**

Get a fresh potato, cutting board, and razor or scalpel. Cut two small cubes of potato (about ½ inch per side) from the inside, white part of the potato (not the peel). Chop *one* of the cubes into very tiny pieces.

Label three clean test tubes 1, 2, and 3. Put about 1 inch (2 to 3 squirts) of hydrogen peroxide into each tube. Then add the following to each tube:

Tube 1: Using a clean spatula, add a small amount of sand.

Tube 2: Add the cube of potato.

Tube 3: Add the chopped potato.

Using the bubbling scale above, record your observations below.

Tube #	Bubbling
1	
2	
3	

- Why did you add sand to one tube?

- Where do the bubbles come from?

Step 2
Cut three cubes of fresh potato the same size as before, and chop them up (keep them in three separate piles). Label three test tubes "acid," "neutral" and "base." Put one chopped potato cube in each tube. Add the following:

"Acid" tube: Add one squirt (about ½ inch) of 0.01 M HCl.

"Neutral" tube: Add one squirt of water.

"Base" tube: Add one squirt of 0.01 M NaOH.

Wait two minutes for the solutions to soak into the potato pieces.
Then add about 1 inch (2-3 squirts) of hydrogen peroxide to each tube.
Observe any bubbling, and record your results in the table below.
Fill in the pH column by referring to your previous tests with the pH strips.

Tube	pH	Bubbling
Acid		
Neutral		
Base		

Post Lab 3 Test:

1. How does the bubbling relate to catalase enzyme activity?

2. The catalase enzyme is most active at pH ____.

3. Enzymes are made of the class of macromolecules called _____

4. The individual building blocks of the macromolecules in #1 are _____

5. What function does rennin serve in mammals?

6. Why do non-mammalian vertebrates not produce rennin?

7. Enzymes may be _____(name of general process) by extreme temperatures or pH.

8. A solution of pH 2 is ___ times more acidic than a solution of pH 3 and ___ times more acidic than a solution of pH 6.

9. Which of the following is most basic? (circle one)

   0.1 M NaOH 0.01 M NaOH water Alka-Seltzer

10. T or F: Enzymes are catalysts

11. What were the results of the catalase & pH experiment? Explain your group's results in your own words.

12. Your stomach produces gastric juices that are about pH 2. This is highly acidic, of course. What prevents your stomach from digesting itself?