In this lab, you’ll investigate some of the properties of enzymes.

**What are enzymes?**
- Enzymes are large protein molecules (macromolecules)
- They catalyze or speed up chemical reactions
- But they are not altered in the reaction
- And they can be used over and over again.

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 An enzyme whose shape is changed and is no longer active is called “denatured”.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”**
- **Follow instructions.** Assign someone in your group of 4 to read the instructions to the rest of the group before you do each part of the experiment.
- Let everyone in your group participate.
- **Clean up after yourself!!!** That means—all spills wiped up, all glassware cleaned, all equipment returned to its place. THANKS!

**Experiment 1: How does TEMPERATURE affect enzymes?**

**Rennin** is an enzyme found in the stomach lining of mammals (we’re mammals—so are any animals that feed milk to their young). 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 3 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 3 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 (5°C), 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 the 37°C water bath. Wait 5 minutes. (Don’t boil the milk!)

6. **Mix the rennin and milk to start the enzyme reactions.**
   a. With a clean squeeze bulb, put 3 drops 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 drops of the rennin solution from the “warm” tube into the “warm” milk. Leave both tubes in the 37°C water bath.
   c. With a clean squeeze bulb, put 3 drops of the rennin solution from the “boiled” tube into the “boiled” tube of milk. Leave both tubes in the 37°C water bath. **Do not boil a second time!**

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

8. **After 30 minutes, record your observations** here.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Observations 30 min after adding rennin to milk (Has the milk curdled or not? Is the enzyme active?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold (5°C)</td>
<td></td>
</tr>
<tr>
<td>Warm (37°C)</td>
<td></td>
</tr>
<tr>
<td>Boiled (100°C)</td>
<td></td>
</tr>
</tbody>
</table>

During the 30 minute incubation period, do Experiment 2 (cell respiration).
**Experiment 2: Effect of exercise on cellular respiration**

Cellular respiration is a chemical reaction that creates energy for your cells. When you are exercising your muscle cells are creating ATP to fuel muscle contraction. Cellular respiration requires a fuel (sugar) and oxygen (which is breathed in) and creates carbon dioxide (which is breathed out). Here’s the chemical reaction:

\[
C_6H_{12}O_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} + 36 \text{ATP (energy)}
\]

This lab will address how exercise (increased muscle activity) affects the rate of cellular respiration. You will measure 3 different indicators of cellular respiration: breathing rate, heart rate, and carbon dioxide production.

You will measure these indicators at rest (with no exercise) and after 1 and 2 minutes of exercise. Breathing rate is measured in breaths per minute, heart rate in beats per minute, and carbon dioxide production in the time it takes bromthymol blue to change color.

Carbon dioxide production can be measured by breathing through a straw into a solution of bromthymol blue (BTB). *When BTB reacts with acid it turns from blue to yellow.*

When carbon dioxide reacts with water, a weak acid (carbonic acid) is formed (see chemical reaction below). The more carbon dioxide you breathe into the BTB solution, the faster it will change color to yellow.

\[
6 \text{CO}_2 + 6 \text{H}_2\text{O} \rightarrow 6 \text{HCO}_3^- + 6 \text{H}^+
\]

**Materials you’ll need:**
- Beaker/Test tube/cup
- straw
- Bromthymol blue solution (BTB)
- stop watch

**Part A: Resting (base-line measurements)**

• **Measuring Carbon Dioxide Production:**
  1. Use a graduated cylinder to measure out 20 mL of tap water and pour it into a small beaker.
  2. Use a dropper to add 8 drops of bromthymol blue to make a BTB solution.
  3. Using a straw, exhale into the BTB solution. (CAUTION: Do not inhale the solution!)
  4. Time how long it takes for the blue solution to turn yellow. Record the time here: ____________.
  5. Wash out the beaker repeat steps 1-4 twice more.
  6. Average the results of the 3 trials. Record in the *Group Lab Report, Table 2.*
• **Measuring Breathing Rate:**
  1. Count the number of breaths (1 breath = inhale + exhale) your lab partner takes in 1 minute. Record this here: ______________.
  2. Repeat this 2 more times.
  3. Average the results of the 3 trials. Record this in **Table 2**.

• **Measuring Heart Rate:**
  1. While you calculate your breathing rate, have your partner take your pulse.
  2. Count the number of beats in 30 seconds and multiply that number by 2. Record this here: ______________.
  3. Repeat this 2 more times.
  4. Average the results of the 3 trials. Record this in **Table 2**.

**PART B: Exercise for 1 min (Increased Muscle Activity)**
  1. Exercise for exactly 1 minute by doing jumping jacks.
  2. While you are exercising, your partner should get the BTB solution ready.
  3. After 1 minute of exercise, immediately exhale through the straw into the BTB solution. Time how long it takes for the BTB to turn yellow. Record this in **Table 2**.
  4. Then quickly measure your breathing and heart rates as you did before. You only need to do this once.
  5. Record these values in **Tables 2**. Remake your BTB solution.

**PART C: Exercise for 2 min (Exercise as you did before for 2 minutes).**
  6. Immediately exhale through the straw into the BTB solution. Time how long it takes for the BTB to turn yellow. Record this in **Table 2**.
  7. Then quickly calculate your breathing and heart rates as you did before. You only need to do this once.
  8. Record these values in **Tables 2**.

If there is time, repeat the entire procedure for your lab group.
Catalase is a common enzyme found in nearly all living things. Its main function is to break down hydrogen peroxide ($H_2O_2$), a toxic chemical produced by cell metabolism, into water and oxygen. Since one of the products is a gas, we can see if catalase is working by looking for oxygen bubbles.

$$2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \text{ (gas)}$$

If oxygen bubbles form that means the catalase is working. Watch the bubbles and note the highest point the bubbles get to in each tube. After one minute, measure the highest point the bubbles reached in each tube (in millimeters). Record this measurement in the charts provided. We will use the thickness of bubbles as a measure of catalase activity. Our source of catalase will be potatoes.

**Step 1**

First, you will observe the effect of adding several different substances to hydrogen peroxide. Get a fresh potato, cutting board, and scalpel. Cut two small cubes of potato (about $\frac{1}{2}$ 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.

Record the thickness of bubbles in the table below.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Bubbles (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (sand)</td>
<td></td>
</tr>
<tr>
<td>2 (cube of potato)</td>
<td></td>
</tr>
<tr>
<td>3 (chopped potato)</td>
<td></td>
</tr>
</tbody>
</table>

- Why did you add sand to one tube?
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. Record the thickness of bubbles in the table below and in Table 1 of the Group Lab Report.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Bubbles (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td></td>
</tr>
</tbody>
</table>
Lab 3: Group Lab Report

Names: ___________________________   _______________________________
                                                 _______________________________
                                                 _______________________________

1. Enzymes are made of the class of macromolecules called ____________________.

2. What is the function of enzymes in living organisms?

3. List 4 things that influence enzyme function
   a. 
   b. 
   c. 
   d. 

Table 1. What affects enzyme reactions in our bodies? (Results and data)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Cold (5°C)</th>
<th>Warm (37°C)</th>
<th>Boiling (100°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(curdling: + √ - )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Acid</td>
<td>Neutral</td>
<td>Base</td>
</tr>
<tr>
<td>Amount of reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(bubble thickness, mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. According to your results, what is the ideal temperature for the rennin enzyme (where it showed greatest reaction)? Why would this be an advantage in mammals?

5. What was the effect of boiling the rennin solution on enzyme activity? Explain what happened to the enzyme.
6. Why did you use bubbling to measure catalase enzyme activity?

7. According to your results, what is the ideal pH for the catalase enzyme (where it showed greatest reaction)? Use your data to explain your conclusion.

8. Each enzyme in your body must work in its environment. That means an enzyme’s “ideal” conditions should match the conditions in the organ where it is found. The relative pH inside different human organs is shown below. Based on this information and your data, where in the body do you think human catalase would work most effectively? Why?

<table>
<thead>
<tr>
<th>Organ</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestine</td>
<td>Basic</td>
</tr>
<tr>
<td>Liver</td>
<td>Neutral</td>
</tr>
<tr>
<td>Stomach</td>
<td>Acidic</td>
</tr>
</tbody>
</table>

Experiment 2: Effects of exercise on cellular respiration

9. Below the equation for cellular respiration, label which items are the substrates (or reactants) and which are the products.

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O} + 36 \text{ATP (energy)} \]

Table 2. Effect of exercise on cellular respiration

<table>
<thead>
<tr>
<th></th>
<th>CO\textsubscript{2} production (time in min)</th>
<th>Breathing rate (breaths/min)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting (average of 3 measurements)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise (1 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise (2 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the space below, make a **bar graph** of the data for this experiment. Use different patterns or colors for each set of data. Make sure your graph has a **title**, and **label the x and y axis**, providing scales and units.

10. Our hearts and lungs spend a lot of time and energy delivering oxygen to our cells. Why bother? Why does your body need oxygen and how exactly is it used?

11. How does an increased heart rate help your cells during exercise?

12. If the resting heart rate and exercise heart rate are about the same, what does that say about the fitness/health of the individual?