Nutrition LAB - What happen during digestion?

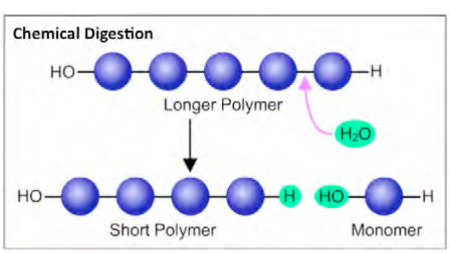
All the important, carbon-based molecules of human life - carbohydrates, lipids, proteins, vitamins, and nucleic acids - have to be built from the stuff we eat. All the energy to build the complex molecules of life and body parts and carry out work in our bodies also must come from the stuff we eat.

After you eat a meal, your body does two important things. First, it digests your food to make it small enough to get into your blood [absorption]. The nutrient molecules can then circulate around as building materials for the molecules, cells, and tissues of your body. Second, your cells pull apart the molecular bonds of your nutrient molecules to harness the energy and create ATP.

Have you ever been around a campfire or a fireplace with wood burning? If so, you've seen what happens when you break the bonds of fuel molecules! In a fire, oxygen helps to pull apart the molecules of wood, which releases lots of energy – most obviously in the forms of heat and light. The mitochondria in your cells help perform a somewhat similar process with your food. Oxygen helps your mitochondria gradually pull apart fuel molecules to release energy – mostly used to make ATP and heat! In science, we measure energy in calories (1 calorie = the energy needed to raise the temperature of 1 gram water by 1 degree C). In biology and nutrition, we generally refer to 1000 calorie units (kcal's). Today, whenever we talk about Calories, we will be referring to kcal's.

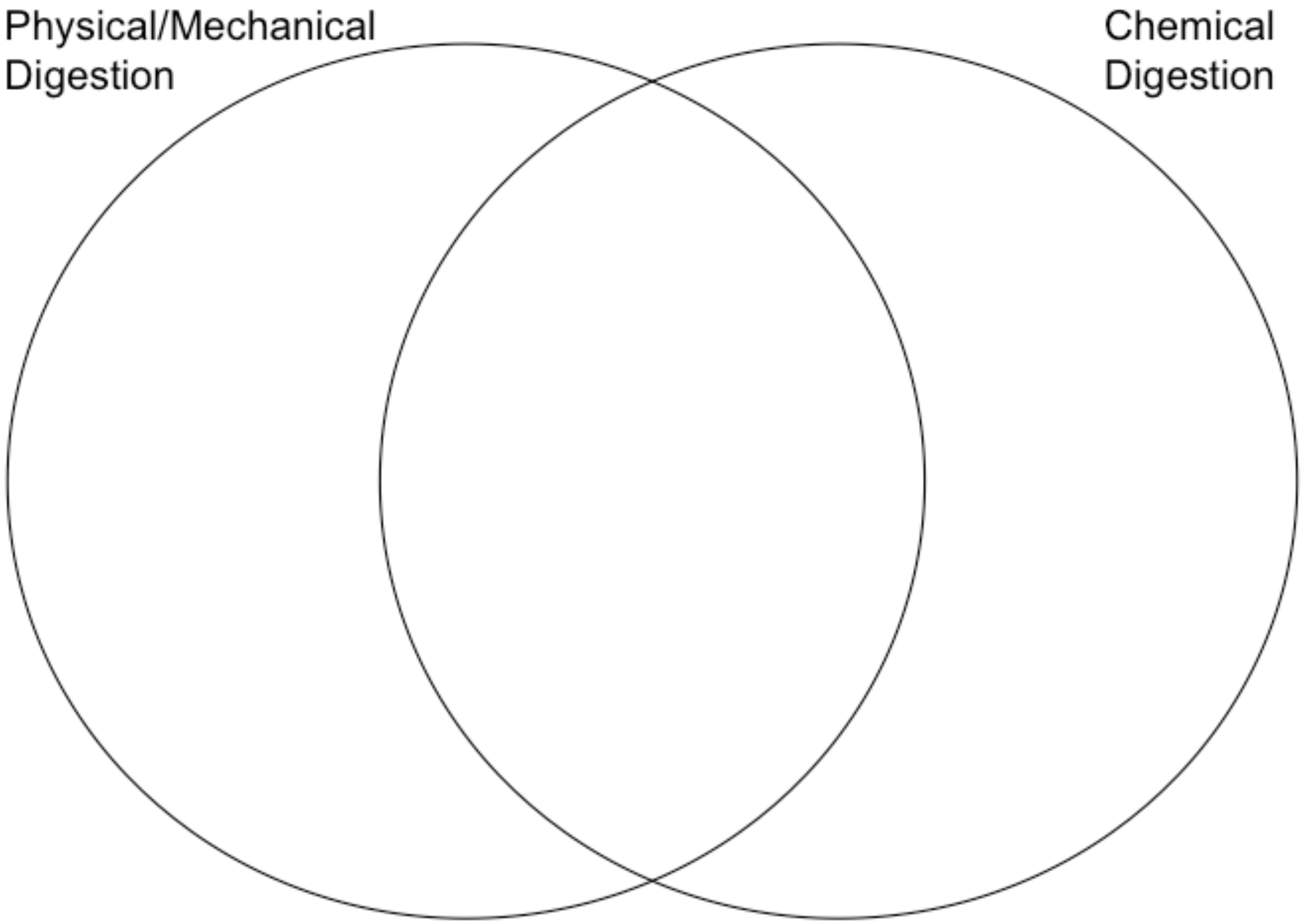
ACTIVITY 1. Doing Digestion

Can a piece of pizza fit into your blood? When you eat a chocolate bar, are there chunks of chocolate floating through your arteries? I don't think so. To get nutrients into your blood, food must first be broken down in two ways – mechanically and chemically. Mechanical digestion *cuts, pounds,* and *grinds* food into smaller food pieces, but does not change the molecular structure of foods. This leaves large nutrient molecules called polymers (ex. proteins, starches, triglycerides) in the digestive system. These polymers generally need to be broken down into small monomer building blocks (ex. amino acids, monosaccharides, fatty acids) to be absorbed. Chemical digestion therefore *breaks bonds* of large carbohydrates, proteins, and lipids, to make smaller molecules that can fit into the blood.



The Organs of Digestion

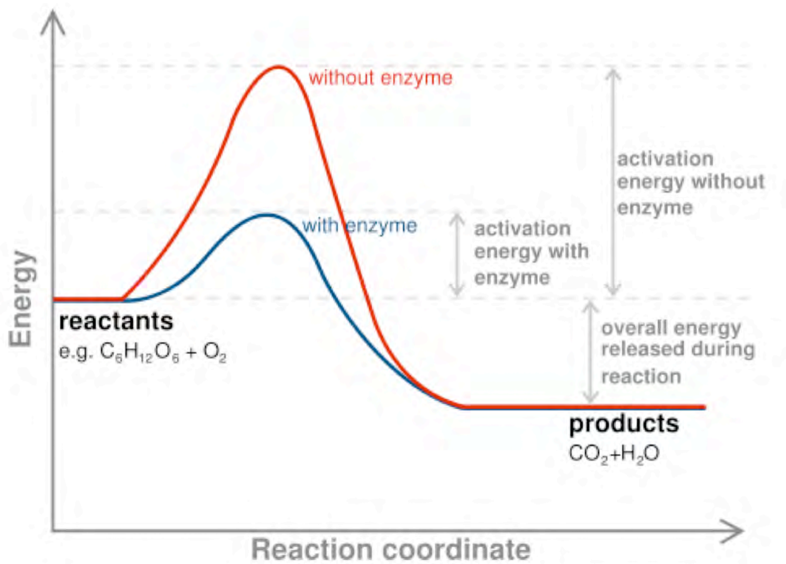
We have completed the digestive system lab. Be able to ID the organs in the picture and torso or other models. Cut out the organs [in figures provided separately] along the dotted lines and place them in the Venn diagram on this page to indicate whether each organ contributes to mechanical digestion, chemical digestion, or both.



What Affects Reactions in Our Bodies?

Our bodies are incredibly busy factories of chemical reactions! Among many other things, we need to break apart food molecules, build new tissues, replicate DNA, detoxify drugs, and the list goes on. All of these activities somehow involve pulling apart or putting together molecules (making and breaking covalent bonds). Many molecules are just happy as they are, though! That means they won't easily or frequently break apart or go together on their own. Luckily, we have special **proteins** called **enzymes** that create more favorable environments for these reactions. Enzymes basically reduce the amount of energy needed to start a reaction, so the reaction can happen much more easily and frequently.

Each of the reactions mentioned above has an enzyme to help it along. Amylase is an enzyme that helps to break apart food molecules, DNA polymerase is an enzyme that helps replicate DNA, etc. There are over 2000 known enzymes. You can often recognize their names, since they often they end with *–ase.* Enzymes are **substrate specific** (only work with certain specific reactant molecules). The enzyme peptidase (which breaks bonds in proteins) will not work on starch (which is broken down by amylase).



An enzyme's shape determines its function. Enzymes have "pockets" that we call **active sites.** Just like pockets on your pants and shirts, active sites can have many different sizes and shapes. Only molecules that fit properly into the enzyme's active site have a chance of going through a reaction in the enzyme. If the active site changes shape, that can quickly affect whether or not a reaction happens! Putting an enzyme in an uncomfortable environment can sometimes **denature** (basically scramble and reshape) an enzyme.

In today's experiment, you will be working with the enzyme **catalase.** Catalase is a common enzyme found in nearly all living things, including humans. Its main function is to help break down its substrate, hydrogen peroxide (H_2O_2), into water (H_2O) and oxygen (O_2). Hydrogen peroxide naturally builds up in our bodies during metabolism. Catalase works very quickly to eliminate hydrogen peroxide, since even small amounts of it could harm our cells and tissues. Since one of the products of this reaction is oxygen, a gas, we can see if catalase is working by looking for oxygen bubbles.

Catalase Reaction: $2H_2O_2$ + Catalase -------------------> $2H_2O$ + O_2 + Catalase

Experimental Procedures:

Preparing Catalase (These *steps done beforehand by instructor)*

1. Cut a cube of liver ~ 5 cm^2
2. Add 1 medium-sized scoop of ice
3. Add 100 ml pH 7 phosphate buffer
4. Grind until smooth in blender (~ 30 - 60 seconds)
5. Pour mixture through a double layer of cheesecloth into a beaker
6. This stock solution should be diluted for class use and stored 4°C
7. Dilute 10 ml of stock solution in 350 - 500 ml distilled water (each class)
8. Keep enzyme suspension on ice

I. Measuring Catalase Activity

1. Place five test tubes in a rack and label them 1, 2, 3, 4 and 5

   - Each group member should prepare one of the test tubes below:
   - Fill test tube # 1 with 4 mL of water
   - Fill test tube # 2 with 1 mL of 3.0% H_2O_2 and 3 mL of water
   - Fill test tube # 3 with 2 mL of 3.0% H_2O_2 and 2 mL of water
   - Fill test tube # 4 with 3 mL of 3.0% H_2O_2 and 1 mL of water
   - Fill test tube # 5 with 4 ml of 3.0% H_2O_2
2. Using a clean transfer pipette, add one pipette full of catalase to each tube. Each person in your group can do this with a different test tube, so that all the tubes start at the same time. Try to make sure the catalase goes directly to the bottom, not down the side of the tube.
3. If oxygen bubbles form, that means the catalase is working. Watch the bubbles and take note of the highest point the bubbles get to in each tube. After one minute, measure the height of bubbles to the highest point the bubbles reached in each tube (in millimeters). Record this measurement in the chart provided on the following page. We will use the thickness of bubbles as a measure of the amount of catalase reactions that take place.
4. Rinse and dry your test tubes so you can reuse them in additional trials later.
5. **Use the graph paper to create a graph to express the data you collected in Part I.**

II. Measure Effect of Temperature on Catalase Activity

1. Place four test tubes in a rack and label them 1, 2, 3 and 4.
2. Using a clean pipette, add one dropper full of catalase to each test tube.
   - Place test tube # 1 in a 5°C ice bath
   - Leave test tube # 2 in the test tube rack at room temperature (~ 22°C)
   - Place test tube # 3 in a 38°C water bath
   - Place test tube # 4 in a 70°C water bath
3. Allow test tubes to incubate at each temperature for 10 minutes before proceeding with the next step of the experiment. Assign one member of your group to be in charge of each test tube.
4. After 10 minutes bring the tubes back to your table. Each person should use a clean pipette to add 2 ml of water to their test tube.
5. Each person should then add 2 ml of 3.0% H_2O_2 to their test tube.
6. After one minute, measure the height of bubbles to the highest point the bubbles reached in each tube (in millimeters). Record this information in the chart provided.
7. **Use the graph paper to create a graph to express the data you collected in Part II.**

III. Measure Effect of pH on Catalase Activity

1. pH is a measure of the concentration of hydrogen ions (H^+) in a solution of water. An **acidic** solution of water has a high concentration of H^+, and a low pH (below 7.0). A **basic** solution of water has a low concentration of H^+, and a high pH (above 7.0). **Spend a few minutes designing an experiment to measure the effect of acids and bases (different pH solutions) on catalase activity**. Plan to test 4 different pH's.

We have 3 prepared solutions of water in class, each with a different pH (4.0, 7.0, 12.0). Since we want to test 4 different pH's, you will need to create a 4th solution on your own. You can combine a couple of the prepared solutions, which should create a new solution with a pH somewhere in the middle. Or you could use tap water or a liquid of your own (soft drink, tea, etc.) as your 4th solution. In any case, you should use the provided pH test paper to find out the pH of your 4th solution.

pH Experiment Procedures:

(**Hints**: We want to know the impact of acids and bases on catalase. That means you should probably expose catalase to the acid and base solutions first, before introducing hydrogen peroxide. You also should make sure to include enough acid or base solution in your test tubes so that it is not simply diluted by the catalase mixture.)

Experimental design by your group: Describe below -

Check your experimental design with your instructor before beginning.

Carry out your experiment and record all your data.

Use the graph paper on the following pages to create a graph to express the data you collected in Part III.

Graph all of your data on the following pages (graph grids are provided). Be sure to give each graph a **title** and each **axis** a **label** and **scale.**

What Affects Reactions in Our Bodies?

Results and Data (Be sure to attach *all 3 graphs*

Substrate (H_2O_2) Volume	**0 mL**	**1 mL**	**2 mL**	**3 mL**	**4 mL**
Amount of Reaction (foam thickness in mm)					

Temperature	**5°C**	**22°C**	**38°C**	**70°C**
Amount of Reaction (foam thickness in mm)				

pH	**pH _____**	**pH _____**	**pH _____**	**pH _____**
Amount of Reaction (foam thickness in mm)				

Questions

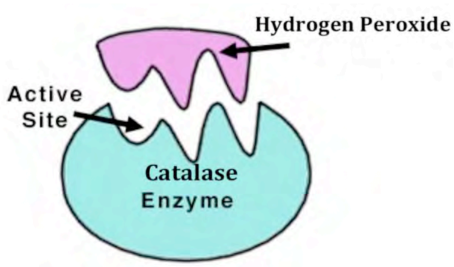
1. According to your results, what is the ideal temperature for the catalase enzyme (where it showed greatest reaction)? Use *your data* to explain your conclusion.

2. 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.

Each enzyme in your body must be able to work in the environment where it resides. That means an enzyme's "ideal" conditions should match the conditions for the organ in which it works. The relative pH's inside different human organs are shown below. Based on this information and your data, where in the body do you think catalase would work most effectively? Why?

organ	pH
small intestine	slightly basic
oral cavity	6.2 - 7.4
stomach	acidic [1.5 - 3.0]
Liver	neutral

3. Pretend that the diagrams below show a molecule of hydrogen peroxide and one of the active sites on catalase under normal conditions. Create another *labeled* diagram to show how you think this would look different if the enzyme was first exposed to an extremely hot environment (ex. 70 degree water).



4. Today we looked at how catalase reactions are affected by different conditions, like temperature and pH. Considering that you have catalase and many other enzymes in your body, what diseases, environmental conditions, and dietary abnormalities might change your body's conditions in ways that could denature your enzymes? In what ways would those things change your body's conditions?

5. Specifically how has this lab changed or strengthened *your original ideas* about the chemical reactions in our bodies?

LAB QUIZ on Digestive System

Answer the following:

1. What are the subunits that make up the proteins?
2. Where does [name the organ] digestion of proteins start? Name other organs where protein digestion takes place.
3. Name all the enzymes by organs that help digestion of proteins.
4. Does the effectiveness of pepsin depend on pH? Explain your answer.
5. What are the subunits that make up carbohydrates?
6. What is the role of amylase in digestion in human? Describe.
7. Name all the organs in the human body where carbohydrate digestion take place.
8. Where does fat digestion take place in human digestive system?
9. What is the role of liver and the gall bladder in fat digestion? Describe briefly.
10. How will removal of the gall bladder affect human digestion? Explain