Chemical and Physical Processes of Digestion

Objective:

To explain in short essays or diagrams how carbohydrates, proteins, and fats are digested into end products that can be absorbed into the blood, at the level of 85% proficiency for each student.

In order to achieve this objective, you will need to be able to:

1. Name the major enzymes that are involved in the digestion of proteins, fats, and carbohydrates and the end products.
2. Measure the digestion of digestion of proteins, fats, and carbohydrates.
3. Explain the role of bile in the digestive process.
4. Discuss the possible role of temperature and pH in the regulation of enzyme activity.
5. Explain the role of the tongue, larynx, and lower esophageal (cardiac) sphincter in swallowing.

Enzyme Action

Materials:

General supply area:

Test tubes and test tube rack
Wax markers
Large beakers
Warm Water bath (37C) (if not available, incubate at room temperature and double the time)
Ice water bath (0C)
Hot plates

Supply area 1:

Spot plates
Dropper bottles of distilled water
250-mi beakers
Boiling chips
Dropper bottles of
- 1% alpha-amylase solution (The alpha-amylase must be a *low-maltose preparation* for good results.)
- 1% boiled starch solution, freshly prepared (Prepare by adding 1 g starch to 100 mi distilled water; boil and cool; add a pinch of salt (NaC1).)
- 1% maltose solution
- Lugol's IKI (Lugol's iodine)
- Benedict's solution

Supply area 2:

Dropper bottles of
- 1% trypsin
- 1% BAPNA solution

Supply area 3:

Parafilm (small squares to cover the test tubes)
Bile salts (sodium taurocholate)
Dropper bottles of
- 1% pancreatin solution
- Litmus cream (fresh cream to which powdered litmus is added to achieve a blue color)
- *O.1NHCI*
- Vegetable oil

Starch Digestion by Salivary Amylase

Methods:

Work in groups of 3 or 4, with each group taking responsibility for setting up and conducting one of the following experiments. Each group should then communicate its results to the rest of the class by recording them in a chart on the chalkboard. All members of the class should observe the controls as well as the positive and negative examples of all experimental results. Additionally, all members of the class should be able to explain the tests used and the results observed and anticipated for each experiment. Note that water baths (37C and 0C) and hot plates are at the general supply area.

1. Use the hot plates to prepare boiling water at the beginning. Place a few boiling chips and about 250 mL water into a 500 mL beaker and bring to a boil.

2. From the general supply area, obtain a test tube rack, 10 test tubes, and a wax marking pencil. From supply area 1, obtain a dropper bottle of distilled water and dropper bottles of maltose, amylase, and starch solutions.

3. Since in this experiment you will investigate the hydrolysis of starch to maltose by salivary amylase (the enzyme produced by the salivary glands and secreted into the mouth), it is important to be able to identify the presence of these substances to determine to what extent the enzymatic activity has occurred. Thus controls must be prepared to provide a known standard against which comparisons can be made. Starch decreases and sugar increases as digestion occurs, according to the following equation:

$$\text{Starch + water} \xrightarrow{\text{Amylase}} \text{X* maltose}$$

3. Chart 1 below outlines the general procedures. Two students should prepare the controls (tubes 1A to 3A) while the other two prepare the experimental samples (tubes 4A to 6A).

    a. Mark each tube with a wax pencil and load the tubes as indicated in Chart 1, using ***3 drops*** of each indicated *additive*.

    b. Boil tube 4A for 4 minutes prior to starting the incubation.

    c. Place all tubes in a rack in the water bath (37C or 0C) for approximately 1 hour.

    d. While these tubes are incubating, proceed to the next section.

4. From supply area get a “spot plate”, and dropper bottles of Lugol's Iodine and Benedict's solution. Use a hot plate as necessary from the general supply area.

5. Mark the spot plate *A* (for amylase) and number six of its depressions 1-6 for sample identification. Make sure that you have boiling water available when the 1 hour incubation time is complete.

6. After the 1 hour incubation time, place about a drop of the sample from each of the tubes into the appropriately numbered spot. Into each sample droplet, place a drop of Lugol's Iodine solution. A blue-black color indicates the presence of starch and is referred to as a **positive starch test.** If starch is not present, the mixture will not turn blue, which is referred to as a **negative starch test.** Record your results (++ for most positive (black), + for less positive (blue), − for negative) in Chart 1.

7. Into the remaining mixture in each tube, place 3 drops of Benedict's solution. Put each tube into the beaker of boiling water for about 5 minutes. If a green to orange precipitate forms, maltose is present; this is a **positive sugar test. A negative sugar test** is indicated by no color change. Record your results in Chart I (++ for most positive (orange), + for less positive (green), − for negative).

Results:

Chart 1 Salivary Amylase Digestion of Starch

Tube no.	1A	2A	3A	4A	5A	6A
Additives	Starch and Water	Amylase and Water	Maltose and Water	Starch and Amylase	Starch and Amylase	Starch and Amylase
Pre-incubation				boil 4 min		
Incubation condition	37C	37C	37C	37C	37C	0C
Lugol's Iodine test						
Benedicts's test						
Carbohydrate Hydrolysis (Y/N)						

Protein Digestion by Trypsin

Trypsin, an enzyme produced by the pancreas, hydrolyzes proteins to small fragments (proteoses, peptones, and peptides). BAPNA is a synthetic (man-made) protein substrate consisting of a dye covalently bound to an amino acid. Trypsin hydrolysis of BAPNA cleaves the dye molecule from the amino acid, causing the solution to change from colorless to bright yellow. Since the covalent bond between the dye molecule and the amino acid is the same as the peptide bonds that link amino acids together, the appearance of a yellow color indicates the presence and activity of an enzyme that is capable of peptide bond hydrolysis. Because the color change from clear to yellow is direct evidence of hydrolysis, additional tests are not required when determining trypsin activity using BAPNA.

Methods:

1. From the general supply area, obtain 5 test tubes and a test tube rack, and from supply area 2 get a dropper bottle of trypsin and one of BAPNA and bring them to your bench.

2. Chart 2 below outlines the general procedures. Two students should prepare the controls (tubes 1T and 2T) while the other two prepare the experimental samples (tubes 3T to 5T).

    a. Mark each tube with a wax pencil and load the tubes as indicated in Chart 2, using **3 drops** of each indicated *additive*.

    b. Boil tube 3T for 4 minutes prior to starting the incubation.

    c. Place all tubes in a rack in the appropriate water bath (37C or 0C) for approximately 1 hour.

    d. While these tubes are incubating, proceed to the next section.

3. Since BAPNA is a synthetic colorigenic (color-producing) substrate, the presence of yellow color indicates a **positive hydrolysis test;** the dye molecule has been cleaved from the amino acid. If the sample mixture remains clear, no detectable hydrolysis has occurred. 1. Record the color of the experimental tubes in Chart 2 (++ for most positive (yellow), + for less positive (faint yellow), − for negative).

Results:

Chart 2 Trypsin Digestion of Protein

Tube no.	1T	2T	3T	4T	5T
Additives	Trypsin and Water	BAPNA and Water	BAPNA and Trypsin	BAPNA and Trypsin	BAPNA and Trypsin
Preincubation			boil 4 min		
Incubation condition	37C	37C	37C	37C	0C
Color change					
Protein Hydrolysis (Y/N)					

Pancreatic Lipase Digestion of Fats and the Action of Bile

Methods:

The fact that some of the end products of fat digestion (fatty acids) are organic acids that decrease the pH provides an easy way to recognize that digestion is ongoing or completed. You will be using a pH indicator called *litmus blue* to follow these changes; it changes from *blue* to *pink* as the test tube contents become acid.

1. From the general supply area, obtain 9 test tubes and a test tube rack, plus one dropper bottle of each of the solutions in supply area 3.

2. Although *bile*, a secretory product of the liver, is not an enzyme, it is important to fat digestion because of its emulsifying action (the physical breakdown of larger particles into smaller ones) on fats. Emulsified fats provide a larger surface area for enzymatic activity. To demonstrate the action of bile on fats, prepare two test tubes and mark them 1E, 2E, and 3E.

    a. To tube 1E add 10 drops of water and 2 drops of vegetable oil.

    b. To tube 2E add 10 drops of water, 2 drops of vegetable oil, and a pinch of bile salts.

    c. To tube 3E add 10 drops of water, 2 drops of vegetable oil, and a small drop of dish detergent

    d. Cover each of these tubes with a small square of Parafilm, shake vigorously, and allow the tubes to stand at room temperature.

3. After 10-15 minutes, observe both tubes. If emulsification has not occurred, the oil will be floating on the surface of the water. If emulsification has occurred, the fat droplets will be suspended throughout the water, forming an emulsion. In which tube has emulsification occurred?

4. Two students should prepare the controls (1L, and 2L,), while the other two students in the group set up the experimental samples (3L to 5L, 4B, and 5B) as indicated in Chart 3.

    a. Mark each tube with a wax pencil and load the tubes using **5 drops** of each indicated *additive*.

    b. A pinch of bile salts should be placed in tubes 4B and 5B.

    c. Temporarily cover each tube with Parafilm and shake to mix the contents of the tube.

    d. Boil tube 3L for 4 minutes prior to starting the incubation.

    e. Remove the Parafilm and place all tubes in a rack in the appropriate water bath (37C or 0C) for approximately 1 hour.

5. The basis of this assay is a pH change that is detected by a litmus powder indicator. Alkaline or neutral solutions containing litmus are blue but will turn reddish in the presence of acid. Since fats are digested to fatty acids (organic acids) during hydrolysis, they lower the pH of the sample they are in. Litmus cream (fresh cream providing the fat substrate to which litmus powder was added) will turn from a bluish color to pink if the solution is acid. Because the effect of hydrolysis is directly seen, additional assay reagents are not necessary.

6. Record the color of the tubes in Chart 3.

7. After recording the data, prepare a color control. Add a drop of 0.1 *N* HC1 to tubes 1L and 2L Tube 1L should not change color. Tube 2L should turn pink.

Results:

Chart 3 Pancreatic Lipase Digestion of Fats

Tube no.	1L	2L	3L	4L	5L	4B	5B
Additives	Lipase *and* Water	Litmus cream *and* Water	Litmus cream *and* Lipase	Litmus cream *and* Lipase	Litmus cream *and* Lipase	Bile salts *and* Litmus cream *and* Lipase	Bile salts *and* Litmus cream *and* Lipase
Pre-incubation			boil 4 min				
Incubation condition	37C	37C	37C	37C	0C	37C	0C
Color change							
Lipid Hydrolysis (Y/N)							
Color control			X	X	X	X	X

Physical Processes: Mechanisms of Food Propulsion and Mixing

Although enzyme activity is a very important part of the overall digestion process, foods must also be processed physically (churning and chewing), and moved by mechanical means along the tract if digestion and absorption are to be completed. Just about any time organs exhibit mobility, muscles are involved, and movements of and in the gastrointestinal tract are no exception. Although we tend to think only of smooth muscles when visceral activities are involved, both skeletal and smooth muscles are involved in digestion. This fact is amply demonstrated by the simple demonstrations that follow.

Materials

Supply area 4

Water pitcher
Paper cups
Stethoscope
Alcohol swabs
Disposable autoclave bag

Deglutition (Swallowing)

Swallowing, or deglutition, which is largely the result of skeletal muscle activity, occurs in two phases: *buccal* (mouth) and *pharyngeal-esophagea1*. The initial phase --the buccal--is voluntarily controlled and initiated by the tongue. Once begun, the process continues involuntarily in the pharynx and esophagus, through peristalsis, resulting in the delivery of the swallowed contents to the stomach.

Methods:

1. Obtain a pitcher of water, a stethoscope, a paper cup, an alcohol swab, and an autoclave bag in preparation for making the following observations.

2. While swallowing a mouthful of water, consciously note the movement of your tongue during the process. Record your observations.

3. Repeat the swallowing process while your laboratory partner watches the externally visible movements of your larynx. (This movement is more obvious in a male, who has a larger Adam's apple.) Record your observations. What do these movements accomplish?

4. Before using the stethoscope your lab partner should clean the earpieces with an alcohol swab. Then, he or she should place the diaphragm of the stethoscope over your abdominal wall, approximately 1 inch below the xiphoid process and slightly to the left, to listen for sounds as you again take two or three swallows of water. There should be two audible sounds--one when the water splashes against the gastro-esophageal (lower esophageal) sphincter and the second when the peristaltic wave of the esophagus arrives at the sphincter and the sphincter opens, allowing water to gurgle into the stomach. Determine, as accurately as possible, the time interval between these two sounds and record it below.
   This interval gives a fair indication of the time it takes for the peristaltic wave to travel down the l0-inch-long esophagus. (Actually the time interval is slightly less than it seems, because pressure causes the sphincter to relax before the peristaltic wave reaches it.)

Results and Discussion:

Describe the movement of your tongue.

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Describe the movements of your larynx.

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Determine the time interval between the sound of the water splashing against the gastro-esophageal (lower esophageal) sphincter and the sound of the water gurgling into the stomach.

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