# Protein Digestion

## Introduction

Every living cell contains from several hundred to several thousand different macromolecules known as proteins. Proteins are vital to most structural components of cells as well as to their chemistry. In order for cells to make the types of proteins they need they must first break down proteins into their building blocks—amino acids. This degradation process is called digestion.

## Concepts

- Amino acids
- Protein structure
- Protein digestion
- Enzymes

## Materials

Egg whites, fresh
Hydrochloric acid solution, 0.1 M, 8 mL
Pepsin solution, 1%, 10 mL
Toluene, several drops
Beaker, 400-mL, or pot for water bath
Forceps
Hot plate
Markers
Metric ruler
Paper towels
Pipets, Beral-type, thin-stem
Scalpel
Scissors
Test tubes, 13 × 100 mm, 4
Water bath

## Preparation

1. Separate the egg whites from the yolks prior to the laboratory work. One egg is required for every 3–4 lab teams.
2. Prepare the hydrochloric acid and pepsin solutions.

## *Safety Precautions*

*This activity requires the use of hazardous components and/or has the potential for hazardous reactions. Use care in working with hot surfaces and substances. Hydrochloric acid can damage the eyes, skin, and clothing. If spilled, rinse thoroughly with excess water. Toluene is poisonous and flammable and should be dispensed by the instructor. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.*

## Procedure

1. Secure two Beral-type pipets. Carefully draw egg white into the pipets, one at a time, until each pipet stem is filled with liquid egg white. Use scissors to cut off the end of each pipet while the stem is still submerged in the egg white (Figure 1). Use paper towels to prevent liquid egg white from getting on the table tops, etc.
2. Immerse one of the pipets into a boiling water bath until the egg white is cooked, i.e., it hardens and turns white. Repeat this procedure for the other pipet. Be careful with the boiling water and allow the pipet to cool before handling the stem.


**Figure 1.** Beral pipet with cooked egg albumin.

3. Use a sharp scalpel or razor blade to cut each filled pipet stem into two 3-cm pieces. The egg white should be flush at both ends of the cut stem.

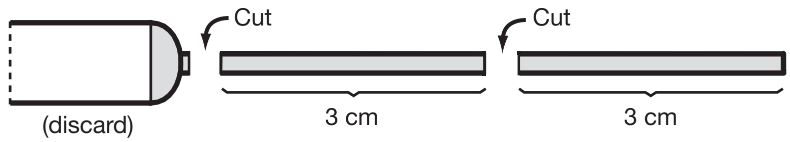


**Figure 2.** Beral pipet cut.

4. Place each of your egg white stems into four separate test tubes.
5. Pour 5 mL of water into the first test tube and label it "W." Pour 5 mL of a 1% pepsin solution into the second test tube and label it "P." Pour 5 mL of a 0.1 *M* hydrochloric acid solution into the third test tube and label it "A." Pour equal amounts of pepsin solution and hydrochloric acid solution into the fourth test tube to equal 5 mL total volume and label it "PA". Be sure the solutions completely cover the stems containing the egg white.
6. Every ten minutes for the rest of this lab period, use a forceps to remove the stems, one at a time, from each test tube and observe them. Place each stem on a separate paper towel and be sure to return the stem to the proper test tube after your observations are complete. Rinse the forceps between each tube. In some of the stems, you may notice that the egg white is no longer flush with the ends of the tubing. With the metric ruler, measure the clear area between one end of the stem and the solid egg white. This distance is a measure of how much of the egg white protein has been digested. (When digested, protein becomes a clear liquid that flows out of the tube). For each reading, measure the same end of the stems. Record your measurements in Data Table 1 on the Protein Digestion Worksheet.
7. Allow digestion to continue overnight. Store the test tubes in a water bath or warm place as directed by your instructor. Bacteria are likely to start to grow on the egg white; therefore, something must be added to retard bacterial growth during the rest of the experiment. Your instructor will add a drop of toluene to each of your test tubes. *Caution:* Toluene is flammable and poisonous. Handle safely.
8. After 24 hours, remove each stem carefully and measure the amount of digestion in each. Record your results in Data Table 1. After 48 hours, record another set of measurements.
9. Plot your measurements of amount of digestion versus time on graph paper. Label each treatment separately (W, P, A, and PA). Answer the questions on the Protein Digestion Worksheet.

## Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Solutions from this experiment can be diluted with water, neutralized, and flushed down the drain with plenty of water according to Flinn Suggested Disposal Method #24b. Solids can be disposed of with normal solid waste removal at your school.

## Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

***Unifying Concepts and Processes: Grades K–12***
Evidence, models, and explanation
***Content Standards: Grades 5–8***
Content Standard C: Life Science, structure and function in living systems
***Content Standards: Grades 9–12***
Content Standard C: Life Science, matter, energy, and organization in living systems

## Tip

- If the end of the pipet is not cut, the egg white will be forced out of the pipet when it is placed in the boiling water. The egg white will harden very quickly when placed in the boiling water.

## Discussion

The importance of proteins in living organisms cannot be overemphasized. They are structural components of cells as well as messengers and receptors of messages between cells. They play an important role in the defense against disease. Their most important role, however, is as enzymes—specialized molecules that facilitate the many reactions occurring in cells. In fact, one protein was digesting another protein in this experiment. Pepsin, an enzyme and protein, is digesting egg white, another common protein.

The early studies of digestion in the stomach are always of interest to students. In 1783, Spallanzani described digestion as a chemical process and concluded that the stomach was acidic. Recall the observation of Dr. Beaumont in 1822 on his patient, Alexis St. Martin, who had an open hole in his stomach from a gunshot wound that healed with an open hole to the outside of his body. Dr. Beaumont looked right into St. Martin's stomach and did experiments through the hole in his side. He collected samples and designed experiments which he performed in St. Martin's stomach. Biologists today often install windows into the stomach of organisms to study digestion.

Two aspects of enzyme activity that are important to cells are illustrated in this lab. Enzyme reactions are faster at higher temperatures (an easy extension to this lab), but the temperature range is a fairly narrow one. Above certain temperatures, enzymes break down. How does the rate of enzyme activity do at 98.6 °F? Enzyme activity also varies with the pH of the environment. Each enzyme has an optimum temperature and pH. Pepsin (operating in the stomach) obviously enjoys an acidic pH range and, in fact, works best in acid conditions. Students could further try different acid concentrations and compare their results to the results from this lab.

### Answers to Questions

1. In which stems did you observe protein digestion? Do these stems have anything in common?

   *Digestion occurred in tubes P and PA. The pepsin is the common ingredient and is a protein-digesting enzyme.*

2. Which stem showed the greatest protein digestion? Suggest a hypothesis to explain this result. How would you test the hypothesis?

   *Tube PA showed the greatest digestion. Hypothesis—pepsin works best in an acid pH range. Possible experiment to test hypothesis—set up a variety of PA tubes in various pHs.*

3. What purpose did the W and A stems serve in the experiment?

   *The W and A test tubes served as controls for the experiment.*

### Extensions

1. This inexpensive laboratory format lends itself readily to continued experimentation. Students can test other enzyme rate variables without great expense. Temperature, light, dark, and other competing chemicals are just a few of the possibilities.
2. Pose this question: If hydrochloric acid can eat away your skin, why doesn't it eat away your stomach? What are antacids?

## Materials for *Protein Digestion* are available from Flinn Scientific, Inc.

| Catalog No. | Description |
|---|---|
| AP1718 | Beral-type Pipets, Thin-Stem |
| AB1018 | Forceps, Polypropylene |
| AP8387 | Hot Plate |
| AP8291 | Pencils, Wax |
| AB1060 | Scissors |
| P0006 | Pepsin, 25 g |
| H0014 | Hydrochloric Acid, HCl, 0.1 M, 500 mL |
| AP1872 | Ruler, Metric |
| AB1047 | Scalpel |
| T0019 | Toluene |
| AP7301 | Water Bath |

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

# Protein Digestion Worksheet

## Data Table

| Tube | Distance Between Egg White and End of Stem (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | O min. | ___ min. | ___ min. | ___ min. | ___ min. | 24 hours | 48 hours |
| W (Water) | | | | | | | |
| P (Pepsin) | | | | | | | |
| A (Acid) | | | | | | | |
| PA (Pepsin/Acid) | | | | | | | |

## Questions

1. In which stems did you observe protein digestion? Do these stems have anything in common?

2. Which stem showed the greatest protein digestion? Suggest a hypothesis to explain this result. How would you test the hypothesis?

3. What purpose did the W and A stems serve in the experiment?