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CHEMICAL COMPOSITION OF CELLS

Learning Objectives

1. Name the organic chemical components of cells studied in this lab and be able to recognize the chemical structure of these components.
2. State the building blocks of specific macromolecules.
3. Cite the tests used to identify the presence of proteins (and polypeptides), starch, sugars (reducing), and lipids.
4. Distinguish tests responses that show a chemical's absence (negative) or presence (positive).
5. Describe the variable response of the Benedict's test.

Introduction

All living things consist of simple substances called **elements**. Elements are composed of discrete units called **atoms**. The atom of each kind of element has a definite number and arrangement of **protons** and **neutrons** within a nucleus and electrons within energy shells about the nucleus. To stabilize the outer shell electron configuration, atoms share or transfer electrons with other atoms. In other words, they bond with other atoms. To major kinds of bonds are formed: **ionic** and **covalent**. **Ionic bonds** result from the transfer of electrons between atoms, and **covalent bonds** result from electron sharing. Ionic bonds are common to inorganic molecules, while covalent bonds are common to organic molecules.

ral classes of organic molecules have biological significance. In this laboratory, you will be studying **proteins, carbohydrates (monosaccharides, disaccharides, polysaccharides), and fats.** As you shall see, amino acids are joined by peptide bonds with a protein. Many glucose (monosaccharide) molecules are joined within a polysaccharide, such as starch. A fat contains one glycerol and three fatty acids.

Various chemicals will be used in this laboratory to test for the presence of these molecules. Most often, you will be looking for a particular color change. If the change is observed, the test is said to be positive because it indicates that a particular molecule is present. If the color change is not observed, the test is said to be negative because it indicates that a particular molecule in not present.

In all of the procedures, you will need water as a control. Usually, a control goes through all the steps of the experiment but lacks one essential factor (the experimental variable). This missing factor allows you to observe the difference between a positive result and a negative result. If the control sample tests positive, you know your test is invalid.

PROTEINS

Proteins are made up of **amino acids** with the structure shown in figure 1. About 20 different common amino acids are found in . All amino acids have an acidic group (-COOH) and an amino group (H_2N-). They differ by the **R group** (remainder group) attached to the carbon atom, as shown in figure 1. The R groups have varying sizes, shapes, and chemical acitivities.

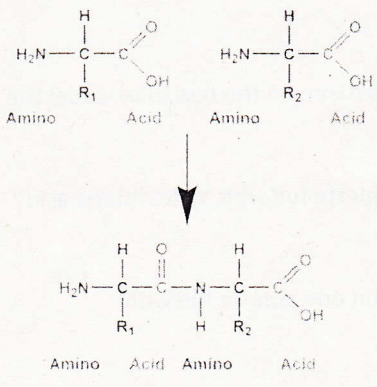

Figure 1 --Peptide bond formation between amino acids creates a dipeptide. Multiple amino acids forming peptide bonds establish the primary structure of a protein.

A chain of several amino acids joined together is called a **peptide**, and if the number of amino acids involved is large, the chain is called a **polypeptide.** The amino acid in a peptide are held together by **peptide bonds:**

In a protein, the polypeptide chain, which contains hundreds of amino acids, twists to form a helix (spiral) due to hydrogen bonding between every fourth peptide linkage. The helix then bends and folds to produce a three-dimensional shape. Some proteins have more than one polypeptide chain (figure 2).

Proteins have numerous functions in cells. One important category of proteins is **enzymes**, organic catalysts that speed up metabolic reactions. In addition, the contractile elements of muscle cells contain protein filaments. **Albumin** is the major protein found in blood. **Pepsin** is a digestive enzyme active in the stomach, where conditions are acidic.

Tests for Proteins

Biuret reagent (blue color) contains a strong solution of sodium or potassium hydroxide (NaOH or KOH) and a very small amount of dilute copper sulfate ($Cu^{++}SO_4$) solution. The reagent changes color in the presence of proteins or peptides because the amino group (H_2N-) of the protein or peptide chemically combines with the copper ions in biuret reagent (see table 1)

Table 1

Test for Protein		
	Protein	**Peptides**
Biuret reagent (blue)	**Violet**	**Pinkish**

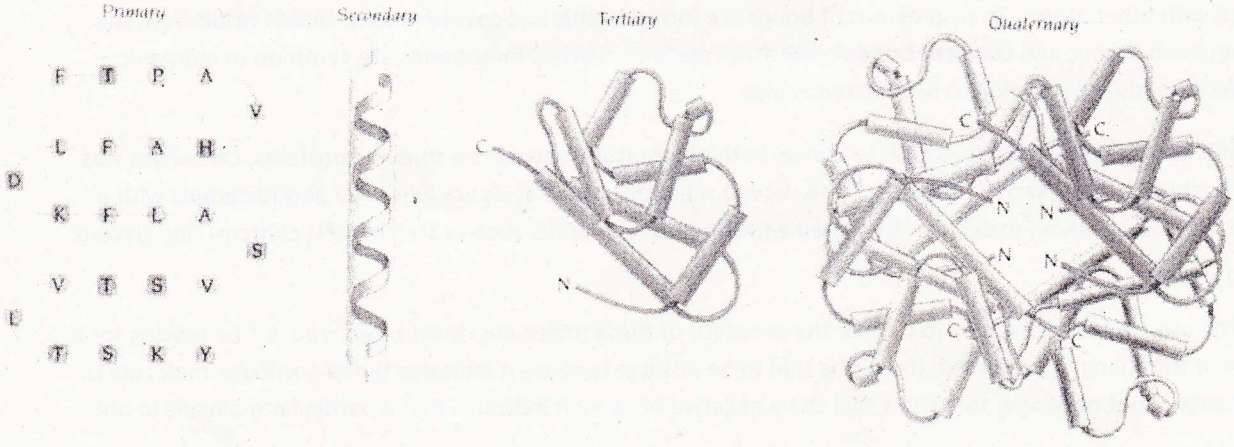

Figure 2 –Levels of organization in the structure of protein. The primary structure is a sequence of the amino acids; the secondary structure is the alpha helix; and the tertiary structure often forms by turning and twisting of the helix. Proteins having more than one polypeptide also have a quaternary structure.

Enzyme Function of Proteins

The enzyme pepsin occurs in the hydrochloric acid (HCl) environment of the stomach. In the experiment that follows, you test the ability of this enzyme to speed up the following reaction:

$$\text{Protein + water} \xrightarrow[\text{(Hydrochloric acid HCl)}]{\text{Pepsin (enzyme)}} \text{peptides}$$

In doing so, you also test whether or not albumin contains peptides.

EXPERIMENTAL PROCEDURES 1

Wrap around the top of two test tubes masking tape. Label the first test tube 1 and put your group name on the test tube. Label the second test tube 2 and put your group name on the test tube.

Tube 1 — Fill one pipette full with albumin solution, one pipette full with water, and one pipette full with hydrochloric acid (0.2% HCl). Gently shake.

Tube 2 — Fill one pipette full with albumin solution, one pipette full with pepsin solution and one pipette full with hydrochloric acid (0.2% HCl). Gently shake.

Place both test tubes at the same time in the hot water bath (37˚C) for 30 minutes. After 30 minutes, carefully remove the test tubes from the hot water bath. Add one pipette full of Biuret reagent to each test tube. Record the color of the test tubes in table 2.

Table 2

Action of Pepsin			
Tube	**Contents**	**Final Color**	**Conclusion (+ or – reaction)**
1	Albumin Water HCl	No color change	–
2	Albumin Pepsin HCl	No color change	–

→ should have turned violet

→ should have turned pink

Conclusions

1. Why was incubation at 37°C? Incubation was at 37°C to simulate the environment of a human stomach.

2. From your test results, conclude what kind of chemical is present and why the results occurred. Enter your conclusions in table 2. Enzymes are specific and speed up only one type of chemical reaction. Considering this, do you predict that pepsin will break down starch? No Why or why not? Pepsin is an enzyme in the stomach that breaks down proteins, not starch

EXPERIMENTAL PROCEDURE 2

With masking tape, mark four test tubes 1 through 4.

Tube 1 Fill one pipette full with water and then add one pipette full with biuret reagent. Record the final color in table 3.

Tube 2 Fill one pipette full with albumin solution and one pipette full with biuret regent. Record the final color in table 3.

Tube 3 Fill one pipette full with pepsin solution and one pipette full with biruet solution. Record the final color in table 3.

Tube 4 Fill one pipette full with starch solution and one pipette full with biruet solution. Record the final color in table 3.

Table 3

Biuret Test			
Tube	**Contents**	**Final Color**	**Conclusion (+ or – reaction)**
1	Water	Blue	Negative
2	Albumin	Violet	Positive
3	Pepsin	~~Blue~~ -1	~~Negative~~ -1
4	Starch	Blue	Negative

Conclusions

1. From your test results, conclude what kind of chemical is present and why the results occurred. Enter your conclusions in table 3.
2. Which of the four tubes is the control sample? 1 What is a control sample? A control sample is a sample that you know does not have the property you are looking for
3. Why do experimental procedures include control sample? Experimental procedures include a control, because it serves as a comparison to the test samples and evaluates the accuracy of a procedure.

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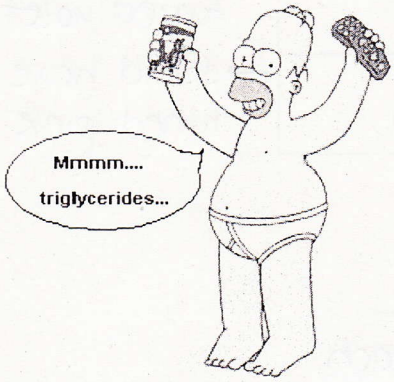
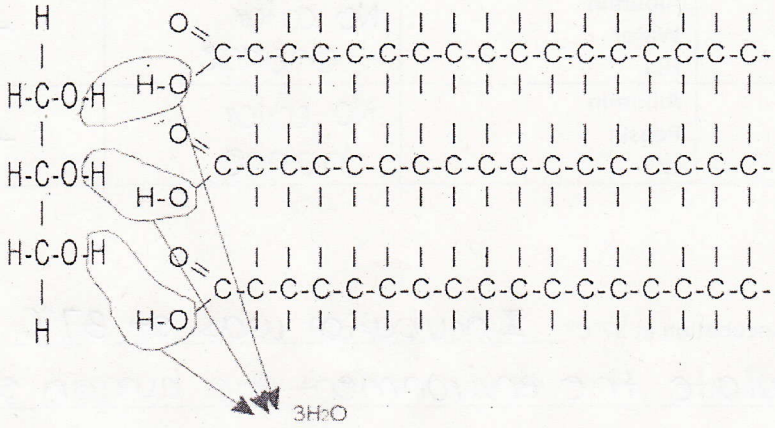
LIPIDS

Lipids are compounds that are insoluble in water and soluble in solvents, such as alcohol and ether. Lipids include fats, oils, phospholipids, steroids, and cholesterol. Typically, fats and oils are composed of three molecules of fatty acids bonded to one molecule of glycerol (figure 3)





Test for Lipids

Fats do not evaporate from brown paper, instead leaving an oily spot.

EXPERIMENTAL PROCEDURE 3

1. Place a small drop of water on a square of brown paper. Describe the immediate effect. Water does not penetrate the paper. It is a drop lying on the surface.
2. Place a small drop of vegetable oil on a square of brown paper. Describe the immediate effect. The oil penetrates the brown paper and spreads on the paper.
3. Wait at least 5 minutes. Evaluate which substance penetrates the paper and which is subject to evaporation. Record your results in table 4

Table 4

Test for Lipids	
Sample	**Results**
Water spot	No lipid (negative)
Oil spot	Lipid (positive)

Emulsification of Lipids

Some molecules are **polar**, meaning that they have charged groups or atoms, and some are **nonpolar**, meaning that they have no charged groups or atoms. A water molecule is polar, and therefore, water is a good solvent for other polar molecules. When charged ends of water molecules interact with the charged groups of polar molecules, these polar molecules disperse in water.

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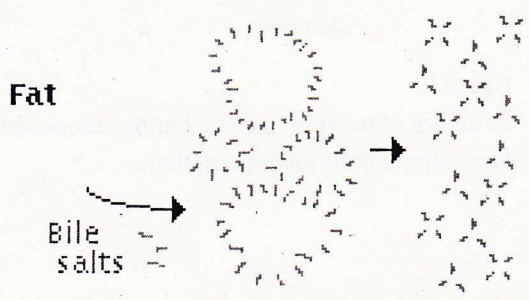
Water is not a good solvent for nonpolar molecules, like a fat. A fat has no polar groups to interact with water molecules. An **emulsifier**, however, can cause a fat to disperse in water. An emulsifier contains molecules with both polar and nonpolar ends. When the nonpolar ends interact with the fat, and the polar ends interact with the water molecules, the fat disperses in water (figure 4)

Bile salts (emulsifiers found in bile produced by the liver) are used in the digestive tract. Tween is commercially produced emulsifier. Experimental procedures 4 illustrates the effect of emulsifiers.



An emulsifier contains molecules with both polar and nonpolar ends. When the nonpolar ends are attached to a nonpolar fat, the polar ends are exposed. Since the polar ends are soluble in water, the fat becomes dispersed.

Figure 4

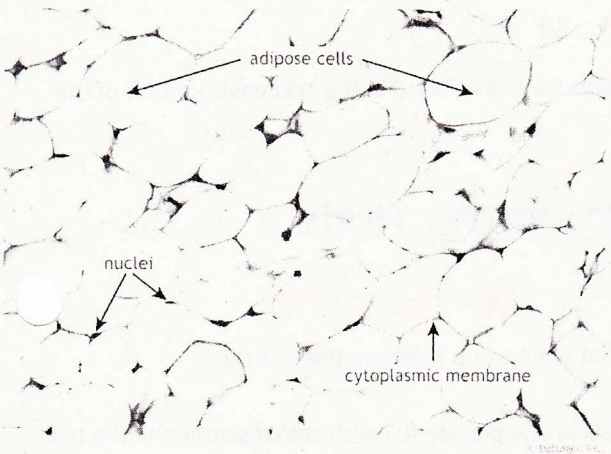
EXPERIMENTAL PROCEDURE 4

el two test tubes 1 and 2 with masking tape.

Tube 1 Place one pipette full of water in tube and one pipette full of vegetable oil. Place thumb over top of test tube and shake. Observe what happens in test tube. Is vegetable oil soluble in water? Forms layer - not soluble

Tube 2 Place one pipette full of water in test tube, place one pipette full of vegetable oil in test tube, and also add one pipette full of emulsifier to test tube. Shake. Describe what happens to the oil. The oil mixes with the water and emulsifier.

Functions of Fat



In animals, **adipose (fat) tissue** is found beneath the skin and around various internal organs. The cells that make up this tissue contain droplets of fat, a molecule that provides long-term energy storage.

Below is a picture of adipose tissue. Notice how the fat droplets push the cytoplasm to the edges of the cells.

CARBOYDRATES

Carbohydrates include sugars and molecules that are chains of sugars. **Glucose**, which has only one sugar unit, is a monosaccharide (figure 5). **Maltose**, which has two sugar units, is a disaccharide (figure 6). Starch is a polysaccharide, a chain of glucose units (figure 7)

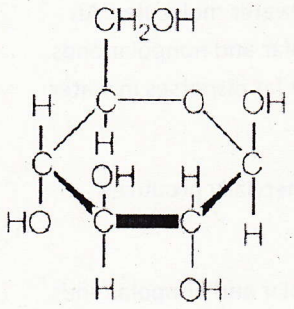


Figure 5

Structure of glucose, a monosaccharide with Six carbon atoms.

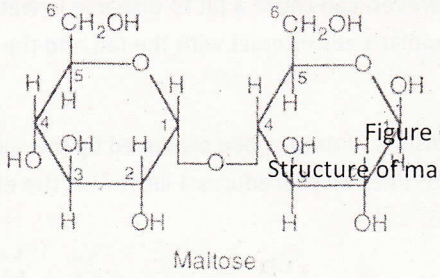


Figure 6
Structure of maltose, a dissacharide composed of two glucose units joined together.

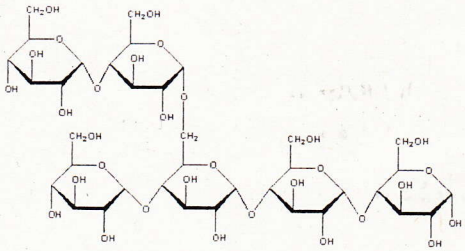


Figure 7

Starch molecule made of repeating units of glucose.

Starch Structure

This experiment is designed to indicate the structure of starch by noting the action of two enzymes on the macromolecule. **Amylase** is an enzyme (a protein that speeds up a reaction) that acts on polysaccharides to produce disaccharides (double sugars):

Starch + water —Amylase (enzyme)→ maltose

Pepsin, as discussed previously, is an enzyme that acts on proteins to produce peptides and is found in the acid environment of the stomach. Which enzyme do you predict will have an effect on starch?

Amylase Why? Amylase is fand in the spit of humans and starts to break down starchy foods.

EXPERIMENTAL PROCEDURE 5

Wrap around the top of 3 test tubes masking tape. Label the test tubes 1-3 and put your group name on the test tube.

Tube 1 One person in your lab group will need to spit into this test tube. Then place one pipette full with starch solution in the test tube. Then place in boiling water bath for 30 minutes. At the end of this time, test for sugar by adding one pipette full of Benedict's reagent to the test tube. Then return the test tube to the boiling water bath for 10 additional minutes. At the end of this time, remove the test tube and record the color of the liquid in table 5.

mono and disaccharides test +

Tube 2 Place one pipette of water into test tube, then add one pipette full of pepsin, and finally one pipette full of hydrochloric acid (0.2% HCl) to simulate the stomach's acidic environment. Then place in boiling water bath for 30 minutes. At the end of this time, test for peptides by adding one pipette full of biuret reagent to the test tube. Record the color of the liquid in table 5.

Tube 3 Place one pipette of starch into test tube, then add one pipette full of pepsin solution, and finally add one pipette full of HCl to simulate the stomach's acid environment. Place test tube in boiling water for 30 minutes. At the end of this time, test for peptides by adding one pipette full of biuret reagent to the test tube. Record the color of the liquid in table 5.

Table 5

Starch Composition				
Tube	**Contents**	**Test**	**Color change**	**Conclusion**
Control	Starch and water	Benedict's reagent	Blue	Negative
1	Starch and amylase	Benedict's reagent	orange	Positive
2	Water, pepsin and HCl	Biuret reagent	No color	Negative → should have changed color
3	Starch, pepsin, and HCl plus heat	Biuret reagent	No color → should have turned violet	Negative

Conclusions

1. From your results, conclude what kind of chemical is present (or absent) and why the results occurred. Enter your conclusions in table 5.
2. Does pepsin act on starch to produce peptides? No
   Why or why not? Pepsin is an enzyme that acts on proteins, not starch. Also, we found that amylase is the enzyme that starts digesting starch.

Test for Starch

In the presence of starch, iodine solution (iodine-potassium iodide (IKI) turns from a brownish color to blue-black.

EXPERIMENTAL PROCEDURE 6

Label two test tubes 1 and 2 with masking tape.

Tube 1 Place one pipette full with starch suspension (1%) and add five drops of iodine solution. Note the final color change and record your results in table 6.

Tube 2 Place one pipette full with water and add five drops of iodine solution. Note the final color change and record your results in table 6.

...to and Onion

1. With a sharp razor blade, slice a very thin piece of potato. Place in a dish. Place two drops of iodine solution onto the potato slice. What color is the potato slice with the iodine? Record your results in table 6.
2. With a sharp razor blade, slice a very thin piece of onion. Place in a dish. Place two drops of iodine solution onto the onion slice. What color is the onion slice with the iodine? Record your results in table 6.

Table 6

Iodine Test for Starch		
Sample	**Color Change**	**Conclusion**
Tube 1: Starch	Black	Starch present
Tube 2: Water	No change	Negative
Potato	Black	Starch present
Onion	No change	Negative

Conclusions

1. From your test results, draw conclusions about what organic compound is present in each tube, and write these conclusions in table 6.
2. Sugars are immediate energy source n cells. In plant cells, glucose (a primary energy molecule) is often stored in the form of starch. Is glucose stored as starch in the potato? Yes Is glucose stored as starch in the onion? No

Test for Sugars

Sugars react with **Benedict's reagent** after being heated in boiling water bath. Increasing concentrations of sugar give a continuum of colored products. This experiment tests for the presence (or absence) of varying amounts of reducing sugars in a variety of materials and chemicals.

Table 7

Benedict's Reagent		
Chemical	**Chemical Category**	**Benedict's Reagent (after heating)**
Water	**Inorganic**	**Blue (no change)**
Glucose	**Monosaccharide (carbohydrate)**	**Varies with concentration:** **Very low – green** **Low – yellow** **Moderate – yellow-orange** **High – orange** **Very high – orange-red**
Maltose	**Disaccharide (carbohydrate)**	**Varies with concentration:** **Same as glucose**
Starch	**Polysaccharide (carbohydrate)**	**Blue (no change)**

EXPERIMENTAL PROCEDURES 7

Wrap around the top of 5 test tubes masking tape. Label the test tubes 1-5 and put your group name on the test tube.

Tube 1 Place one pipette full with water and then add one pipette full of Benedict's reagent. Then place in boiling water bath for 10 minutes. Note any color change and record in table 8.

Tube 2 Place one pipette full with glucose solution and then add one pipette full of Benedict's reagent. Then place in boiling water bath for 10 minutes. Note any color change and record in table 8.

Tube 3 Place a few drops of onion juice in test tube. Obtain juice by crushing a small piece of onion with a mortar and pestle. Then add one pipette full with water and then add one pipette full of Benedict's reagent. Then place in boiling water bath for 10 minutes. Note any color change and record in table 8.

Tube 4 Place a few drops of potato juice in test tube. Obtain juice by crushing a small piece of potato with a mortar and pestle. Then add one pipette full with water and then add one pipette full of Benedict's reagent. Then place in boiling water bath for 10 minutes. Note any color change and record in table 8.

Tube 5 Place one pipette full with starch solution and then add one pipette full of Benedict's reagent. Then place in boiling water bath for 10 minutes. Note any color change and record in table 8.

Table 8

Benedict's Reagent Test			
Tube	**Contents**	**Color (after heating)**	**Conclusions**
1	Water	Blue	No change
2	Glucose solution	Dark orange	very high monosaccharide concentration
3	Onion juice	Yellowish orange	Low monosaccharide concentration
4	Potato juice	Yellow green	very low monosaccharide concentration
5	Starch suspension	Blue	No change

Conclusions

From your test results, conclude what kind of chemical is present and why the results occurred. Enter your conclusions in table 8

Testing Chemical Composition of Everyday Material and an Unknown

EXPERIMENTAL PROCEDURE 10

1. I will provide you with several everyday materials. Use the tests for the carbohydrates (reducing sugars and starches), proteins, and lipids in this laboratory to determine the macromolecules present in these materials.
2. Write the name of each known material, and assign a letter to any unknowns (Unknown A, Unknown B, etc)
3. Record your results in table 11.

Table 11

Everyday Materials and Unknowns				
Sample name	Reducing Sugar (Benedict's)	Starch (Iodine)	Protein (Biuret)	Lipid (Brown paper)
Unknown 1	–	–	–	+
Unknown 2	orange/brown	–	–	–
Unknown 3	orange	–	+	–
Unknown 4	Black	–	–	–
Unknown 5	–	–	–	–

* don't know why #4 was black

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LABORARTORY REVIEW QUESTIONS

1. What macromolecules studied today are present in cells?
   Proteins, lipids, sugars, starch
2. You have been assigned the task of constructing a protein. What type of building block would you use? Amino acids
3. Digestive enzymes break down starch to what disaccharide studied in this laboratory? Maltose
4. How would you test an unknown solution for each of the following:
   a. Sugars Add Benedict's solution
   b. Fat Put the solution on brown paper
   c. Starch Add Iodine
   d. Protein Add Biuret reagent
5. Assume that you have tested an unknown sample with both biuret solution and Benedict's solution and that both tests result in a blue color. What have you learned? This means that neither sugars or proteins are present
6. What purpose is served when a test is done using water instead of a sample substance? The water serves as a control we can compare our results with
7. A test tube contains starch, hydrochloric acid, and water. The biuret test is negative. Explain. Biuret changes color only when a protein or peptide is present.
8. A test tube contains albumin and pepsin. After 1 hour, the test for protein is positive. Explain. Biuret will test positive when proteins/peptides are present and albumin and pepsin fall into that category.
   The test for peptides is negative. Explain. This means that albumin and pepsin are proteins, not peptides.

& no HCl / ↓pH (2) for enzyme to work

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