LAB TOPIC 3

Macromolecules

Introduction

Living organisms are composed of molecules that come in diverse shapes and sizes and serve a variety of purposes. Some molecules form the structure of an organism's body—for example, the cellulose that makes up the cell walls in plants, the proteins and phospholipids that comprise cell membranes, and the fibers that make up animal muscles.

There is also a wide array of molecules that perform all the functions of life. For example, enzymes catalyze the chemical reactions necessary for biological processes, neurotransmitters convey information from one brain cell to another, and visual pigments absorb light so that you can read the words on this page.

In this laboratory you will study three classes of the largest biological molecules, called **macromolecules:** carbohydrates, lipids, and proteins. A fourth class of macromolecules, the nucleic acids, will not be studied in this laboratory.

Outline

EXERCISE 3.1

Carbohydrates

Objectives

After completing this exercise, you should be able to

1. Define monosaccharide, disaccharide, and polysaccharide and give examples of each.
2. Name the monosaccharide components of sucrose and starch.

3. Describe the test that indicates the presence of most small sugars.
4. Describe the test that indicates the presence of starch.
5. Define hydrolysis and give an example of the hydrolysis of carbohydrates.

Most **carbohydrates** contain only carbon (C), oxygen (O), and hydrogen (H). The simplest form of carbohydrate molecules are the **monosaccharides** ("single sugars"). One of the most important monosaccharides is glucose ($C_6H_{12}O_6$), the end product of photosynthesis in plants. It is also the molecule that is metabolized to produce another molecule, ATP, whose energy can be used for cellular work. There are many other common monosaccharides, including fructose, galactose, and ribose.

Some **disaccharides** ("double sugars") are also common. A disaccharide is simply two monosaccharides linked together. For example, maltose consists of two glucose molecules, lactose (milk sugar) consists of glucose and galactose, and sucrose (table sugar) consists of glucose and fructose. Can you discern a rule used in naming sugars?

Carbohydrates are also found in the form of **polysaccharides** ("many sugars"), which are long chains of monosaccharide subunits linked together.

Starch, a polysaccharide composed of only glucose subunits, is an especially abundant component of plants. Most of the carbohydrates we eat are derived from plants. What was the last starch you ate?

Starch is the plant's way of storing the glucose it makes during photosynthesis. When you eat starch, you are consuming food reserves that the plant has stored for its own use. The starch of potatoes and root vegetables, for example, would be used the next spring for the plant's renewed growth after the winter die-back. All perennial plants (those that come up year after year, such as tulips) have some kind of food storage for overwintering. Beans, on the other hand, contain starch in the seeds. Beans are annual plants; they will die at the end of the growing season. So the seeds are stocked with starch to use when they have a chance to germinate the next spring.

Animals store glucose in **glycogen,** which is another form of polysaccharide. Although starch and glycogen are both composed of glucose subunits, the glucose molecules are bonded together in different ways, so these polysaccharides are not identical. Glucose subunits are bonded together a third way in the polysaccharide **cellulose.** While starch and glycogen are meant to be metabolized for energy, cellulose, which is the most abundant carbohydrate in the world, is a structural molecule that is designed *not* to be metabolized. Cellulose makes up the cell walls of plants and is a primary component of dietary fiber. For most animals it is completely indigestible. Those that can digest it, such as termites and cows, do so only with the assistance of organisms such as bacteria, fungi, or protistans.

Most disaccharides and polysaccharides can be broken down into their component monosaccharides by a process called **hydrolysis**, which is accomplished in organisms by digestive enzymes. This process is important in seeds. If the seed's food resource is starch, it must be able to convert the starch to glucose. The glucose is then used to generate ATP, which in turn is used to provide the growing plant embryo with energy for metabolic work. Hydrolysis of starch begins when the seed takes up water and begins to germinate.

Germination of barley seeds is part of the process of brewing beer. When the barley is germinated, the starch-to-sugar conversion begins. In the breakdown of starch, disaccharide maltose molecules are formed before the final product, glucose, is obtained. At a certain point in the germination, the barley is dried so that no further hydrolysis takes place. The maltose sugar is extracted and used in the brewing process. That's the "malt" listed on the beer can as an ingredient. The process of germinating the barley is called malting.

A chemical hydrolysis can be done in the laboratory by heating the molecules with acid in the presence of water. You will perform a chemical hydrolysis in this exercise.

Wear safety glasses throughout the lab session.

Activity A: Monosaccharides and Disaccharides

You will use **Benedict's reagent** as a general test for small sugars (monosaccharides and disaccharides). When this reagent is mixed with a solution containing single or double sugars and then heated, a colored precipitate (solid material) forms. The precipitate may be yellow, green, orange, or red. If no monosaccharide or disaccharide is present, the reaction mixture remains clear. However, Benedict's reagent does not react with all small sugars. For example, sucrose gives a negative Benedict's reaction.

Glucose will be used in this laboratory to demonstrate a positive Benedict's test (Figure 3.1). What should be used as a negative control for this test?

Procedure

1. Make a boiling water bath by filling a beaker about half full of water and heating it on a hot plate. Put six or seven boiling chips in the beaker. You will need to use this water bath in several activities.

Set the hot plate where it will not be in your way as you work. Be careful—it will be very hot!

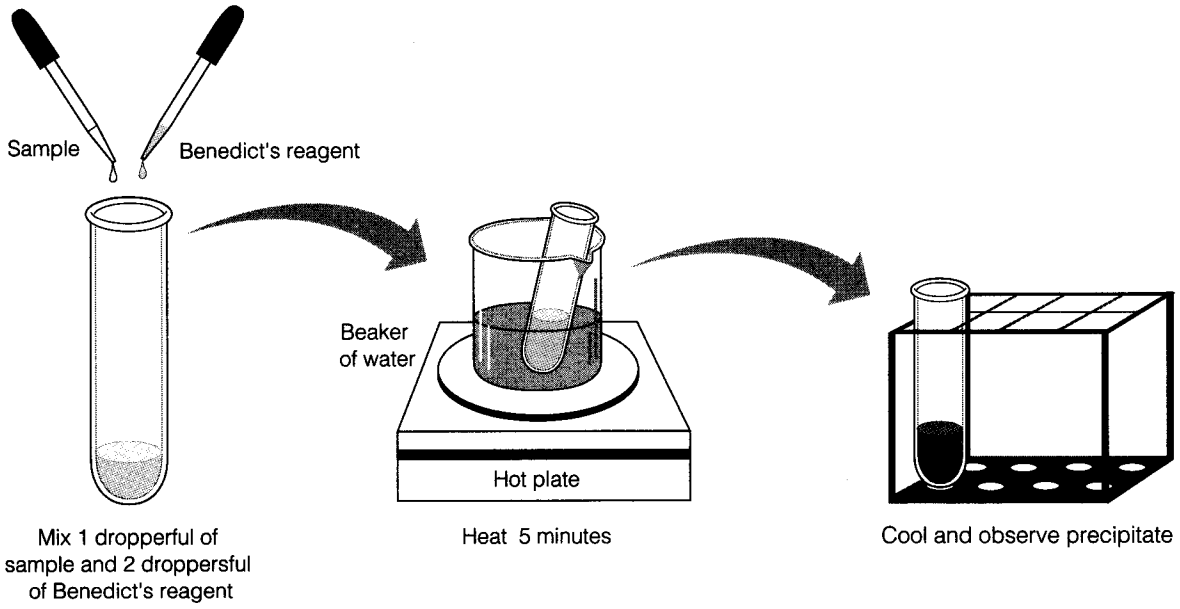


Figure 3.1.
Benedict's test for detecting small sugars.

2. Get two test tubes and label them 1 and 2 with a wax pencil.

Make heavy marks so that they don't melt off in the water bath.

3. Put 1 dropperful of glucose into Tube 1. Tube 1 is the positive control.
4. Tube 2 is the negative control. What substance goes in it? How much should be used?

5. Add 2 droppersful of Benedict's reagent to each tube.
6. Place the tubes in the boiling water bath and let them heat for 5 minutes.
7. After 5 minutes, remove the tubes from the water bath.

Use a test tube holder to retrieve test tubes from the boiling water.

8. Allow the tubes to cool at room temperature for several minutes in the test tube rack while you go on to the next procedure.

9. Record your observations below.
   Tube 1 (glucose):

   Tube 2 (negative control):

Interpretation of Results

Describe a positive Benedict's test.

What are the limitations of this test?

Activity B: Starch

Starch is tested by using **iodine reagent** (I_2KI—iodine potassium iodide). A dark blue color indicates the presence of starch (Figure 3.2).

You will use a solution of potato starch to demonstrate a positive test. What negative control should be used for this test?

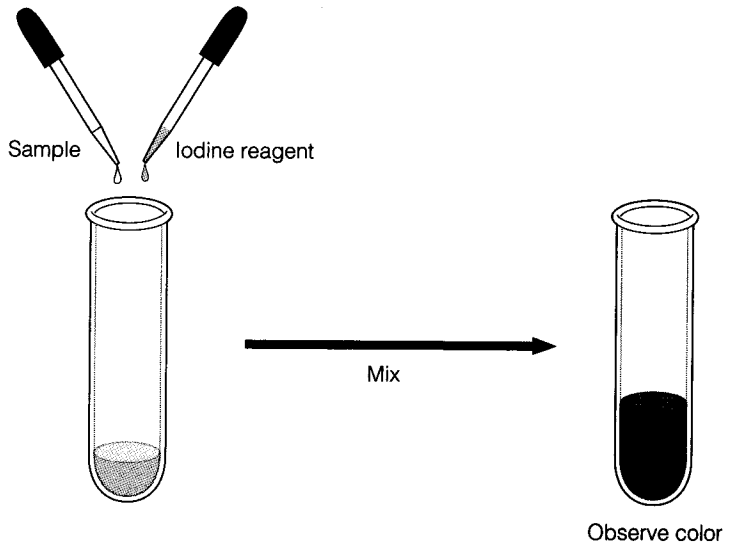


Figure 3.2.
The iodine test for detecting starch.

Procedure

1. Get two test tubes and label them 1 and 2.
2. Put a dropperful of starch solution in Tube 1. This is the positive control.
3. Tube 2 is the negative control. What substance goes in it? How much should be used?

4. Put 3 or 4 drops of iodine reagent into each tube.
5. Record the results below.
   Tube 1 (starch):

   Tube 2 (negative control):

Interpretation of Results

Describe a positive test for starch.

What are the limitations of this test?

Activity C: Hydrolysis of Carbohydrates

As discussed earlier, disaccharides are composed of two monosaccharides linked together. Polysaccharides are long chains of monosaccharides. The bonds joining these subunits can be broken in a process called hydrolysis. In this procedure, you will hydrolyze sucrose and starch by heating them with acid.

What monosaccharides will result from the hydrolysis of sucrose?

What monosaccharide will result from the hydrolysis of starch?

The hydrolysis reactions will be carried out in two large test tubes. As Figure 3.3 shows, one contains sucrose and hydrochloric acid (HCl) and

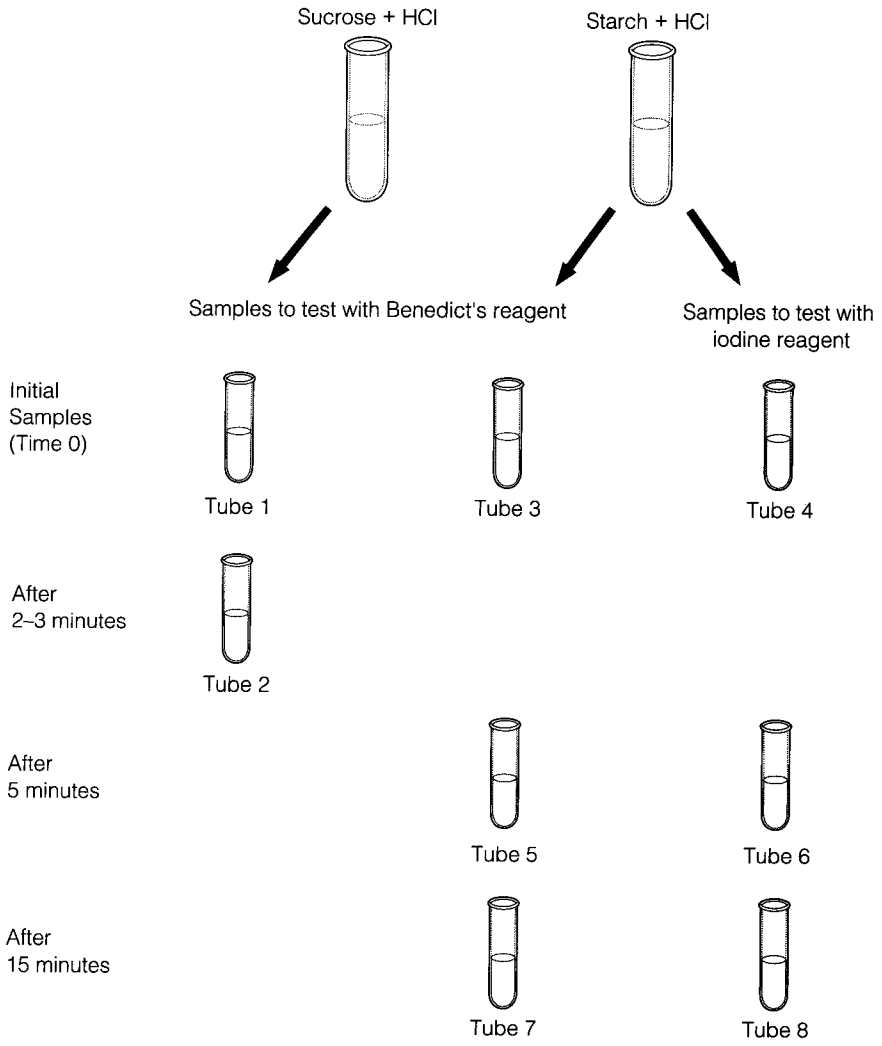


Figure 3.3.
Sampling hydrolysis products of sucrose and starch.

the other contains starch and HCl. You will sample the sucrose tube twice: once before the hydrolysis has begun and again after 3 minutes. You will take six samples from the starch tube: two before the hydrolysis has been done, two after 5 minutes of hydrolysis, and two after 15 minutes. Two samples are needed at each time so that one can be tested for small sugars (Benedict's test) and one can be tested for starch (iodine test).

Procedure

Check your boiling water bath and add water if needed.

1. Get eight test tubes and label them 1 through 8. Line up the test tubes in order in a test tube rack.
2. Get two extra large test tubes and label them starch and sucrose. Use an empty beaker as a test tube holder if the test tubes don't fit in the rack.

Hydrolysis of Starch and Sucrose

3. Pipet 10 mL starch solution and 5 mL 2*N* HCl into the tube labeled starch.

HCl is a strong acid. Handle it with caution. After you use the pipet, replace it in its holder. Do not lay it down on the bench.

4. Pipet 10 mL sucrose solution and 2 mL 2*N* HCl into the tube labeled sucrose.
5. Swirl each tube gently to mix the contents.

Sampling

6. Use a pasteur pipet to draw 1 pipetful of solution from the sucrose tube and put it in Tube 1.
7. Using a *different* pasteur pipet, draw 1 pipetful of solution from the starch tube and put it in Tube 3. (Skip Tube 2 for now.)
8. Draw an additional pipetful of solution from the starch tube and put it in Tube 4.
9. Place the extra-large starch and sucrose tubes in your boiling water bath. Note the time:

   _____

10. After 2 or 3 minutes, draw 1 pipetful of solution from the sucrose tube and put it in Tube 2. You are now finished with the sucrose solution. You may remove it from the water bath.
11. After 5 minutes, draw 1 pipetful of solution from the starch tube and put it in Tube 5.
12. Put a second pipetful of starch solution in Tube 6.
13. Wait 10 more minutes and then repeat steps 11 and 12, putting the solution in Tubes 7 and 8.

Do Exercise 3.2 during the waiting period.

Testing for Starch and Sugar

14. Add two droppersful of Benedict's reagent to Tubes 1, 2, 3, 5, and 7. Place these tubes in the boiling water bath for 5 minutes.
15. Add 3 or 4 drops of iodine reagent to Tubes 4, 6, and 8. Record the results in Table 3.1 on the next page.

Table 3.1

	Tube Number							
	Sucrose		Starch					
	1	2	3	4	5	6	7	8
Time (min)	0	2–3	0	0	5	5	15	15
Benedict's reagent								
Iodine reagent								

16. Remove the tubes from the water bath and wait 5 minutes for them to cool. Record the results in Table 3.1.

Interpretation of Results

Explain the results you obtained using the Benedict's test on the sucrose solution.

Explain the results you obtained using the iodine reagent test with starch.

Explain the results you obtained using the Benedict's test with starch.

Why does hydrolysis of starch take longer than hydrolysis of sucrose?

EXERCISE 3.2

Lipids

Objectives

After completing this exercise, you should be able to

1. Define lipid and give examples.
2. Describe the test that indicates the presence of lipids.

Lipids are compounds that contain mostly carbon and hydrogen. They are grouped together solely on the basis of their insolubility in water. The lipids we will consider in this laboratory are fats and oils, which are generally used as storage molecules in both plants and animals. You are no doubt already familiar with the fact that your body converts excess food into fat. This fat is stored in your adipose tissue until your food intake is lower than your metabolic needs, at which time the fat can be metabolized to generate ATP, whose energy can be used for cellular work. Plants, too, can store fats. Seeds are often provisioned with fats that can be metabolized by the developing embryo when germination time comes. Thus we obtain corn oil, peanut oil, sunflower oil, and others by pressing the seeds.

You will use the **paper test** (Figure 3.4) to indicate the presence of lipids in various foods. Although this test is not very sophisticated, it is quick and convenient.

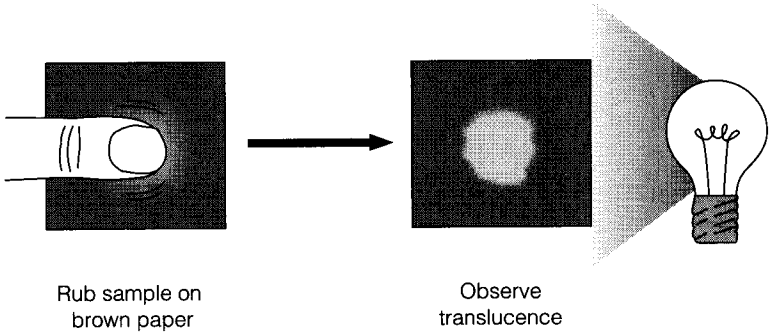


Figure 3.4.
Brown paper test for lipids.

Procedure

1. Get a small square of brown paper. Write "oil" on one half and "water" on the other.
2. Put a tiny drop of salad oil on the half of the paper labeled oil. Rub it gently with your fingertip.
3. As a negative control, put a tiny drop of water on the half of the paper labeled water. Rub it gently with a different fingertip to avoid contamination.
4. Allow the spots to dry. This may take quite a while, so go on to another exercise while you wait.
5. When the spots are dry, hold the paper up to the light.

Interpretation of Results

Describe a positive test for lipids.

What are the limitations of this test?

EXERCISE 3.3
Proteins

Objectives

After completing this exercise, you should be able to

1. Define protein and give examples.
2. Explain why the structure of a protein is important for its function.
3. Describe the test that indicates the presence of protein.

A **protein's** structure is determined by the amino acid subunits that make up the molecule. Although there are only 20 different naturally occurring amino acids, each protein molecule has a unique sequence. The amino acids are linked by fairly tight bonds, and the side groups that are part of the amino acids also interact with each other to help shape the molecule.

Proteins have a greater diversity of roles than either carbohydrates or lipids. The shape of a protein is key to its purpose: Proteins work by selectively binding to other molecules.

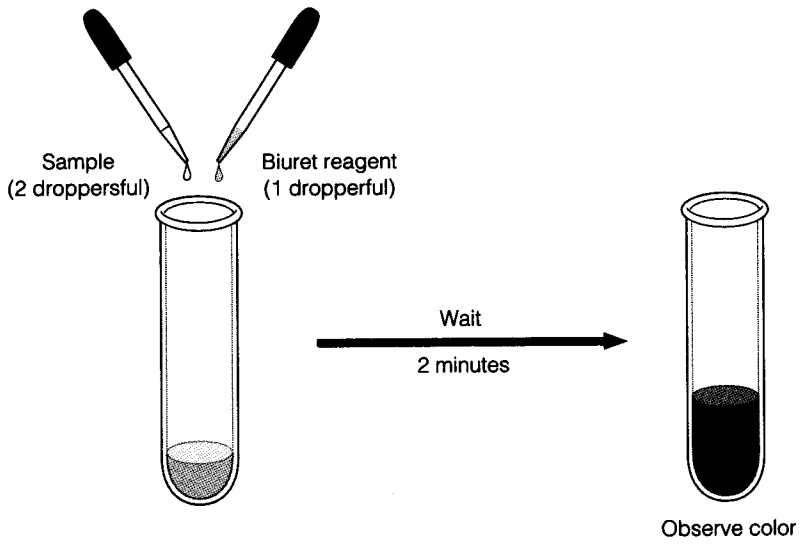
You will use **biuret reagent** as a test for proteins (Figure 3.5). This reagent, which is blue, reacts with proteins to give a light violet or lavender color.

You will use a solution of egg albumin (a protein extracted from egg whites) to demonstrate a positive biuret test. What negative control should be used for this test?

Procedure

1. Get two test tubes and label them 1 and 2.
2. Put two droppersful of egg albumin into Tube 1.

Figure 3.5.
Biuret test for protein.



3. Tube 2 is the control. What substance goes in it? How much should be used?

4. Put 1 dropperful of biuret reagent into each tube and swirl gently to mix.
5. After 2 minutes, record the color in each tube.
   Tube 1 (egg albumin):

   Tube 2 (negative control):

Interpretation of Results

Describe a positive biuret test.

What are the limitations of this test?

EXERCISE 3.4

Macromolecules in Food

Objectives

After completing this exercise, you should be able to

1. Explain how the components of butter are distributed in two fractions by the process of clarification.
2. Interpret the results of tests that indicate the presence of sugar, starch, lipid, and protein in an unknown sample.

We metabolize food in order to release energy to produce the ATP needed for cellular work. We also break down food molecules in order to use their subunits as raw materials for synthesizing our own macromolecules. In this exercise, you will investigate certain foods to learn which macromolecules are present in each.

Activity A: Separation of Butter

Most foods are complex mixtures of substances. Butter, for example, may appear to be solid fat, but it is actually a mixture of proteins, carbohydrates, and lipids. It is an emulsion, which means that the lipids occur in very small droplets dispersed throughout the water-soluble portion. (You will learn more about emulsions in Lab Topic 14, Digestion.)

The lipid can be separated from the water-soluble, protein-containing part of the butter in a process called clarification. Butter is often clarified for use in cooking. Once the water-soluble part has been removed, the lipid that remains can be used to fry at higher temperatures than for whole butter because it is the protein in butter that scorches first. It is also the protein that spoils most readily, so clarified butter keeps longer.

Procedure

1. Fill a 250-mL beaker approximately to the 75-mL mark with water.
2. Put the beaker on a hot plate and let the water come to a boil.
3. Cut approximately 1 tablespoon of butter into smaller chunks and put them in a 50-mL beaker.
4. When the water boils, remove the beaker from the hot plate with a potholder and set it on a paper towel on the lab bench. Choose a spot where it will not be disturbed.
5. Put the 50-mL beaker into the 250-mL beaker (water bath).
6. Leave the butter undisturbed for at least 15 minutes. While you wait, continue with steps 7–9.

7. Get approximately 1 teaspoon of butter from your instructor and put it into a test tube. (A teaspoon is $\frac{1}{3}$ of a tablespoon.)
8. Put the test tube in a warm water bath to melt the butter. (You can use the water bath the 50-mL beaker is in, but don't disturb the beaker.)
9. When the butter in the test tube has melted, perform the four tests that were introduced in this laboratory and record your results in Table 3.2. (The test procedures are reviewed in the next section.)
10. After 15 minutes, gently pick up the 50-mL beaker and set it in ice. Leave it undisturbed for about 10 minutes. Work on Activity B while the lipid (upper) layer solidifies.
11. The upper layer (clarified butter) should now be solid or semisolid. Remove it with a spatula and put it on a paper towel. Pat the bottom of the butter dry with a paper towel to remove any contaminants from the lower layer.
12. Place the clarified butter in a test tube and melt it in a warm water bath.
13. Perform the four tests on the melted clarified butter and record the results in Table 3.2.
14. Perform the four tests on the lower (liquid) layer of the clarified butter and record the results in Table 3.2. If you do not have enough liquid to perform all the tests, arrange to share results with another lab group.

Table 3.2

	Benedict's (sugar)	**Iodine (starch)**	**Paper (lipid)**	**Biuret (protein)**
Whole butter				
Clarified butter, upper layer				
Clarified butter, lower layer				

Interpretation of Results

Describe what happens to butter as a result of the clarification procedure.

Clarified butter lacks the "butter" flavor. What does this tell you about the molecules responsible for the taste of butter?

Some students try this procedure to clarify margarine. They warm the margarine and leave it on ice for 10 minutes as specified in the directions. They find that the margarine has resolidified but there is no liquid lower layer. What does this tell them about the margarine?

Activity B: Tests with Food

Test each of the items in Table 3.3 for the presence of simple sugars, starch, lipid, and protein. Your instructor may want to modify the list. The procedures for the tests are reviewed below.

Benedict's Test (sugar)

Put 1 pasteur pipetful of sample into a test tube. Add 2 droppersful of Benedict's reagent; mix. Heat in a boiling water bath for 5 minutes. Allow to cool and observe the precipitate.

Some samples may require extra cooling time, so don't be too hasty in recording results.

Iodine Test (starch)

Put a pipetful of sample into a test tube and add 4 or 5 drops of iodine reagent; mix.

In some foods, the starch is still contained in granules inside the cells. You may see these dark granules suspended in the yellow solution instead of seeing the entire solution turning blue.

Paper Test (lipid)

If the sample is whole (for example, a peanut), rub a piece of it directly on the paper. If the sample is liquid, put a small drop on the paper.

Remember to wait for the paper to dry before you record the results.

Biuret Test (protein)

Put 1 pipetful of sample into the test tube and add 1 dropperful of biuret reagent; mix.

Allow at least 2 minutes for the reaction to occur. Some samples may take 5 minutes to react.

Some of the foods to be tested are solids. Use a razor blade to mince approximately 1 cm^3 (about the size of a pea) of the sample. Put it in a test tube with 10 mL distilled water. Put your thumb over the top of the test tube and shake it vigorously for 1 minute. Perform the tests using the liquid (except the lipid test). Record your results in Table 3.3. Be sure to rinse off the razor blade and cutting board between samples to avoid contamination.

Table 3.3

	Benedict's (sugar)	**Iodine (starch)**	**Paper (lipid)**	**Biuret (protein)**
Banana				
Coconut				
Milk				
Peanut				
Potato				

Interpretation of Results

Which results confirmed your expectations about the composition of foods?

Which results were unexpected?

What factors might result in a false negative test (that is, the food does contain a molecule but the tests results are negative)?

Why might a plant storage organ (such as a fruit or tuber) contain both starch and sugar?

If you have tested foods in addition to the ones listed in Table 3.3, compare the results from those tests with the results for the foods listed in Table 3.3.

Questions for Review

1. What subunits make up
   a. Carbohydrates?

   b. Proteins?

2. Why is each test done initially using water as well as a known sample?

3. Why might a substance taste sweet, yet give a negative reaction with the Benedict's test?

   What procedure could you use to check your answer to the previous question?

4. You have been given an unknown solution. Describe how you would test it for the presence of

   a. Starch:

   b. Lipid:

   c. Sugars:

   d. Protein:

5. You have tested an unknown sample with biuret and Benedict's reagents. The solution mixed with biuret reagent is blue. The solution boiled with Benedict's reagent is also blue. What does this tell you about the sample?

6. Whole butter gives only a slightly positive test for protein (and may show no reaction at all). When the same butter is clarified, however, the liquid lower layer is definitely positive for protein. Explain why these different results might have been obtained.

7. Since potatoes have starch in them, why don't they taste sweet after they are boiled?

Acknowledgments

Procedures for the macromolecule tests were adapted from the following sources:

Armstrong, W. D., and C. W. Carr. Physiological Chemistry Laboratory Directions, 3rd ed. Minneapolis: Burgess Publishing, 1963.

Dotti, L. B., and J. M. Orten. Laboratory Instructions in Biochemistry, 8th ed. St. Louis: C. V. Mosby, 1971.

Oser, B. L., ed. *Hawk's Physiological Chemistry*, 14th ed. New York: McGraw-Hill, 1965.