BIOLOGY

Exercise 6

Biologically Important Molecules

Carbohydrates, Proteins, Lipids, and Nucleic Acids

Learning Objectives

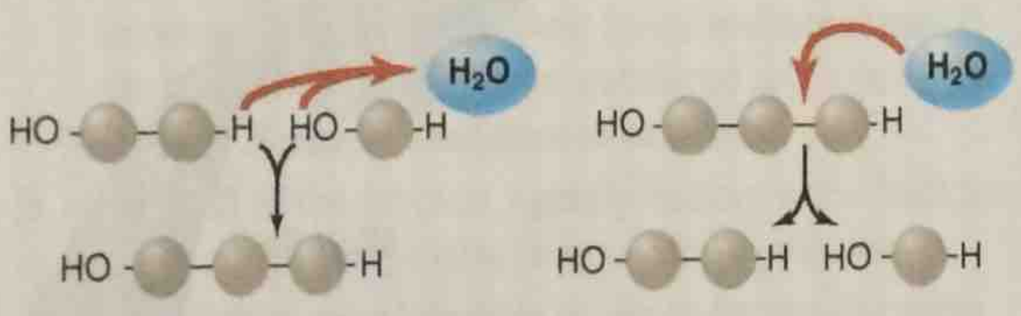
By the end of this exercise you should be able to:

1. Perform tests to detect the presence of biologically important carbohydrates, proteins, lipids, and nucleic acids.
2. Explain the importance of a positive and a negative control in biochemical tests.
3. Use biochemical tests to identify an unknown compound.

Please visit connect.mheducation.com to review online resources tailored to this lab.

Most organic compounds in living organisms are **carbohydrates, proteins, lipids,** or **nucleic acids.** Each of these macromolecules is made of smaller subunits. These subunits are linked by **dehydration synthesis,** which is an energy-requiring process in which a molecule of water is removed and the two subunits are bonded covalently (fig. 6.1). Similarly, breaking the bond between the subunits requires the addition of a water molecule and releases energy. This energy-releasing process is called **hydrolysis.**

The subunits of macromolecules are held together by covalent bonds and have different structures and properties. For example, lipids (made of fatty acids) have many C—H bonds and relatively little oxygen, while proteins (made of amino acids) have amino groups ($—NH_2$) and carboxyl (—COOH) groups. These characteristic subunits and groups impart different chemical properties to macromolecules—for example, monosaccharides such as glucose are polar and soluble in water, whereas lipids are nonpolar and insoluble in water.



(a) **Dehydration synthesis** (b) **Hydrolysis**

Figure 6.1 Making and breaking macromolecules.
(*a*) **Dehydration synthesis.** Biological macromolecules are polymers formed by linking subunits together. The covalent bond between the subunits is formed by dehydration synthesis, an energy-requiring process that creates a water molecule for every bond formed.
(*b*) **Hydrolysis.** Breaking the bond between subunits requires the returning of a water molecule with a subsequent release of energy, a process called hydrolysis.

CONTROLLED EXPERIMENTS TO IDENTIFY ORGANIC COMPOUNDS

Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments: (1) an **unknown solution** to be identified, and (2) **controls** to provide standards for comparison. As its name implies, an unknown solution may or may not contain the substance that the investigator is trying to detect. Only a carefully conducted experiment will reveal its contents. In contrast, controls are known solutions. We use controls to validate that our procedure is detecting what we expect it to detect and nothing more. During the experiment we compare the unknown solution's response to the experimental procedure with the control's response to that same procedure.

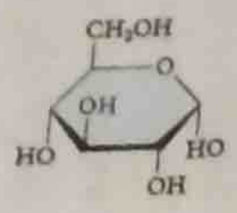
A **positive control** contains the variable for which you are testing; it reacts positively and demonstrates the test's ability to detect what you expect. For example, if you are testing for protein in unknown solutions, then an appropriate positive control is a solution known to contain protein. A positive reaction shows that your test reacts as expected; it also shows you what a positive test looks like.

A **negative control** does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

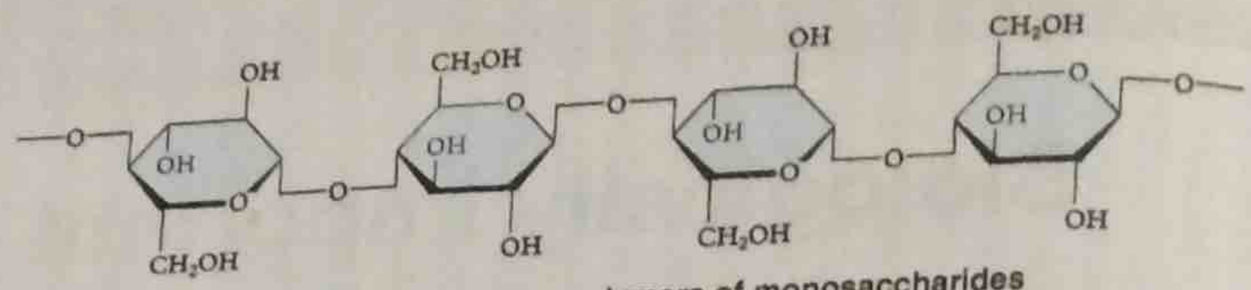
CARBOHYDRATES

Benedict's Test for Reducing Sugars

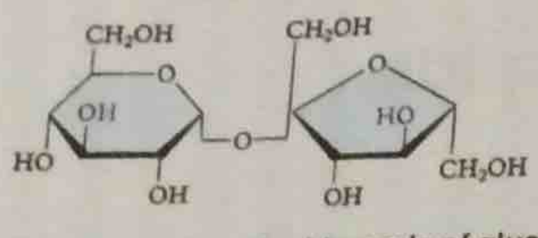
Carbohydrates are molecules made of C, H, and O in a ratio of 1:2:1 (e.g., the chemical formula for glucose is $C_6H_{12}O_6$). Carbohydrates are made of **monosaccharides,** or simple sugars (fig. 6.2). Paired monosaccharides form **disaccharides**—for example, sucrose (table sugar) is a



Glucose, a monosaccharide



Polysaccharides are polymers of monosaccharides



Sucrose, a disaccharide made of glucose (left) and fructose (right)

Figure 6.2 Carbohydrates consist of subunits of mono- or disaccharides. These subunits can be combined by dehydration synthesis (see fig. 6.4) to form polysaccharides.

disaccharide of glucose linked to fructose. Similarly, linking three or more monosaccharides forms a **polysaccharide** such as starch, glycogen, or cellulose (fig. 6.3).

Question 1
Examine figure 6.2. Which groups of a glucose molecule are involved in forming a polysaccharide? Shade the groups with a pencil.

As already mentioned, the linkage of subunits in carbohydrates, as well as other macromolecules, involves the removal of a water molecule (dehydration). Figure 6.4 depicts how dehydration synthesis is used to make maltose and sucrose, two common disaccharides.

Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free aldehyde (–CHO) or ketone (–C=O) groups that reduce weak oxidizing agents such as the copper in Benedict's reagent. **Benedict's reagent** contains cupric (copper) ion complexed with citrate in alkaline solution. Benedict's test identifies reducing sugars based on their ability to reduce the cupric (Cu^{2+}) ions to cuprous (Cu^{+}) oxide at basic (high) pH. Cuprous oxide is green to reddish orange.

Oxidized Benedict's reagent (Cu^{2+}) + Reducing sugar (R-COH)

(blue)

Heat
High pH ↓

Reduced Benedict's reagent (Cu^{+}) + Oxidized sugar (R-COOH)

(green to reddish orange)

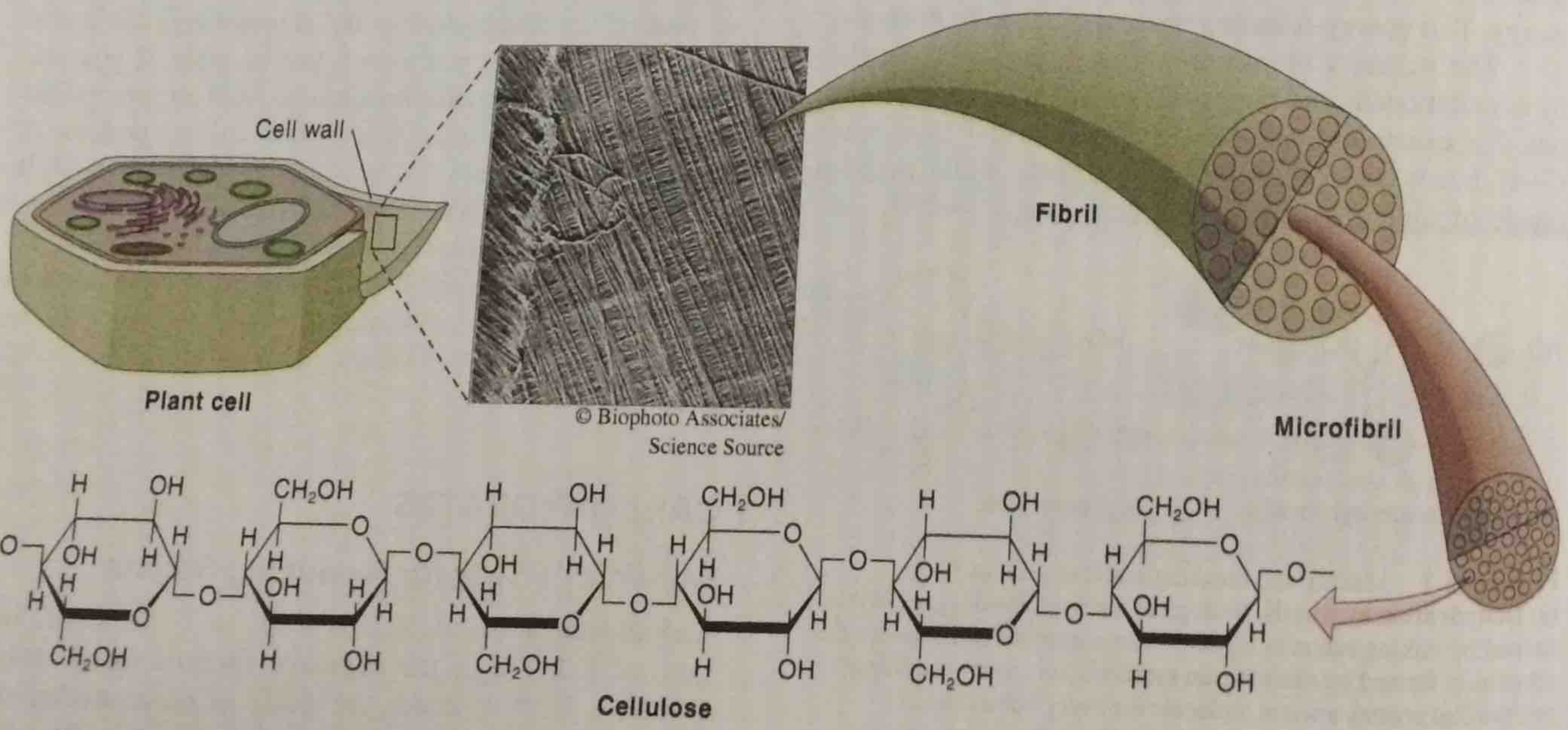


Figure 6.3 Plant cell walls made of cellulose arranged in fibrils and microfibrils. The scanning electron micrograph shows the fibrils in a cell wall of the green alga *Chaetomorpha* (30,000×).

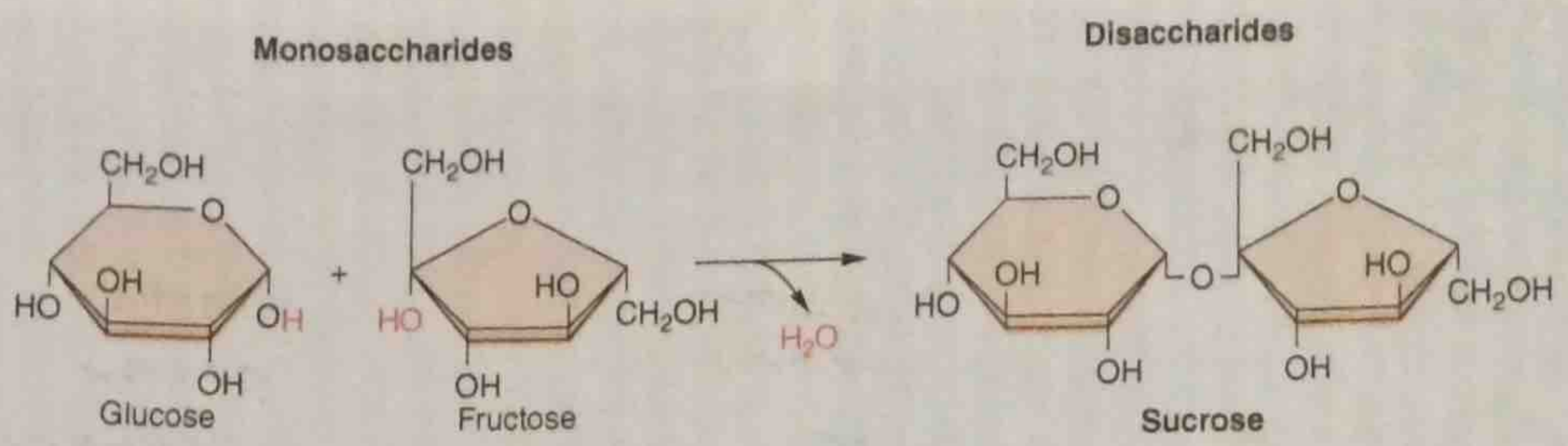


Figure 6.4 Dehydration synthesis is used to link monosaccharides (such as glucose and fructose) into disaccharides. The disaccharides shown here are maltose (malt sugar) and sucrose (table sugar).

A green solution indicates a small amount of reducing sugars, and reddish orange indicates an abundance of reducing sugars. Nonreducing sugars such as sucrose produce no change in color (i.e., the solution remains blue).

SAFETY FIRST Before coming to lab, you were asked to read this exercise so you would know what to do and be aware of safety issues. In the space below, briefly list the safety issues associated with today's procedures. If you have questions about these issues, contact your laboratory assistant before starting work.

Procedure 6.1 Perform the Benedict's test for reducing sugars

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (the quantities of these materials are listed in table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes. Add 2 mL of Benedict's solution to each tube.
3. Place all of the tubes in a gently boiling water-bath for 3 min and observe color changes during this time.
4. After 3 min, use a test-tube holder to remove the tubes from the water-bath. After giving the tubes ample time to cool to room temperature, record the color of their contents in table 6.1.
5. When you are finished, dispose of the contents of each tube as instructed by your instructor.

Question 2

a. Which of the solutions is a positive control? Negative control?

positive: 1, 2, 4, 6

Negative: 3, 5, 7, 8

b. Which is a reducing sugar, sucrose or glucose? How do you know?

glucose because it change to red color so it has long amount of reducing sugar

c. Which contains more reducing sugars, potato juice or onion juice? How do you know?

onion juice has more because orange is moderate amount and green is a small

d. What does this tell you about how sugars are stored in onions and potatoes?

there are more sugrs in onions then potato

Iodine Test for Starch

Staining by iodine (iodine-potassium iodide, I_2KI) distinguishes starch from monosaccharides, disaccharides, and other polysaccharides. The basis for this test is that starch is a coiled polymer of glucose; iodine interacts with these coiled molecules and becomes bluish black. Iodine does not react with carbohydrates that are not coiled and remains yellowish

unknown
1 - shake
2 Electrolyte Drink
3 - Soup

TABLE 6.1

SOLUTIONS AND COLOR REACTIONS FOR (1) BENEDICT'S TEST FOR REDUCING SUGARS AND (2) IODINE TEST FOR STARCH

TUBE	SOLUTION	BENEDICT'S COLOR REACTION	IODINE COLOR REACTION
1	10 drops onion juice	Yellow	Negative
2	10 drops potato juice	green	negative
3	10 drops sucrose solution	blue	negative
4	10 drops glucose solution	red	negative
5	10 drops distilled water	blue	negative
6	10 drops reducing-sugar solution	red	negative
7	10 drops starch solution	blue	positive
8	unknown	blue	negative
9			

brown. Therefore, a bluish-black color is a positive test for starch, and a yellowish-brown color (i.e., no color change) is a negative test for starch. Glycogen, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Procedure 6.2 Perform the iodine test for starch

1. Obtain seven test tubes and number them 1–7.
2. Add to each tube the materials to be tested (table 6.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add three to six drops of iodine to each tube.
4. Record the color of the tubes' contents in table 6.1.

Question 3

a. Which of the solutions is a positive control? Which is a negative control?

strach pustive

nigative: 1, 2, 3, 4, 5, 6, 7

b. Which colors more intensely, onion juice or potato juice? Why?

Potaito juice is orang

c. In what parts of a plant is the most starch typically stored?

plant cell wall

d. What are the functions of carbohydrates in living organisms?

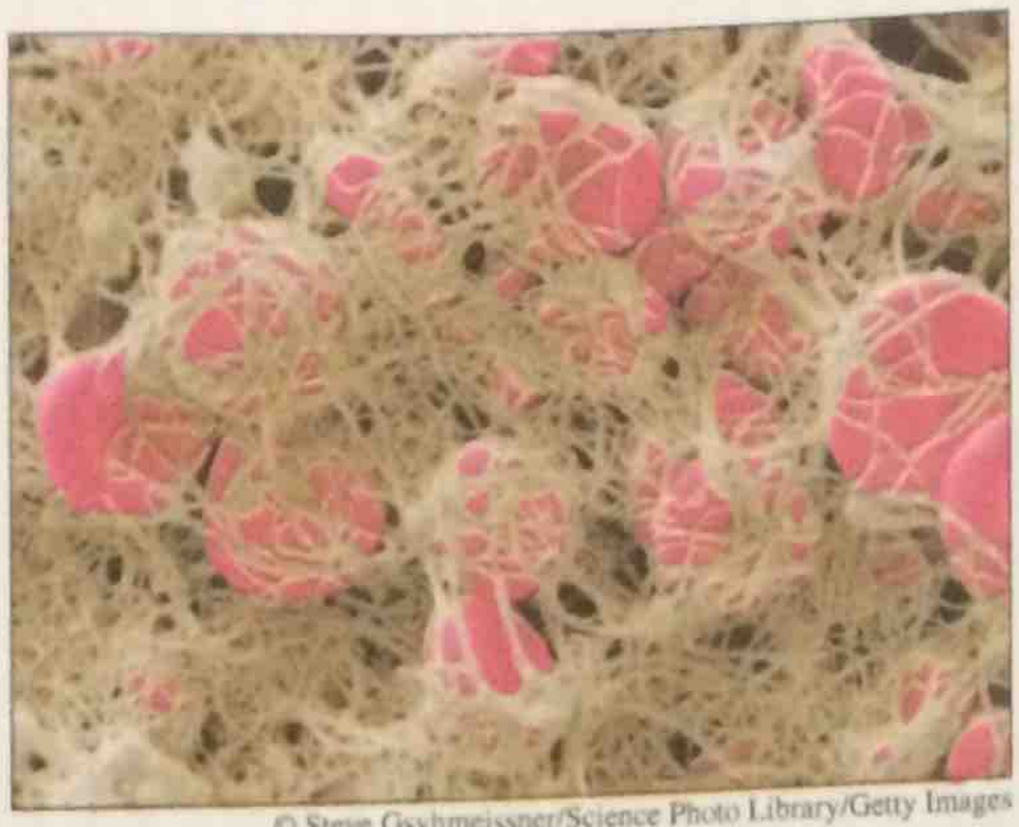
PROTEINS

Proteins are remarkably versatile structural molecules found in all life forms (fig. 6.5). Proteins are made of amino acids (fig. 6.6), each of which has an amino group (—NH_2), a carboxyl (acid) group (—COOH), and a variable side chain (—R). A **peptide bond** (fig. 6.7) forms between the amino group of one amino acid and the carboxyl group of an adjacent amino acid and is identified by a **Biuret test.** Specifically, peptide bonds (C—N bonds) in proteins complex with Cu^{2+} in Biuret reagent and produce a violet color. A Cu^{2+} must complex with four to six peptide bonds to produce a color; therefore, individual amino acids do not react positively. Long-chain polypeptides (proteins) have many peptide bonds and produce a positive reaction.

Biuret reagent is a 1% solution of $CuSO_4$ (copper sulfate). A violet color is a positive test for the presence of protein; the intensity of color relates to the number of peptide bonds that react.

Question 4

Examine figure 6.6. Shade with a pencil the reactive amino and carboxyl groups on the three common amino acids shown.



(a) Fibrin © Steve Gsvhmeissner/Science Photo Library/Getty Images

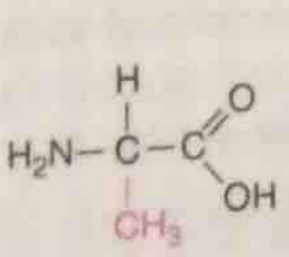
(b) Collagen © Craig Veltri/Getty Images

(c) Keratin © Jim Wehtje/Brand X Pictures/Punchstock

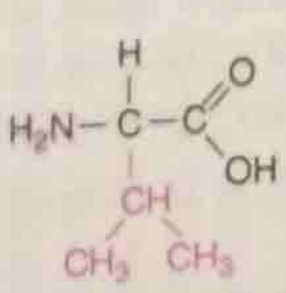
(d) Spider silk © Index Stock/Alamy

(e) Hair © Armina-Udovenko/Getty Images

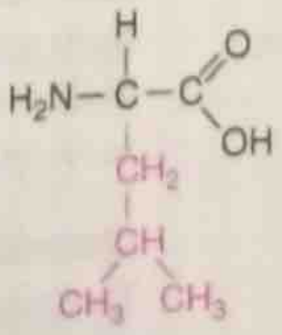
Figure 6.5 Common structural proteins. (*a*) Fibrin. This electron micrograph shows a red blood cell caught in threads of fibrin (800×). Fibrin is important in the formation of blood clots. (*b*) Collagen. The so-called "cat-gut" strings of a tennis racket are made of collagen. (*c*) Keratin. This type of protein makes up bird feathers, such as this peacock feather. (*d*) Spider silk. The web spun by this agile spider is made of protein. (*e*) Hair. Hair is also a protein.



Alanine



Valine



Leucine

Figure 6.6 Structures of three amino acids common in proteins. Each amino acid has one carbon bonded to both an amine group (—NH_2) and a carboxyl group (—COOH). The side chains that make each amino acid unique are shown in red.

Procedure 6.3 Perform the Biuret test for protein

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.2.
3. Add 2 mL of 2.5% sodium hydroxide (NaOH) to each tube.

TABLE 6.2

SOLUTIONS AND COLOR REACTIONS FOR THE BIURET TEST FOR PROTEINS

TUBE	SOLUTION	COLOR
1	2 mL egg albumen	porple
2	2 mL honey	blue
3	2 mL amino acid solution	blue
4	2 mL distilled water	blue
5	2 mL protein solution	prple
6		blue
7		

Figure 6.7 A peptide bond joins two amino acids, and peptide bonds link many amino acids to form polypeptides, or proteins. The formation of a peptide bond (i.e., between the carbon of one amino acid's carboxyl group and the nitrogen of another amino acid's amino group) liberates a water molecule. The R in these amino acids represents a variable side chain that characterizes each type of amino acid.

Do not spill the NaOH—it is extremely caustic. Rinse your skin if it comes in contact with NaOH.

4. Add three drops of Biuret reagent to each tube and mix.
5. Record the color of the tubes' contents in table 6.2.

Question 5

a. Which of the solutions is a positive control? Which is a negative control?

solutions positive 1,2
negative 6,5,4,3

b. Which contains more protein (C—N bonds), egg albumen or honey? How can you tell?

egg albumen it change to purple which is positive

c. Do free amino acids have peptide bonds?

d. What are the functions of proteins in living organisms?

LIPIDS

Lipids include a variety of molecules that dissolve in nonpolar solvents such as ether, acetone, methanol, or ethanol, but not as well in polar solvents such as water. Triglycerides (fats) are abundant lipids made of glycerol and three fatty acids (fig. 6.8). Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

Question 6

Examine figure 6.8. What are the reactive groups of the fatty acids?

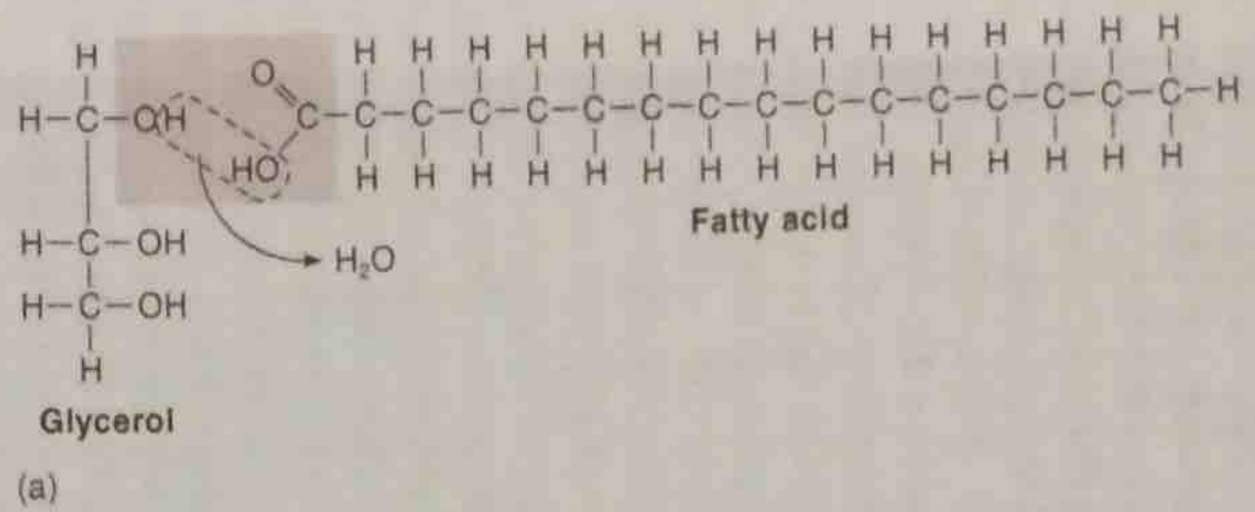


Figure 6.8 The structure of a fat includes glycerol and fatty acids. (*a*) An ester linkage forms when the carboxyl group of a fatty acid links to the hydroxyl group of glycerol, with the removal of a water molecule. (*b*) Triacylglycerides are fats whose fatty acids vary in length and vary in the presence and location of carbon–carbon double bonds.

Handle acetone carefully; it is toxic.

Procedure 6.4 Solubility of lipids in polar and nonpolar solvents

1. Obtain two test tubes. To one of the tubes, add 5 mL of water. To the other tube, add 5 mL of acetone.
2. Add a few drops of vegetable oil to each tube.

Question 7
What do you conclude about the solubility of lipids in polar solvents such as water? In nonpolar solvents such as acetone?

Procedure 6.5 Perform the Sudan IV test for lipid

1. Obtain five test tubes and number them 1–5. Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
2. Add the materials listed in table 6.3.
3. Add five drops of water to tube 1 and five drops of Sudan IV to each of the remaining tubes. Mix the contents of each tube. Record the color of the tubes' contents in table 6.3.

Question 8
a. Is salad oil soluble in water?

No