WEEK 4
Biologically Important Molecules: Carbohydrates and Lipids

Objectives:

1. Understand how to test for the presence of carbohydrates, lipids, proteins, and nucleic acids.
2. Understand the importance of a control in biochemical tests.
3. Use biochemical tests to identify an unknown compound.
4. Know the elemental composition of the molecules.
5. Distinguish between subunits and macromolecules.
6. Identify the important functional groups.
7. Appreciate the important functions of the macromolecules.
8. Understand the process by which macromolecules are formed and broken down.

INTRODUCTION

Most organic compounds in living organisms can be classified as either **carbohydrates**, **proteins**, **lipids**, or **nucleic acids**. Each of these macromolecules are made of smaller subunits . These subunits of macromolecules are held together with 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 groups impart different chemical properties to macromolecules --- for example. Monosaccharides such as glucose are polar and soluble in water, while lipids are nonpolar and insoluble in water.

CONTROLLED EXPERIMENTS TO IDENTIFY ORGANIC COMPOUNDS

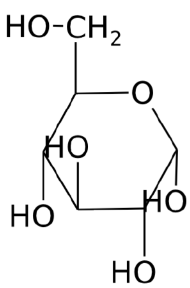
Scientists have devised several biochemical tests to identify the major types of organic compounds in living organisms. Each of these tests involves two or more treatments; an **unknown solution** to be identified and **controls.** As its name implies, an unknown solution may or may not contain the substance 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 indeed 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 that 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 known solution of protein. A positive reaction proves that your test reacts correctly, and shows you exactly what a positive test looks like.

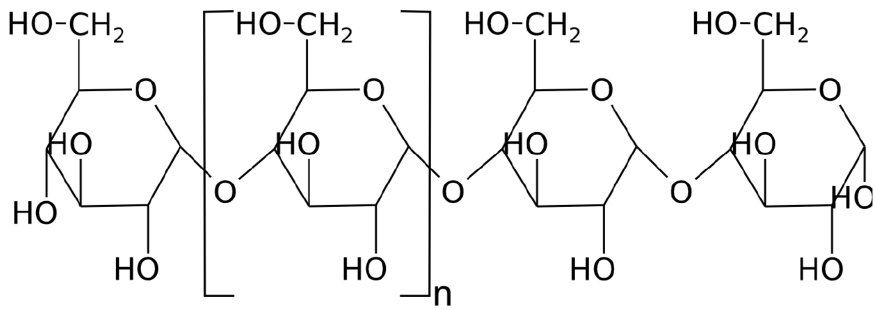
A **negative control** does not contain the variable for which you are searching. It contains only the solvent (often distilled water with no solute) and does not react in the test. A negative control shows you what a negative result looks like.

CARBOHYDRATES:

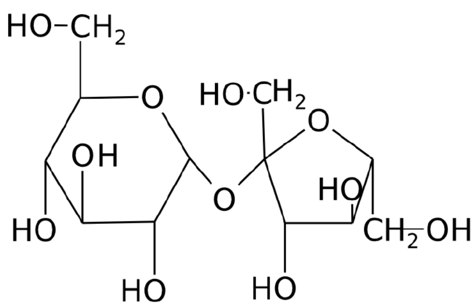
Carbohydrates are molecules made of the elements C, H, and O in a ratio of 1 : 2 : 1 (e.g., the chemical formula for glucose is $C_6H_{12}O_6$). Carbohydrates are made of **monosaccharides (**subunits**),** or simple sugars (Fig. 4.1). Paired monosaccharides form **disaccharides** --- for example, sucrose is a disaccharide of glucose linked to fructose. Similarly, linking three or more monosaccharides forms a **polysaccharide** such as starch, glycogen, or cellulose. The polysaccharides are made by the removal of H_2O. This process is termed **dehydration synthesis**. The addition of H_2O to a polysaccharide to break it down to it's monosaccharide subunits is termed **hydrolysis**.



Glucose, a monosaccharide



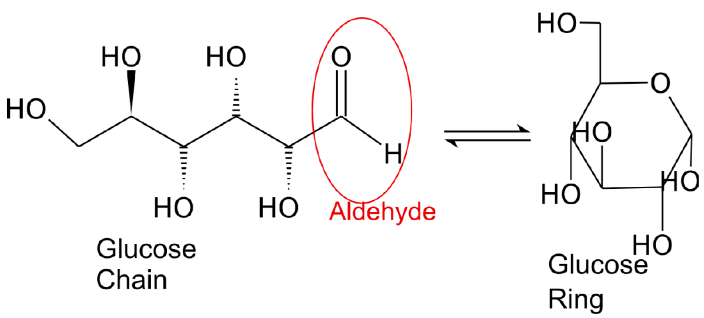
Amylose (Starch), a polysaccharide



Sucrose, a disaccharide

Figure 4.1 Carbohydrate macromolecules are made of sub units of mono or disaccharides.

Many monosaccharides such as glucose and fructose are **reducing sugars,** meaning that they possess free **aldehyde** or **ketone** 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 oxide at basic (high) pH. Cuprous oxide is green to reddish orange.



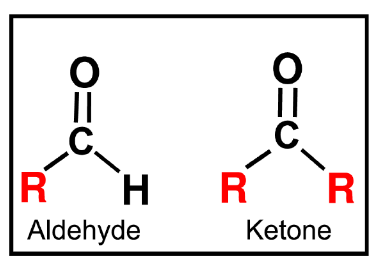


Figure 4.2: Isomerization between chain and ring forms of monosaccharides. The chain form contains a free aldehyde that makes glucose monomers *reducing sugars*.

Oxidized Benedict's reagent (Cu^{2+}) + Reducing sugar (R-C=O)
(blue)

↓ Heat, High pH

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

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

Exercise 4.1 Benedict's Test For Reducing Sugars

1. Obtain 7 test-tubes and number them 1-7.
2. Add to each tube the materials to be tested (Table 4.1). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add 2 ml Benedict's solution to each tube.
4.
5. Place all of the tubes in a boiling water bath for 3 min and observe color changes during this time.
6. After 3 min, remove the tubes from the water bath and let them cool to room temperature. Record the color of their contents in Table 4.1.

Table 4.1 Solution And Color Reactions For Benedict's Test For Reducing Sugars

TUBE	SOLUTION	BENEDICT'S COLOR REACTION
1	10 drops onion juice	
2	10 drops potato juice	
3	10 drops sucrose solution	
4	10 drops glucose solution	
5	10 drops distilled water	
6	10 drops reducing-sugar solution	
7	10 drops starch solution	

Iodine Test for Starch

Iodine (iodine-potassium iodide, I_2KI) staining 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 other carbohydrates that are not coiled, and remains yellowish 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. Notably, **glycogen**, a common polysaccharide in animals, has a slightly different structure than does starch and produces only an intermediate color reaction.

Exercise 4.2 Iodine Test For Starch

1. Obtain 7 test-tubes and number them 1-7.
2. Add to each tube the materials to be tested (Table 4.2). Your instructor may ask you to test some additional materials. If so, include additional numbered test tubes.
3. Add 3-5 drops of iodine to each tube.
4. Record the color of the tubes' contents in Table 4.2.

Table 4.2 Solution And Color Reactions For Iodine Test For Starch

TUBE	SOLUTION	Iodine Starch Test
1	10 drops onion juice	
2	10 drops potato juice	
3	10 drops sucrose solution	
4	10 drops glucose solution	
5	10 drops distilled water	
6	10 drops reducing-sugar solution	
7	10 drops starch solution	

LIPIDS:

Lipids are made of glycerol and fatty acids (Fig. 4.3). Lipids are insoluble in polar solvents such as water, and are soluble in nonpolar solvents such as ether and acetone. Tests for lipids are based on a lipid's ability to selectively absorb pigments in fat-soluble dyes such as Sudan IV.

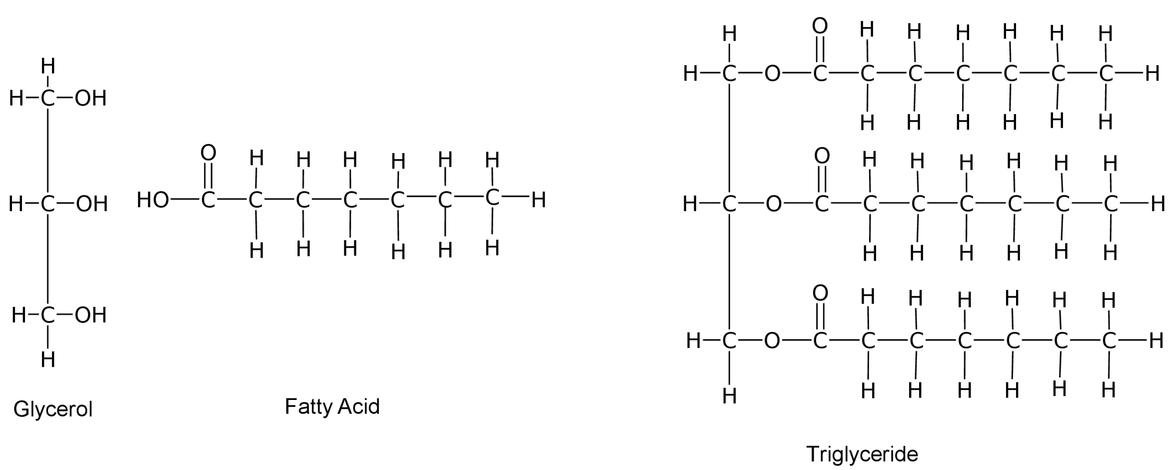


Figure 4.3 Lipids (triglycerides) are macromolecules made from subunits of glycerol and three fatty acids.

Exercise 4.3 Sudan IV Test For Lipid

1. Obtain 5 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 4.3
3. Add 3 ml of water to each tube.
4. Add 5 drops of water to tube 1, and 5 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 4.3

Table 4.3 Solutions and Color Reactions for Sudan IV Test for Lipids

TUBE	SOLUTION	REACTION DESCRIPTION
1	1 ml salad oil + water	
2	1 ml salad oil + Sudan IV	
3	1 ml honey + Sudan IV	
4	1 ml distilled water + Sudan IV	
5	1 ml known lipid solution + Sudan IV	

Grease-spot Test for Lipids

A simpler test for lipids is based on their ability to produce translucent grease-marks on unglazed paper.

Exercise 4.4 Grease-Spot Test For Lipids

1. Obtain a piece of brown wrapping paper from your lab instructor.
2. Use an eye-dropper to add a drop of salad oil near one corner of the piece of paper.
3. Add a drop of water near the opposite corner of the paper.
4. Let the fluids evaporate.
5. Look at the paper as you hold it up to a light.
6. Test in a similar way other food products and solutions available in the lab and record your results in Table 4.4.

Table 4.4 Materials And Grease-Spot Reactions as a Test For Lipid Content

FOOD PRODUCT	DISCRIPTION OF GREASE-SPOT REACTION
1	
2	
3	
4	
5	
6	

Questions:

1. a. Which is a reducing sugar, sucrose or glucose?
   b. Which contains more reducing sugars, potato juice or onion juice?
   c. What does this tell you about how sugars are stored in onions and potatoes?
2. a. Which colors more intensely, onion juice or potato juice?
   b. What does this tell you about how these plants store carbohydrates?
3. a. Is salad oil soluble in water?
   b. Compare tubes 1 and 2. What is the distribution of the dye with respect to the separated water and oil?
   c. What observation indicates a positive test for lipid?
   d. Does honey contain much lipid?
   e. Lipids supply more than twice as many calories per gram as carbohydrates. Based on your results, which contains more calories, oil or honey?
4. Which food products contain large amounts of lipid?