Carbohydrates and Lipids

Lab III-2

EQUIPMENT AND MATERIALS

You'll need the following items to complete this lab session. (The standard kit for this book, available from *www.thehomescientist.com*, includes the items listed in the first group.)

MATERIALS FROM KIT

- Goggles
- Barfoed's reagent
- Beaker, 250 mL
- Benedict's reagent
- d-glucose (dextrose)
- graduated cylinder, 10 mL
- Gram's iodine stain
- Hydrochloric acid
- Pipettes
- Reaction plate, 24-well
- Seliwanoff's reagent
- Slides (flat) and coverslips
- Sudan III stain
- Test tubes
- Test tube rack

MATERIALS YOU PROVIDE

- Gloves
- Butane lighter (or other flame source)
- Butter
- Diet sweetener
- Fruit juice (unsweetened)
- Hair dryer (optional)
- Honey
- Isopropanol
- Marking pen
- Microscope
- Microwave oven
- Milk (whole)
- Non-dairy creamer
- Onion
- Paper bag (brown)
- Peanut (or cashew, etc.)
- Potato
- Soft drink, colorless (Sprite or similar)
- Sucrose (table sugar)
- Vegetable oil
- Water, distilled

BACKGROUND

In this lab session we'll investigate two classes of *biologically important molecules*, carbohydrates and lipids.

CARBOHYDRATES (SACCHARIDES)

Carbohydrates, also known as *saccharides* (sack'-uh-rides), are a fundamental building block of life. Carbohydrates perform many key biological functions, notably storing and transporting energy and providing physical structure.

Carbohydrates contain only carbon, hydrogen, and oxygen, and have the empirical formula $C_n(H_2O)_m$, where some number of carbon atoms is combined with some number of water molecules. (In fact, the structure of carbohydrates is considerably different than a simple grouping of carbon atoms with water molecules, but they were named carbohydrates because the empiric ratio of atoms in their structures corresponds to hydrated carbon.) Carbohydrates are categorized as members of the following groups.

CHIRALITY

Chirality is the property of handedness. For example, the structures of a right glove and a left glove are identical but the gloves are chiral because each is the mirror image of the other. Many organic molecules, including monosaccharides, are chiral. The *dextrorotary* form of a chiral molecule rotates the plane of polarized light to the right, and the *levorotary* form to the left.

The naturally occurring form of glucose rotates polarized light to the right, and is called *D-glucose* or *dextrose*. L-glucose is useless biologically because hexokinase, the enzyme that metabolizes D-glucose, is itself chiral and cannot operate on L-glucose.

Monosaccharides

Monosaccharides, also called *simple sugars*, are the smallest and simplest carbohydrates, and are important both for themselves and as the fundamental building blocks of larger, more complex carbohydrates. (You can think of complex carbohydrates as a brick wall in which monosaccharides are the bricks.) *Glucose*, *fructose*, *galactose*, *ribose*, and *xylose* are examples of biologically important monosaccharides.

Monosaccharides are classed by the number of carbon atoms and the functional group they contain. A *triose* contains three carbon atoms, a *tetrose* four, a *pentose* five, a *hexose* six, and a *heptose* seven. Monosaccharides that contain an aldehyde group—a carbonyl group (C=O) bonded to a hydrogen atom and a carbon atom—are *aldoses*, and those that contain a ketone group—a carbonyl group bonded to two other carbon atoms—are *ketoses*.

These two important characteristics may be combined to describe both the number of carbon atoms and the type of functional group in one term. For example, because ribose contains five carbon atoms and an aldehyde functional group, it is an *aldopentose*. Fructose contains six carbon atoms and a ketone functional group, and so is a *ketohexose*.

Disaccharides

Disaccharides, also called *compound sugars*, comprise two bound monosaccharides, which may be the same or different. For example, the disaccharide *lactose* contains glucose bound to galactose, *sucrose* contains glucose bound to fructose, and *maltose* contains two bound glucose molecules.

Disaccharides can be *hydrolyzed* (split) into their component monosaccharides using enzymes, heat, or an acid or base catalyst. For example, sucrose can be hydrolyzed into its component monosaccharides, glucose and fructose, by heating it in a dilute hydrochloric acid solution.

Oligosaccharides

An *oligosaccharide* is a polysaccharide polymer made up of more than 2 but fewer than 10 monosaccharide units. Many oligosaccharides are biologically important molecules. Oligosaccharides are components of many proteins, glycoproteins, and glycolipids, where they often function as chemical markers. For example, blood types A and B contain different oligosaccharides; blood type AB contains both of those oligosaccharides; blood type O contains neither.

Polysaccharides

A *polysaccharide* is a *macromolecule* (large molecule) made up of 10 or more (often *many* more) monosaccharide and/or disaccharide units. Two familiar polysaccharides are *cellulose*, which plants use as a structural element, and *starch*, which plants use to store energy. (Animals store energy using *glycogen*, which is structurally similar to starch.)

Some polysaccharides are easily hydrolyzed into their component mono- or disaccharides. For example, the glucose (dextrose) sold in drugstores and health-food stores is produced by hydrolyzing starch. Other polysaccharides are very difficult to hydrolyze. For example, while it is possible in principle to hydrolyze cellulose into glucose—which would immediately make biofuels cheap and universally available—in practice it has so far proven impossible to do so economically.

REDUCING SUGARS

A *reducing sugar* (more properly, *reducing saccharide*) is one that, when in solution, exposes a reactive ketone or aldehyde group. All monosaccharides (ketoses and aldoses) are, by definition, reducing sugars. Many di-, oligo-, and polysaccharides are reducing saccharides. Sucrose is one example of a nonreducing disaccharide. The "reducing" part of the name refers to the ability of these saccharides to react with mild oxidants such as Fehling's, Benedict's, or DNSA reagent to yield a color change or precipitate that identifies the presence of a reducing sugar.

Biologists frequently use four color-test reagents to discriminate among types of sugars. All of these reagents are used in the same way: transfer a small amount of the reagent to a test tube, add a few drops of the specimen, and place the tube in a boiling water bath for a few minutes.

Barfoed's reagent

Barfoed's reagent is used to discriminate monosaccharides. A brick-red precipitate forms within five minutes if a monosaccharide is present. Disaccharides generally cause no precipitate even after 10 to 15 minutes of heating.

Benedict's reagent

Benedict's reagent is used to discriminate reducing sugars. A precipitate forms within five minutes if a reducing sugar is present. The amount and color of the precipitate vary with the amount of reducing sugar present. With increasing concentration of the reducing sugar, the precipitate color varies from green to yellow to orange to brick-red, with the brick-red color generally occurring at concentrations of 1% or higher.

Seliwanoff's reagent

Seliwanoff's reagent is used to discriminate ketoses from aldoses. An orange to red color forms within five minutes if a ketose is present. If the ketose is present in low concentration, the color may be anything from a straw yellow to yellowish-orange. Aldoses yield no color change even after 10 to 15 minutes of heating.

If you're using the kit, you can make up Seliwanoff's reagent by adding one part 6 M hydrochloric acid to one part of Seliwanoff's Reagent A solution. For example, to make up 3 mL of Seliwanoff's Reagent, use a graduated pipette to transfer 1.5 mL of 6 M hydrochloric acid to 1.5 mL of Seliwanoff's Reagent A.

Bial's reagent, which we won't use in this session, is used to discriminate pentoses from hexoses. Bial's reagent forms a green, greenish-blue, or blue color in the presence of pentoses, but shows no color change for hexoses.

Starches can be detected using a dilute aqueous solution of iodine, such as Gram's stain. The brown iodine solution reacts with starch to form an intense blue complex. Adding a drop of iodine solution to a few mL of even extremely dilute starch solution causes a blue coloration to appear; with more concentrated starch solutions, the blue is so intense it may appear black. Iodine is also used to stain cells for microscopic examination. Any starch present in the cell is stained black.

THE MOLISCH GENERAL TEST FOR CARBOHYDRATES

In a biology lab, a quick test is sometimes needed to determine if carbohydrates of any type are present in a specimen. Molisch's reagent is widely used for this purpose.

LIPIDS

Lipids are members of a large group of biomolecules that includes *oils*, *fats*, *waxes*, *sterols*, *fatty acids*, and other classes. Lipids perform many key biological functions. Like carbohydrates, lipids are widely used for storing energy and providing physical structure in cell membranes. Many important signaling molecules are lipids, as are several vitamins.

Some lipids are *hydrophobic* (water-hating), which means they do not mix with water or aqueous solutions. For example, vegetable oil contains hydrophobic lipids. If you mix vegetable oil with water and agitate the liquid, it initially forms a suspension of tiny globules of vegetable oil suspended in the water. If you allow the liquid to sit undisturbed, it eventually separates into two layers, with the denser water forming the bottom layer and the vegetable oil the top layer.

Other lipids are *amphiphilic*, which means they mix well with both water and other lipids. The molecular structure of these lipids has a hydrophobic group on one end of the molecule and a *hydrophilic* (water-loving) group on the other end. Amphiphilic lipids called *phospholipids* form the structure of the *bi-layer membranes* found in many cells. These phospholipids arrange themselves into a double layer with their hydrophobic groups in the center of the layer and their hydrophilic groups facing outward toward the aqueous solutions on both sides of the membrane, thereby isolating the aqueous areas with a hydrophobic lipid layer that is impermeable to water.

Lipophilic dyes (fat-loving dyes) such as Sudan III are the best general test for the presence of lipids. These dyes are nearly insoluble in water but readily soluble in lipids (such as oils and fats). When a lipophilic dye is applied to a specimen that contains lipids, it is selectively attracted to the lipids, staining them and leaving parts of the specimen that do not contain lipids unstained. This property is useful for discriminating lipids both macroscopically and microscopically.

THE GREASE SPOT TEST

Lipids also have the property of forming "grease spots" on kraft paper, which is the basis for a frequently used quick test for the presence of lipids. You can use a liquid specimen as is. Dissolve a solid specimen in alcohol, and apply a few drops of the liquid to kraft paper, such as a brown paper bag. When the solvent (if any) evaporates—you can use a hair dryer to help it along—any lipids that were present in the specimen form a translucent grease spot on the paper.

We will attempt to resolve the following:

- The type or types of sugars present in the various specimens.
- Whether it is possible to hydrolyze sucrose into its component monosaccharide(s) and, if so, how closely you might categorize the component monosaccharide(s).
- The presence or absence of starch in the various specimens.
- Whether it is possible to hydrolyze starch into its component monosaccharide(s) and, if so, how closely you might categorize the component monosaccharide(s).
- Whether it is possible to identify the presence of starch as a component of plant cells and, if so, how.
- The presence or absence of lipids in the various specimens.
- Whether it is possible to identify the presence of lipids as a component of plant cells and, if so, how.

PROCEDURE III-2-1: INVESTIGATING SUGARS

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Prepare a hot water bath by filling the 250 mL beaker about one third full of tap water and heating it in the microwave until it comes to a gentle boil. Alternatively, simply bring a pot of water to a gentle boil on the stove, and use that pot as your source of boiling water during this procedure.

WARNING: USE EXTREME CAUTION

A microwave oven can actually heat water above its boiling point without causing the water to boil, a phenomenon called superheating. The slightest disturbance can cause superheated water to boil violently, expelling it from the container.

The water needn't be actually boiling, as long as its temperature is close to 100 °C. As the hot water bath cools during these procedures, periodically replace the water with freshly boiled water to maintain the temperature near 100 °C.

3. Label a test tube for each of your first six specimens, including one tube for distilled water, and place the tubes in the rack.

4. Prepare your specimens as described in the footnotes of Table III-2-1. Any specimens that do not include preparation instructions can be used as-is.

5. Transfer about 0.5 mL of Barfoed's reagent to each test tube.

6. Add three drops of each specimen to the corresponding tube and swirl the tubes to mix the contents.

7. Transfer the first six tubes to the boiling water bath and allow them to remain for five minutes.

8. Record your observations in your lab notebook.

9. After the tubes have cooled, dispose of their contents by flushing them down the drain with plenty of water. Rinse and then wash the tubes.

10. Repeat steps 5 through 9 for your other six specimens.

11. Repeat steps 5 through 10 using Benedict's reagent and Seliwanoff's reagent. (We'll fill out the Gram column in the next procedure.)

Retain the prepared specimens in the reaction plate and the (clean) labeled test tubes for the following procedure.

Table III-2-1: *Detecting and Classifying Saccharides—experimental observations*

#	Specimen	Barfoed	Benedict	Seliwanoff	Gram
1	distilled water				
2	diet sweetener[a]				
3	fruit juice				
4	glucose solution				
5	honey[b]				
6	milk (whole)				
7	nondairy creamer[c]				
8	onion water[d]				
9	potato water[e]				
10	Sprite soft drink				
11	sucrose[f] solution				
12	hydrolyzed sucrose[g]				

a. Dissolve a pinch of diet sweetener in about 2 mL of distilled water in a reaction plate well.

b. Dissolve two or three drops of honey in about 2 mL of distilled water in a reaction plate well.

c. Dissolve a pinch of non-dairy creamer in about 2 mL of distilled water in a reaction plate well.

d. Mash bits of onion with a stirring rod in about 2 mL of distilled water in a reaction plate well.

e. Mash bits of potato with a stirring rod in about 2 mL of distilled water in a reaction plate well.

f. Dissolve a pinch of table sugar in about 2 mL of distilled water in a reaction plate well.

g. Dissolve a pinch of sugar in about 2 mL of distilled water in a test tube and add two drops of hydrochloric acid. Heat for 10 minutes in the hot water bath, allow to cool, and transfer to a reaction plate well.

If you don't use them normally, you can obtain diet sweetener and nondairy creamer in small packets from any restaurant.

PROCEDURE III-2-2: INVESTIGATING STARCHES

This procedure has two parts. In the first, we'll test the specimens we used in the last procedure for the presence of starch. In the second part, we'll test for starch at the cellular level of some solid specimens.

TESTING SPECIMENS FOR THE PRESENCE OF STARCH

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Transfer about 0.5 mL of each of the first six specimens listed in Table III-2-1 to the corresponding test tubes and place the tubes in the rack.
3. Add one drop of Gram's stain to each of the test tubes.
4. Record your observations in your lab notebook and in Table III-2-1.
5. Dispose of the contents of the tubes by flushing them down the drain with plenty of water. Rinse and then wash the tubes.
6. Repeat steps 2 through 5 for each of the remaining six specimens.

Retain the prepared specimens in the reaction plate and the (clean) labeled test tubes for the following procedure.

TESTING FOR STARCH AT THE CELLULAR LEVEL

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Transfer a tiny amount of solid potato to a slide, add one drop of distilled water, and use the stirring rod to crush and spread the potato to make a smear mount. Heat-fix the smear mount.
3. Repeat step 2 using a tiny amount of solid onion.
4. Repeat step 2 using a tiny amount of solid peanut.

WARNING

Skip step 4 if you are or anyone else in the vicinity is allergic to peanuts.

5. Place one drop of Gram's iodine stain on the smear area of each slide. Allow the stain to work for 30 seconds and then rinse gently with distilled water to remove excess stain. Allow the slides to dry naturally or use a hair dryer set to low to speed drying.

6. Examine each slide at low magnification to locate a cluster of cells. Examine those cells at medium and high magnifications. Record your observations, including sketches, in your lab notebook. Retain the slides for use in the next procedure.

PROCEDURE III-2-3: INVESTIGATING LIPIDS

This procedure has four parts. In the first, we'll test the solubility of lipids in water and isopropanol. In the second, we'll use the grease-spot test to detect lipids in our various liquid specimens. In the third, we'll investigate the effect of Sudan III stain (a lipophilic dye) on lipids. In the fourth, we'll use Sudan III stain to stain the slides we made in the previous procedure to determine if we can detect lipids at the cellular level.

SOLUBILITY OF LIPIDS

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Transfer 5.0 mL of water to one test tube and 5.0 mL of isopropanol to a second test tube. Place the tubes in the rack.
3. Use a pipette to add 0.5 mL of vegetable oil to each of the test tubes. Agitate the tubes and replace them in the rack.
4. Allow the tubes to remain undisturbed for a minute or so, and then record your observations in your lab notebook.

Retain the contents of the isopropanol test tube for the following sections.

THE GREASE-SPOT TEST FOR LIPIDS

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Draw a 4x4 grid of 5 cm squares on a brown paper bag. Label the squares for each of the 12 solutions you tested earlier, plus a thirteenth square for the mixture of isopropanol and vegetable oil.
3. Transfer one drop of each of the 13 liquids to the corresponding square. Allow the paper to dry naturally, or use a hair dryer set on low to speed drying.
4. Hold the paper up to a window or bright lamp, and examine it for translucent grease spots, which indicate the presence of lipids. Record your observations in your lab notebook.

THE EFFECT OF SUDAN III STAIN ON LIPIDS

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Label four test tubes. Transfer 1.0 mL of distilled water to the first tube, 1.0 mL of honey to the second, 1.0 mL of vegetable oil to the third, and 0.5 mL of vegetable oil and 0.5 mL of distilled water to the fourth.
3. Add two drops of Sudan III stain to each of the test tubes, and agitate the tubes to mix their contents. Replace the tubes in the rack and allow them to sit undisturbed for a minute or so. Record your observations in your lab notebook.

OBSERVING LIPIDS AT THE CELLULAR LEVEL

1. If you have not already done so, put on your goggles, gloves, and protective clothing.
2. Add one drop of Sudan III stain to the smear areas of the slides you prepared earlier and stained with Gram's iodine. Allow the Sudan III stain to work for 30 seconds and then rinse gently with distilled water to remove excess stain. Allow the slides to dry naturally or use a hair dryer set to low to speed drying.

3. Examine each slide at low magnification to locate a cluster of cells. Examine those cells at medium and high magnifications. Record your observations, including sketches, in your lab notebook.

REVIEW QUESTIONS

Q1: In procedure III-2-1, what is the purpose of the test tube that contains only distilled water?

Q2: If you obtain a positive result with one of the reagents when testing distilled water, what can you conclude? What action would you take?

Q3: Which of the specimens you tested in procedure III-2-1 contained a monosaccharide? How do you know?

Q4: Which of the specimens you tested in procedure III-2-1 contained a reducing sugar? How do you know?

Q5: Which of the specimens you tested in procedure III-2-1 contained a ketose? How do you know?

Q6: Based on your tests of hydrolyzed sucrose in procedure III-2-1, what can you conclude about the monosaccharide hydrolyzation products present?

Q7: Based on your tests in procedure III-2-2, which of your specimens contained starch?

Q8: Based on your microscopic examinations in procedure III-2-2, which of the three specimens contained starch and in what amounts? Was the starch evenly distributed throughout the cells or localized? How can you tell?

Q9: Based on your tests in procedure III-2-3, are the lipids in vegetable oil more soluble in water or isopropanol? Which of your 13 specimens contained lipids?

Q10: Based on your microscopic examinations in procedure III-2-3, which of the three specimens contained lipids and in what amounts? Were the lipids evenly distributed throughout the cells or localized? How can you tell?

Q11: What do you conclude about the similarities and differences in how potatoes, onions, and peanuts store food energy?