Experiment 1 – Carbohydrates

Carbohydrates in our diet are a major source of energy and comfort! Foods high in carbohydrates include the most delicious kinds, such as potatoes, bread, and pasta. If we take in more carbohydrate than needed for energy, the excess is converted to glycogen or fat. The carbohydrate family is organized into three general classes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides contain C, H, and O in units of $(CH_2O)_n$. The most common monosaccharides have six carbon atoms (hexoses) with five alcohol groups and one carbonyl group. Hexoses are found in fruit juices, honey, and corn syrup. Either the monosaccharide itself is present, or it is part of a disaccharide and is accessed *via* hydrolysis. **Aldohexoses** contain an aldehyde group on C1 while **ketoses** contain a ketone on C2. Aldohexoses (along with all other aldoses) are known as **reducing sugars**, since the aldehyde can be further oxidized to a carboxylic acid. Ketoses can isomerize into aldoses and thus are also called reducing sugars.

The main point of interest for hexoses is their metabolic fate: **Glycolysis**. Metabolism is an organism's way of breaking down nutrients into simpler, more versatile components. Catabolism is the opposite – building complex molecules from small molecules. Almost every hexose we ingest in our diet is enzymatically converted to D-glucose, also known as blood sugar, so that glycolysis can occur. Through this process, the energy stored within the sugar is released. The ten steps of glycolysis release a net 85 kJ (20 kcal) of energy per mole of glucose.* D-Glucose is broken down into two three-carbon units called pyruvate (**Figure 1**). The fate of pyruvate depends on the presence of oxygen (aerobic vs. anaerobic), type of organism, and the metabolic needs of the organism. In some cases, pyruvate is converted into CO_2, which is passed on to plants, wherein CO_2 is used as a building block in plant catabolism. Let us take a moment to appreciate the great carbon-circle of life!

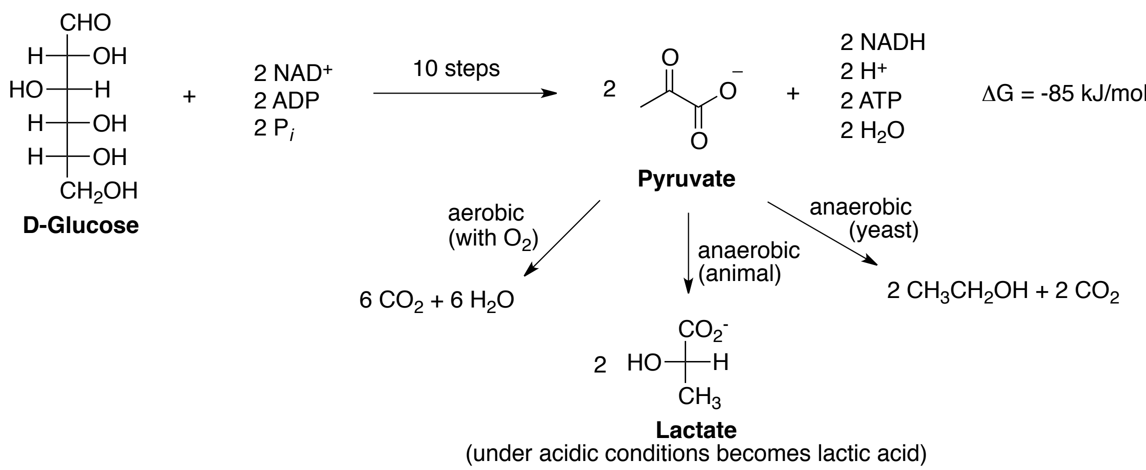

Figure 1. Overview of glycolysis and examples of the fates of pyruvate.

Hexoses contain 3 or 4 chiral centers. There are 8 possible stereoisomers for ketohexoses and 16 possible for aldohexoses, each of which have a slightly different taste due to different receptors on the tongue! Each stereoisomer is metabolized differently, even if the processes differ by only one enzymatic reaction (glycolysis uses 10 different enzymes). This is narrowed down by the high prevalence of **D-sugars** in nature. The "D" designation is given to the penultimate (next to last) carbon on the chain and limits the configuration of that chiral center to "*R*" (**Figure 2**). An "L" designation is given to a sugar with an "*S*" configuration at the penultimate carbon. Monosaccharides are typically draw in open chain form using a Fischer projection. Fischer projections are drawn with straight lines (no wedges or dashes), but this convention still implies 3D shape and specific configuration to each chiral center.

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* For frame of reference, running a mile burns about 200 kcal (dietary "calories" are really kilocalories). Assuming glycolysis is the only source of energy, the body uses almost 10 moles of glucose (1773 g) to run a mile and goes through the steps of glycolysis 6.022×10^{24} times!

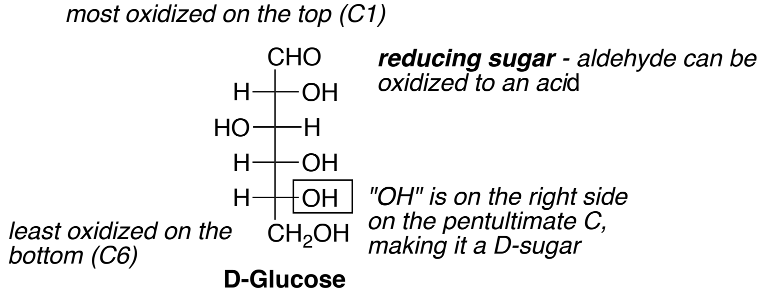

Figure 2. Structural features of glucose; conventions for drawing monosaccharides.

The open chain form of hexoses is in a dynamic equilibrium with the closed form. Aldohexoses tend to close into pyranoses (six-membered rings) while ketohexoses form furanoses (five-membered rings). In these cases, the penultimate OH attacks the carbonyl carbon (pay attention to the numbering in **Figure 3**). Aldohexoses could also close into furanoses and ketohexoses into pyranoses, but we will leave these possibilities aside for the sake of simplicity. The closed-form sugars are commonly shown as Haworth projections, a simpler version of chair conformations.

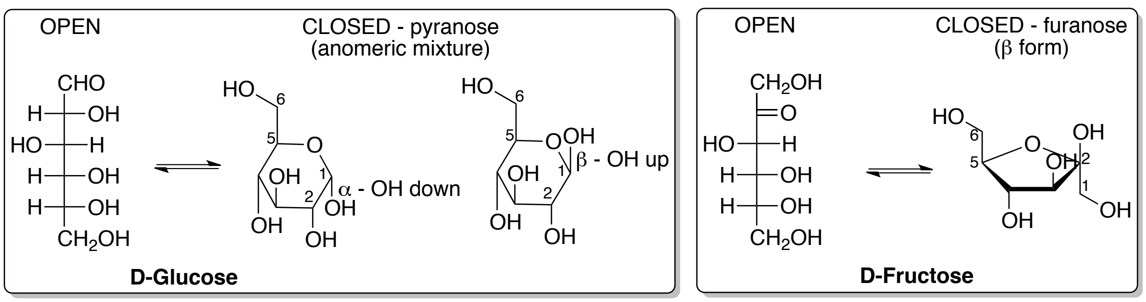

Figure 3. Glucose (an aldohexose) in closed (pyranose) form; fructose (a ketohexose) in closed (furanose) form.

Disaccharides contain two of the common monosaccharides. Some common disaccharides include maltose, sucrose (table sugar), and lactose (milk sugar).

Disaccharide	Sources	Monosaccharides
Maltose	Germinating grains, starch hydrolysis	Glucose + glucose
Lactose	Milk, yogurt, ice cream	Glucose + galactose
Sucrose	Sugar cane, sugar beets	Glucose + fructose

In a disaccharide, two monosaccharides form a **glycosidic** bond with the loss of water. For example, in maltose, two glucose units are linked by an α-1,4-glycosidic bond.

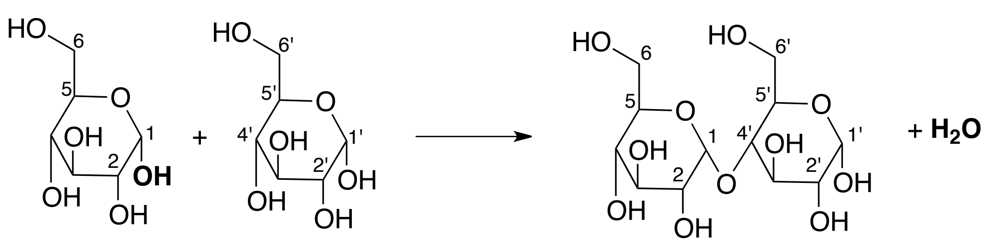

Figure 4. Condensation of glucose into α-Maltose.

Polysaccharides are long-chain polymers that contain many thousands of monosaccharides (usually glucose units) joined together by glycosidic bonds. Three important polysaccharides are starch, cellulose, and glycogen. These examples all contain glucose units, but differ in the type of glycosidic bonds and the amount of branching in the molecule.

Starch is an insoluble storage form of glucose found in rice, wheat, potatoes, beans, and cereals. Starch is composed of two kinds of polysaccharides, amylose and amylopectin. Amylose, which makes up about 20% of starch, consists of α-D-glucose molecules connected by α-1,4-glycosidic bonds in a continuous chain (**Figure 5a**). A typical polymer of amylose may contain from 250 to 4000 glucose units.

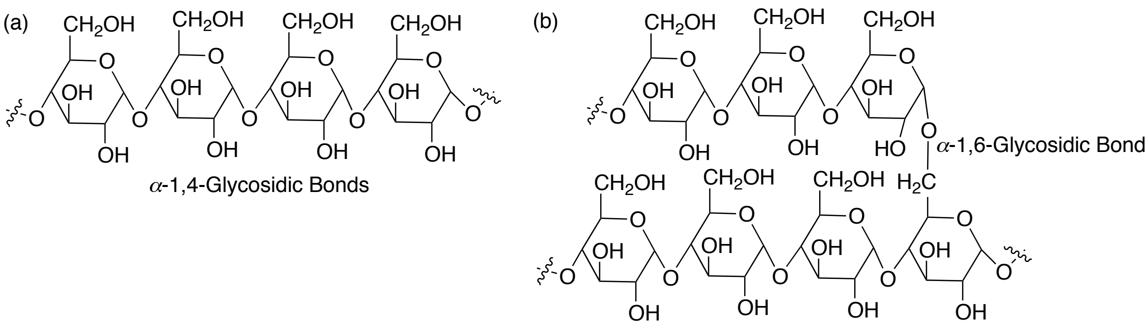

Figure 5. Portions of (a) amylose and (b) amylopectin.

Amylopectin is a branched-chain polysaccharide that makes up as much as 80% of starch. In amylopectin, α-1,4-glycosidic bonds connect most of the glucose molecules. However, at about every 25 glucose units, there are branches of glucose molecules attached by α-1,6-glycosidic bond (**Figure 5b**).

Cellulose is the major structural material of wood and plants. Cotton is almost pure cellulose. In cellulose, glucose molecules form a long unbranched chain similar to amylose except that β-1,4-glycosidic bonds connect the glucose molecules. The β isomers are aligned in parallel rows that are held in place by hydrogen bonds between the rows. This gives a rigid structure for cell walls in wood and fiber and makes cellulose resistant to hydrolysis.

Chemical Tests for Carbohydrates

The following chemical tests are used to distinguish between the different types of carbohydrates described above. You will be given a 2% solution of an unknown carbohydrate. The chemical tests will be run on the unknown alongside standards: glucose, fructose, sucrose, lactose, starch, and water as a control. *Students will work in pairs.* Use your observations to determine the type of carbohydrate in your unknown solution.

Notebook Preparation

Turn in with lab report (after 'experimentation' not before). Each of Parts A-D should start with a new notebook page containing the following:

- General *reaction scheme* for a positive test (given within procedure and/or lecture)
- *Reagent table* – read through procedure for list of chemicals; include amount (# of drops, mg, or mL), MW, bp or mp, and one-word hazards where applicable (refer to Clean-up & Safety Table at the end of the procedures). Provide properties for sugars, unknown, and water, only once then refer to previous pages in the following parts.
- *Procedure* (remote) – hand-drawn 'comic strip' with diagrams of all equipment and chemicals with amounts. Include pertinent notes from the Clean-up & Safety Table.
- *Data Table* – re-create tables shown after each procedure
- *Clean-up & Safety* – (copy pertinent notes from the Clean-up & Safety Table within procedure section)

A. Benedict's Test for Reducing Sugars

All of the monosaccharides and most of the disaccharides can be oxidized. When the cyclic structure opens, the aldehyde group is available for oxidation. Reagents such as Benedict's reagent contain a Cu^{2+} ion that is reduced. Therefore, all the sugars that react with Benedict's reagent are called reducing sugars. Ketoses also acts as reducing sugars because the ketone group on C2 isomerizes through two keto-enol tautomerizations to give an aldehyde group on C1. A solution of Benedict's solution contains copper (II) sulfate dehydrate, sodium carbonate, and sodium citrate dehydrate. Essentially, this is a basic copper solution. When oxidation of a sugar occurs, Cu^{2+} is reduced to Cu^{+}, which forms a red precipitate of copper (I) oxide (**Figure 6**). The color of the precipitate varies from green to gold to red depending on the concentration of the reducing sugar. Sucrose is an example of a disaccharide that is not a reducing sugar because it cannot revert to the open-chain form that would provide the aldehyde group needed to reduce the cupric ion.

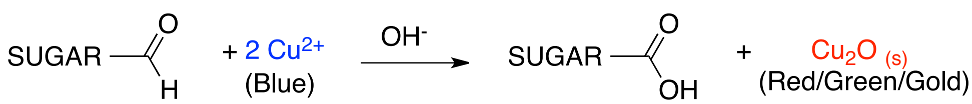

Figure 6. Benedict's test for reducing sugars.

PROCEDURE: Place 5 drops of solutions of glucose, fructose, sucrose, lactose, starch, the unknown, and water in separate test tubes. Label each test tube. Add 1 mL of Benedict's reagent to each sample. Place the test tubes in a boiling water bath for 3-4 minutes (careful the labels don't fall off!). Record your observations and classify each as a reducing (R) or non-reducing (NR) sugar.

Table A. Observations of Benedict's Test

Glucose	Fructose	Sucrose	Lactose	Starch	Water	Unknown

B. Seliwanoff's Test for Ketoses

Seliwanoff's test is used to distinguish between ketohexoses and aldohexoses. The reagents consist of resorcinol (3-hydroxyphenol) and hydrochloric acid. The acid causes the dehydration of the ketose to eliminate 3 equivalents of water. The dehydrated ketose reacts with resorcinol in another series of condensation reactions to form a highly conjugated molecule with a deep cherry red color (you can find this structure in the lecture notes). With ketoses, a red color is formed rapidly. Aldoses give a light pink color that takes a longer time to develop. The test is most sensitive for fructose, a ketohexose.

PROCEDURE: Place 5 drops of each carbohydrate solution in separate test tubes. Add 1 mL of Seliwanoff's reagent to each. *The reagent contains concentrated HCl – use carefully!* Place the test tubes in a boiling hot water bath (careful the labels don't fall off!) and note the time. After 1 minute, observe the colors in the test tubes. Record your observations (fast, slow, or no color change) along with whether the carbohydrate is a ketose (K) or not a ketose (NK).

Table B. Observations of Seliwanoff's Test

Glucose	Fructose	Sucrose	Lactose	Starch	Water	Unknown

C. Iodine Test for Polysaccharides

When molecular iodine is added to amylose, the helical shape of the unbranched polysaccharide traps iodine molecules, producing a deep blue-black complex. Amylopectin and cellulose react with iodine to give red to brown colors. Glycogen produces a reddish-purple color. Monosaccharides and disaccharides are too small to trap iodine molecules and do not form dark colors with iodine.

PROCEDURE: Using a spot plate, place 5 drops of each carbohydrate solution and water in the wells. Be sure to carefully label the wells. Add 1 drop of iodine solution to each sample. Record your observations and whether each is a polysaccharide (P) or not (NP).

Table C. Observations of Iodine Test

Glucose	Fructose	Sucrose	Lactose	Starch	Water	Unknown

D. Hydrolysis of Disaccharides and Polysaccharides

Disaccharides hydrolyze in the presence of an acid to give the individual monosaccharides. In the lab, we use water and acid to hydrolyze starches, which produce smaller saccharides such as maltose. Eventually, the hydrolysis reaction converts maltose to glucose molecules. In the body, enzymes in our saliva and from the pancreas carry out the hydrolysis. Complete hydrolysis produces glucose, which provides about 50% of our nutritional calories.

PROCEDURE: Place 3 mL of 2% starch in two test tubes and 3 mL of 2% sucrose solution in two more test tubes. If at this point, you suspect your unknown to be a disaccharide or polysaccharide, do the same with the unknown. To one sample each of sucrose and starch (and the unknown if applicable), add 20 drops of 10% HCl. To the other samples of sucrose and starch, add 20 drops of H_2O. Label the test tubes and heat in a boiling water bath for 10 minutes.

Remove the test tubes from the water bath and let them cool. To the samples containing HCl, add 10% NaOH until one drop of the mixture turns litmus paper blue (about 20 drops), indicating the HCl has been neutralized. Test the samples for hydrolysis using the iodine test and Benedict's test. Record your observations and determine whether hydrolysis occurred.

Table D. Observations of Hydrolysis Tests

Results	**Sucrose/H_2O**	**Sucrose/HCl**	**Starch/H_2O**	**Starch/HCl**	**Unk/H_2O**	**Unk/HCl**
Iodine test						
Benedict's test						

Table 1. Clean-up and Safety

Clean-up	**Safety**
Clean and return all shared glassware to reagent counters.	Wear gloves. Remove gloves before leaving.
Liquid waste: contents of test tubes – carefully transfer into waste using a pipet.	HCl, Benedict's reagent, Seliwanoff's reagent, and NaOH are corrosive
Wipe down benchtops and leave the lab just as your found it. Thank you!	Benedict's reagent, potassium iodide, and all sugars are irritants.
ALL STUDENTS IN ANY SECTION WITH GLASSWARE (INCLUDING PIPETS) IN ANY OF THE TRASHCANS WILL **LOSE 5 POINTS** FROM THEIR LAB REPORT	

Introduction: Pre-Lab Questions

These questions are incorporated into a Canvas quiz due the Monday before lab.

1. Provide the sugar classification and draw representations of D-glucose as indicated.
   (a) Fischer projection
   (b) Haworth projections: α-D-Glucopyranose and β-D-Glucopyranose

2. Provide the sugar classification and draw representations of D-fructose as indicated.
   (a) Fischer projection
   (b) Haworth projections: α-D-Fructofuranose and β-D-Fructofuranose

3. What are the specific criteria for a carbohydrate to be considered a reducing sugar?

4. Draw the structure of α-D-Maltose and show the complete equation for its hydrolysis with aqueous HCl. Indicate the type of glycosidic bond in maltose.

5. Briefly describe the expected observation in each chemical test for each sugar (re-create this table in your word processing document).

Table x. Expected Chemical Test Results

	A. Benedict	**B. Seliwanoff**	**C. Iodine**
Glucose			
Fructose			
Sucrose			
Lactose			
Starch			

6. What carbohydrate(s) would have the following test results?

   (a) Produces a reddish-orange solid with the Benedict's test and a red color with Seliwanoff's reagent in 1 minute.

   (b) Gives a color change with Benedict's test and a light orange color with Seliwanoff's reagent after 5 minutes.

   (c) Gives no color change with Benedict's or Seliwanoff's test, but turns a blue-black color with the iodine reagent.

EXPERIMENT 1 LAB REPORT

Abstract – See the *Technical Writing Guidelines* online. The main experimental objective is unknown determination. Describe the methods by chemical names of standards and chemical test name. Include only one or two test results (positive or negative for ___) that was most helpful in unknown determination. The conclusion sentence for each student should read: "The unknown sugar was determined to be _______."

In-Lab Questions - Typed responses in complete sentences turned in with the lab report. It is recommended that students write the in-lab questions into the notebook before lab.

1. Re-create and complete the following tables in a word processing document to summarize your results.

Table x. Expected Chemical Test Results

	A. Benedict	**B. Seliwanoff**	**C. Iodine**
Glucose			
Fructose			
Sucrose			
Lactose			
Starch			
Water			
Unknown: ______			

D. Hydrolysis Results

Results	**Sucrose/H_2O**	**Sucrose/HCl**	**Starch/H_2O**	**Starch/HCl**	**Unk/H_2O**	**Unk/HCl**
Iodine test						
Benedict's test						
Hydrolysis products present?						

2. Discuss the results of the Benedict, Seliwanoff, and iodine tests: List the reducing sugars, ketoses, and polysaccharides. Were results as expected?

3. How do the results of the Benedict's test performed after the hydrolysis test indicate that hydrolysis of sucrose and starch (and the unknown, if applicable) occurred?

4. How do the results of the iodine test indicate that hydrolysis of starch (and the unknown) occurred?

5. Provide the unknown code and identity of your unknown (glucose, fructose, sucrose, lactose, or starch). Summarize the results of the chemical tests for the unknown and how this was used to determine the identity of the unknown.

6. Go to pubs.acs.org and perform a search on any one of the tests from this experiment. *Choose any journal article* (not a book, website, or other type of source) and provide the citation in the standard ACS format (see **Technical Writing Guidelines** – pay attention to punctuation and what to put in **bold** or *italics*). Describe 2-3 new things you learned from that paper.