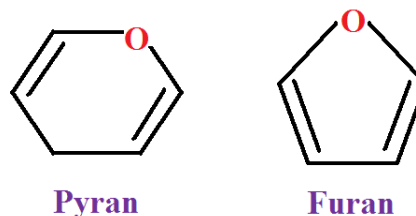


Carbohydrates

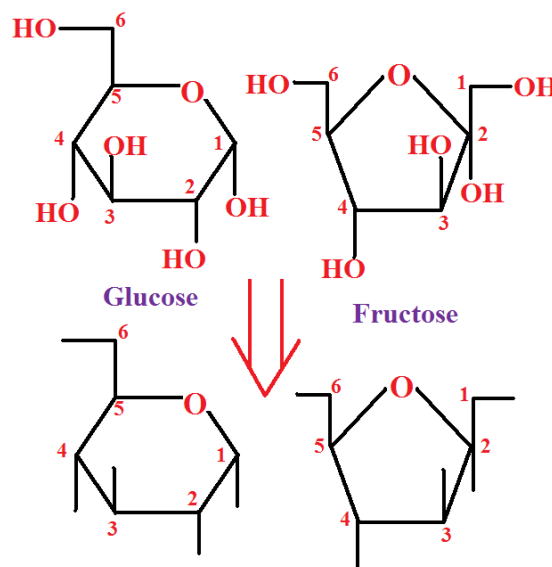
There are, for all intents and purposes, 2 classes of carbohydrates: pyranoses and furanoses ("ose" is the suffix for a sugar). These two classes are so called because their carbon skeletons are based off of pyran and furan (figure at right). Note that both pyran and furan each have an oxygen atom in the closed ring. At each corner is a carbon atom.



Carbohydrates are generally seen as sources of quick energy. They consist of carbon, hydrogen and oxygen. In the old days, they were named carboHYDRATES as the ratio of hydrogen to oxygen was thought to be 2:1. We now know differently, although the name has stuck throughout time. There are three categories of carbohydrates in which we have interest: monosaccharides, disaccharides and polysaccharides (saccharide is Greek for sugar).

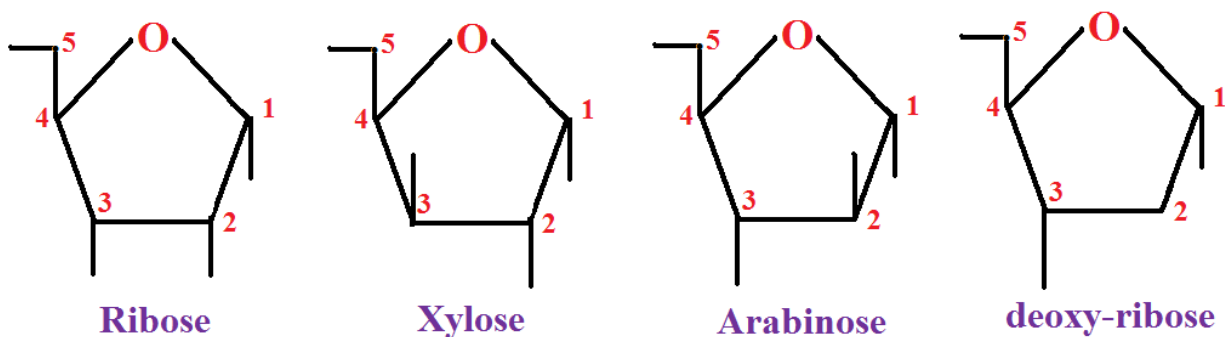
Haworth Projections

Two of the simplest carbohydrates are glucose and fructose, the sugar in our blood and fruit sugar. As you can see in the figure at right, both have unique geometric shapes in their base structures and each has a unique orientation of the -OH groups upon the base structure. Remember that at each corner, there is a carbon atom and that carbon takes 4 bonds. The hydrogen atoms that are by themselves are not shown but are understood to be present.



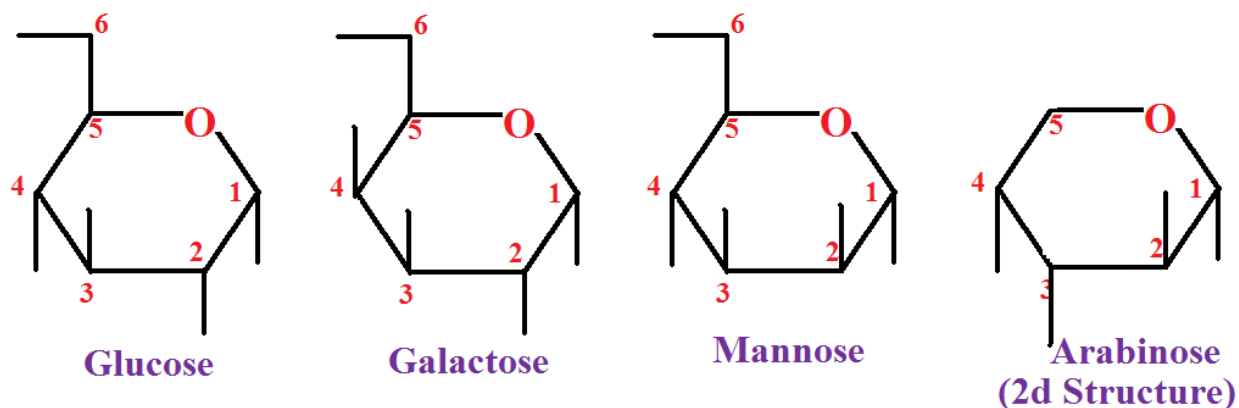
Biochemists developed a short-hand for quickly sketching carbohydrates called the Haworth projections. The graphics on the right of each of the above two sugars show the Haworth projections (bottom of right graphic), where the -OH's are replaced with lines going in the correspondingly correct directions. Each of the above carbohydrates has a base (fundamental or elementary) structure that contains one oxygen atom in its ring.

Glucose and fructose are both hexoses (6-carbon sugars); glucose is a pyranose and an aldose and fructose is a furanose and a ketose. The sugars are so called because their base ring structures are based upon furan and ketones and pyran and aldehydes. These two compounds



(furan and pyran, top previous page) provide the framework for numerous monosaccharides (single sugars).

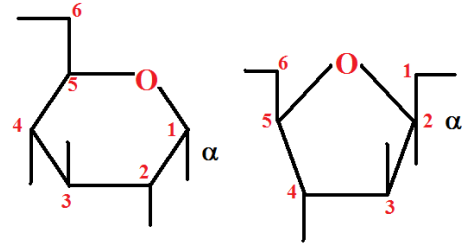
Four pentoses are pin the figure, top center, this page. Ribose is found in ribonucleic acid (RNA); xylose is wood sugar and is non-fermentable; arabinose is gum sugar -- it may sometimes be inconsistently found in urine. A form of ribose is found in deoxyribonucleic acid (DNA): deoxy-ribose. Ribose must lose the -OH on the 2' carbon to become deoxy-ribose. Note that ribose, xylose and arabinose are pentoses (5 carbon sugars). In addition, the proper names of the above furanoses are: D-ribofuranose, D-xylofuranose and D-arabinofuranose.



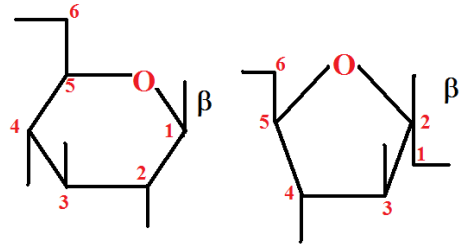
Four pyranoses are illustrated immediately above (Note the 2d arabinose structure – it can be either a furanose OR a pyranose!) Glucose, we've briefly discussed. Galactose is of significance in that it forms half of the disaccharide (double sugar) lactose (milk sugar). Mannose is a plant sugar. Note that these sugars differ in their geometric structure simply by the orientation of the -OH groups. Indeed, the orientation of the -OH groups -- only differing by the position of 1 - OH group -- changes the sweetness of the sugar.

To remember these sugars, I've got three mnemonics for you. They are based off the first 4 carbons (1,2,3,4) and #5 carbon has no -OH group on it and #6 carbon is always up and to the left for our purposes. To remember glucose, the mnemonic is DDUD: down, down, up, down.

This describes the orientation of the -OH groups on the first four carbons. Galactose is DDUU, while mannose is DUUD. The proper names for the first three of the above pyranoses are: D-glucopyranose, D-galactopyranose and D-mannopyranose.



We can go another step in naming monosaccharides based upon the position of the -OH on carbon #1 (#2 in fructose). This carbon is called the anomeric carbon. An anomer is a special type of epimer (a class of isomers that are non-superposable, non-mirror images of one another). It is an isomer of a saccharide in the cyclic form (Hayworth projection) that differs only in its configuration at the hemiacetal carbon in aldoses or hemiketal carbon in ketoses, which is also called the **anomeric carbon**.

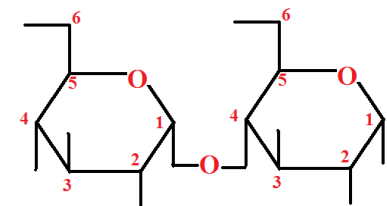


If the the hydroxyl group on the anomeric carbon is in the down position of glucose, then the sugar is an alpha (α) anomer. If, however, that hydroxyl group is in the up position, then the sugar is a beta (β) anomer. For example, α -D-glucopyranose and β -D-glucopyranose, the two cyclic forms of glucose, are anomers. Humans metabolize monosaccharides in the α -configuration. The above graphic illustrates glucose in both configurations.

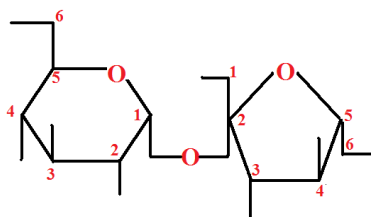
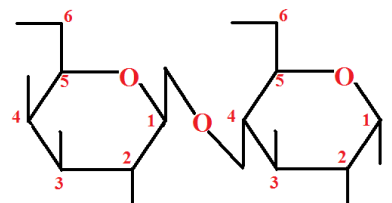
The Disaccharides

We are interested in only three of them:

Maltose (figure at immediate right) is also known as malt sugar; Maltose consists of two glucose molecules bonded together;



Lactose (at lower right) is milk sugar; Lactose consists of one molecule of galactose and one molecule of glucose – (pretty clever considering that young animals living on mother's milk use the glucose for quick energy and send the galactose to their livers where it will stored for future energy needs as



glycogen) – are bonded together.

Sucrose (at left) is table sugar. Sucrose consists of one molecule of glucose and one molecule of fructose bonded together.

Glycoside Bonds

The bonds that hold these sugars together are called glycoside bonds.

An oxygen atom between the first and 4th carbons of each respective glucose molecule (see above) connects the two glucose molecules linked together in maltose (previous page). Since the linkage is from an -OH group on the left glucose molecule that is in the α -configuration, this is called an α 1 to 4 link, or α 1 \rightarrow 4 link.

Since the linkage between the galactose molecule and the glucose molecule starts in the β -configuration and is also between the 1st and 4th carbons via an oxygen atom, this is called a β 1 \rightarrow 4 link, or β 1 \rightarrow 4 link.

The linkage between the glucose and fructose molecules in sucrose occurs through the 1st and 2nd carbons of glucose and fructose, respectively. This is an α 1 to 2 link, or α 1 \rightarrow 2 link.

Refer to the graphics on the previous page for clarification of the shapes of these bonds.

Remember, also, that there are NO carbons in the actual glycoside bond: ONLY an oxygen atom links the monosaccharides together to form the disaccharides.

The Polysaccharides

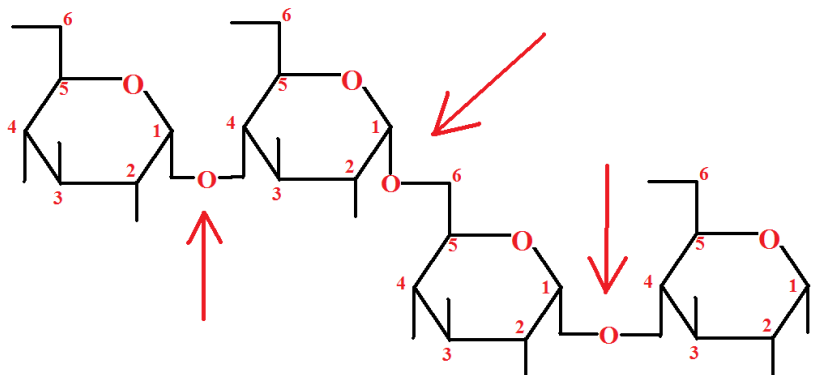
We are interested in three polysaccharides: starch, glycogen and cellulose.

Starch is found in PLANTS. Starch consists of two forms of complex carbohydrates: amylose and amylopectin.

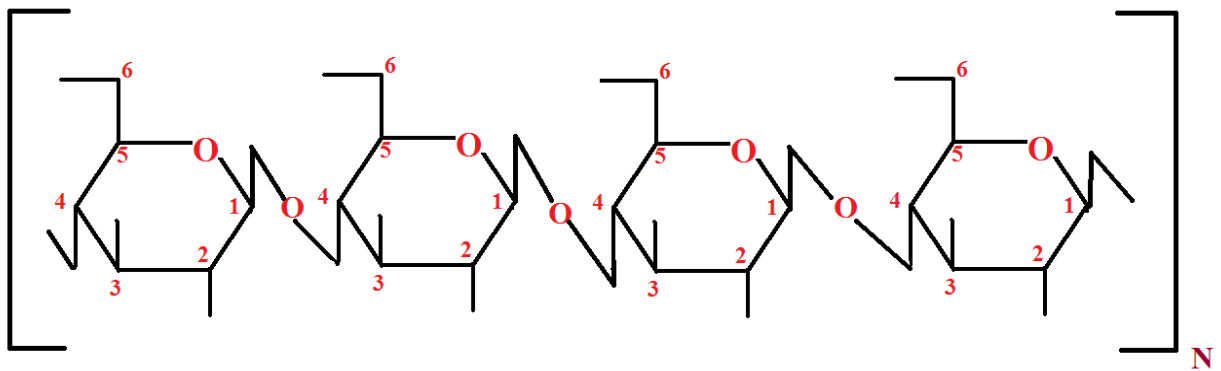
Amylose is the less abundant form and forms an α helix. Analytically, iodine "crawls" into the helix and forms inclusion compounds which turns a dark blue. Amylose is hydrolyzed by amylase in our mouths.

Amylopectin is the more abundant form in starch. Forms BOTH α 1 \rightarrow 4 and α 1 \rightarrow 6 links (shown in graphic below right)

and is similar to glycogen due to the branching caused by the α 1 \rightarrow 6 links. Amylopectin has lesser helix amount, hence less iodine binding; the color obtained is a red-violet color.



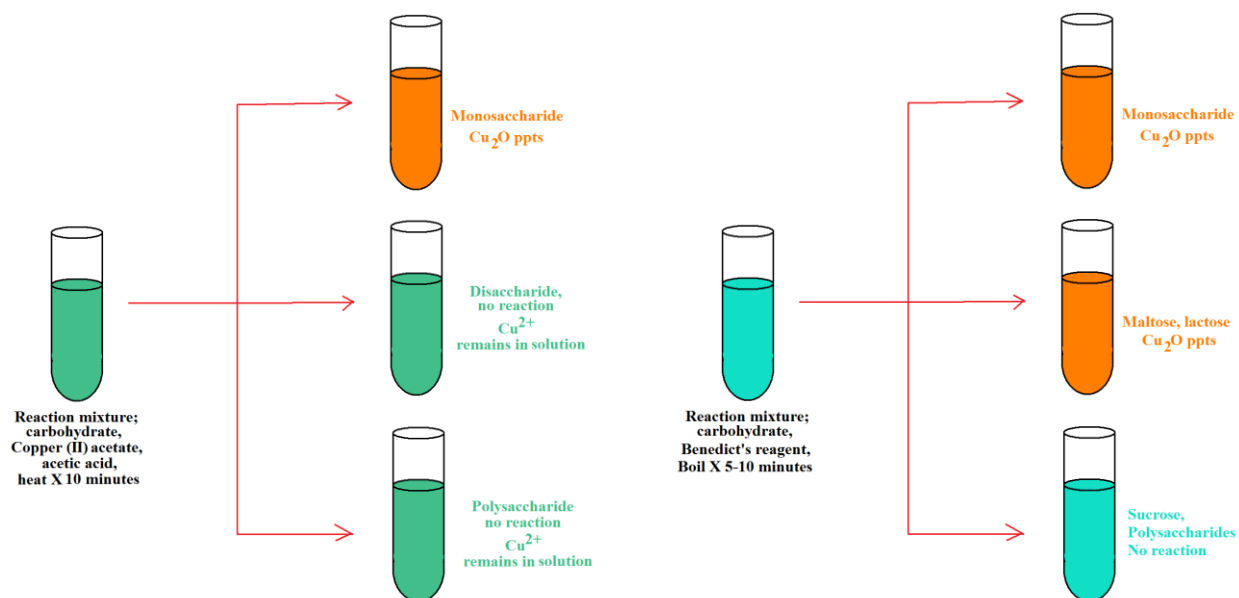
Glycogen is found in ANIMALS, specifically in the skeletal muscle and liver of animals. It is also found in fetal hearts and fetal lungs. Fetal hearts run off glycogen while adult hearts run off lipids. The glycogen in fetal lungs is necessary to form surfactant to make oxygen passage into the body from the lungs easier when the fetus is in room air rather than the womb. Glycogen branches due to the same sort of linkages found in amylopectin (α 1 \rightarrow 6 links). Having both 1 to 4 and 1 to 6 links lets the glycogen molecule become very dense and be very efficient for storage. Approximately 1/3 of the weight of the human liver is glycogen. The branches that are formed are then "de-formed" by an enzyme called the "debranching enzyme" when glycogen is needed for energy. Although glycogen has some helix, it is more like amylopectin: it forms less inclusion compounds with iodine. The color obtained is amber red and may be stabilized with the addition of the dihydrate of calcium chloride. Each glycogen molecule contains approximately 100,000 molecules of glucose per molecule of glycogen.



Cellulose, our last carbohydrate (image above), is a bit different. In order to hold the glucose molecules together, they are linked by β 1 \rightarrow 4 linkages. Humans can not metabolize these β links, while ruminants can. Ruminants digest cellulose because they have bacteria in their stomachs that contain the enzyme cellulase that hydrolyzes these links. Cellulose is the most abundant carbohydrate on earth; humans utilize it as dietary fiber.

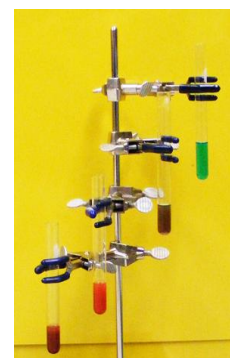
Reducing Sugars

Reducing Sugars are defined as follows: ANY carbohydrate which can reduce an alkaline solution of Cu(II); ALL monosaccharides ; Lactose and Maltose; NOT sucrose!!!!!! IN GENERAL: if a free -OH is on the **ANOMERIC CARBON**, the carbohydrate is a reducing sugar.



The Barfoed test is used to differentiate BETWEEN mono- and disaccharides. In acid solution, Cu(II) is a WEAKER oxidizing agent. If the solutions are heated for > 10 minutes, false positive results will be obtained. Only mono-saccharides give the positive cuprous oxide, orange, precipitate, figure top left.

Benedict's test (an alkaline solution of Cu(II), figure top right, this page) is the basis for the Clinitest: urine test for urinary sugar excretion in diabetes. As a rule, if the solution obtained from the Clinitest is green that means there is NO glucose in the patient's urine. If, however, the solution is Brick Red, that means there is more than 2 g glucose/100 mL urine. Typical results for fructose, glucose, lactose, sucrose from bottom of left image going up are illustrated at right.

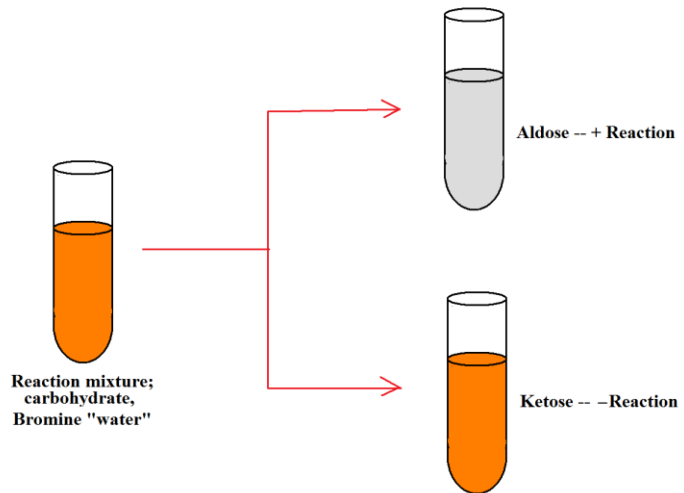


Tollen's test (alkaline solution of Ag(I) with ammonia) is also known as the silver mirror test because reducing sugars reduce the ammoniacal silver (I) to elemental silver that plates out as a mirror on the walls of the test tube (image at left shows sucrose, lactose, glucose and fructose reactions from top to bottom). Why, though, is ALKALINE so important? Alkaline Conditions favor ENEDIOL formation of BOTH glucose and fructose.



How does this work? Base (alkali) catalyzes enediol formation. Classically, aldehydes, but NOT ketones react with Tollen's Reagent. This is due to TAUTOMERIZATION, therefore, fructose CAN react with Tollen's Reagent.

Tautomerization is simply a rapid reorientation of isomers with movement of an “H” and a double bond. Fructose (keto) and Eneiol (enol) are tautomers. Hence, ketoses react with Benedict’s, Fehling’s and Tollen’s reagents as well as do aldoses, anyways. To reiterate: Ketose → Eneiol ← Aldose .



Bromine water (image at top right) oxidizes aldoses but NOT ketoses. Note that “bromine water” does NOT

refer to bromine dissolved in water. It refers to the state that the bromine is purchased in: a liquid that resembles amber colored “water”.

Physiological Enzymology of Carbohydrates

In terms of catabolic enzymes that drive carbohydrate digestion, we’ve already mentioned α -amylase for hydrolyzing amylose. Three enzymes in the small bowel, maltase, lactase and sucrose hydrolyase, respectively to their respective monosaccharides for easier uptake. The pancreas secretes its own amylase into the small bowel to handle any amylose that made it past the oral amylase OR to handle amylose from those people who are deficient in orally-derived α -amylase ... a sort of “back-up” enzyme.

Experimental: Supplies

Disposable test tubes	Disposable pipets	Beaker for water bath
Vortex	2 rings -- identical	Ring stand
Glucose	Fructose	Sucrose
Lactose	Benedict’s Reagent	Tollen’s Reagent
Bunsen burner and tubing	<u>GOGGLES!!!!!!!!!!!!!!!!!!!!</u>	

Preparation of Benedict’s Reagent: add 173 grams of sodium citrate and 100 grams of sodium carbonate to 800 mL warm water. Mix well, then filter. Bring the volume up to 850 mL with water. At the same time, add 17.3 grams copper sulfate to 100 mL water. Add enough water to this last solution to make up to 150 mL total volume. Slowly mix the two solutions together with stirring. Dispense as directed, below.

Preparation of Tollen's Reagent: mix equal parts of 10% AgNO_3 with 10% NaOH . Add enough ammonia to dissolve the Ag_2O precipitate that forms. Dispense as directed, below.

CAUTION!!!!!! This solution (Tollen's Reagent) MUST be acidified and completely and correctly disposed of after use: it is HIGHLY explosive when dried out!

Benedict's Test

Obtain 4 test tubes. Label them. Place about a quarter the size of a small pea of glucose, fructose, sucrose or lactose into the appropriately labeled tube, e.g., tube #1 has glucose, tube #2 has fructose, ad nauseum, in it. Add about 20 gtts water to each of the 4 tubes. Get your water bath boiling. When the bath is boiling, add 20 drops of Benedict's reagent to each of the four tubes and heat them to boiling, as well. Let boil for 5 minutes. Record your results below (orange is positive, blue is negative for reducing sugars):

Benedict's Test Results			
Glucose	Fructose	Sucrose	Lactose

Tollen's Test

Obtain 4 test tubes. Label them. Place about a quarter the size of a small pea of glucose, fructose, sucrose or lactose into the appropriately labeled tube, e.g., tube #1 has glucose, tube #2 has fructose, ad nauseum, in it. Add about 30 gtts of Tollen's Reagent to each of the 4 tubes. Vortex immediately, place on the lab bench and do not disturb for at least 15 minutes at room temperature. Observe and record the order of mirroring – or lack thereof – in the table below:

Tollen's Test Results			
Glucose	Fructose	Sucrose	Lactose

When you have completed your testing, dump the solutions down the sink with lots of water. Rinse your tubes out with water and put them in the rack for your professor to dispose of.

Which sugars are reducing sugars?

What was the order of mirroring, i.e., which tube mirrored first, last, never?

Using some source, e.g., library, web, find and draw the structures for 6 mono-saccharides.

Based upon your results in the Tollen's test, explain what happened in each tube based on the carbohydrate chemistry of each saccharide you used.