BIOCHEMISTRY 1

2ND CLASS

UNIVERSITY OF ANBAR
COLLEGE OF SCIENCE
BIOLOGY DEPARTMENT

2020-2021

Carbohydrates

Polysaccharides

Lecture Two(2)

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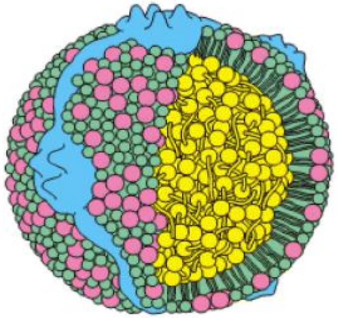
وزارة التعليم العالي والبحث العلمي
جامعة الموصل

الكيمياء الحياتية

الجزء الأول



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المرحلة الثالثة

تأليف
الاستاذة الدكتورة خولة احمد آل فليح

Textbook of Medical Biochemistry

Eighth Edition

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Harper's Illustrated Biochemistry

Twenty-Eighth Edition

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Principles of Biochemistry

Geoffrey L. Zubay
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William W. Parson
University of Washington

Dennis E. Vance
University of Alberta

WCB Wm. C. Brown Publishers
Dubuque, Iowa • Melbourne, Australia • Oxford, England

References:

Harper's Illustrated Biochemistry

Lippincott Biochemistry

Lehninger Principles of Biochemistry

Stryer Biochemistry

SYLABUSE

1- Carbohydrates

2- Amino Acids, Peptides and Proteins.

3- Lipids.

4- Enzymes.

5- Vitamins and Coenzymes.

6- Nucleotides and Nucleic acids.

7- Biological Oxidation.

Carbohydrates

Major Concepts

A. What are carbohydrates? Their general properties and biomedical importance.
B. List the monosaccharides of biological importance and learn their properties.
C. List the disaccharides of biological importance and learn their properties.
D. Study the chemistry and properties of various polysaccharides.

Learning outcomes

- To know the formation of carbohydrates
- To understand the nature of glycosidic bonds
- To understand the structural organisation of carbohydrates
- To appreciate the various functions of carbohydrates

Polysaccharide

- **Polysaccharides** are long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds.
- They range in structure from linear to highly branched.
- Examples include storage polysaccharides such as starch and glycogen, and structural polysaccharides such as cellulose and chitin.
- Polysaccharides have a general formula of $C_x(H_2O)_y$ where x is usually a large number between 200 and 2500.
- the general formula can also be represented as $(C_6H_{10}O_5)_n$

- Polysaccharides (glycans) serve as stored fuel and as structural components of cell walls and extracellular matrix.

- The homopolysaccharides starch and glycogen are stored fuels in plant, animal, and bacterial cells. They consist of D-glucose with linkages, and all three contain some branches.

TABLE 7–2 Structures and Roles of Some Polysaccharides

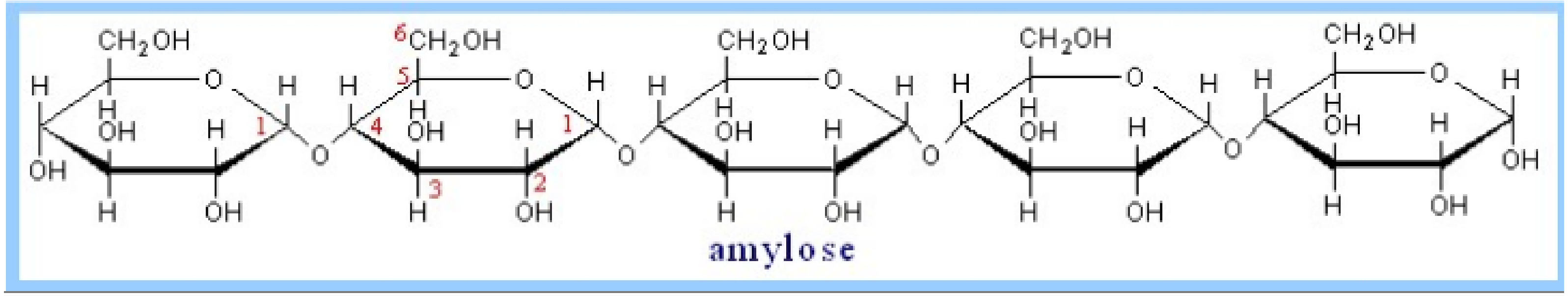
Polymer	*Type**	*Repeating unit†*	*Size (number of monosaccharide units)*	*Roles/significance*
Starch				Energy storage: in plants
Amylose	Homo-	(α1→4)Glc, linear	50-5,000	
Amylopectin	Homo-	(α1→4)Glc, with (α1→6)Glc branches every 24-30 residues	Up to 10^6	
Glycogen	Homo-	(α1→4)Glc, with (α1→6)Glc branches every 8-12 residues	Up to 50,000	Energy storage: in bacteria and animal cells
Cellulose	Homo-	(β1→4)Glc	Up to 15,000	Structural: in plants, gives rigidity and strength to cell walls
Chitin	Homo-	(β1→4)GlcNAc	Very large	Structural: in insects, spiders, crustaceans, gives rigidity and strength to exoskeletons
Dextran	Homo-	(α1→6)Glc, with (α1→3) branches	Wide range	Structural: in bacteria, extracellular adhesive
Peptidoglycan	Hetero-; peptides attached	4)Mur2Ac(β1→4) GlcNAc(β1	Very large	Structural: in bacteria, gives rigidity and strength to cell envelope
Agarose	Hetero-	3)D-Gal(β1→4)3,6-anhydro-L-Gal(α1	1,000	Structural: in algae, cell wall material
Hyaluronate (a glycosaminoglycan)	Hetero-; acidic	4)GlcA(β1→3) GlcNAc(β1	Up to 100,000	Structural: in vertebrates, extracellular matrix of skin and connective tissue; viscosity and lubrication in joints

Starch

- The reserve carbohydrate of plants. Occurs as granules in the cell. Made of amylose and amylopectin.

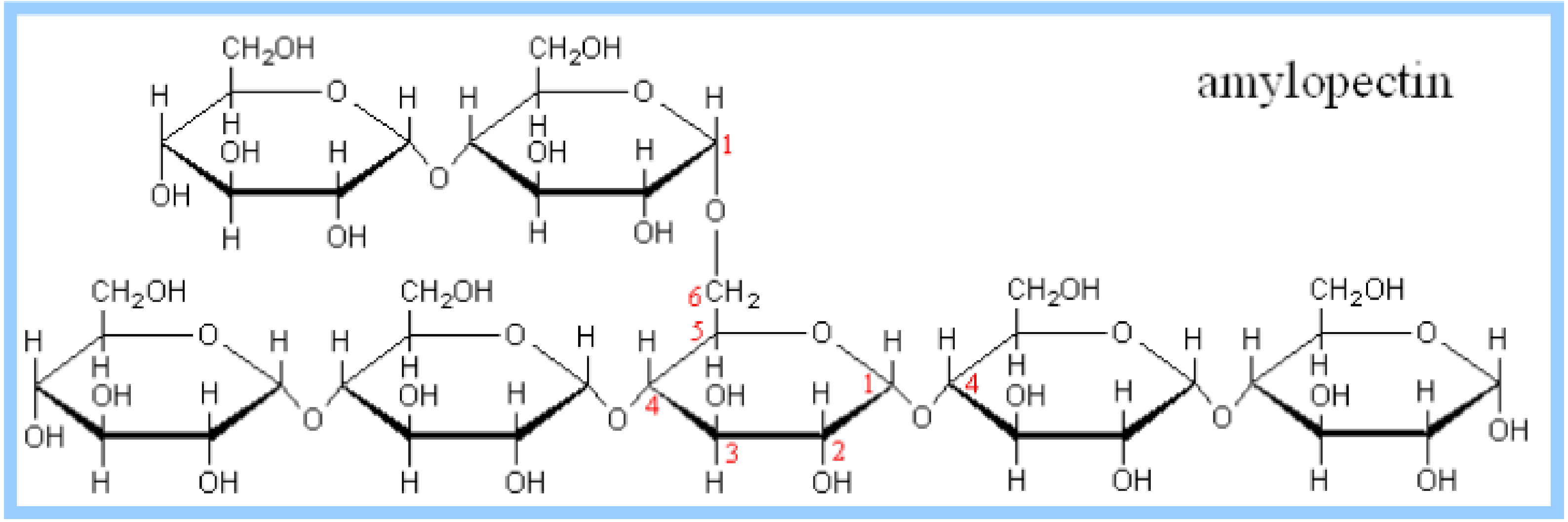
- **Amylose** is a glucose polymer with **$\alpha(1\rightarrow4)$** linkages.

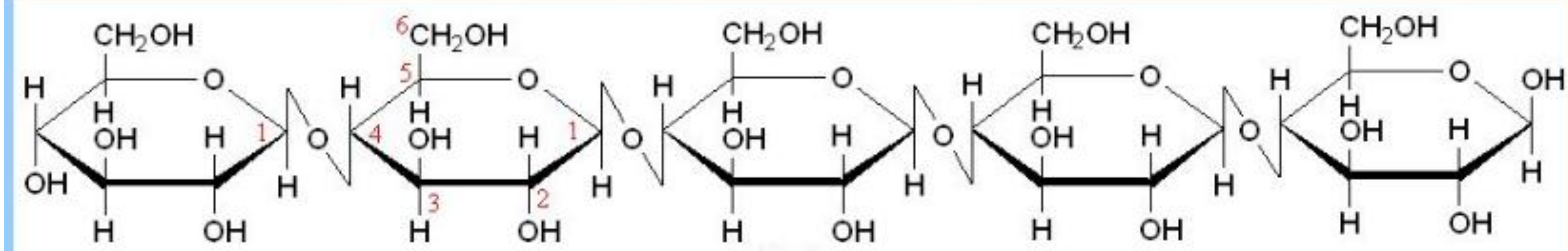
- The end of the polysaccharide with an anomeric C1 not involved in a glycosidic bond is called the **reducing end**.



• **Amylopectin** is a glucose polymer with mainly α(1→4) linkages, but it also has **branches** formed by α(1→6) linkages. Branches are generally longer than shown above.

• The branches produce a compact structure & provide multiple chain ends at which enzymatic cleavage can occur.

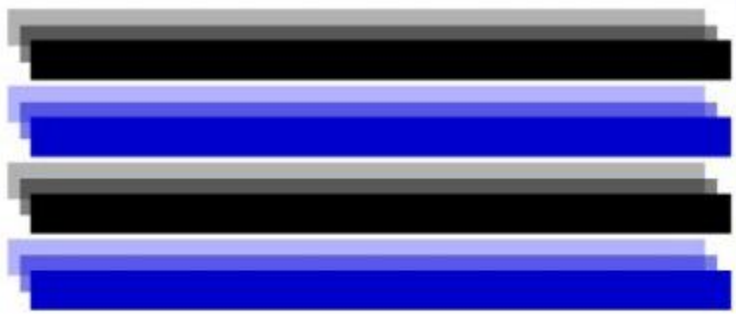




Cellulose, a major constituent of plant cell walls, consists of long linear chains of glucose with β(1→4) linkages.

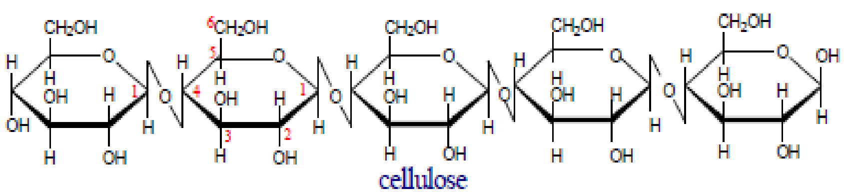
Every other glucose is flipped over, due to β linkages.

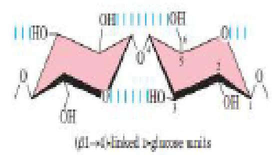
This promotes intra-chain and inter-chain H-bonds and van der Waals interactions, that cause cellulose chains to be straight & rigid, and pack with a crystalline arrangement in thick bundles - microfibrils.

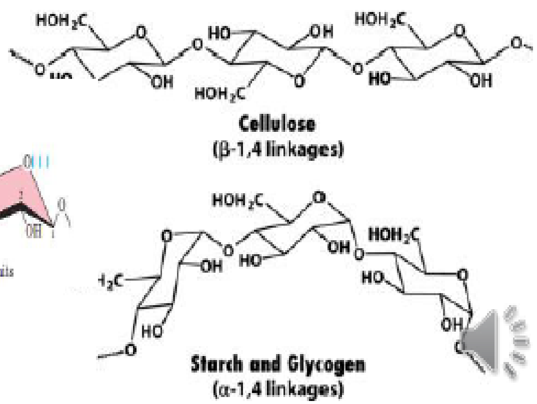


Schematic of arrangement of cellulose chains in a microfibril.

- **Cellulose** is a polysaccharide of glucose found in **plants**, consists of linear chains of glucose units. It is an **unbranched polymer** of glucose residues joined by **β-1,4 linkages**.
- The β configuration allows cellulose to form very long, straight chains. Fibrils are formed by parallel chains that interact with one another through hydrogen bonds.
- The α-1,4 linkages in glycogen and starch produce a very different molecular architecture from that of cellulose. A hollow helix is formed instead of a straight chain.
- These differing consequences of the α and β linkages are biologically important. The straight chain formed by β linkages is optimal for the construction of fibers having a high tensile strength.
- **Mammals lack cellulase** and therefore cannot digest wood and vegetable fibers.







Glycogen

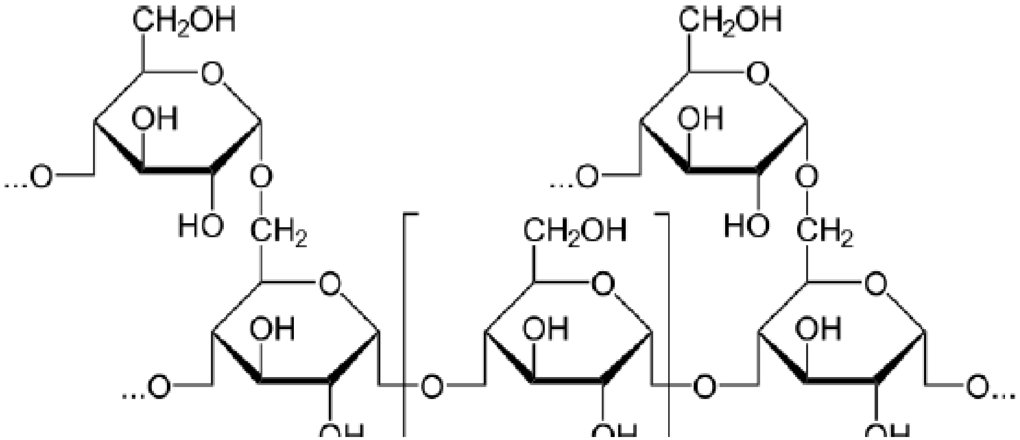
Having a similar structure to amylopectin of starch, but more branches. and is commonly referred to as animal starch.

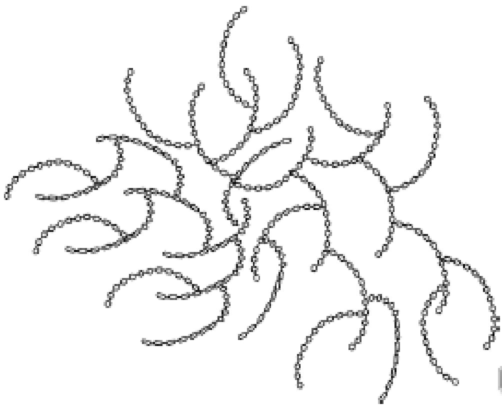
Glycogen does not possess a **reducing end**.

The „reducing end" glucose residue is not free but is covalently bound to a protein termed **glycogenin**

Main storage of glucose in **liver and skeletal muscle.**

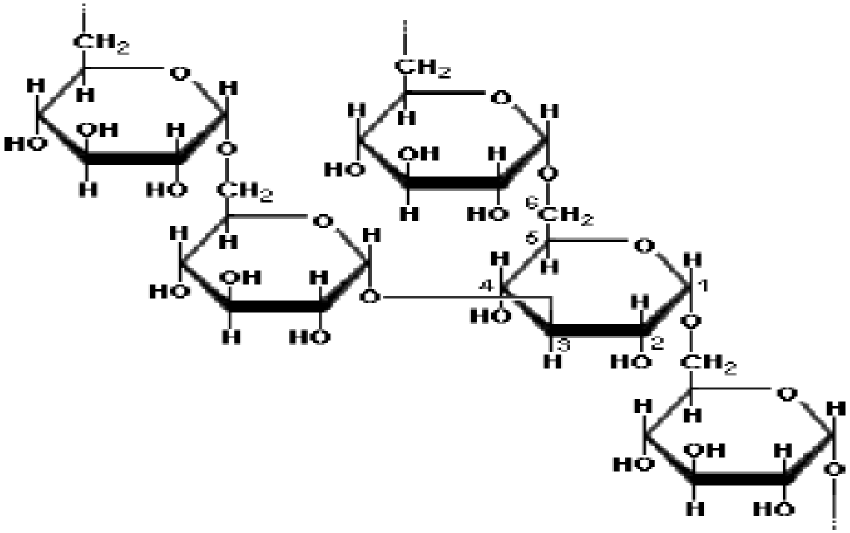
The glycogen granules contain both glycogen and the enzymes of glycogen synthesis (**Glycogenesis**) and degradation (**Glycogenolysis**).





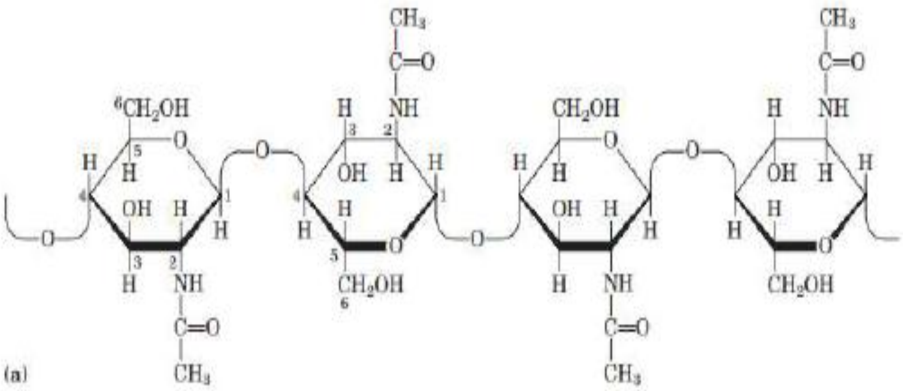
Dextrans

- **Dextrans** are bacterial and yeast polysaccharides made up of **(α1→6)-linked poly-D-glucose**; all have **(α1→3)** branches, and some also have **(α1→2) or (α1→4) branches**.
- Dental plaque, formed by bacteria growing on the surface of teeth, is rich in dextrans. Synthetic dextrans are used in several commercial products (for example, Sephadex) that serve in the fractionation of proteins by size-exclusion chromatography.



Chitin

- (a) A homopolymer of N-acetyl-D-glucosamine units in **β-1,4** linkage, strengthens the exoskeletons of arthropods
- (b) A spotted beetle (Pellidnota punetatia), showing its surface armor (exoskeleton) of chitin.



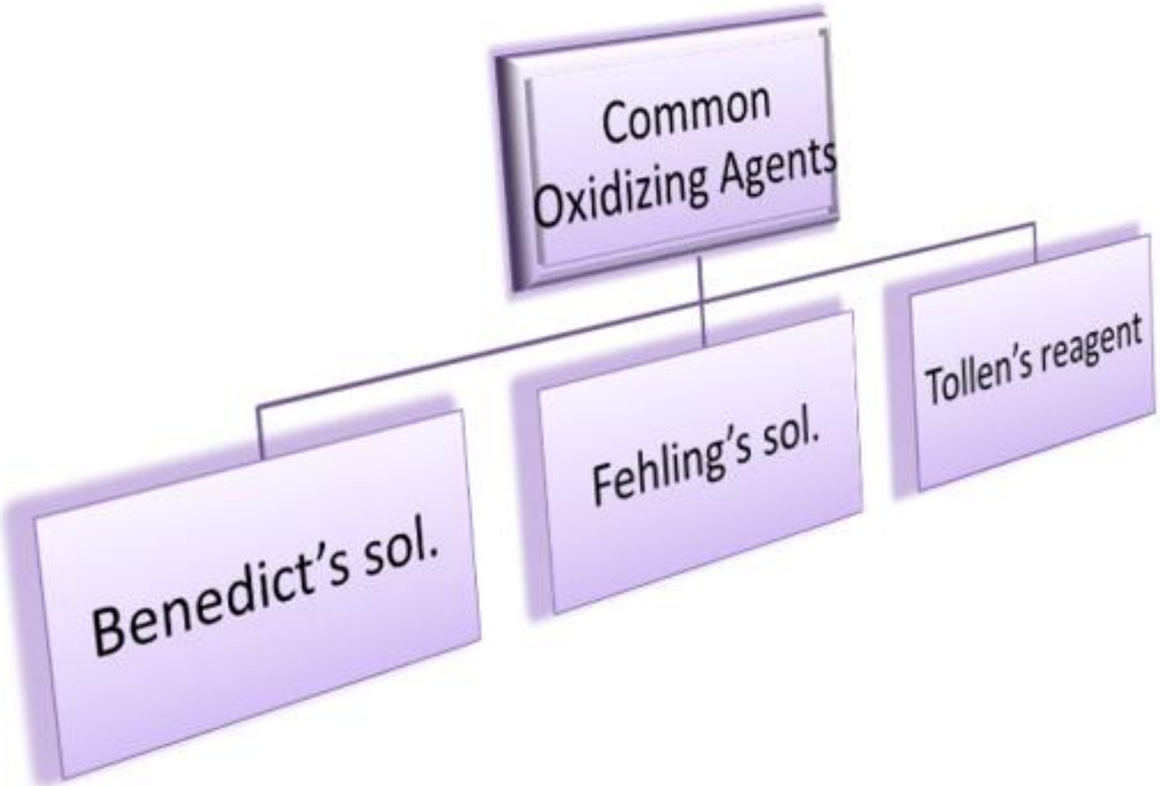
(a)

(b)

Classification upon reducing end

Reducing sugars
- All monosaccharides
- Maltose, Lactose

Non-reducing sugars
- Sucrose
- All polysaccharides


Common
Oxidizing Agents
Benedict's sol.
Fehling's sol.
Tollen's reagent

Oxidizing Reagent	Benedict's Solution	Fehling's Solution	Tollen's Reagent
Composition	copper sulfate in alkaline citrate	copper sulfate in alkaline tartrate	silver nitrate in aqueous ammonia
Color of Solution	deep blue	deep blue	colorless
Color After Reaction with a Reducing Sugar	brick red precipitate $Cu_2O_{(s)}$	brick red precipitate $Cu_2O_{(s)}$	silver mirror forms $Ag_{(s)}$
Species Being Reduced (the oxidant)	Cu^{2+} $Cu^{2+} + e \rightarrow Cu^{+}$	Cu^{2+} $Cu^{2+} + e \rightarrow Cu^{+}$	Ag^{+} $Ag^{+} + e \rightarrow Ag_{(s)}$
Species Being Oxidized (the reductant)	reducing sugar oxidized to carboxylate	reducing sugar oxidized to carboxylate	reducing sugar oxidized to carboxylate

In-lab Experiments

- Benedict's Test For reducing sugars
- Barfoed's Test for monosaccharides
- Bial's (Orcinol) Test for pentoses
- Seliwanoff's Test for Ketoses
- Picric Acid Test For reducing sugars

Benedict's Test .1
(positive for reducing sugars)

- Principle:

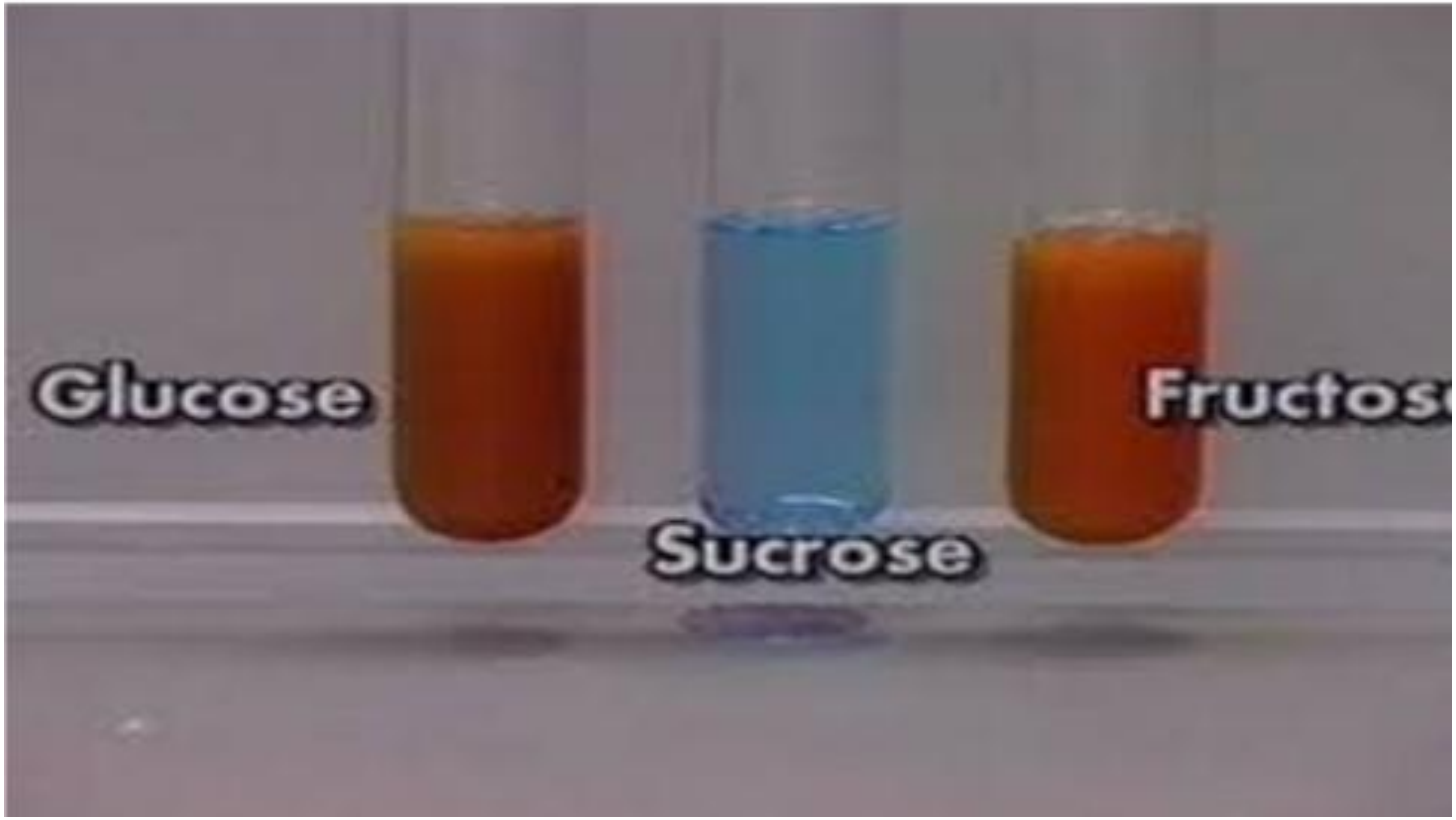
  Benedict's reagent contains cupric ions, which in an alkaline environment, oxidize the aldehyde group to a carboxylic acid. Cupric ions are reduced to cuprous oxide, which forms a red precipitate

  $RCHO + 2Cu^{2+} + 4OH^{-} \text{---->} RCOOH + Cu_2O + 2H_2O$

Procedure

1. Place 1mL of the following 1% carbohydrate solutions in separate, labeled test tubes: glucose, fructose, sucrose, lactose, maltose, and starch.
2. Also place 1 ml of distilled water in another tube to serve as a control.
3. To each tube, add 1 ml of Benedict's reagent and heat the tubes in a boiling water bath for 5 minutes.
4. Remove the tubes from water bath. Note and record the results.

- In the presence of a reducing sugar a precipitate which may be red, yellow or green will form.


Glucose
Sucrose
Fructos

Barfoed's Test .2

(Used to distinguish between mono- & di-saccharides)

- Principle

  Barfoed's reagent reacts with mono-saccharides to produce cuprous oxide at a faster rate than disaccharides do:

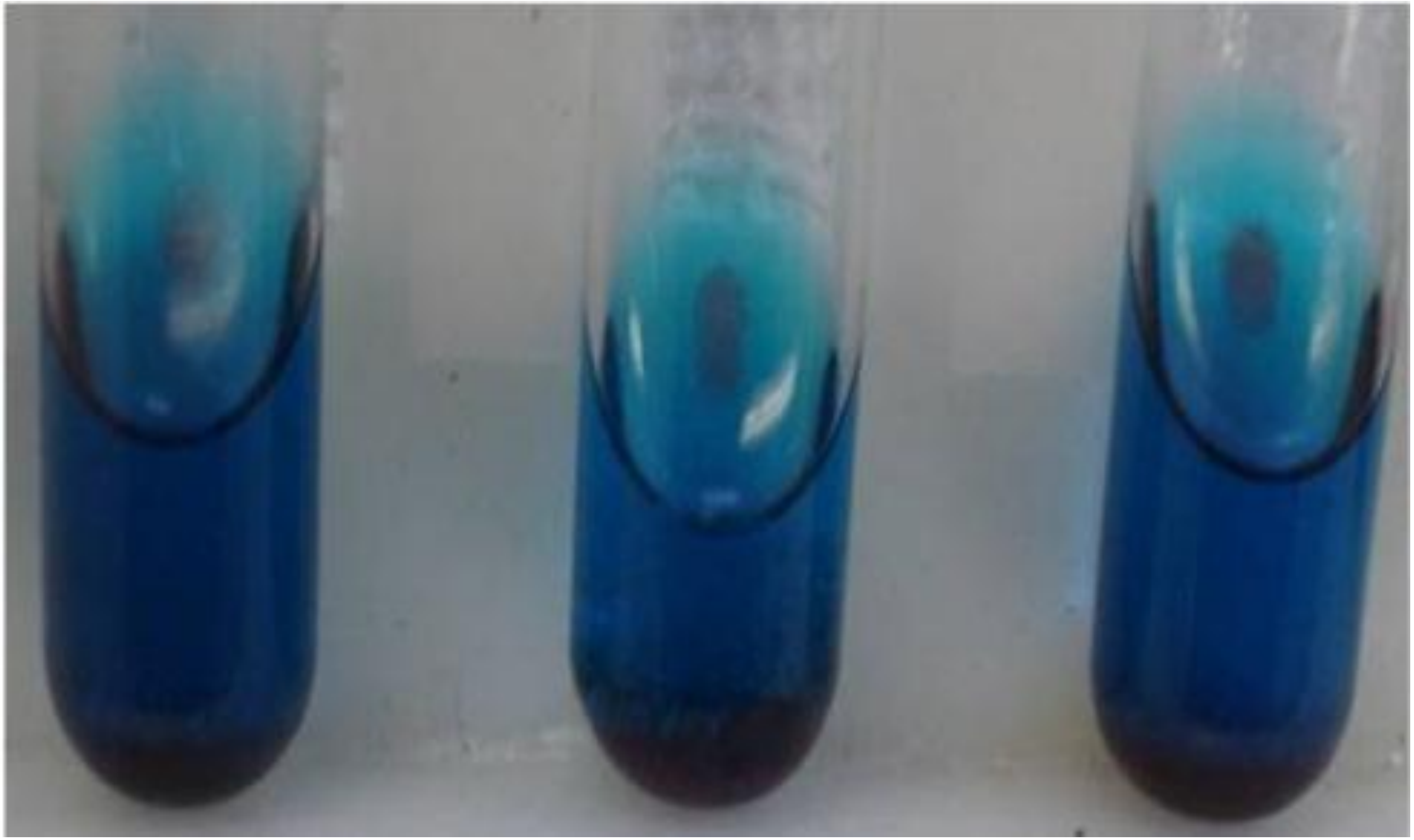
- $RCHO + 2Cu^{2+} + 2H_2O \dashrightarrow RCOOH + Cu_2O + 4H^+$

Procedure

- **Procedure:**

1. Place 1 mL of the following 1% carbohydrate solutions in separate, labeled test tubes: glucose, fructose, sucrose, lactose, and maltose.
2. To each tube, add 1 ml of Barfoed's reagent, and heat in a boiling water bath for 10 minutes.
3. Remove the tubes from water bath. Note and record your observations.

❖ A red precipitate will form if the test is positive.

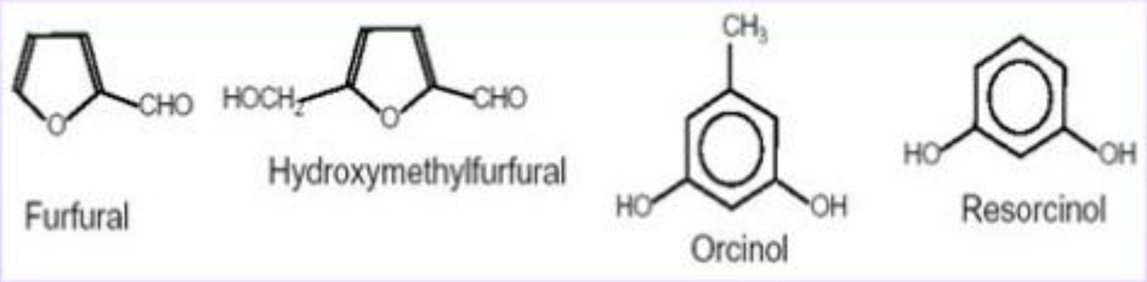


Bial's (Orcinol) Test for pentoses .3

(for the detection of pentoses)

Principle

- Pentoses are converted to furfural by this reagent, which forms a blue green color with orcinol.

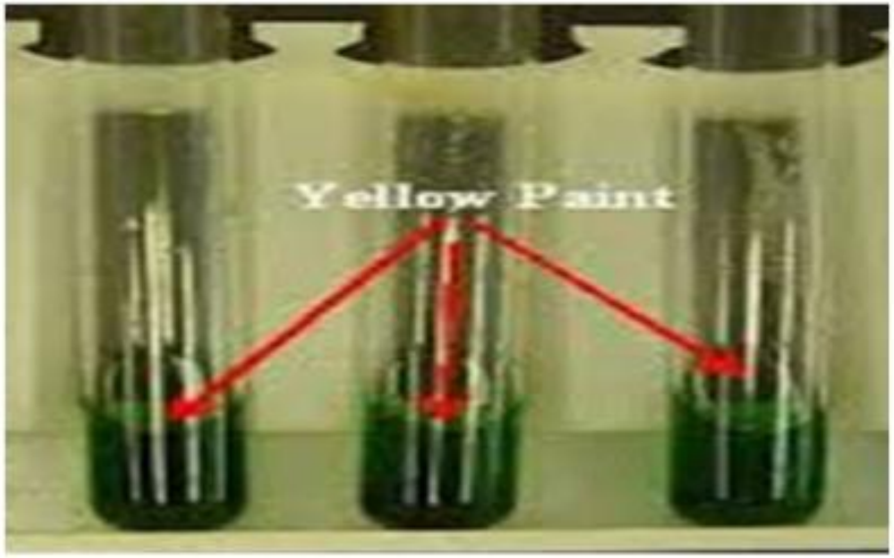


Procedure

1. Add about 1 ml of 1% xylose, glucose, fructose, maltose, arabinose, and xylose solution to their respective labeled test tubes.
2. Add 1.5 ml of Bial's reagent to each tube and mix well.
3. Carefully heat each tube (with some agitation) directly over the burner flame. Hold the tube at a diagonal and heat along the sides of the tube rather than at the bottom to prevent eruption of the liquid from the tube. Move the tube diagonally in and out of the flame, until the mixture just begins to boil. Stop heating when the mixture begins to boil.

- A blue-green color indicates a positive result. Prolonged heating of some hexoses yields hydroxymethyl furfural which also reacts with orcinol to give colored complexes.

Bial's reagent (0.1 % orcinol in concentrated HCl containing 0.1 % $FeCl_3.6H_2O$).



Seliwanoff's (Resorcinol) Test .4

(used for detection of Ketoses)

- Principle

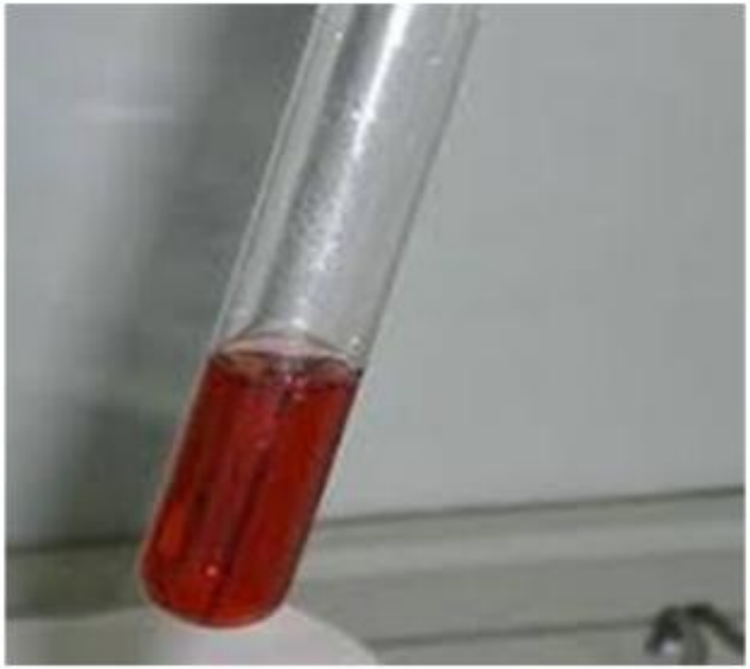
Ketohexoses (such as fructose) and disaccharides containing a ketohexose (such as sucrose) form **a cherry-red** condensation product. Other sugars (e.g. aldose) may produce yellow to faint pink colors.

Procedure

1. Add about 3 ml of Seliwanoff's reagent to each labeled test tube.
2. Add 1 drop of the respective sugar solution to the appropriate test tubes, and mix well.
3. Place all the test tubes in the boiling water bath at the same time and heat for 3 min after the water begins to boil again. Record your observations.

❖ A positive result is indicated by the formation of a red color with or without the separation of a brown-red precipitate.

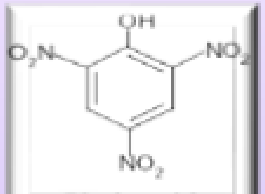
Seliwanoff's reagent (0.5 % resorcinol in 3N HCl).



Picric Acid Test .5
(for reducing sugars)

- Principle

Picric acid (2,4,6-trinitrophenol) or TNP reacts with reducing sugars to give a red colored picramic acid $C_6H_2.OH.NH_2(NO_2)_2$



Picric acid

Procedure

1. Into a test tube add 1 ml of maltose solution, into the second tube, 1ml of sucrose solution.
2. Add into each tube 1 ml of a saturated solution of picric acid, and then add into each tube 0.5 ml of sodium hydroxide solution.
3. Heat both samples in a boiling water bath.

- In the presence of reducing sugars, the solution stains red; a sodium salt of picric acid is formed.

References:

Harper's Illustrated Biochemistry

Lippincott Biochemistry

Lehninger Principles of Biochemistry

Stryer Biochemistry

THANKS FOR LISTENING