Carbohydrates

Definition: The term carbohydrates are polyhydroxy aldehydes or ketones having general formula $C_n(H_2O)_n$ commonly known as sugars. Carbohydrates are produced from CO_2 and H_2O by plants through the process of photosynthesis.

Importance:

❖ Carbohydrates are the major food supply and dietary energy source required for various metabolic activities. It also serves as stored energy source as glycogen in animals and starch in plants.

❖ They form structural and protective components, like in the cell wall of plants and microorganisms. In animals, they are an important constituent of connective tissues.

❖ Carbohydrates are intermediates in the biosynthesis of fats and proteins.

❖ Carbohydrates get associated with lipids (glycolipids) and proteins (glycoproteins) to form surface antigens, receptor molecules, vitamins, and antibiotics those are important in cell-cell communication and in interactions between cells and other elements in the cellular environment.

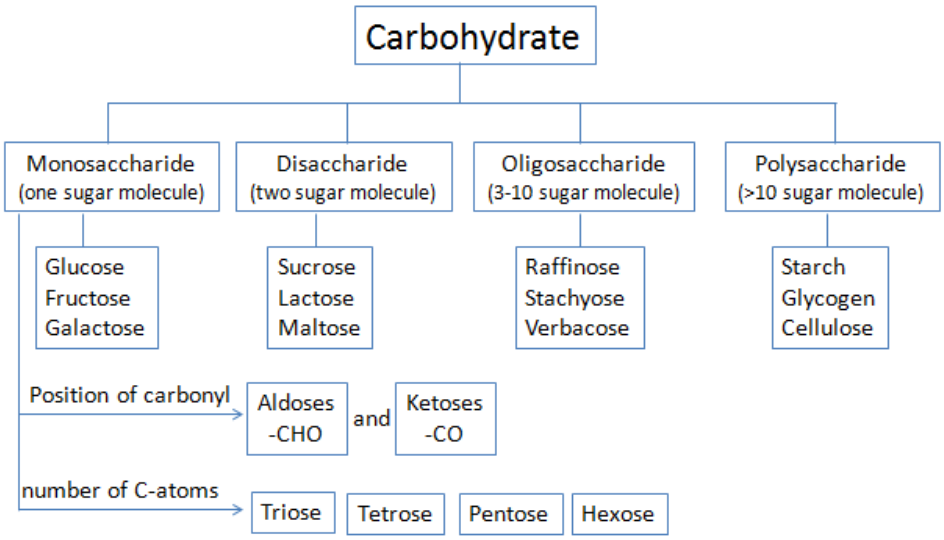
❖ They plays important role in formation of the structural framework of RNA and DNA.

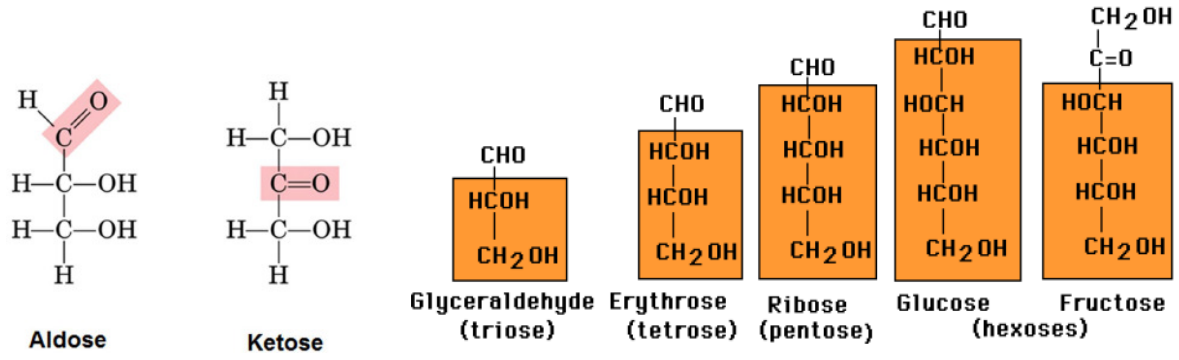
❖ Indigestible carbohydrates known as dietary fiber is beneficial to human nutrition, helping reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation. Carbohydrates also contribute to the sweetness, appearance and textural characteristics of many foods.

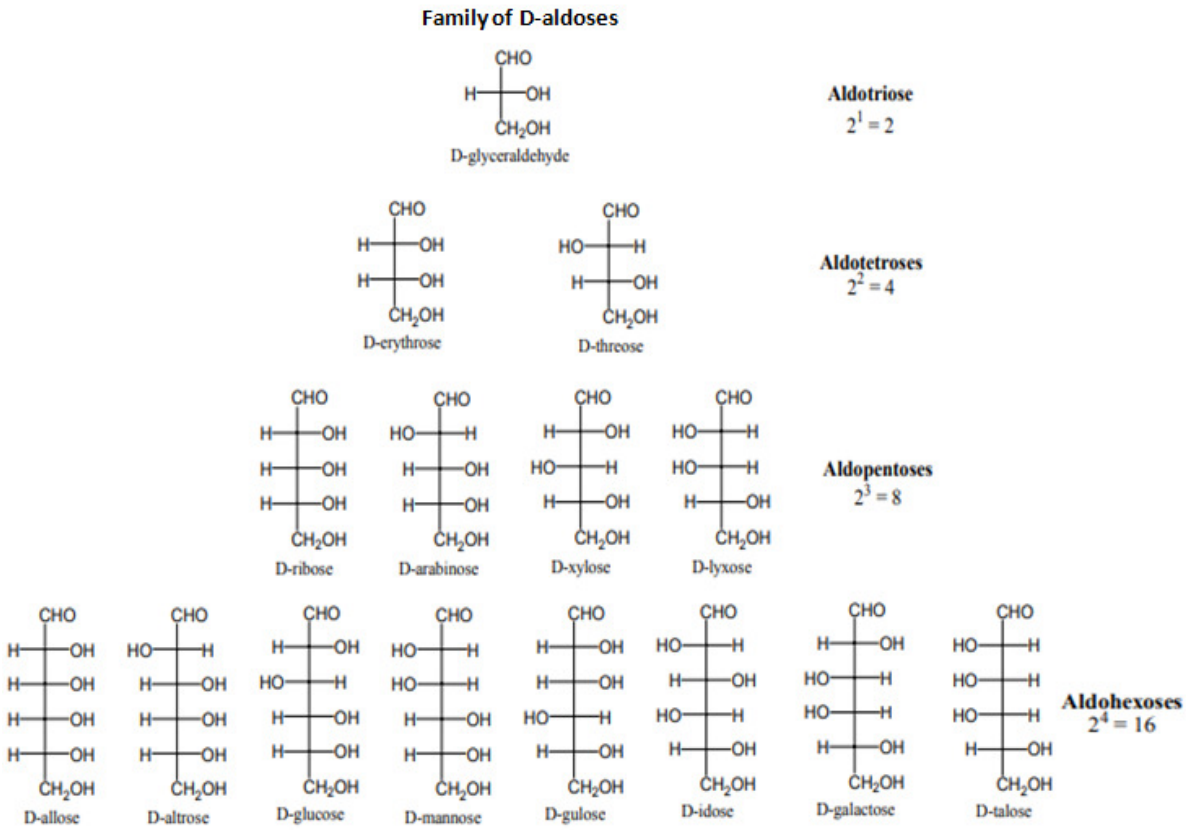
Classification of Carbohydrates

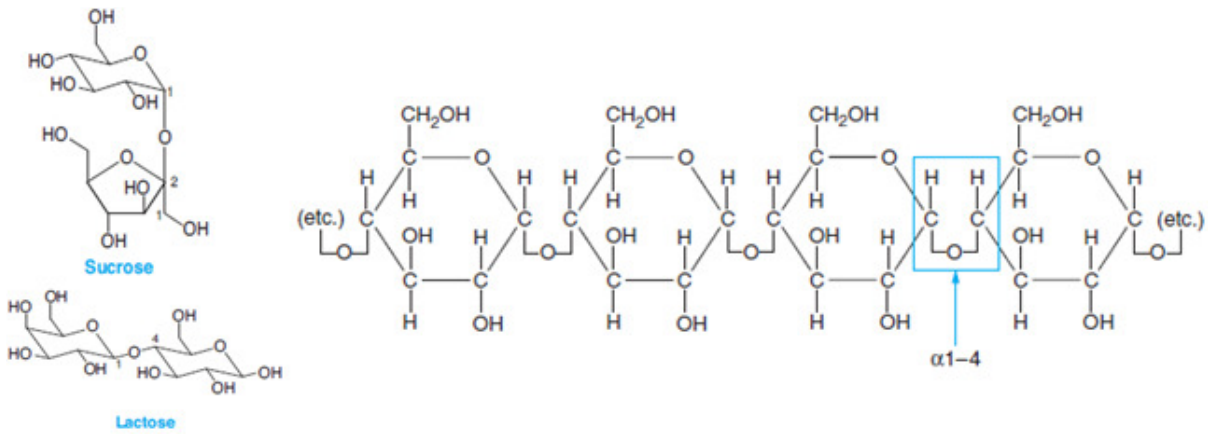
According to chemical composition:

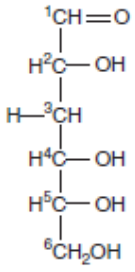
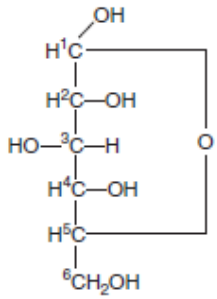
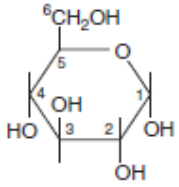
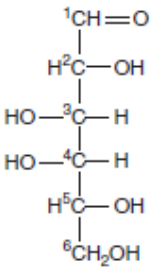
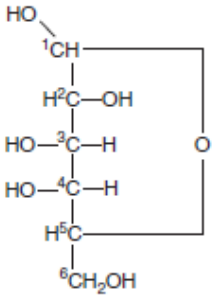
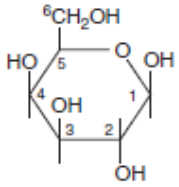
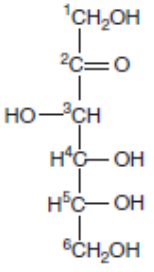
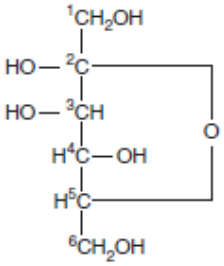
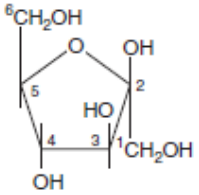
Simple carbohydrates, often called monosaccharides or simple sugars, contain one saccharide unit and **complex** carbohydrates are those containing more than one saccharide group.




Aldose
Ketose
Glyceraldehyde (triose)
Erythrose (tetrose)
Ribose (pentose)
Glucose (hexoses)
Fructose


Family of D-aldoses
D-glyceraldehyde
Aldotriose
$2^1 = 2$
D-erythrose
D-threose
Aldotetroses
$2^2 = 4$
D-ribose
D-arabinose
D-xylose
D-lyxose
Aldopentoses
$2^3 = 8$
D-allose
D-altrose
D-glucose
D-mannose
D-gulose
D-idose
D-galactose
D-talose
Aldohexoses
$2^4 = 16$


Sucrose
Lactose
(etc.)
(etc.)
α1–4

Hexose	Chemical Formula	Fisher Projection	Cyclized Fisher Projection	Haworth
α-D-glucose	$C_6H_{12}O_6$			
β-D-galactose	$C_6H_{12}O_6$			
β-D-fructose	$C_6H_{12}O_6$			

Pyranose: The aldehyde group (-CHO) combines with hydroxyl group (-OH) of 5th carbon atom producing a 6 membered heterocyclic ring containing 5 carbons and one oxygen is called pyranose ring. This linkage is called 'hemiacetal' linkage.

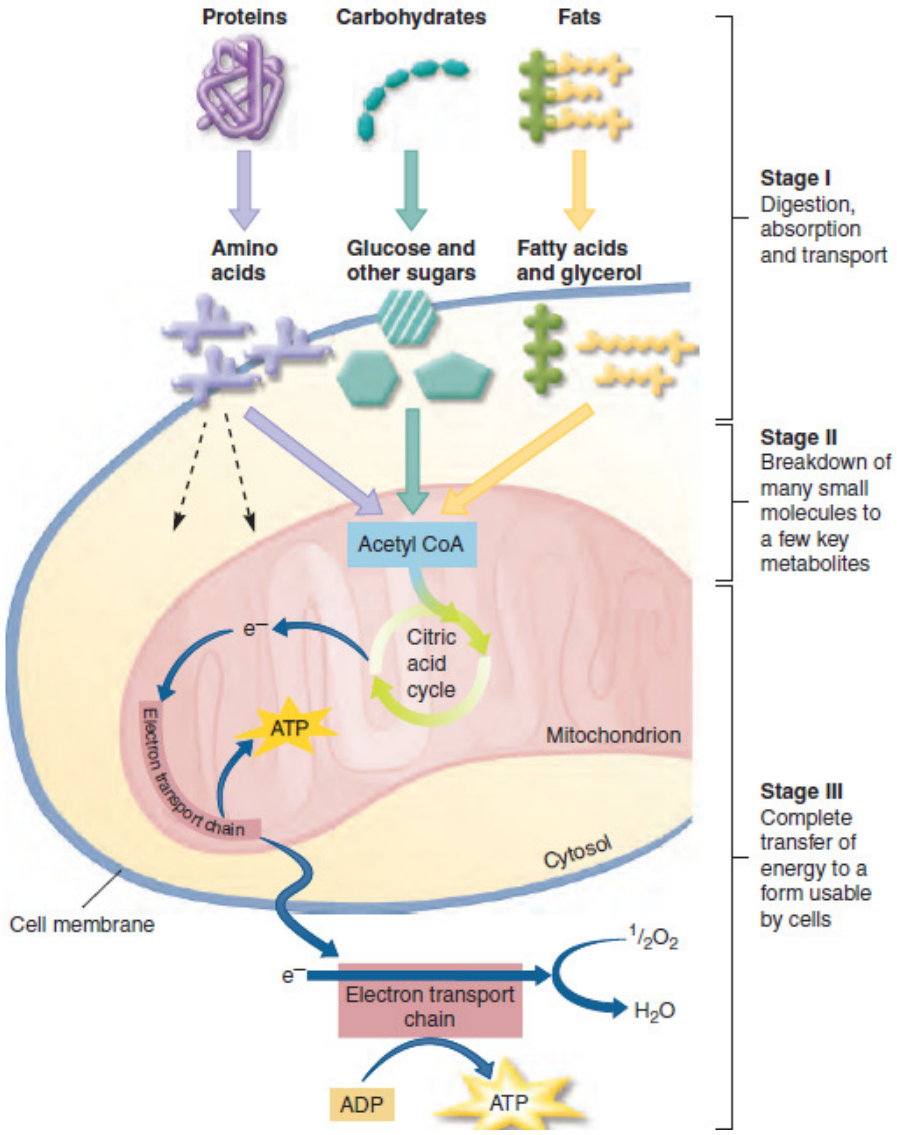
Furanose: Similarly, a keto group (>CO) combines with hydroxyl group on 5th carbon atom generating a 5 membered ring called furanose ring. The linkage is called 'hemiketal' linkage.

Due to formation of such ring forms, a new asymmetric carbon is created known as the anomeric carbon and the two possible configurations (α-form and β-form) are possible called anomers. The α-form has the OH group to the right of the anomeric carbon or below the plane and the β- form has the OH group to the left of the anomeric carbon or above the plane.

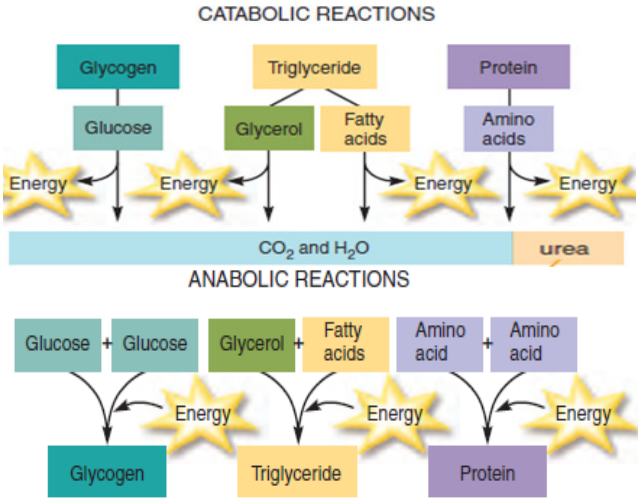
Epimers: The compounds having same bonding connectivity with more than one asymmetric carbon but differs only in the configuration around one carbon is called epimer.

D-Glucose and D-mannose are epimers at C2. Similarly, D-Glucose and D-galactose are epimers at C4. **Anomers** are epimers at C1 of cyclic aldoses and C2 of cyclic ketoses.

Metabolism



Metabolism is defined as the chemical processes by which cells produce the substances and energy needed to sustain life. It is subdivided into: Catabolism and Anabolism.



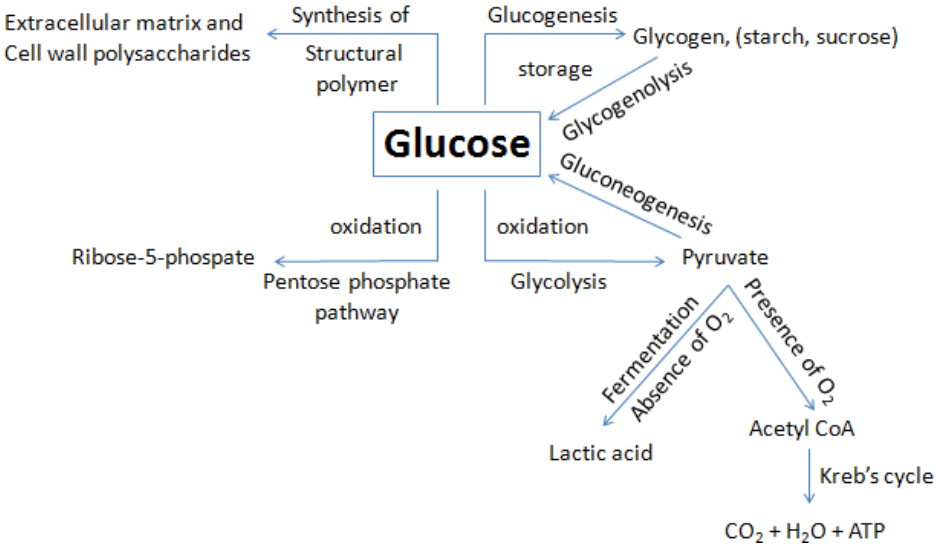
Catabolism: The metabolic breakdown of complex (larger) molecules into simpler (smaller) ones, often resulting in a release of energy i.e. **exergonic** in nature.

Anabolism: The phase of metabolism in which complex (large) molecules, such as the proteins and fats are formed from simpler (smaller) ones. They are usually **endergonic** in nature.

Carbohydrates impart crucial roles in the metabolic processes of living organisms. They act as energy sources (storage). The digestible carbohydrates provide 4 kcal/g. They also serve as structural elements (cellulose of plant, exoskeleton of insects, glycoproteins and glycolipids of cell membrane and receptor) in living cells. Excess carbohydrate is converted to fat.

During carbohydrate digestion, disaccharides, oligosaccharides and polysaccharides are hydrolyzed to form monosaccharides (primarily glucose, fructose, and galactose) which are absorbed into the bloodstream through the lining of the small intestine and transported to the liver. The liver acts as a regulator of blood glucose level where fructose and galactose are rapidly converted to glucose or to compounds that are metabolized by the same pathway as glucose.

In animals, glucose has four major fates as shown below.



Glycolysis

Glycolysis is the process of enzymatic break down of one molecule of glucose (6 carbon) into two pyruvate molecules (3 carbon) with net production of two molecules of ATP.

•Glycolysis is an almost universal central pathway of glucose catabolism. It is anaerobic process. During glycolysis some of the free energy is released and conserved in the form of ATP and NADH.

•In most of the organisms, the pyruvate formed by glycolysis is further metabolised via one of the three catabolic routes. (1) Under aerobic conditions, glucose is oxidized all the way to CO_2 and H_2O. (2) Under anaerobic conditions, the pyruvic acid can be fermented to lactic acid or to (3) ethanol plus CO_2 as shown below.

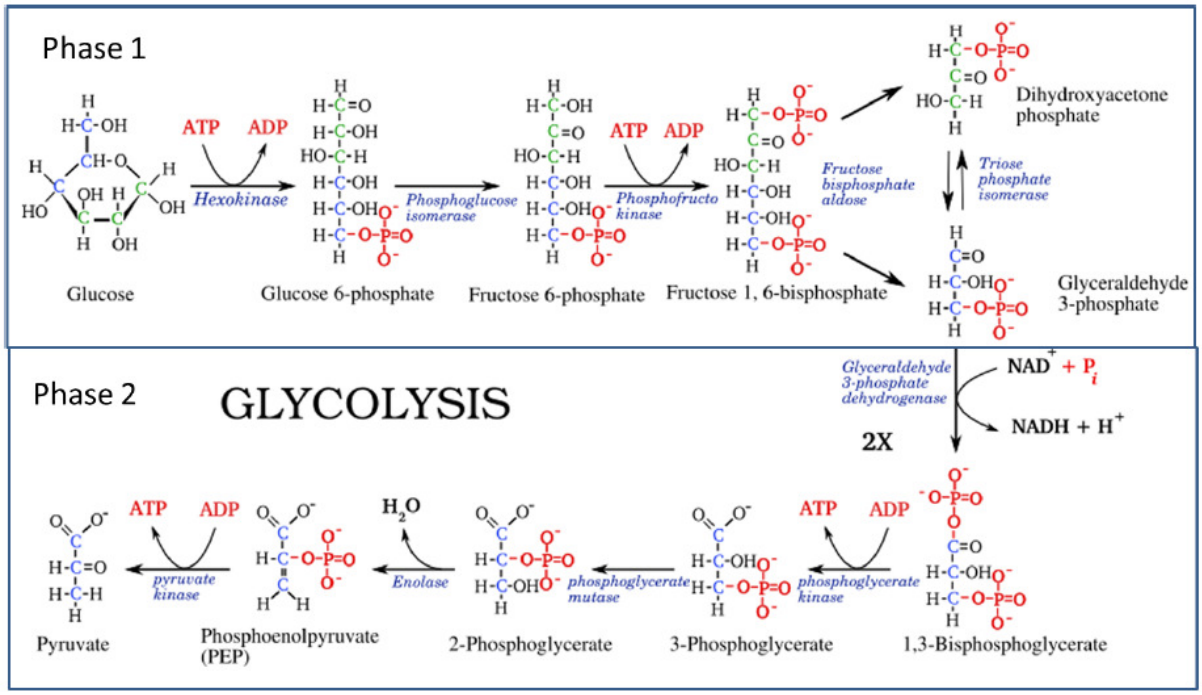
•Glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cells (RBCs, Brain, Renal medulla and Sperm cell).

Process of Glycolysis:

Glycolysis occurs in TEN steps. The breakdown of six carbon glucose into two molecules of the three carbon pyruvate occurs in a series of 10 enzyme catalyzed reactions. The process is divided into two phases.

Phase 1: Preparatory phase → Two molecule of ATP are invested and hexose chain is cleaved into two triose phosphates i.e. one glucose molecule is converted to two molecule of glyceraldehydes-3-phosphate. The energy is invested in the process of phosphorylation of glucose. The first five reactions constitute the preparatory phase.

Phase 2: Payoff phase → Two molecules of glyceraldehydes-3-phosphate is converted to two molecules of pyruvate accompanied by the formation of four ATP molecule from ADP. However, the net yield of ATP per molecule of glucose is only two, because two molecules were invested in preparatory phase. The remaining five reactions constitutes payoff phase.



Step I: Phosphorylation of glucose

Glucose is phosphorylated at -OH group of C6 in which one ATP is consumed. The reaction is catalysed by the hexokinase enzyme in the presence of Mg^{2+} ion. This step is irreversible under intracellular condition.

Step II: Isomerization of glucose-6 phosphate to fructose-6-Phosphate: This step involves the reversible isomerisation of glucose 6-phosphate, an aldose, to fructose 6-phospahte, a ketose. This reaction is catalysed by the enzyme phosphoglucose isomerase.

Step III: Phosphorylation of Fructose-6-phosphate to Fructose-1, 6-bisphosphate: Fructose-6-phosphate is phosphorylated at -OH group of C1 in which one ATP is consumed and fructose-1,6-bisphosphate is formed. This reaction is catalysed by phosphofructokinase (PFK) in the presence of Mg^{2+} ion.

Step IV: Cleavage of Fructose 1,6-bisphosphate: The fructose-1,6-bisphosphate is cleaved yielding two different triose phosphates. One is glyceraldehydes-3-phosphate, an aldose, and another is dihydroxyacetone phosphate, a ketose. The enzyme aldolase (fructose 1,6-diphosphate aldolase) is catalyzed this reversible reaction.

Step V: Conversion of dihydroxyacetone phosphate to glyceraldehydes-3-phosphate: Only glyceraldehyde-3-phosphate can be directly degraded into the subsequent steps of glycolysis. The other product, dihydroxyacetone phosphate, is rapidly and reversibly converted to glyceraldehyde-3-phosphate by the enzyme triose phosphate isomerase.

Step VI: Oxidation of glyceraldehyde-3-phosphate: The first step of payoff phase is the oxidation of glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate in the presence of enzyme glyceraldehydes-3-phosphate dehydrogenase (GAPDH). In this reaction, one molecule of NADH is released.

Step VII: Transfer of phosphoryl group from 1,3-bisphosphoglycerate to ADP: The enzyme phosphoglycerate kinase (PGK) transfer phosphoryl group from 1,3-bisphosphate glycerate to ADP forming ATP and 3-phosphoglycerate. This reaction is an example of substrate level phosphorylation in which phosphoryl group is transfer from substrate i.e., 1,3-bisphosphoglycerate to ADP to form ATP.

Step VIII: Conversion of 3-phosphoglycerate to 2-phoshoglycerate: The enzyme phosphoglycerate mutase catalyses reversible shift of phosphoryl group between C2 and C3 of phosphoglycerate. Mg^{2+} is essential for this reaction.

Step IX: Dehydration of 2-phosphoglycerate (Removal of H_2O from 2-phosphoglycerate): Enolase promotes reversible removal of water from 2-phosphoglycerate forming phosphoenolpyruvete (PEP).

Step X: Transfer of phosphoryl group from phosphoenolpyruvete to ADP: This reaction is catalyzed by the pyruvate kinase (PK) enzyme in the presence of K^+ and Mg^{2+} or Mn^{2+} ions. This is also a substrate level phosphorylation in which phosphoryl group is transferred from phosphoenolpyruvete to ADP forming ATP and Pyruvate.

In glycolysis, one molecule of glucose is break down into two molecules of pyruvate releasing 2 ATP and 2 NADH. The overall equation of aerobic glycolysis is:

$$Glucose + 2NAD^+ + 2ADP + 2Pi \rightarrow 2pyruvate + 2ATP + 2NADH + 2H_2O + 2H^+$$

Regulation of glycolysis: Two major needs of the cell influence the flow of material from glucose to pyruvate is: (a) for ATP (energy) and (b) for building blocks for biosynthesis.

The adjustment of rate of glycolysis is achieved at multiple levels including ATP Consumption, NADH regeneration, and allosteric regulation of enzymes. In metabolic pathways, control is focused on those steps in the pathway that are irreversible. In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase and pyruvate kinase are virtually irreversible and acts as regulatory components.

Hexokinase activity is regulated by high concentration of glucose 6-phosphate. ATP and citrate are allosteric inhibitor of phosphofructokinase whereas ATP, Acetyl CoA, Alanine allosterically inhibits pyruvate kinase to slow glycolysis.

Gluconeogenesis: The biosynthesis of glucose from non-carbohydrate precursors is called gluconeogenesis.

This process is required to maintain a constant level of glucose (prevent hypoglycemia). Some cells such as brain cells and red blood cells are primarily dependent on glucose for their energy requirements. For humans, the average consumption of glucose by brain is about 120 grams per day. The liver stores about 190 g of glucose as glycogen. Gluconeogenesis is not exactly the reverse of glycolysis, but the 7 out of 10 reaction sequences are reversed. The gluconeogenic pathways convert pyruvate into glucose.

The major non-carbohydrate precursors are (a) Pyruvate, (b) Lactate, (c) Glucogenic amino acids, (d) Glycerol. Major sites of gluconeogenesis are (a) Liver (90%), (b) Kidney (10%). Reactions of gluconeogenesis take place in the cytosol except for, pyruvate carboxylase (mitochondria) and Glucose-6-phosphatase (endoplasmic reticulum).

The net reaction of gluconeogenesis is:

$2\text{pyruvate} + 2\text{NADH} + 4\text{ATP} + 2\text{GTP} + 6\text{H}_2\text{O} + 2\text{H}^+ \rightarrow \text{Glucose} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{GDP} + 6\text{Pi}$

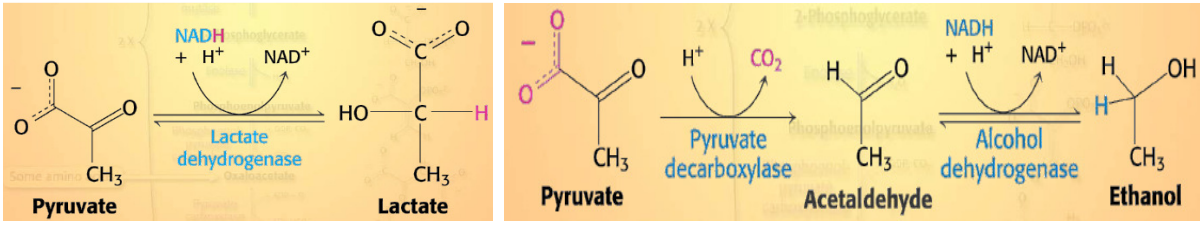
The reactions of gluconeogenesis beginning with pyruvate

Number	Reaction
1	$\text{Pyruvate} + \text{CO}_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i$
2	$\text{Oxaloacetate} + \text{GTP} \rightleftharpoons \text{phosphoenolpyruvate} + \text{CO}_2 + \text{GDP}$
3	$\text{Phosphoenolpyruvate} + \text{H}_2\text{O} \rightleftharpoons \text{2-phosphoglycerate}$
4	$\text{2-Phosphoglycerate} \rightleftharpoons \text{3-phosphoglycerate}$
5	$\text{3-Phosphoglycerate} + \text{ATP} \rightleftharpoons \text{1,3-bisphosphoglycerate} + \text{ADP}$
6	$\text{1,3-Bisphosphoglycerate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{glyceraldehyde-3-phosphate} + \text{NAD}^+ + \text{P}_i$
7	$\text{Glyceraldehyde-3-phosphate} \rightleftharpoons \text{dihydroxyacetone phosphate}$
8	$\text{Glyceraldehyde-3-phosphate} + \text{dihydroxyacetone phosphate} \rightleftharpoons \text{fructose-1,6-bisphosphate}$
9	$\text{Fructose-1,6-bisphosphate} + \text{H}_2\text{O} \rightarrow \text{fructose-6-phosphate} + \text{P}_i$
10	$\text{Fructose-6-phosphate} \rightleftharpoons \text{glucose-6-phosphate}$
11	$\text{Glucose-6-phosphate} + \text{H}_2\text{O} \rightleftharpoons \text{glucose} + \text{P}_i$

Fermentation

Any metabolic process that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor

For the glycolytic pathway to continue, NAD^+ has to be regenerated as only catalytic quantities of NAD^+ is present in cell. NAD^+ is regenerated from NADH through fermentation pathways where pyruvate converted to lactate or ethanol under anaerobic conditions.



Lactic acid Fermentation

• Lactic acid fermentation is use by bacteria and human muscles and produces lactate.

•Formation of lactate catalyzed by lactate dehydrogenase:

$CH_3\text{-}CO\text{-}COOH + NADH + H^+ \rightleftharpoons CH_3\text{-}CHOH\text{-}COOH + NAD^+$

• In highly active muscle, there is anaerobic glycolysis because the supply of O_2 cannot keep up with the demand for ATP.

• Lactate builds up causing a drop in pH which inactivates glycolytic enzymes. End result is energy deprivation and cell death; the symptoms being pain and fatigue of the muscle.

• Lactate is transported to the liver where it can be reconverted to pyruvate by the LDH reverse reaction.

Ethanol fermentation

• Ethanol fermentation is use by yeast and produces ethanol and CO_2

•Formation of ethanol catalyzed by 2 enzymes.

• Pyruvate decarboxylase catalyzes the first irreversible reaction to form acetaldehyde:

$CH_3\text{-}CO\text{-}COOH \rightarrow CH_3\text{-}CHO + CO_2$

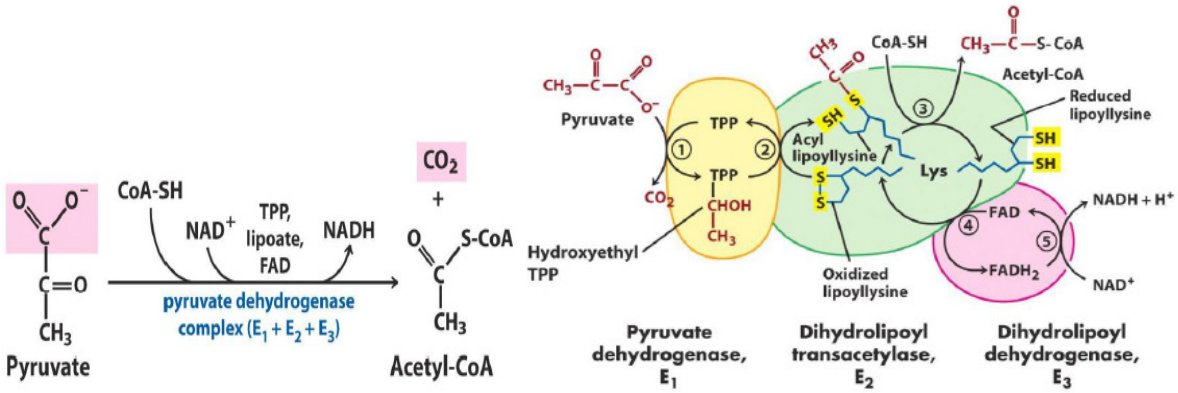
• Acetaldehyde is then reduced by alcohol dehydrogenase which is a reversible reaction:

$CH_3\text{-}CHO + NADH + H^+ \rightleftharpoons CH_3CH_2OH + NAD^+$

• Ethanol fermentation is used during wine-making.

Conversion of Pyruvic acid to Acetyl-CoA:

By the oxidative decarboxylation process pyruvate is converted to Acetyl-CoA using pyruvate dehydrogenase. Pyruvate dehydrogenase complex is an enzyme assembly of three types of subunits (E1, E2, and E3) and 5 coenzymes which are vitaminB1-thiamine pyrophosphate (TPP), lipoate, panthanoic acid derivative (CoA), riboflavin derivative (FAD), and Niacin derivative (NAD^+).



This conversion of Pyruvate→ Acetyl-CoA occurs in 3 steps:

1) Carboxyl group removed and given off as CO_2

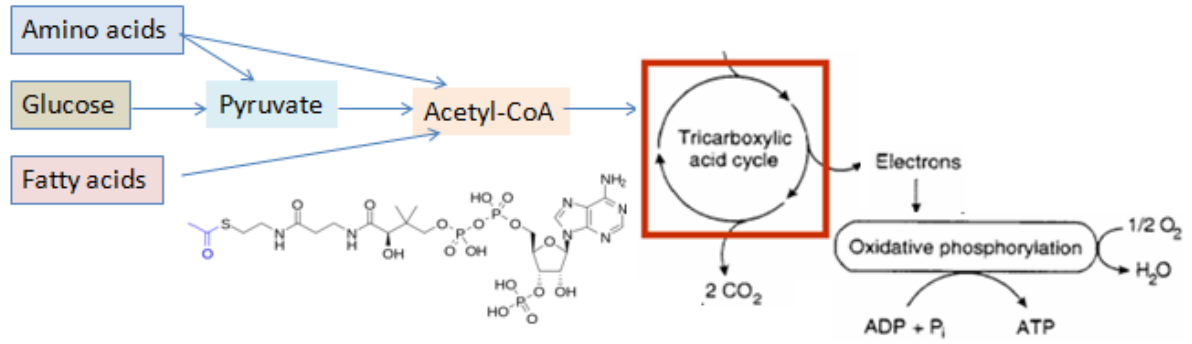
2) Each remaining 2-C fragment is oxidized forming acetate; the extracted electrons are transferred to NAD^+, forming NADH.

3) Coenzyme A (from vitamin B_5) is attached to acetate to form acetyl-CoA.

Kreb's Cycle:

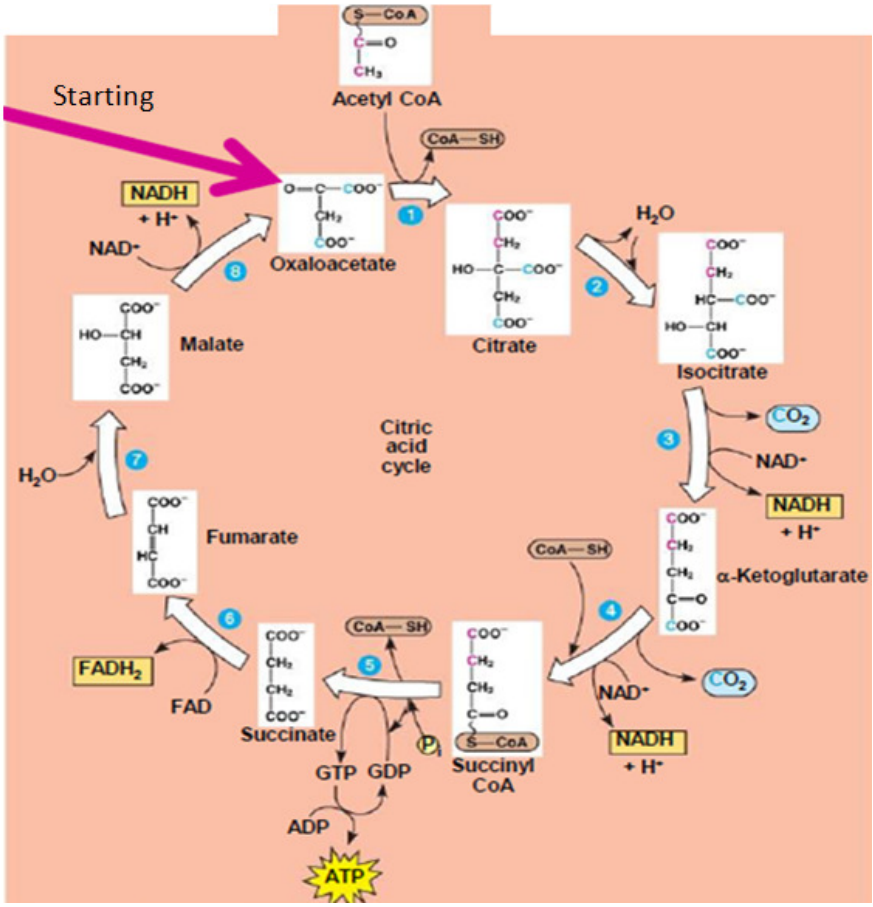
Krebs cycle also known as citric acid cycle (CAC) or tricarboxylic acid cycle (TCA cycle) is a series of chemical reactions that occur in aerobic condition to release stored energy through the oxidation of acetyl-CoA which is derived from carbohydrates, fats, and proteins. In addition, the precursors of certain amino acids, as well as the reducing agent NADH, $FADH_2$ are produced.

The cycle is named by the name of Hans Adolf Krebs who received the Nobel Prize for Physiology or Medicine in 1953**.**



Enzymes

1. Cytrate synthase
2. Aconitase
3. Isocytrate dehydrogenase
4. α-ketoglutarate dehydrogenase complex
5. Succinyl CoA synthetase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase



$$\text{Oxaloacetate} \xrightarrow{1} \text{Citrate} \xrightarrow{2} \text{Isocitrate} \xrightarrow{3} \alpha\text{-Ketoglutarate} \xrightarrow{4} \text{Succinyl-CoA} \xrightarrow{5} \text{Succinate} \xrightarrow{6}$$
$$\text{Fumarate} \xrightarrow{7} \text{Malate} \xrightarrow{8} \text{Oxaloacetate}$$

Step 1-Condensation: The acetyl-CoA combined with oxaloacetate (four-carbon molecule) producing citrate, a six-carbon molecule with releasing the CoA group.

Step 2-Isomerization of Citrate: Citrate is converted into its isomer, isocitrate. It is a two-step process, involving sequential dehydration and hydration reaction. That's why the citric acid cycle is sometimes described as nine step process.

Step 3-First Oxidative Decarboxylation: Isocitrate is oxidized to α-ketoglutarate with removal of carbon dioxide. During this step NAD^+ is reduced to NADH.

Step 4-Second Oxidative Decarboxylation: α-ketoglutarate is oxidized with releasing carbon dioxide and NAD^+ reduces to NADH. The remaining four-carbon molecule combined with CoA, forming the unstable compound succinyl CoA.

Step 5-Substrate-Level Phosphorylation: The unstable succinyl CoA is converted to Succinyl-Pi complex, which is finally transformed to Succinate. During this conversion one GTP is formed from GDP and Pi. Finally, GTP is transformed to ATP by nucleoside diphosphokinase enzyme.

Step 6-Flavin Dependent Dehydrogenation: Succinate is oxidized to fumarate by FAD and one $FADH_2$ molecule is formed.

Step 7-Hydration of a Carbon-Carbon Double Bond: Water is added to fumarate, converting it into malate.

Step 8-Dehydrogenation Reaction to Regenerate Oxaloacetate: In the last step, oxaloacetate is regenerated by dehydrogenation of malate and another molecule NADH is formed.

A single turn of the cycle involves:

1. Two carbon of acetyl CoA is entered and two molecules of carbon dioxide are released.

2. Three molecules of NADH and one molecule $FADH_2$ are generated.

3. One molecule of ATP or GTP is produced.

4. Since, each glucose molecule produces two acetyl CoA molecules, so catabolic oxidation of each glucose molecule produces four CO_2, two ATP, two $FADH_2$ and six NADH molecules.

5 Kreb's cycle doesn't produce much ATP directly. However, it can make a lot of ATP indirectly, through the generation of NADH, $FADH_2$ which are electron carriers.

The overall reaction is:

$CH_3CO\text{-}CoA + 3NAD^+ + FAD + ADP + Pi + 2H_2O \rightarrow CoA + 3NADH + FADH_2 + 3H^+ + ATP + 2CO_2$

Yield of ATP:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$

$Glucose + 2NAD^+ + 2ADP + 2Pi \rightarrow 2Pyruvate + 2NADH + 2H^+ + 2H_2O + 2ATP$

$2Pyruvate + 2CoA + 2NAD^+ \rightarrow 2Acetyl\text{-}CoA + 2CO_2 + 2NADH$

$2Acetyl\text{-}CoA + 4H_2O + 6NAD^+ + 2Pi + 2ADP + 2FAD \rightarrow 4CO_2 + 6NADH + 2ATP + 2CoA + 2FADH_2 + 4H^+$

$Glucose + 10NAD^+ + 4ADP + 2H_2O + 4Pi + 2FAD \rightarrow 6CO_2 + 10NADH + 4ATP + 2FADH_2 + 6H^+$

At this point, 4 moles ATP is formed per mole of Glucose as it passes through the Krebs cycle. However, NADH and $FADH_2$ are energy rich molecules. Their oxidation is highly exergonic and is coupled with the production of ATP from ADP.

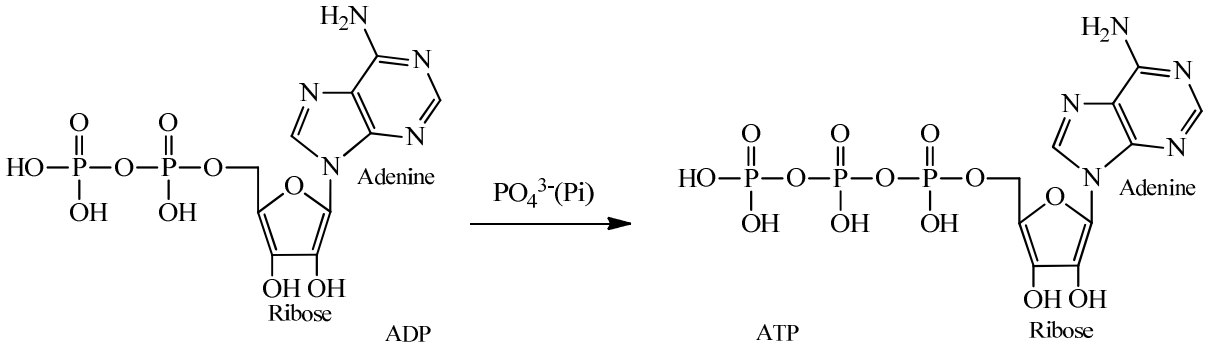
1 mole NADH produces 3 moles ATP whereas 1 mole $FADH_2$ produces 2 moles ATP

Thus total ATP yield = (10 x 3) + (2 x 2) + 4 = 38 moles ATP per mole Glucose.

Cellular currency of energy (ATP)

ATP – Adenosine tri-phosphate is called the energy currency of the cell. It is the organic compound composed of the phosphate groups, adenine, and the sugar ribose. These molecules provide energy for various biochemical processes in the body. Therefore, it is called "Energy Currency of the Cell". These ATP molecules are synthesized by Mitochondria, therefore it is called powerhouse of the cell.

The energy is transferred to the bonds of ATP which stores and releases the energy in usable amounts (packets) to be used by the cell.



ATP is synthesized in two Modes:

(1) Oxidative phosphorylation: This mode of ATP synthesis is powered by redox reactions which transfer electrons from Food → O_2 (O_2 is converted to CO_2 and H_2O)

(2) Substrate-level phosphorylation: It involves the enzyme-catalyzed transfer of inorganic phosphate from a molecule to ADP to form ATP. In this pathway, total four ATP is produced, two each from glycolysis and Krebs cycle.

Functions of ATP:

1) ATP molecule provides energy for both the exergonic and endergonic processes.

2) It is the only energy, which can be directly used for different metabolic process. Other forms of chemical energy need to be converted into ATP before they can be used.

3) It is used by various enzymes and structural proteins in cellular processes like biosynthetic reactions, cell divisions, etc.

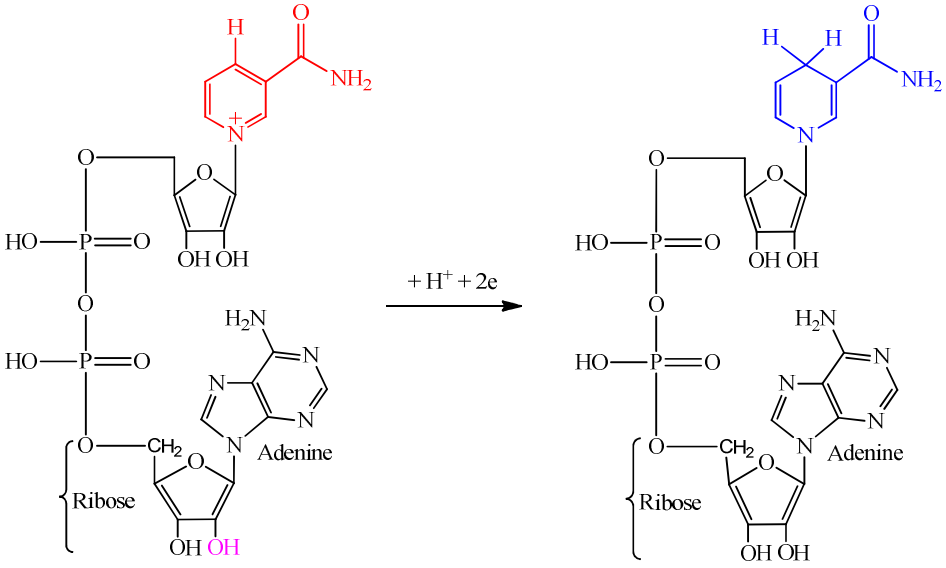
4) It plays an important role in the Metabolism – A life-sustaining chemical reactions including cellular division, fermentation, photosynthesis, photophosphorylation, aerobic respiration, protein synthesis, exocytosis, endocytosis and motility.

5) ATP molecule is also used as a switch to control chemical reactions and acts as a neurotransmitter to send messages.

6) Other functions of ATP include supplying the energy required for the muscle contraction, circulation of blood, locomotion and various body movements.

Electron carriers:

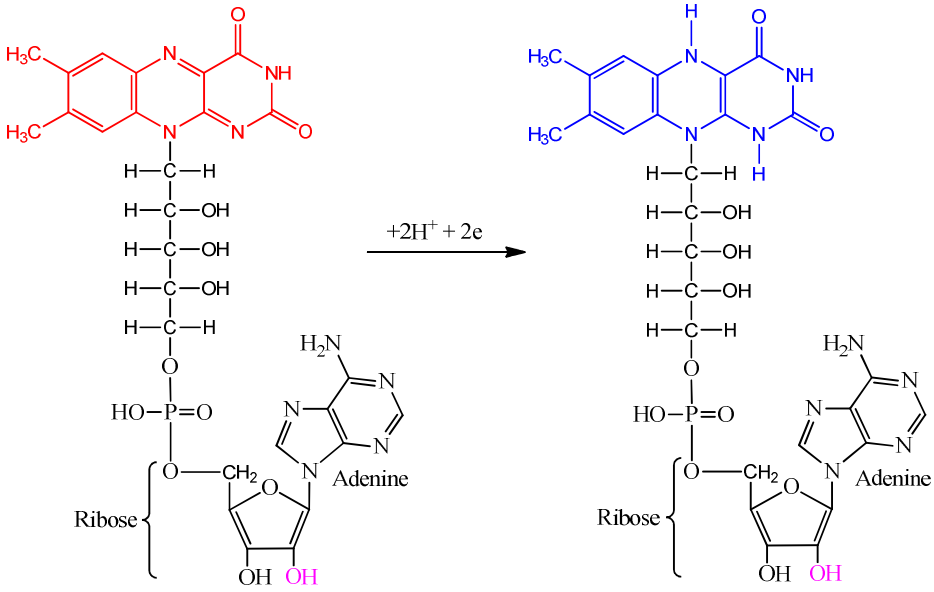
Acts as electron carriers in a multitude of redox reactions



NAD^+ NADH

$NADP^+$ $(OH = PO_4^{2-})$

Nicotinamide adenine dinucleotide phosphate



FAD Flavin Adenine Nucleotide $FADH_2$

Chemical Properties of Carbohydrates:

Molisch Test: specific for carbohydrates.

Benedict's Test: presence of reducing sugars

Fehling's Test: presence of reducing sugars

Barfoed's Test: distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.

Bial's Test: distinguish between pentose and hexose monosacharides

Seliwanoff's Test: distinguish between aldoses and ketoses

Molisch Test: It is a group test for all carbohydrates, whether free or in combined form. Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.

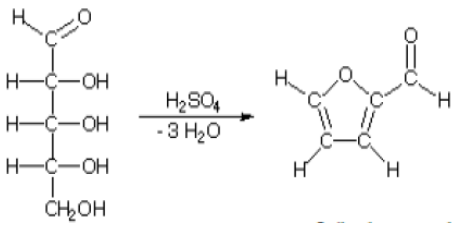
Molisch's reagent: 5%, w/v alpha-naphthol in alcohol.

Principle: Concentrated H_2SO_4 dehydrates carbohydrates to form furfural and its derivatives. This product combines with sulphonated alpha-naphthal to give violet-purple colour.

Procedure: Add 2 drops of Molisch's reagent to about 2 ml of test solution and mix well. Then add conc. H_2SO_4 carefully along the sides of the inclined test tube.

Observation: Red-cum-violet coloured ring is formed at the junction of the two layers. Presence of carbohydrate is confirmed.

Reaction: The colour formed is due to the reaction of alpha-naphthol with furfural and/or its derivatives formed by the dehydration of sugars by concentrated H_2SO_4.



Benedict's Test:

Benedict's reagent is used as a test for the presence of reducing sugars. All monosaccharides are reducing sugars; they all have a free reactive carbonyl group. Some disaccharides have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution. Starches are also non-reducing sugars.

Benedict's solution: 17.3 g of sodium citrate and 10 g of sodium carbonate are dissolved in 75 ml of water. 1.73 g of $CuSO_4.5H_2O$ is dissolved in 20 ml of water. The $CuSO_4$ solution is

mixed with the alkaline citrate solution with continuous stirring, and finally the whole volume is made up to 100 ml with distilled water.

Principle: The copper sulfate ($CuSO_4$) present in Benedict's solution reacts with the aldehyde or ketone group of the reducing sugar. Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.

Cu^{2+} + reducing sugar → Cu^{+} → Cu_2O(red)

Procedure: 1 ml of a sample solution is placed in a test tube. 2 ml of Benedict's reagent is added. Finally, the solution is heated in a boiling water bath for few minutes.

Observation: The formation of a reddish precipitate of Cu_2O, confirms the presence of reducing sugar.

Fehling's Test

It distinguishes reducing sugar from non-reducing sugar.

Fehling's reagent: Fehling's solution A: Dissolve 35 g of $CuSO_4 \cdot 5H_2O$ in water and make the volume to 500 ml. Fehling's solution B: Dissolve 120 g of KOH and 173 g Na-K tartrate (Rochelle salt) in water and make the volume to 500 ml. Mix equal volumes of Fehling's solution A and B prior to use.

Principle: The blue alkaline cupric hydroxide present in Fehling's solution, when heated in the presence of reducing sugars, gets reduced to yellow or red cuprous oxide and it gets precipitated.

Procedure: To 1 ml of Fehling's solution 'A', add 1 ml of Fehling's solution 'B' and a few drops of the test solution. Boil for a few minutes.

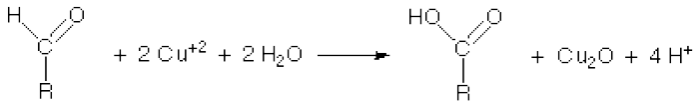
Observation: Formation of yellow or brownish red precipitate indicates the presence of reducing sugars in the test solution.

Barfoed's Test:

This test is performed to distinguish between reducing monosaccharides, reducing disaccharides and non-reducing sugar.

Barfoed's reagent: 13.3 g of copper acetate in 200 ml of water and add 2 ml of glacial acetic acid.

Principle: Barfoed's test used copper (II) ions in a slightly acidic medium, reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper(I) oxide within three minutes. Reducing disaccharides undergo the same reaction, but do so at a slower rate. The non-reducing sugars give negative result.



Procedure: 1 ml of a sample solution is taken in a test tube. 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid is added to it. Heat the solution in a boiling water bath for three minutes.

Observation: Formation of reddish precipitate indicates the presence of reducing sugars in the test solution.

Bial's Test:

This test is used to distinguish between pentose and hexose monosacharides.

Bial's reagent: Dissolve 300 mg of orcinol in 100 ml of concentrated HCl.

Principle: Bial's test uses conc. HCl as a dehydrating acid and orcinol + traces of ferric chloride as condensation reagent.

The test reagent dehydrates pentoses to form furfural. Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish or green product, while hexoses yield muddy-brown to grey condensation product.

Procedure: To 2–3 ml of the test solution, 5 ml of Bial's reagent is added. The contents are heated gently. When bubbles rise to the surface, it is cooled under the tap water.

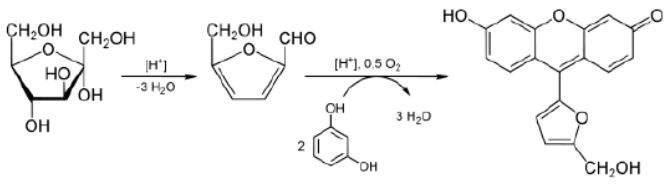
Observation: Appearance of blue or green colour precipitate indicates the presence of pentose sugar. In the presence of ferric ion orcinol and furfural condense to yield a coloured product.

Seliwanoff's Test:

This test is used to distinguish between aldoses (like glucose) and ketoses (like fructose).

Seliwanoff's reagent: Dissolve 50 g of resorcinol in 100 ml of conc. HCl in the ratio of 1:2.

Principle: Seliwanoff's Test uses 6M HCl as dehydrating agent and resorcinol as condensation reagent. The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product. Aldohexoses react to form the same product, but do so more slowly giving yellow to faint pink colour.



Procedure: To 1 ml of the test solution, 3 ml of Seliwanoff's reagent is added and the contents are boiled in water bath for 2 min.

Observation: Cherry red colour is obtained indicating ketoses (e.g., sucrose). In concentrated HCl, ketoses undergo dehydration to yield furfural derivatives more rapidly than do aldoses. These derivatives form complexes with resorcinol to yield deep red colour.

Iodine test

This test is performed to distinguish polysaccharides from mono- and disaccharides.

Principle

Iodine forms a coloured absorption complex with polysaccharides due to the formation of micellae aggregate. Iodine will form a polysaccharide inclusion complex.

Procedure: Add a few drops of iodine solution to about 1 ml of the test solution and observe the colour change.

Observation: (i) Appearance of deep blue colour indicates the presence of starch in solution. ii) Appearance of dark brown colour indicates the presence of polysaccharide (glycogen) in solution.

Remarks: The blue colour is due to the formation of starch-iodine complex, and dark brown colour indicates the presence of polysaccharide.

Test for sucrose

Principle: Sucrose present in the unknown solution is hydrolyzed by acid to glucose and fructose. The resulting fructose formed in this solution is then tested by Seliwanoff's reagent.

Procedure: To about 2-3 ml of test solution, add 1-2 drops of conc. HCl and boil in a water bath for 8-10 min. Then add about 5 ml of Seliwanoff's reagent and again keep it in a water bath for 1 min.

Observation: Appearance of red colour indicates the presence of fructose which is the hydrolytic product of sucrose.

Reaction: In concentrated HCl, fructose undergoes dehydration to yield furfural derivatives. These derivatives form complexes with resorcinol to yield deep red colour.

Qualitative analysis of carbohydrates

Molisch Test: It is a group test for all carbohydrates, whether free or in combined form.

Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.

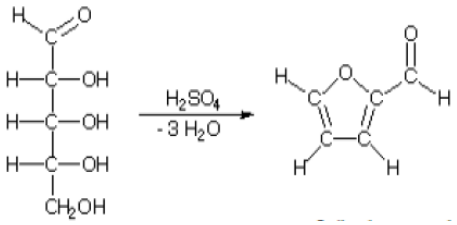
Molisch's reagent: 5%, w/v alpha-naphthol in alcohol.

Principle: Concentrated H2SO4 dehydrates carbohydrates to form furfural and its derivatives. This product combines with sulphonated alpha-naphthal to give violet-purple colour.

Procedure: Add 2 drops of Molisch's reagent to about 2 ml of test solution and mix well. Then add conc. H_2SO_4 carefully along the sides of the inclined test tube.

Observation: Red-cum-violet coloured ring is formed at the junction of the two layers. Presence of carbohydrate is confirmed.

Reaction: The colour formed is due to the reaction of alpha-naphthol with furfural and/or its derivatives formed by the dehydration of sugars by concentrated H_2SO_4.



Quantitative analysis of carbohydrates

Estimation of Total Carbohydrates by Anthrone Method

Objective

Estimation of the concentration of total carbohydrates present in a given sample by Anthrone method.

Principle

Carbohydrates are first hydrolysed into simple sugars using sulphuric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. Anthrone reagent is used as a colouring agent that reacts with furfural derivative to form a blue-green complex. Absorbance of these compounds is measured by a spectrophotometer at 630 nm.

$$\text{Glucose} + H_2SO_4 \rightarrow \text{hydroxymethyl furfural}$$

$$\text{hydroxymethyl furfural} + \text{anthrone} \rightarrow \text{Blue-green complex}$$

Reagents

1. Test sample

2. 2.5 N HCl: Prepare the solution fresh

3. Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95% H2SO4. Prepare fresh before use.

4. Standard glucose: Working standard: Dissolve 100 mg in 100 ml water. Store at 4°C after adding a few drops of toluene.

Procedure:

1. Take clean and dry test tubes and mark all the tubes as per the protocol.

2. Pipette out 0.1-0.5 ml of glucose standard solution in duplicate test tubes.

3. In one test tube take only 1 ml of distilled water and mark it as blank.

4. Make up the volume to 1 ml in each test tube by adding distilled water.

5. Then add 3 ml of anthrone reagent to each test tube and mix thoroughly.

6. Heat the test tubes for 8 min. in a boiling water bath.

7. Cool rapidly and read the green to dark green colour at 630 nm.

8. Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.

9. From the graph calculate the amount of carbohydrate present in the sample tube.

Test tube	Glucose solution (mL)	Conc. of glucose mg/mL	Distilled water	Anthrone reagent solution	absorbance
1	0	0 (blank)	10	3	
2	1	0.1	9	3	
3	2	0.2	8	3	
4	3	0.3	7	3	
5	4	0.4	6	3	
6	5	0.5	5	3	
7	6	0.6	4	3	
8	7	0.7	3	3	
9	8	0.8	2	3	
10	9	0.9	1	3	
	V ml	unknown	0	3	

Calculation:

Amount of carbohydrate present in 100 mg of the sample = $\frac{\text{mg of glucose x100}}{\text{Volume of test sample}}$