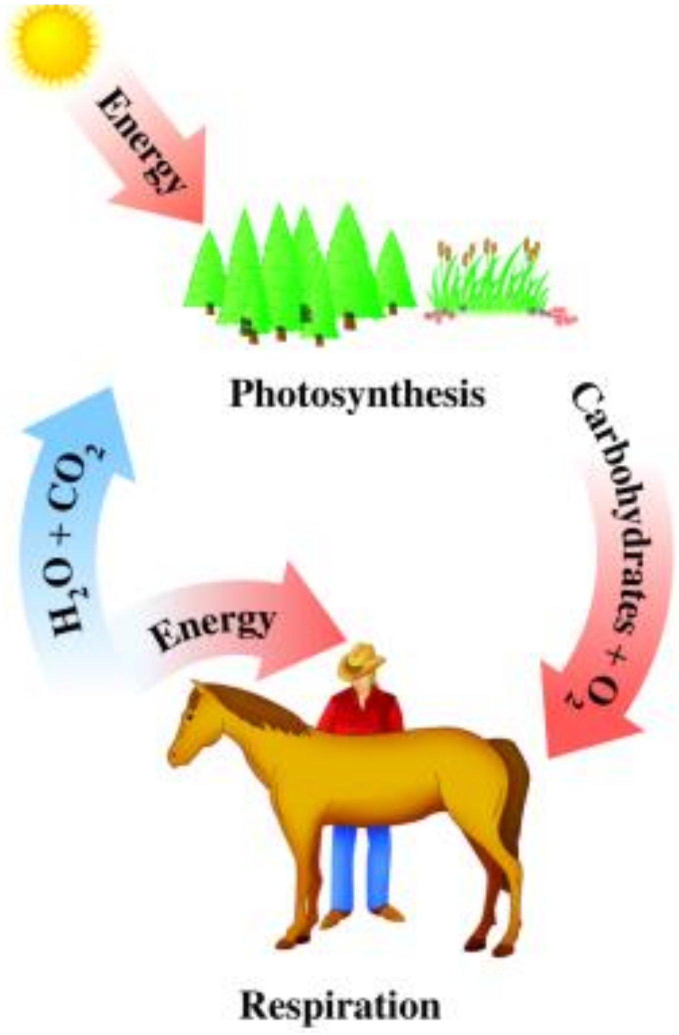
SEC-A2-Analytical clinical biochemistry

Carbohydrates: Biological importance of carbohydrates, Metabolism, Cellular currency of energy (ATP), Glycolysis, Alcoholic and Lactic acid fermentations, Krebs cycle. Isolation and characterization of polysachharides.

Carbohydrates

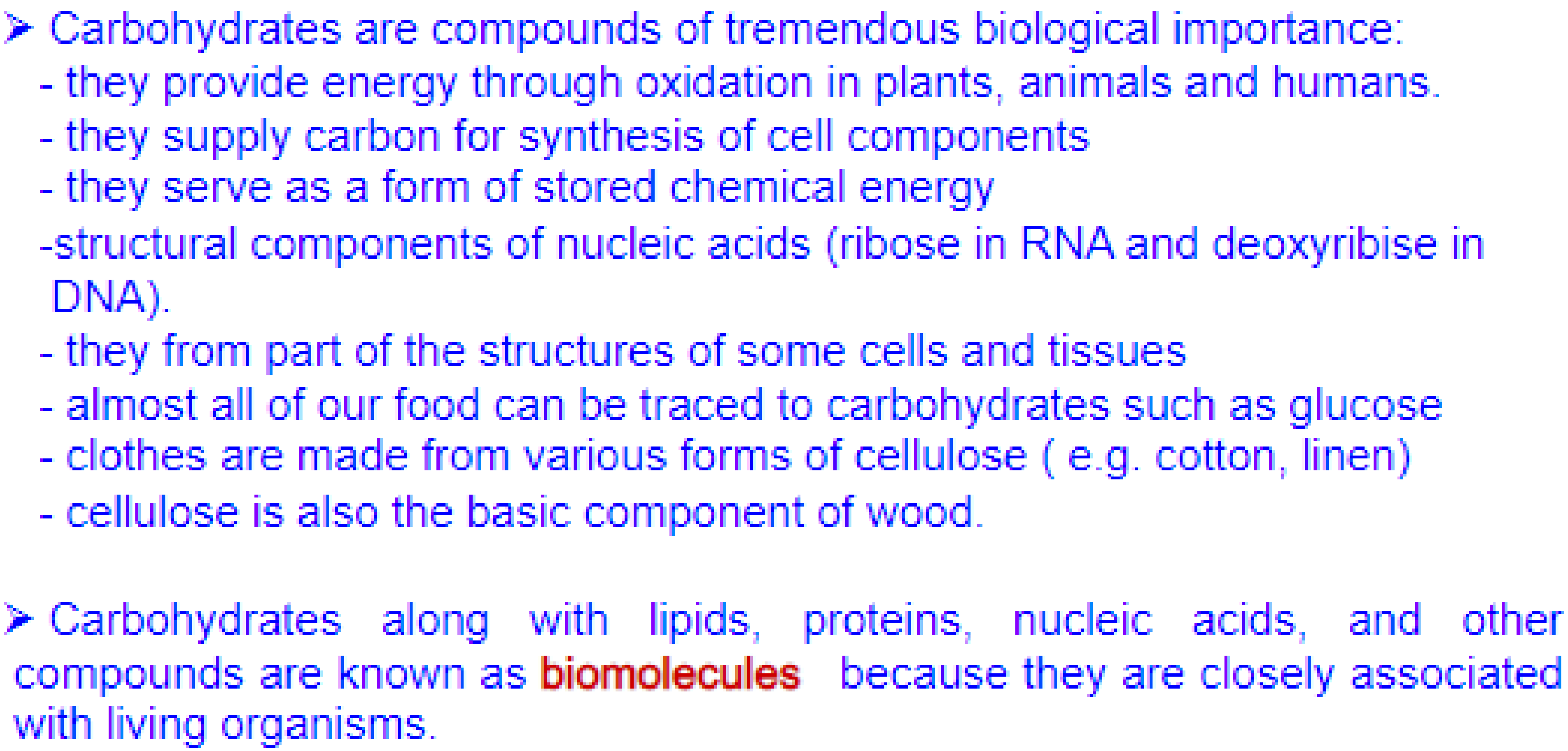


- Produced by photosynthesis in plants.
- The major source of energy from our diet.
- Composed of the elements C, H, and O.
- The name carbohydrate was originally derived from the general formula $C_x(H_2O)_y$ formally to hydrates of carbon.
- Modern science define carbohydrates as polyhydroxy aldehydes or ketones or substances that produce such adehydes and ketones (however, they generally exist as hemiacetal/acetal of hemiketal/ketal) on acidic or enzymatic hydrolysis.
- Some $-NO_2$ containing carbohydrates are also known.
- Simple carbohydrates are referred to as simple sugars and are often sweet to the taste.

$$6CO_2 + 6H_2O + \text{energy} \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftarrows}} C_6H_{12}O_6 + 6O_2$$

glucose

Biological importance of carbohydrates



- Carbohydrates are compounds of tremendous biological importance:
  - they provide energy through oxidation in plants, animals and humans.
  - they supply carbon for synthesis of cell components
  - they serve as a form of stored chemical energy
  - structural components of nucleic acids (ribose in RNA and deoxyribise in DNA).
  - they from part of the structures of some cells and tissues
  - almost all of our food can be traced to carbohydrates such as glucose
  - clothes are made from various forms of cellulose (e.g. cotton, linen)
  - cellulose is also the basic component of wood.

- Carbohydrates along with lipids, proteins, nucleic acids, and other compounds are known as **biomolecules** because they are closely associated with living organisms.

Classification of Carbohydrates on the basis of their nature of hydrolysis

- **Monosaccharides** contain a single polyhydroxy aldehyde or ketone unit (*saccharo* is Greek for "sugar") (e.g., glucose, fructose).

- **Disaccharides** consist of two monosaccharide units linked together by a covalent bond (e.g., sucrose).

- **Oligosaccharides** contain from 3 to 10 monosaccharide units (e.g., raffinose)

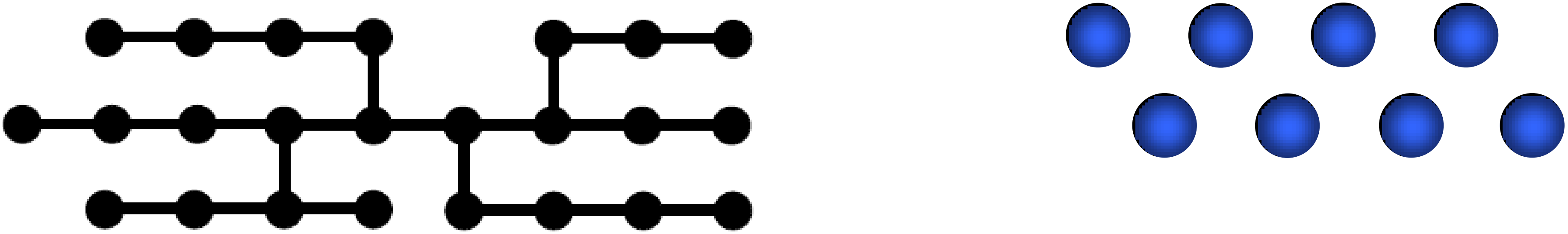
- **Polysaccharides** contain very long chains of hundreds or thousands of monosaccharide units, which may be either in straight or branched chains (e.g., cellulose, glycogen, starch).

1. Monosaccharide + H_2O $\xrightarrow{H^+ \text{ or enzyme}}$ no hydrolysis

2. Disaccharide + H_2O $\xrightarrow{H^+ \text{ or enzyme}}$ two monosaccharide units

3. Oligosaccharide + many H_2O $\xrightarrow{H^+ \text{ or enzyme}}$ many monosaccharide units

3. Polysaccharide + many H_2O $\xrightarrow{H^+ \text{ or enzyme}}$ many monosaccharide units



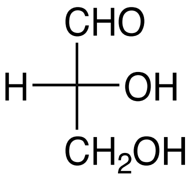
Nomenclature & classification of monosaccharides

Aldose: a monosaccharide containing an aldehyde group. E.g.-Glucose.

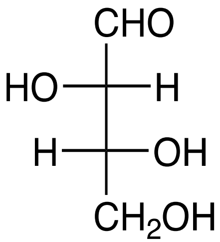
Ketose: a monosaccharide containing a ketone group. E.g.-Fructose.

Carbon atoms	Examples	Functional groups
Trioses (3 carbon)	Glyceraldehyde Dihydroxy acetone	Aldehyde (aldotriose) Ketone (Ketotriose)
Tetroses (4 carbon)	Erythrose	Aldehyde (aldotetrose)
Pentoses (5 carbon)	Ribose Xylose Xylulose	Aldehyde(Aldopentose) Aldehyde(Aldopentose) Ketone (Ketopentose)
Hexoses (6 carbons)	Glucose Galactose Fructose	Aldehyde (Aldohexose) Aldehyde (Aldohexose) Ketone (Ketohexose)

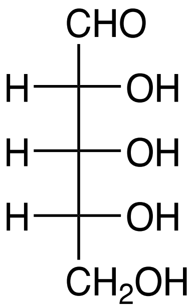
Some examples with structures….



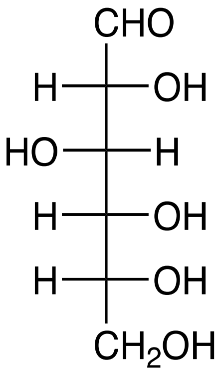
**glyceraldehyde
(triose)
(aldotriose)**



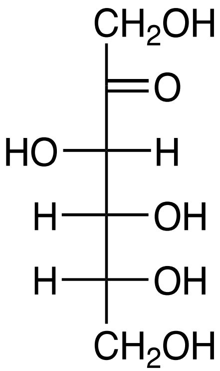
**threose
(tetrose)
(aldotetrose)**



**ribose
(pentose)
(aldopentose)**



**glucose
(hexose)
(aldohexose)**



**fructose
(hexose)
(ketohexose)**

D- / L- designation of monosaccharides

R/S (absolute) versus D/L (relative)

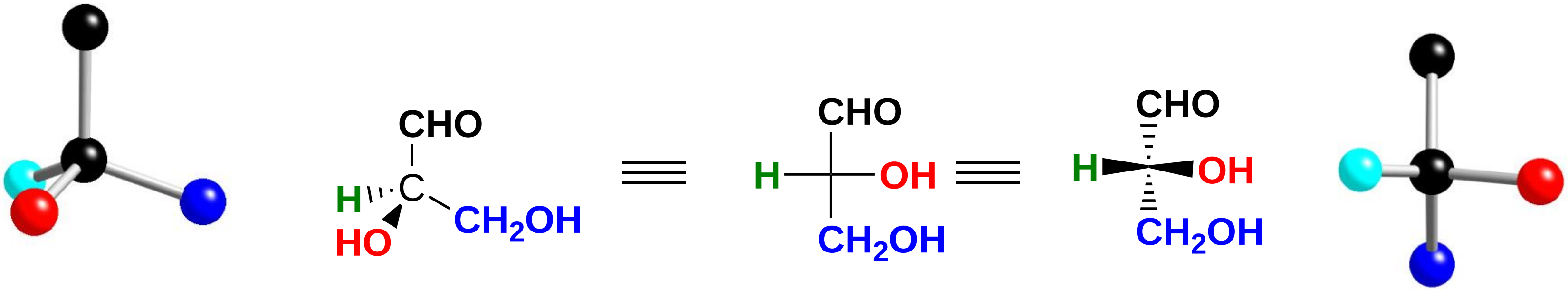
There is NO direct relationship between the configurational descriptors R/S or D/L

and

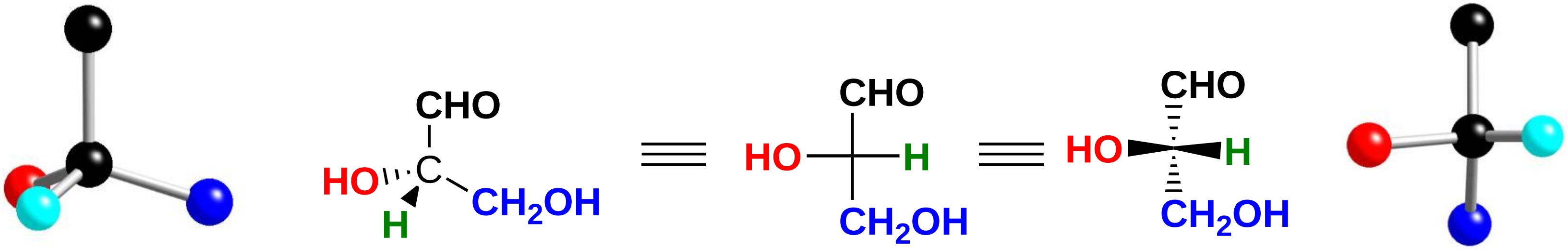
The sign of rotation

Fischer Projections and the D-L Notation:

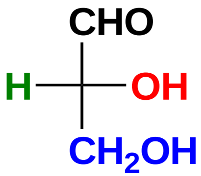
(*R*)-(+)-glyceraldehyde



(*S*)-(-)-glyceraldehyde

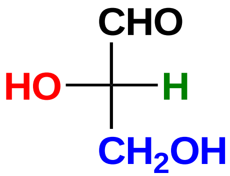


- Before the *R*/*S* convention, stereochemistry was related to (+)-glyceraldehyde



$$\begin{array}{c} \mathrm{CHO} \\ \mathrm{H}-\!\!\!+\!\!\!-\mathrm{OH} \\ \mathrm{CH_2OH} \end{array}$$

D-glyceraldehyde
R-(+)-glyceraldhyde
(+)-rotation = dextrorotatory = D



$$\begin{array}{c} \mathrm{CHO} \\ \mathrm{HO}-\!\!\!+\!\!\!-\mathrm{H} \\ \mathrm{CH_2OH} \end{array}$$

L-glyceraldehyde
S-(-)-glyceraldhyde
(-)-rotation = levorotatory = L

- D-carbohydrates have the -OH group of the highest numbered chiral carbon pointing to the right in the Fisher projection as in R-(+)-glyceraldhyde

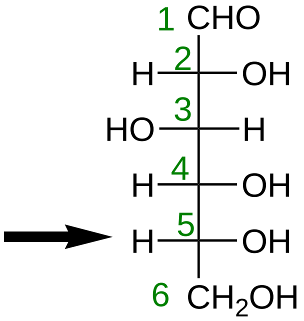
- For carbohydrates, the convention is to arrange the Fischer projection with the carbonyl group at the top for aldoses and closest to the top for ketoses. The carbons are numbered from top to bottom.

- Carbohydrates are designated as D- or L- according to the stereochemistry of the highest numbered chiral carbon of the Fischer projection.

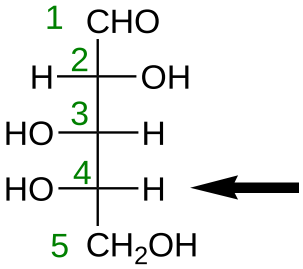
- If the hydroxyl group of the highest numbered chiral carbon is pointing to the right, the sugar is designated as **D** (*Dextro*: Latin for *on the right side*). If the hydroxyl group is pointing to the left, the sugar is designated as **L** (*Levo*: Latin for *on the left side*).

- Most naturally occurring carbohydrates are of the D-configuration.

highest numbered "chiral" carbon



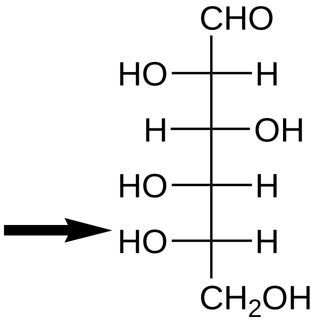
D-Glucose



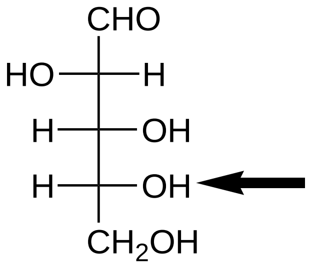
highest numbered "chiral" carbon

L-Arabinose

highest numbered "chiral" carbon



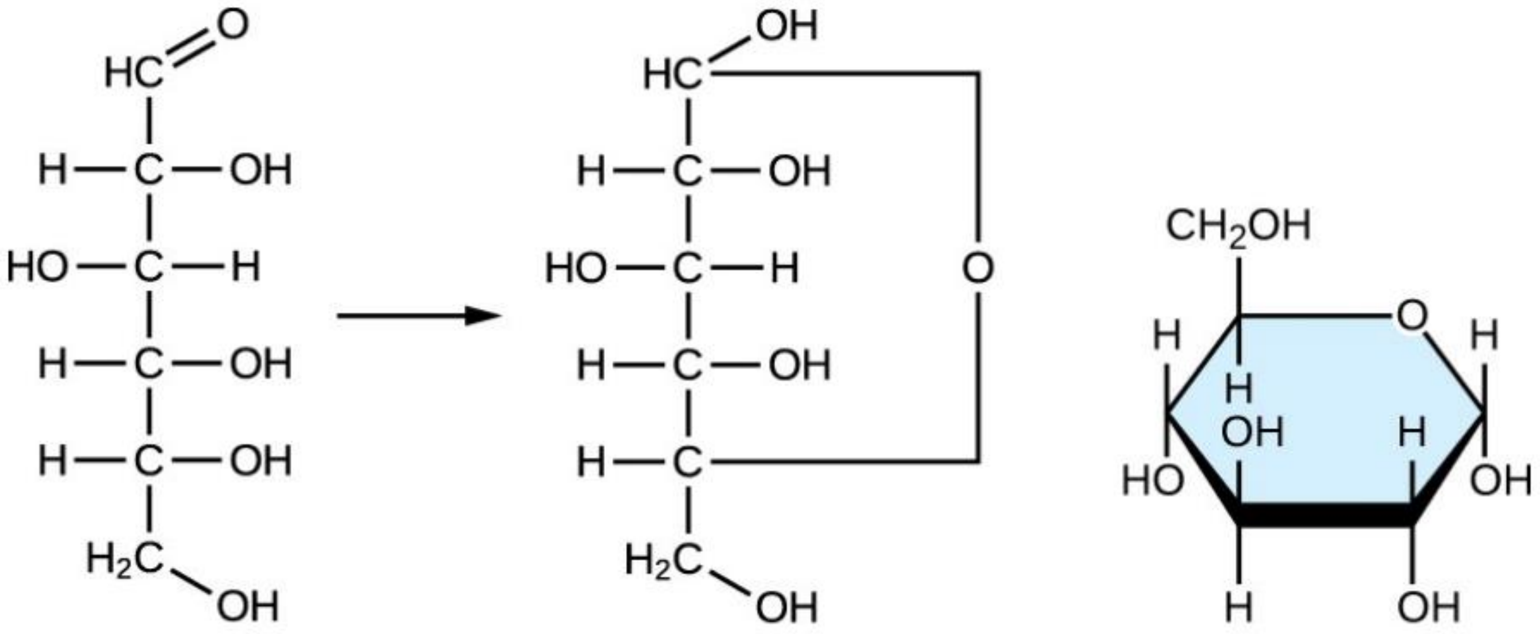
L- glucose



highest numbered "chiral" carbon

D-Arabinose

Cyclic structure of carbohydrates



Polysaccharides:

- Polysaccharides functions as an important source of energy.
- Straight chained ones are linear polysaccharides, and branched ones are branched polysaccharides

Characteristics:

- They are not sweet in taste.
- Many are insoluble in water.
- They are hydrophobic in nature.
- They do not form crystals on desiccation.
- Can be extracted to form a white powder.
- They are high molecular weight carbohydrates.
- Inside the cells, they are compact and osmotically inactive.

Polysaccharides are categorized into two types:

Homopolysaccharides: A polysaccharide that contains the same type of monosaccharides:

- **Glycogen**: It is made up of a large chain of molecules. It is found in animals and fungi.
- **Cellulose**: The cell wall of the plants is made up of cellulose. It comprises long chains of β-glycosides.

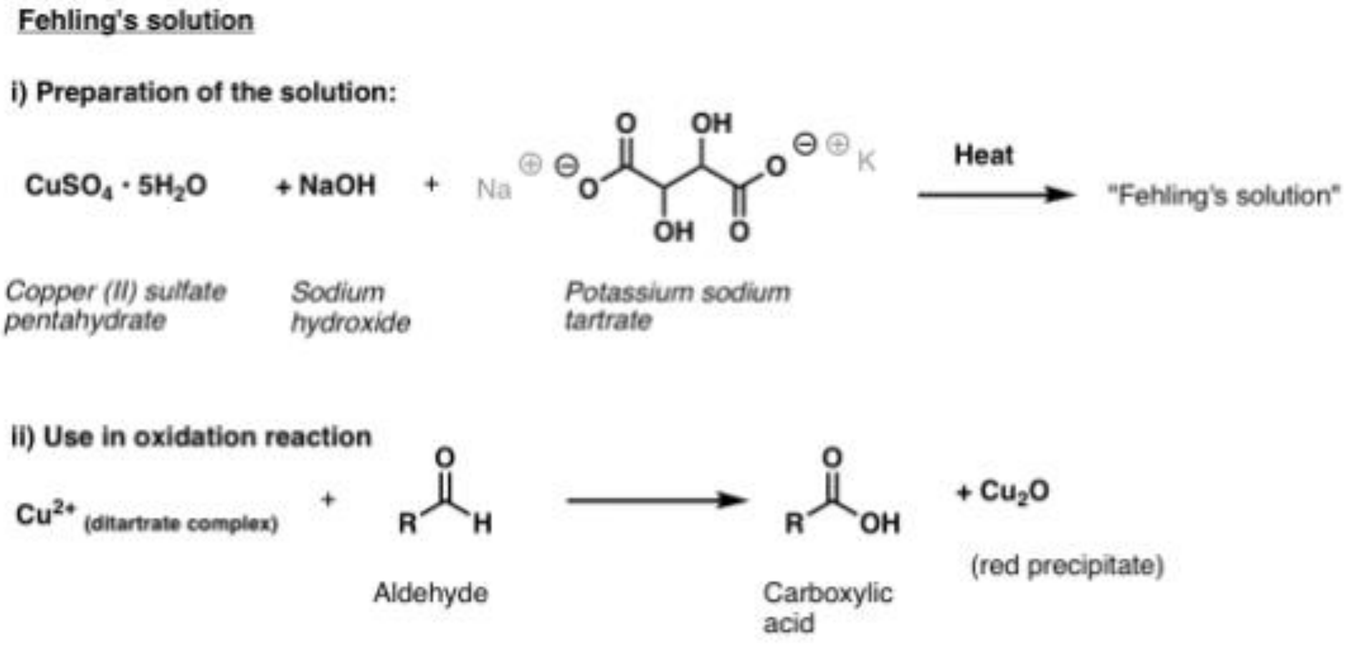
- **Starch**: It is formed by the condensation of amylose and amylopectin. It is found largely in plants, fruits, seeds, etc.
- **Insulin**: It is made up of a number of fructofuranose molecules linked together in chains. It is found in the tubers of dahlia, artichoke, etc.

Heteropolysaccharides: A polysaccharide that contains different types of monosaccharides:

- **Hyaluronic Acid:** It is made up of D-glucuronic acid and N-acetyl-glucosamine. It is found in connective tissues and skin.
- **Heparin:** It is made up of D-glucuronic acid, L-iduronic acid, N-sulfo-D-glucosamine and is largely distributed in mast cells and blood.
- **Chondroitin-4-sulfate:** Its component sugars are D-glucuronic acid and N-acetyl-D-galactosamine-4-O-sulfate. It is present in the cartilages.
- **Gamma globulin:** N-acetyl-hexosamine, D-mannose, D-galactose are the component sugars of this polysaccharide. It is found in the blood.
- **Pectin:** A heteropolysaccharide, contained in the primary cell walls of terrestrial plants. It is used in food as a gelling agent,particularly in jams and jellies

Reducing and non-reducing Sugars:

- Reducing sugars have a hemiacetal functional group and give positive tests with Fehling’s soluton, Benedict’s solution and Tollen’s reagent, since they are in equilibrium with an open-chain aldehyde. But, the non-reducing sugars doesn’t.
    - Fehling's solution, where an aldehyde changes the color of a blue Cu(II) solution to red Cu(I) *[as Cu_2O].*
    - Benedict's solution a slightly modified version of Fehling's solution
    - Tollens' test, where aldehyde oxidation results in a beautiful “mirror” of silver metal to precipitate on the reaction vessel.



Fehling’s solution

Control (blue)

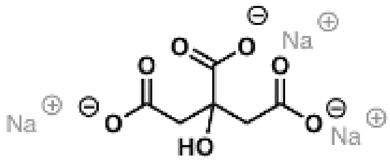
Positive test (red)

Benedict's Solution

(longer shelf-life than Fehling's solution)

i) Preparation of the solution:

$CuSO_4 \cdot 5H_2O$ **+ Na_2CO_3** +



Copper sulfate *Sodium carbonate* *Sodium citrate*

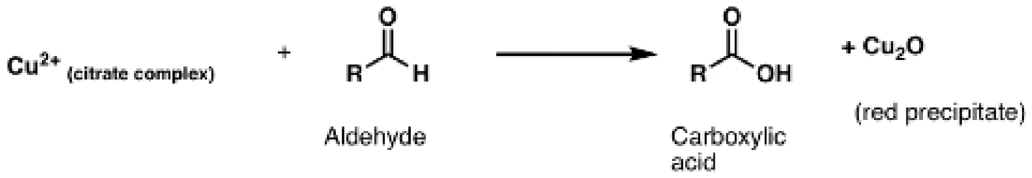
Note: for the quantitative test, potassium thiocyanate is also added

Benedict's test

Control (blue)

Positive test (red)

ii) Use in oxidation reaction



Tollens Test

i) Preparation of the solution:

$AgNO_3$ + NaOH → **AgOH** + NaNO3

Silver nitrate *Sodium hydroxide* *Silver hydroxide* *Sodium nitrate*

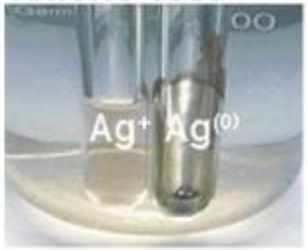
2 AgOH → **Ag_2O** + H_2O

Silver hydroxide *Silver oxide* *water*

Ag_2O + 4 NH_3 + H_2O → **2 $Ag(NH_3)_2^{\oplus}$ + 2 $OH^{\ominus}$**

Silver oxide *ammonia* *water* *Silver ammonia complex* *hydroxide ion*

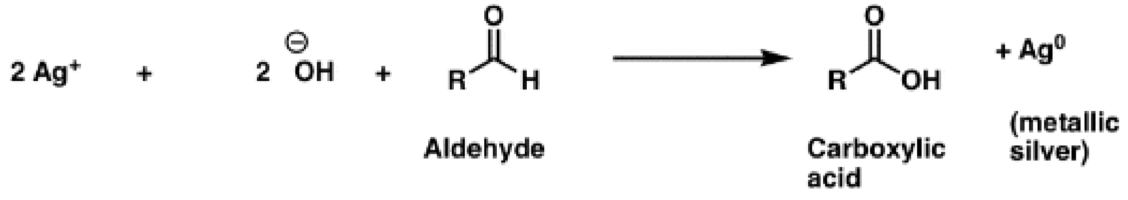
Tollens Test



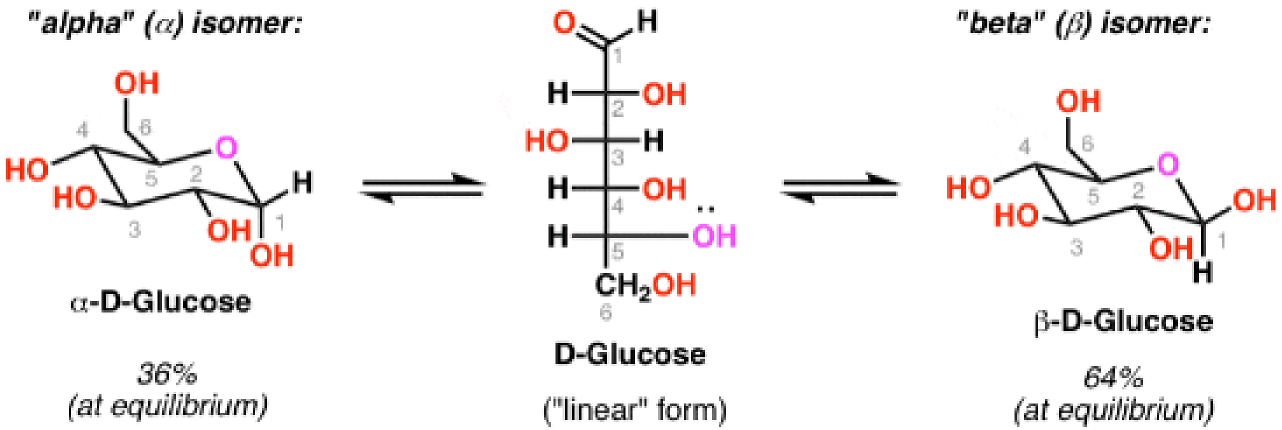
Control (clear)

Positive test (silver mirror)

ii) Use in oxidation reaction



The cyclic forms of glucose are in equilibrium with a linear form containing an aldehyde:



"alpha" (α) isomer:

α-D-Glucose

36%
(at equilibrium)

D-Glucose

("linear" form)

"beta" (β) isomer:

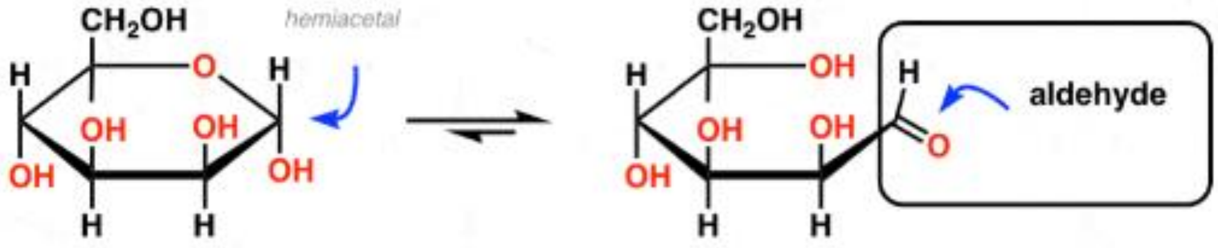
β-D-Glucose

64%
(at equilibrium)

The concentration of aldehyde at any given time is small (<1%), but long-lived enough to be trapped with the right reagent.

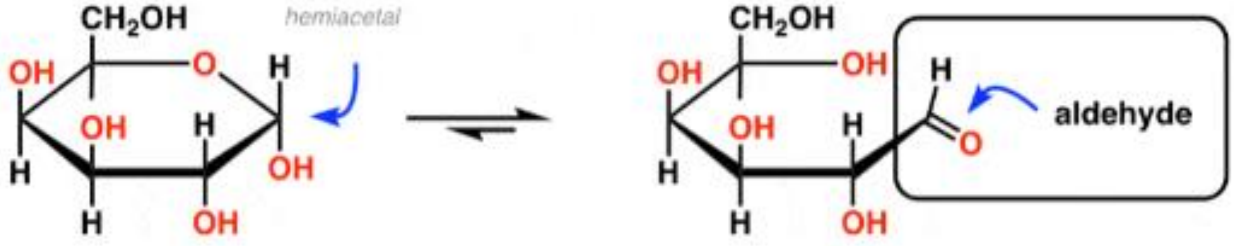
This means that glucose will give a positive test with Benedicts' reagent, Fehlings solution, or the Tollens test, and the aldehyde will be oxidized to a carboxylic acid.

Monosaccharides with a hemiacetal are also "reducing sugars" since their open-chain form contains an aldehyde (or alpha-hydroxy ketone)



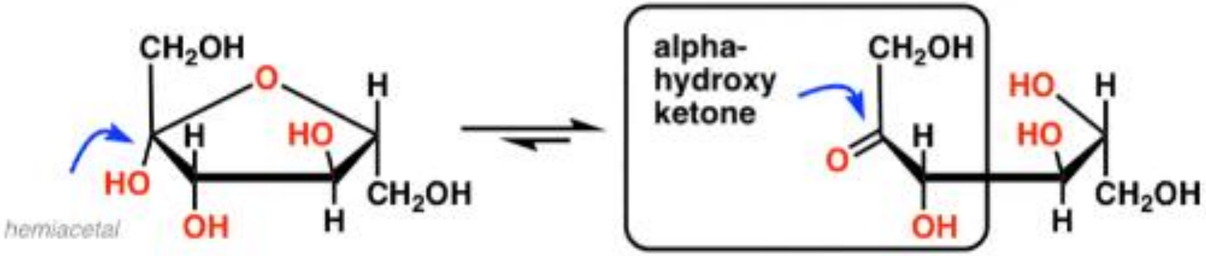
D-Mannose

D-Mannose (open-chain aldehyde)



D-Galactose

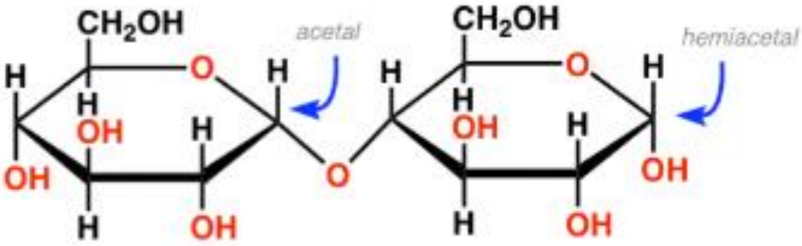
D-Galactose (open-chain aldehyde)



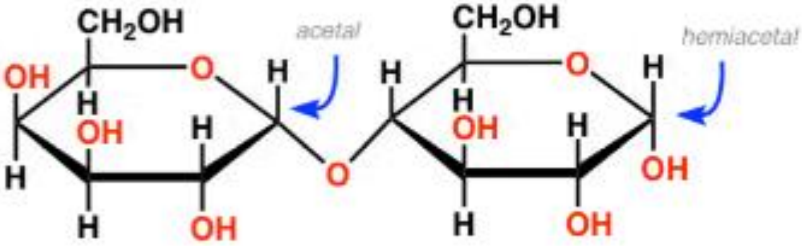
Likewise, some disaccharides such as maltose and lactose contain a hemiacetal. They are also reducing sugars that give a positive Fehlings, Benedict, or Tollens test (picture of lactose positive test is further below).

Lactose and Maltose are also reducing sugars and give a positive Benedict test

Maltose:



Lactose:

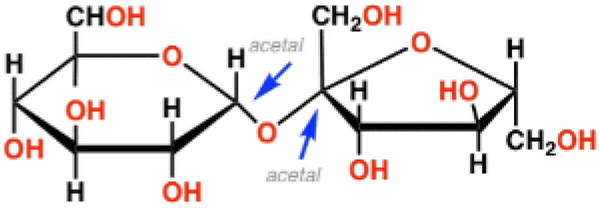


So what *isn't* a reducing sugar?

Two main cases:

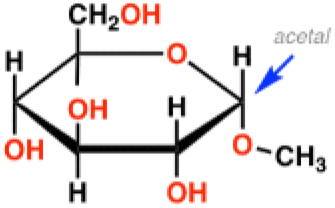
- mono and di-saccharides which lack a hemiacetal
- polysaccharides where the ratio of hemiacetals to acetal linkages is very low (e.g. starch)

Sucrose is a non-reducing sugar



The structure lacks any hemiacetal functional groups and is therefore "locked" in its cyclic form

Another example of a non-reducing sugar: a "glucoside" of glucose

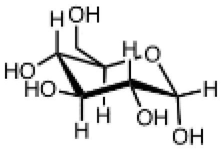


α-D-glucose
methyl glucoside

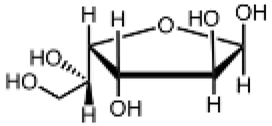
no hemiacetal

Quiz: Reducing Sugar or Non Reducing Sugar?

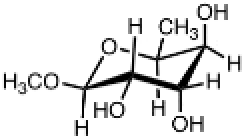
A)



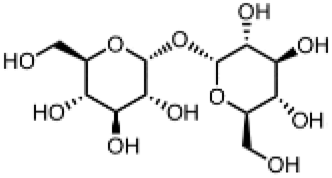
B)



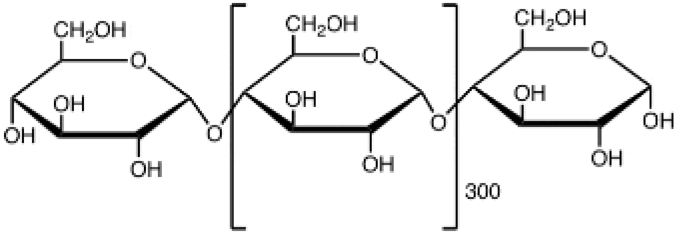
C)



D)



E)

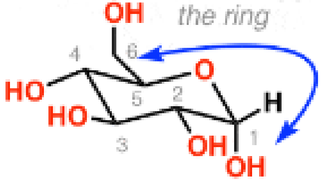


Mutarotation in carbohydrates:

Alpha (α) and beta (β) isomers ("anomers") differ in the orientation of the OH at the C-1 hemiacetal carbon

Example: D-glucose

"alpha" (α) isomer:



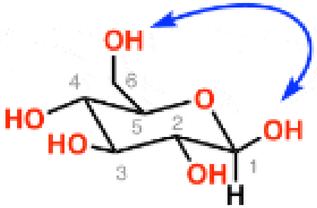
*C_5-CH_2OH (up) and C_1-OH (down) are on **opposite** faces of the ring*

α-D-Glucose

drawn as "chair"

Specific rotation: $[\alpha]_D^{20}$ + 112°

"beta" (β) isomer:



*C_5-CH_2OH (up) and C_1-OH (up) are on the **same** face of the ring*

β-D-Glucose

drawn as "chair"

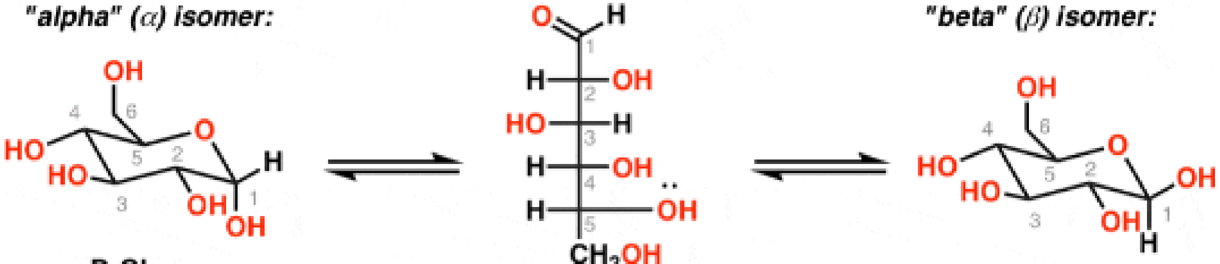
Specific rotation: $[\alpha]_D^{20}$ + 18.7°

↖ **Note different specific rotations!** ↗

The alpha and beta anomers are each in equilibrium with the "linear" form, and therefore with each other.

"alpha" (α) isomer:

"beta" (β) isomer:



α-D-Glucose

36%
(at equilibrium)

6

D-Glucose

("linear" form)

β-D-Glucose

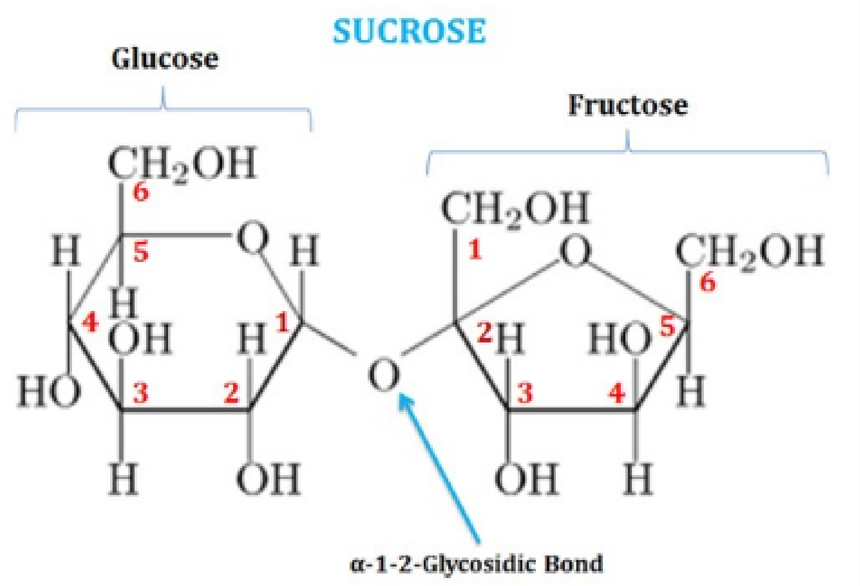
64%
(at equilibrium)

At equilibrium the mixture consists of 36% α-D-Glucose, 64% β-D-Glucose, and traces of the linear and furanose forms.

Examples of some disaccharides:

(1). Sucrose

- Sucrose is also called as 'Table Sugar'.
- It is a disaccharide of Glucose and Fructose joined by **α-1-β2-glycosidic linkage**.
- C1 of the glucose is bonded with the C2 of fructose (both are anomeric carbons).
- The chemical formula of sucrose is $C_{12}H_{22}O_{11}$.



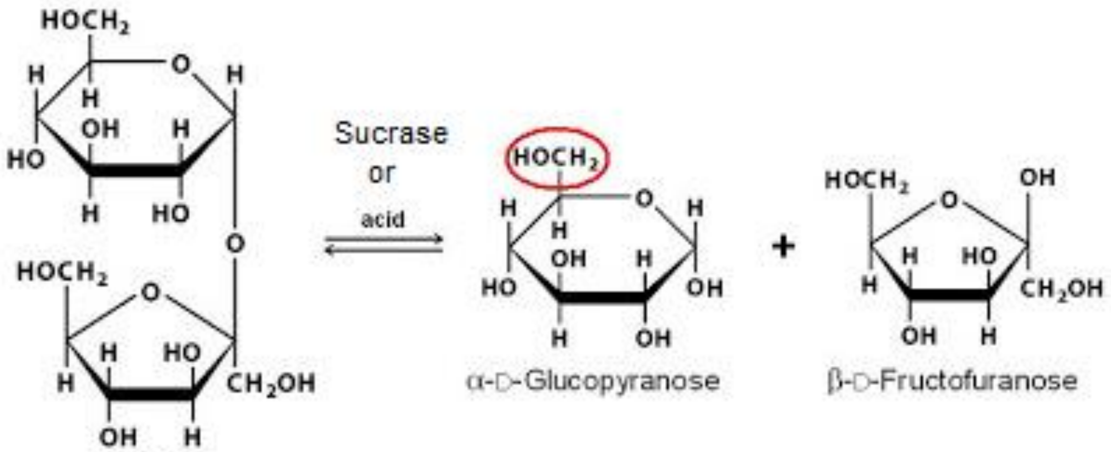
- The systematic name of sucrose is O-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside.
- In sucrose, the glucose residue is in six-membered ring form (Pyranose form) and the

- The systematic name of sucrose is O-α-D-glucopyranosyl-(1→2)-β-D-fructofuranoside.

- In sucrose, the glucose residue is in six-membered ring form (Pyranose form) and the fructose residue is in five-membered ring form (Furanose form).

- Sucrose is a non-reducing sugar since the anomeric carbon atoms of both glucose and fructose are involved in glycosidic bond formation.
- Sucrose is formed only in plants. No animals are known to produce sucrose.
- Sucrose is the major intermediate product of photosynthesis.
- It is the major form of sugar transport in plants.
- Sucrose is hydrolyzed by the enzyme Invertase into glucose and fructose.

Inversion of sugar:



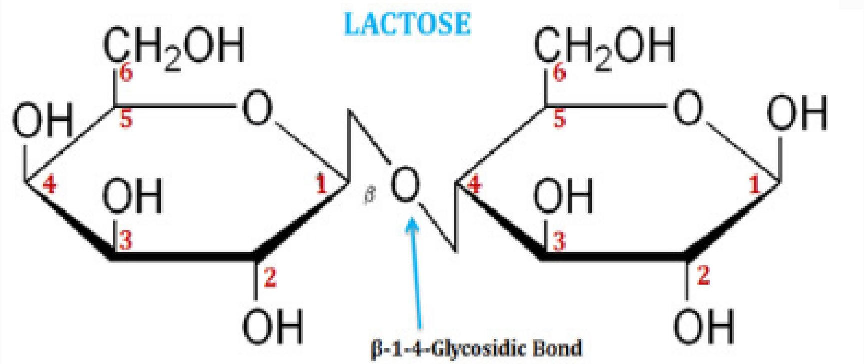
Sucrose
(α-D-Glucopyranosyl-(1-2)-β-D-Fructofuranoside)

α-D-Glucopyranose

β-D-Fructofuranose

(2). Lactose

- Lactose is the milk sugar, present naturally only in milk.
- In milk, the concentration of lactose varies from 2 – 8% in different species.



- It is a disaccharide of Galactose and Glucose connected by **β(1-4) glycosidic linkage.**
- The systematic name of lactose is O-β-D-galactopyranosyl-(1→4)-D-glucopyranose.
- The chemical formula of lactose is $C_{12}H_{22}O_{11}$.
- In lactose, the anomeric carbon on the glucose is free and thus it a reducing sugar.
- Lactose cannot be absorbed directly into the bloodstream of animals.
- First, it should be hydrolyzed into its monomer units (galactose and glucose) by the activity of the enzyme β-Galactosidase commonly called as lactase.
- In mammals, the lactase enzyme is produced only by young milk feeding individuals.
- As the individuals mature the production of lactase enzyme decreases.
- Most adult humans, except certain groups in Africa and northern Europe, produce only very low levels of lactase.

- Thus, the adults cannot utilize lactose as a food source. For most individuals, this is not a problem, but some cannot tolerate lactose and experience intestinal pain and diarrhea upon consumption of milk.

- This condition is called Lactose Intolerance.

- The lactose intolerance is caused by the fermentation of lactose inside the colon of adult individuals by some gut bacteria.

- Lactase fermentation in the colon produced CO_2, H_2, and some irritating organic acids.

- These compounds cause the irritation, painful stomach upset and diarrhea.

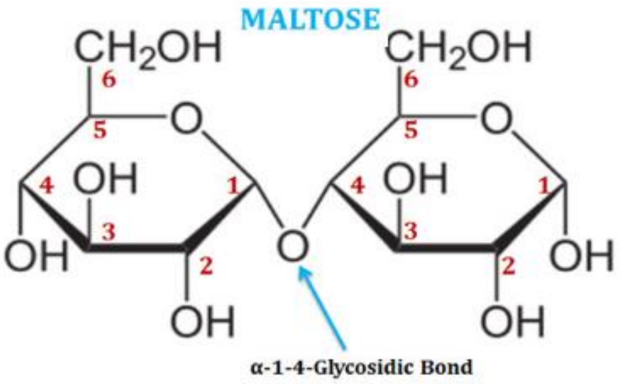
(3). Maltose

- Maltose is also called as malt sugar or maltobiose.

- It is a disaccharide of two glucose residues connected through **α(1-4) glycosidic linkage**.

- Maltose possesses the free anomeric carbon atom at the second glucose and thus it is a reducing sugar.

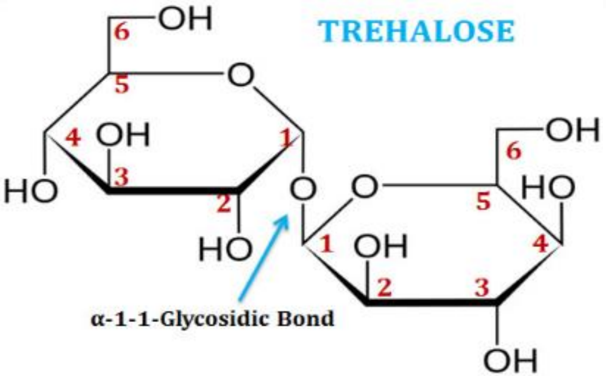
- Maltose is produced from starch by the activity of the enzyme β-amylase.



- β-amylase enzyme produces two-unit groups from the starch.
- Maltose is the two-unit member of the amylose part of starch.
- Maltose is the major component of the 'Malt'.
- The 'Malt' is formed when starchy grains are allowed to germinate by soaking in water.
- An enzyme called diastase, produced during the germination of seeds catalyzes the hydrolysis of starch to maltose.
- Maltose is used in beverages industry for the production of beer by yeast fermentation.

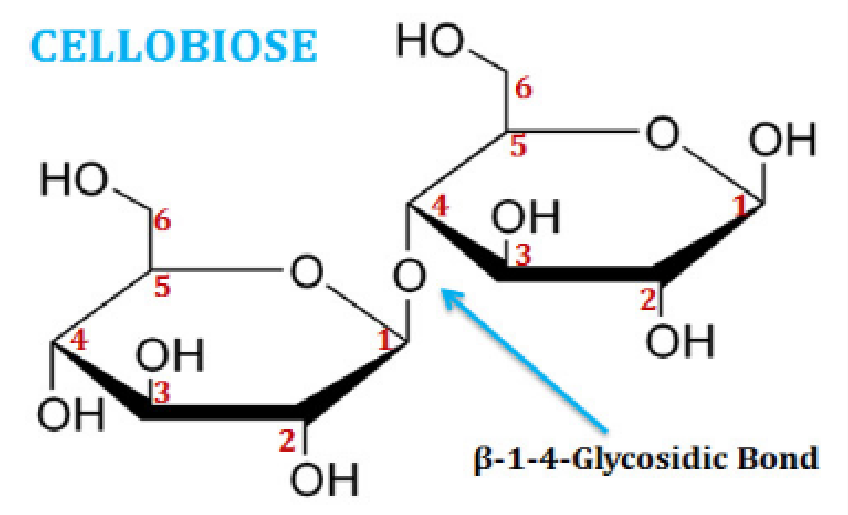
(4). Trehalose

- Trehalose (also called mycose) is a disaccharide of two glucose residues connected through **α(1-1)-glycosidic linkage**.
- The systematic name of trehalose is α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside.
- In trehalose, both the anomeric carbon atoms are involved in glycosidic bond formation. Thus, it is a non-reducing sugar.
- Trehalose is the major constituent of hemolymph of insects.
- Trehalose can be synthesized by bacteria, fungi, plants and some invertebrates.
- Trehalose helps plants to survive during desiccations.



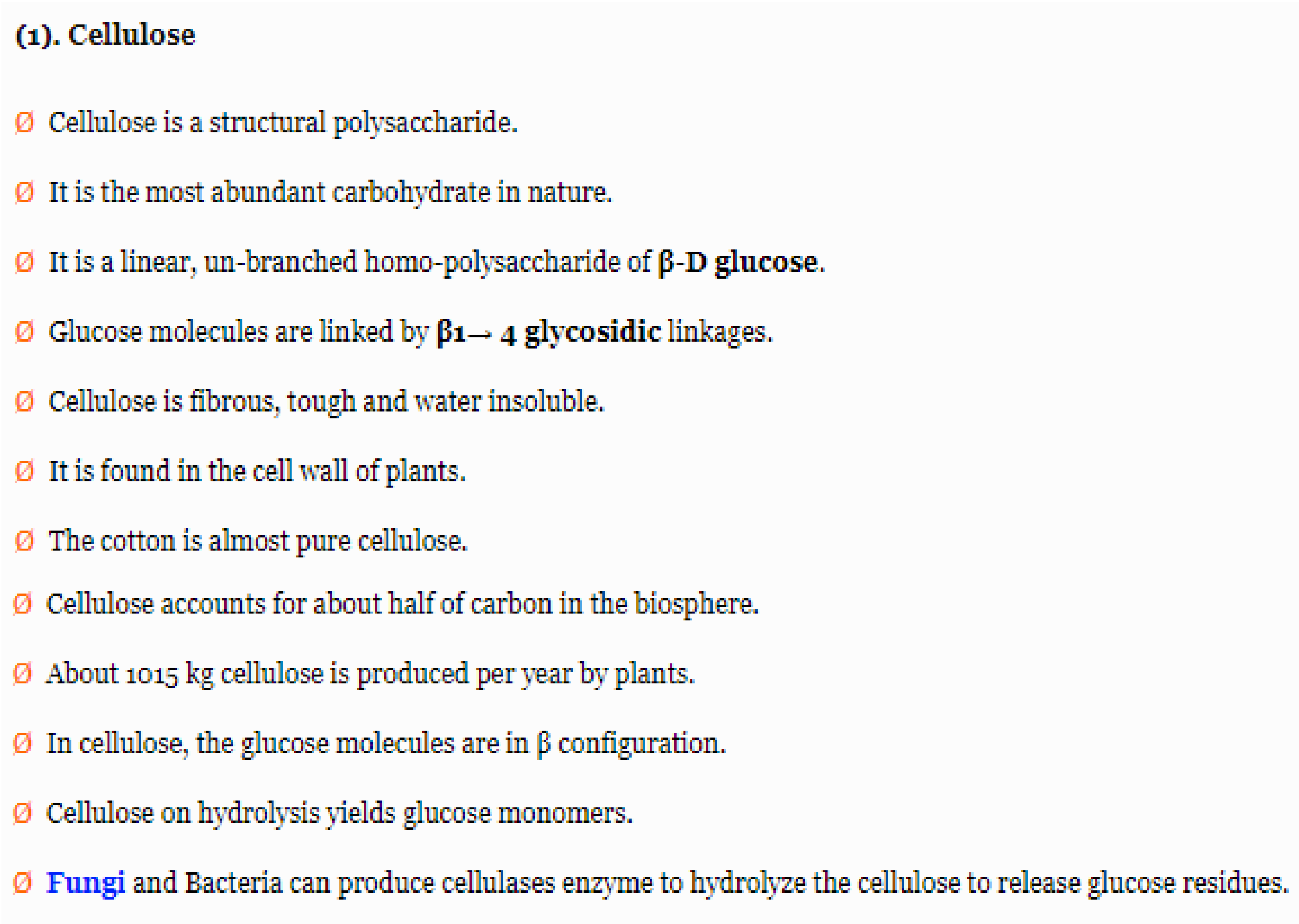
(5). Cellobiose

- Cellobiose is a disaccharide of two glucose molecules connected through **β(1→4) glycosidic linkage**.
- It is a reducing sugar since the anomeric carbon atom of second glucose is free.



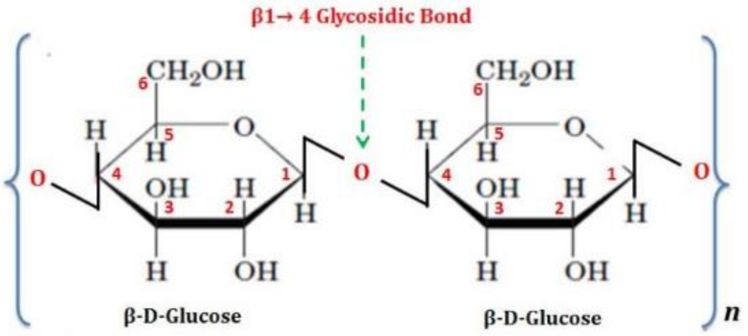
β-1-4-Glycosidic Bond

Examples of some polysaccharides:

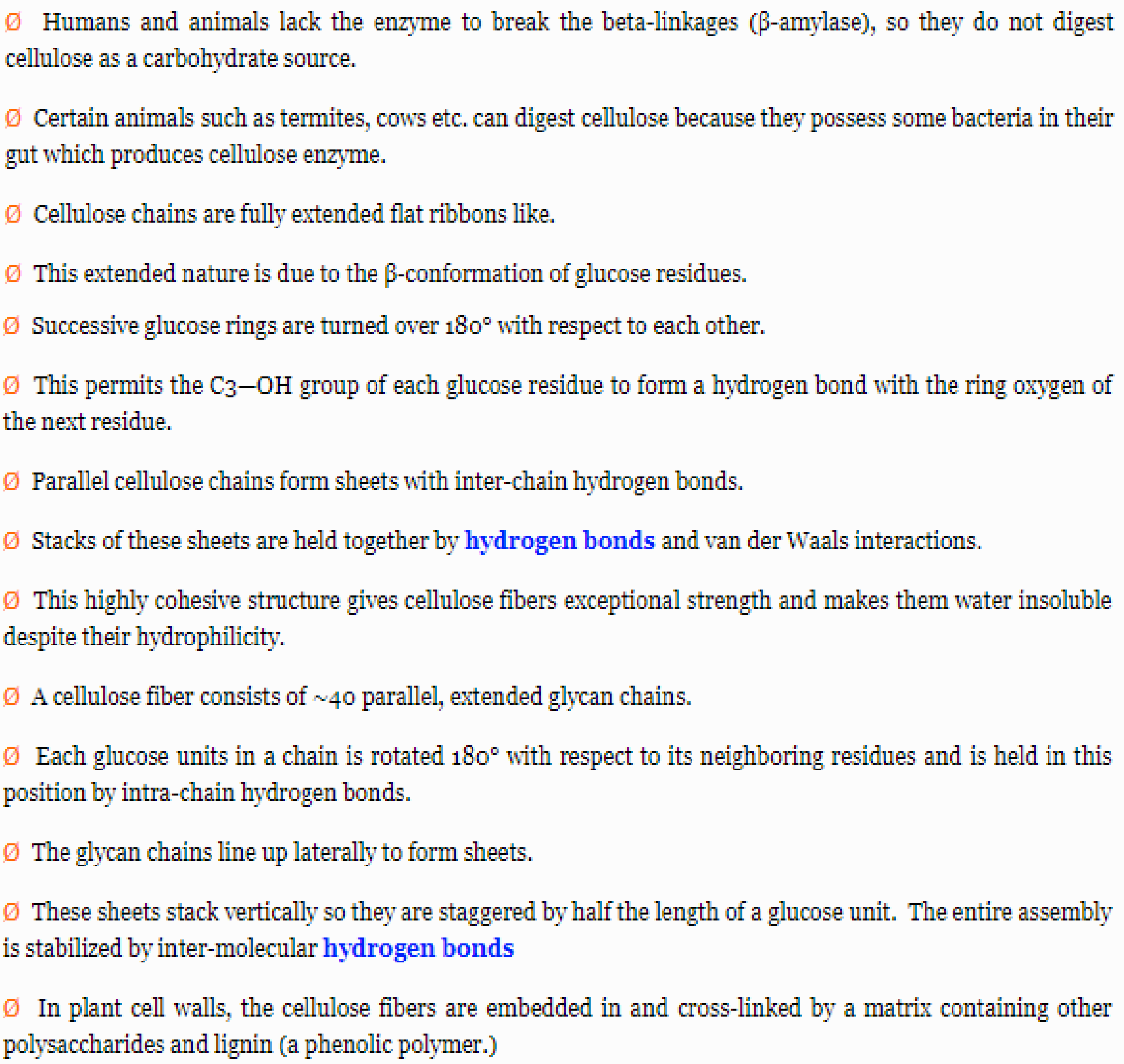


(1). Cellulose

- Cellulose is a structural polysaccharide.
- It is the most abundant carbohydrate in nature.
- It is a linear, un-branched homo-polysaccharide of **β-D glucose**.
- Glucose molecules are linked by **β1→ 4 glycosidic** linkages.
- Cellulose is fibrous, tough and water insoluble.
- It is found in the cell wall of plants.
- The cotton is almost pure cellulose.
- Cellulose accounts for about half of carbon in the biosphere.
- About 1015 kg cellulose is produced per year by plants.
- In cellulose, the glucose molecules are in β configuration.
- Cellulose on hydrolysis yields glucose monomers.
- **Fungi** and Bacteria can produce cellulases enzyme to hydrolyze the cellulose to release glucose residues.



Cellulose



- Humans and animals lack the enzyme to break the beta-linkages (β-amylase), so they do not digest cellulose as a carbohydrate source.
- Certain animals such as termites, cows etc. can digest cellulose because they possess some bacteria in their gut which produces cellulose enzyme.
- Cellulose chains are fully extended flat ribbons like.
- This extended nature is due to the β-conformation of glucose residues.
- Successive glucose rings are turned over 180° with respect to each other.
- This permits the C3—OH group of each glucose residue to form a hydrogen bond with the ring oxygen of the next residue.
- Parallel cellulose chains form sheets with inter-chain hydrogen bonds.
- Stacks of these sheets are held together by **hydrogen bonds** and van der Waals interactions.
- This highly cohesive structure gives cellulose fibers exceptional strength and makes them water insoluble despite their hydrophilicity.
- A cellulose fiber consists of ~40 parallel, extended glycan chains.
- Each glucose units in a chain is rotated 180° with respect to its neighboring residues and is held in this position by intra-chain hydrogen bonds.
- The glycan chains line up laterally to form sheets.
- These sheets stack vertically so they are staggered by half the length of a glucose unit. The entire assembly is stabilized by inter-molecular **hydrogen bonds**
- In plant cell walls, the cellulose fibers are embedded in and cross-linked by a matrix containing other polysaccharides and lignin (a phenolic polymer.)

Ø The resulting composite material can withstand large stresses because the matrix evenly distributes the stresses among the cellulose reinforcing elements.

Ø The difficulty of removing these other substances, however, is one of the main reasons that the cellulose in wood and agricultural waste, despite its abundance, cannot be easily converted to biofuels.

Ø Vertebrates themselves do not possess an enzyme capable of hydrolyzing the β(1→4) linkages of cellulose

Ø However, the digestive tracts of herbivores (and termites) contain symbiotic microorganisms that secrete a series of **enzymes**, collectively known as cellulases, that can hydrolyze cellulose

(2). Starch

Ø Starch is a homo-polysaccharide of α-D-glucose

Ø It is a storage polysaccharide

Ø It is a long branched polysaccharide.

Ø Starch contains 2 types of polymer : **amylose** and **amylopectin**

  $ Amylose chain (20 – 25%): Long, un-branched chain, composed of glucose molecules joined by α1→4 glycosidic linkage.

  $ Amylopectin chain (75 – 80%): Long, highly branched and composed of glucose molecules joined by α1→4 glycosidic linkage

Ø The branches are in α1→6 glycosidic linkage.

Ø Branch points occur at every 24 to 30 residues.

Ø α-amylose is an isomer of cellulose, however, it has very different structural properties.

Ø Cellulose's β-glycosidic linkages cause it to assume a tightly packed, fully extended conformation

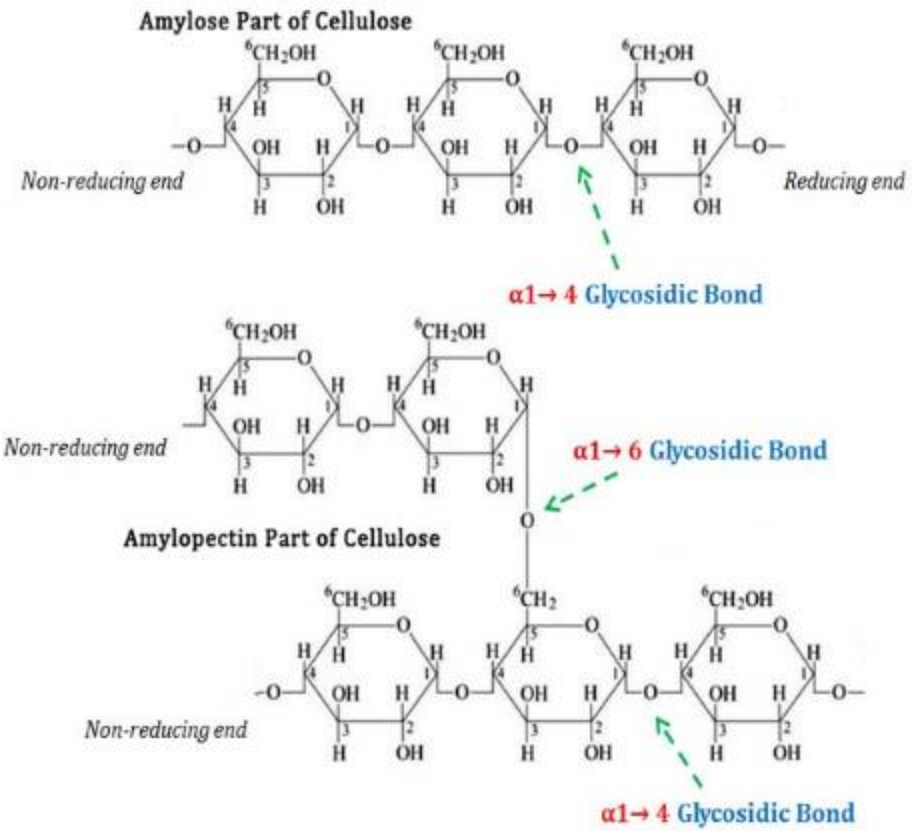
- Whereas, α-amylose's α-glycosidic bonds cause it to adopt an irregularly aggregating left-handed helically coiled conformation

- This helically coiled conformation, amylose can accommodate iodine molecules in the helix to produce an intense blue colour (iodine test)

- Plants synthesize starch as their main energy reserve.

- Starch is deposited in chloroplasts of plant cells as insoluble granules.

Starch



Ø Storage of glucose as starch greatly reduces the large intracellular osmotic pressure that would result from its storage in monomeric form, because osmotic pressure is proportional to the number of solute molecules in a given volume.

Ø Starch is a reducing sugar

Ø Both amylose and amylopectin possess a single reducing site at the end called reducing end

Ø Digestion of starch (main carbohydrate source) begins in the mouth.

Ø Saliva contains an α-amylase enzyme (salivary amylase), which randomly hydrolyzes the α (1→4) glycosidic bonds of starch.

Ø Starch digestion continues in the small intestine under the influence of pancreatic amylase, which degrades starch to a mixture of small oligosaccharides.

Ø Further hydrolysis by an α-glucosidase, which removes one glucose residue at a time, and by a de-branching enzyme, which hydrolyzes α(1→6) as well as α(1→4) bonds, produce monosaccharides that are absorbed by the intestine and transported to the bloodstream.

(3). Glycogen

Ø Glycogen is the major storage polysaccharide in animal cells.

Ø It is a polymer of α1-4 linked glucose with α1-6 branching.

Ø Structurally glycogen is similar to amylopectin part of the starch.

Ø Different from amylopectin: glycogen is heavily branched

Ø In glycogen, branching occurs at every 8 to 12 residues.

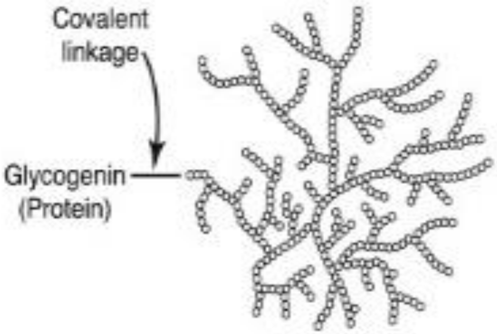
Ø Thus, glycogen is more compact than starch.

Ø Glycogen is present in all cells, prevalent in skeletal muscle and in liver

Ø Glycogen is stored in the liver of animal, stored as granules.

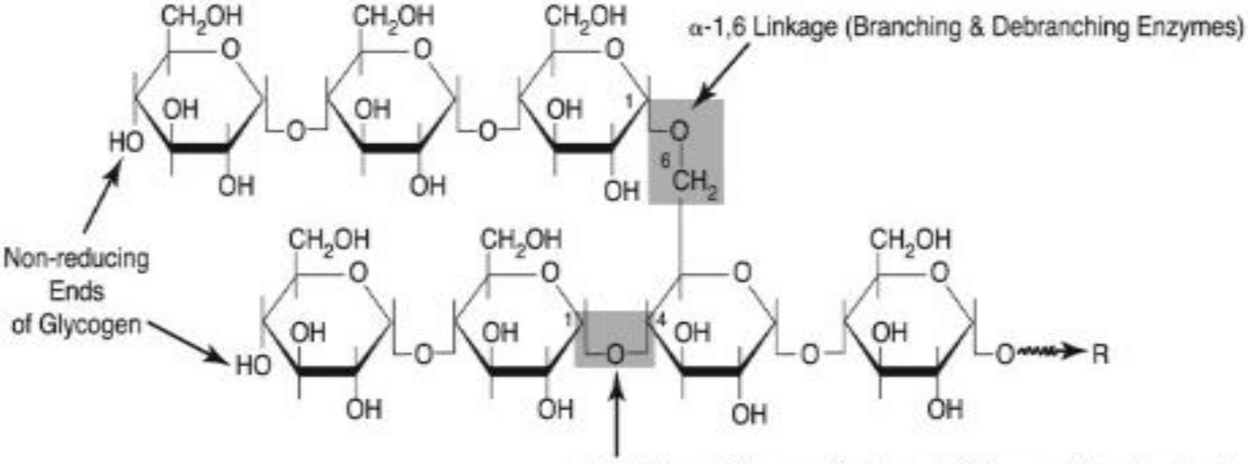
Ø Glycogen constitutes 7% of the wet weight of hepatocytes.

Structure



Glycogen

The α-1,6 glycosidic bonds in glycogen are produced by the non-regulatory brancing enzyme (and broken by debranching enzyme), while α-1,4 glycosidic bonds in glycogen are produced by the regulatory glycogen synthase (and broken by glycogen phosphorylase).



α-1,6 Linkage (Branching & Debranching Enzymes)

α-1,4 Linkage (Glycogen Synthase & Glycogen Phosphorylase).

- Glycogen is degraded by Glycogen phosphorylase enzyme, which phosphorolytically cleaves glycogen's α(1→4) bonds sequentially inward from its non-reducing ends.

- Glycogen's highly branched structure, which has many non-reducing ends, permits the rapid mobilization of glucose in times of metabolic need.

- A glycogen molecule with n branches has n + 1 non-reducing ends, but only one reducing end.

- The α(1→6) branches of glycogen are cleaved by glycogen debranching enzyme.

- Hepatocytes store glycogen equivalent to a glucose concentration of 0.4 M.

- The actual concentration of glycogen, which is insoluble and contributes little to the osmolarity of the cytosol, is about 0.01 μM.

- If the cytosol contained 0.4 M glucose, the osmolarity would be threateningly elevated, leading to osmotic entry of **water** that might rupture the cell.

- Furthermore, with an intra-cellular glucose concentration of 0.4 M and an external concentration of about 5 mM (~blood glucose level of a mammal) the free-energy change for glucose uptake into cells against this very high concentration gradient would be prohibitively large.

(4). Chitin

- Chitin is a linear homo-polysaccharide of N-acetyl glucosamine in β1-4 linkage.

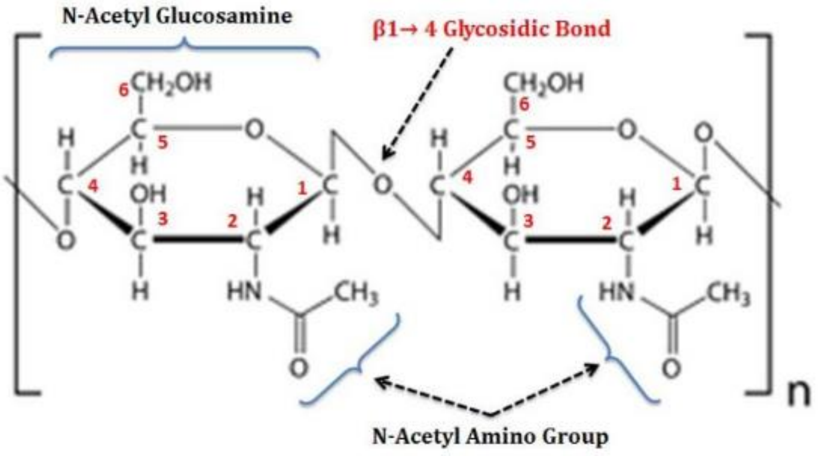
- It is difference from cellulose: the hydroxyl group at C-2 is replaced by an acetylated amino group.

- Chitin is the principle component of the cell wall of fungi.

- Chitin also forms the exo-skeleton of **arthropods**.

- Chitin is not digested by animals.

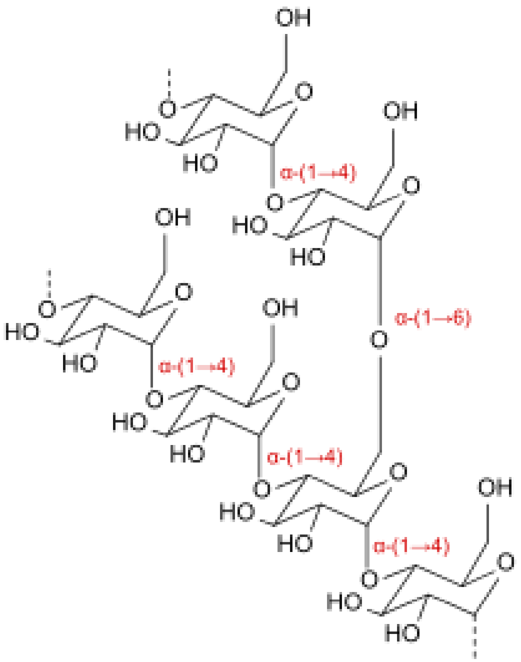
- Chitin is the second most abundant polysaccharide in the biospehere.



Chitin

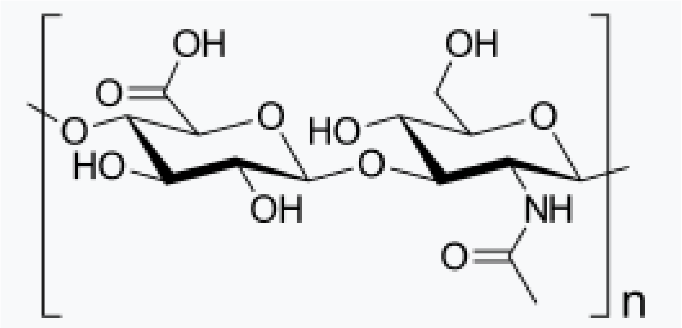
5. Dextrines:

- Dextrins are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch or glycogen.
- Dextrins are mixtures of polymers of D-glucose units linked by α-(1→4) or α-(1→6) glycosidic bonds.
- Dextrins can be produced from starch using enzymes like amylases, as during digestion in the human body and during malting and mashing.
- Dextrins are white, yellow, or brown powder that are partially or fully water-soluble, yielding optically active solutions of low viscosity.
- White and yellow dextrins from starch roasted with little or no acid are called British gum.



HYALURONIC ACID:-

- *Important GAG found in the ground substance of synovial fluid of joints and vitreous humor of eyes.*
- *Present in connective tissue and forms a gel around*
- *Composed of alternative units of D-Glucoronic acid and N-acety*
- *These two molecules formed disaccharides units held together by β-(1-3) Glycosidic bonds.*

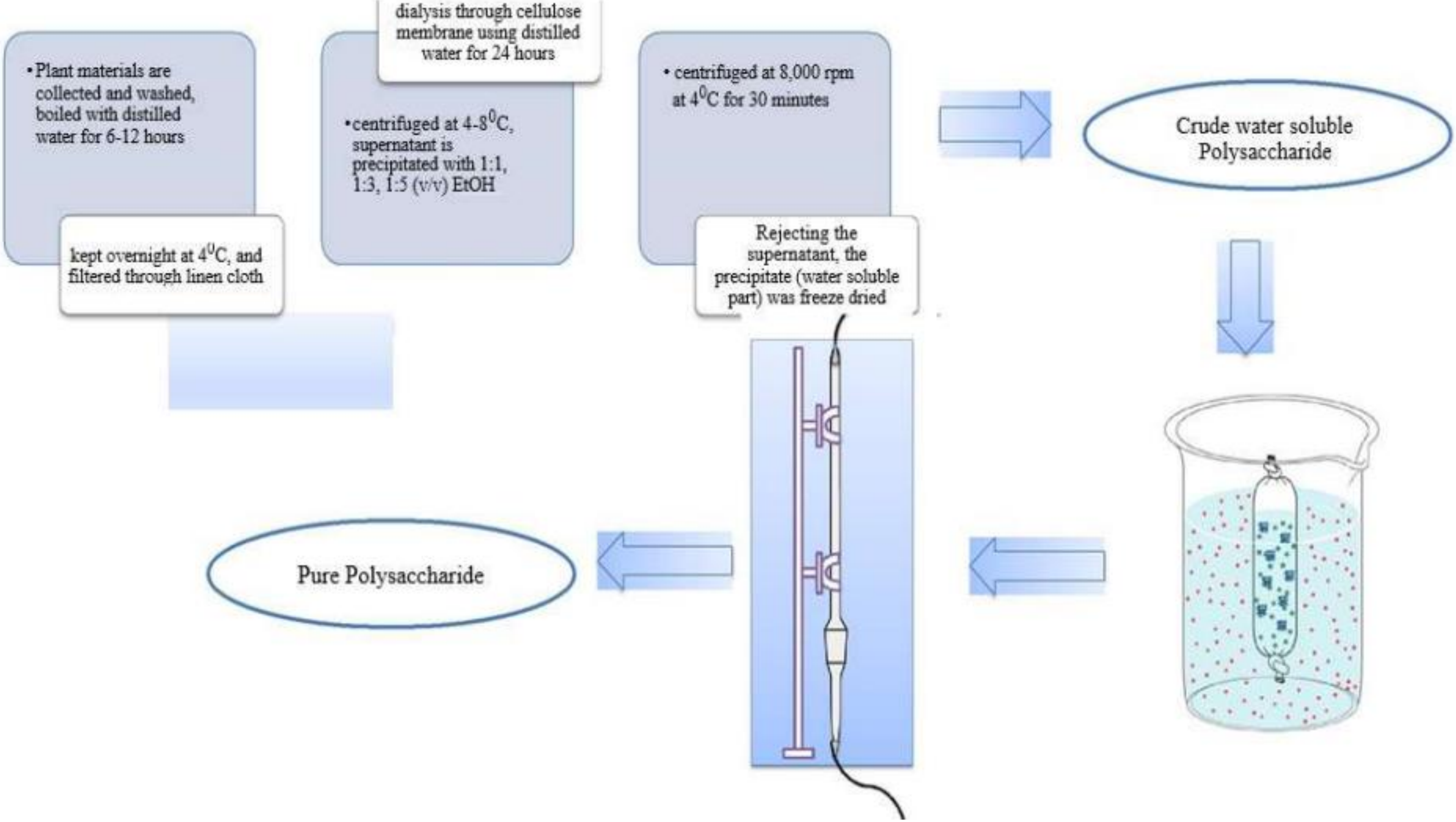


Functions Of Polysaccharides:

- The polysaccharides are important structural part of animal and plants.
- They store energy in organisms.
- They influences the uptake of nutrients and water by the cells.
- Some polysaccharides are covalently bonded with lipids and proteins to form glycolipids and glycoproteins. These are used to send signals between and within the cells.
- They provide support to the cells. The cell wall of plants is made up of polysaccharide cellulose, which provides support to the cell wall of the plant. In insects and fungi, chitin plays an important role in providing support to the extracellular matrix around the cells.

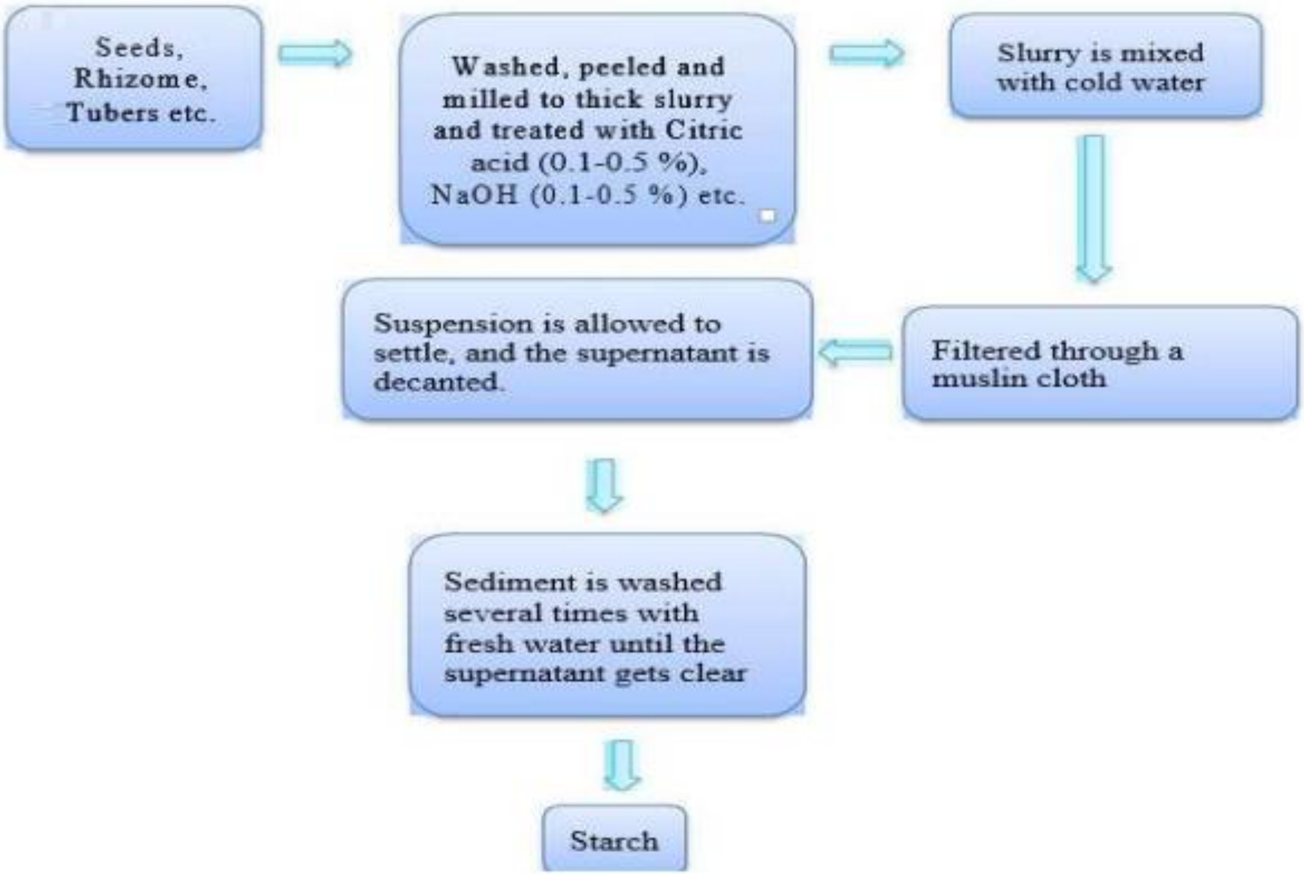
ISOLATION OF WATER SOLUBLE POLYSACCHARIDES:

- Water soluble polysaccharides are isolated from various parts of the plants (fruits, roots, pulp , seeds, leaf) using a hot water extraction regime followed by ethanolic precipitation.
- Generally for isolation of water soluble polysaccharides raw materials are coarsely grounded and boiled/washed with hot water for different time periods and then cold centrifugation is done at 4 ºC- 8 ºC at 8000 rpm for 30 min.
- After centrifugation the supernatant is stored at 4 ºC in a freezer for at least 12 hours and the supernatant is precipitated using alcohol/acetone. Precipitated polysaccharides are then removed by centrifugation.
- For further purification the crude polysaccharide is subjected to dialysis, followed by Gel Permeation Chromatography/ Size exclusion chromatography using different chromatographic column.


• Plant materials are collected and washed, boiled with distilled water for 6-12 hours
kept overnight at 4⁰C, and filtered through linen cloth
dialysis through cellulose membrane using distilled water for 24 hours
• centrifuged at 4-8⁰C, supernatant is precipitated with 1:1, 1:3, 1:5 (v/v) EtOH
• centrifuged at 8,000 rpm at 4⁰C for 30 minutes
Rejecting the supernatant, the precipitate (water soluble part) was freeze dried
Crude water soluble Polysaccharide
Pure Polysaccharide

ISOLATION OF WATER INSOLUBLE POLYSACCHARIDES:

- Water insoluble polysaccharides are divided into two groups e.g. Starch and Non-starch polysaccharides.
- Isolation of water insoluble polysaccharides is done through solvent extraction method. This method is needed to needed to be optimized for each sample.
- Starch is a storage plant polysaccharide. It is isolated using aqueous extraction method or through chemical treatment.



Seeds, Rhizome, Tubers etc.

Washed, peeled and milled to thick slurry and treated with Citric acid (0.1-0.5 %), NaOH (0.1-0.5 %) etc.

Slurry is mixed with cold water

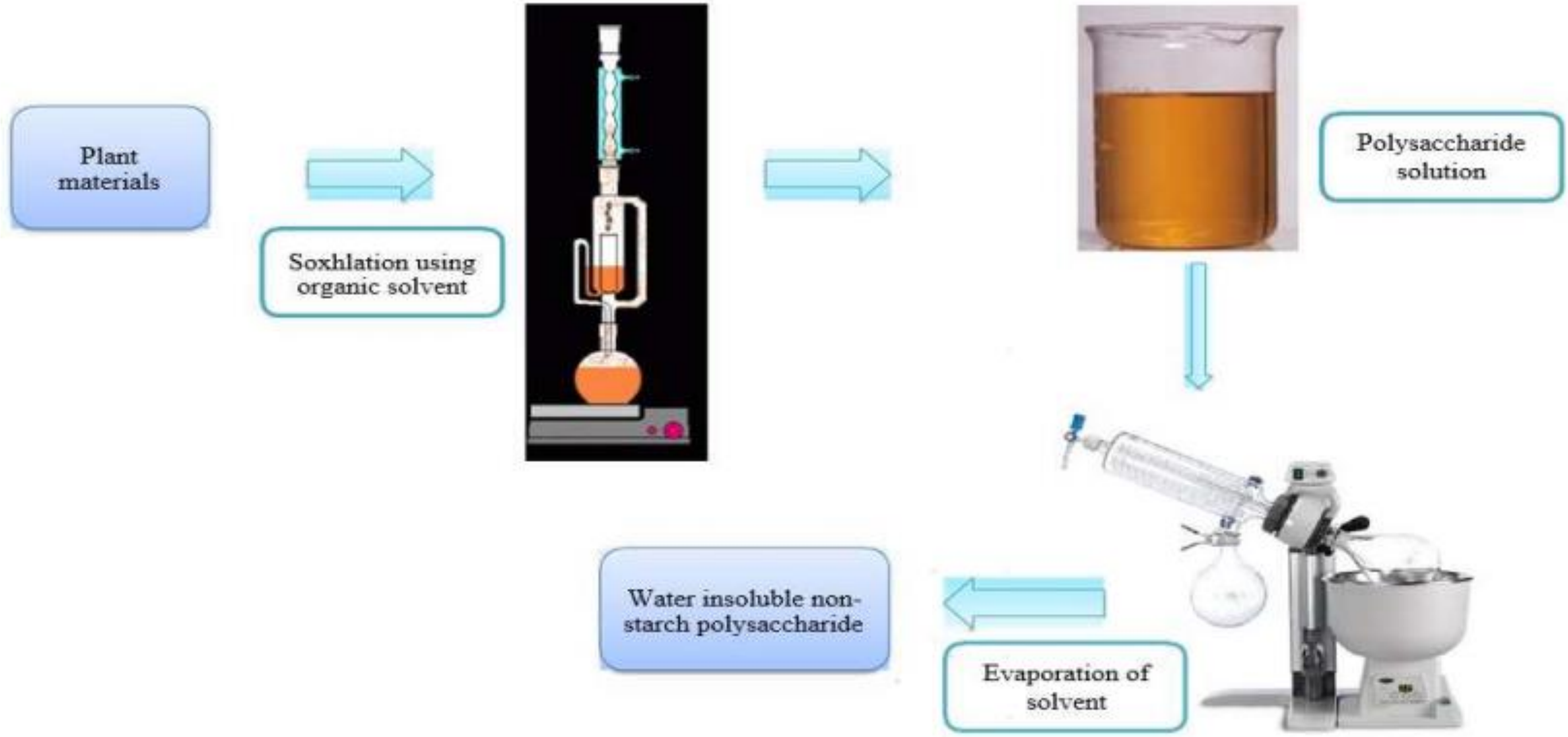
Filtered through a muslin cloth

Suspension is allowed to settle, and the supernatant is decanted.

Sediment is washed several times with fresh water until the supernatant gets clear

Starch

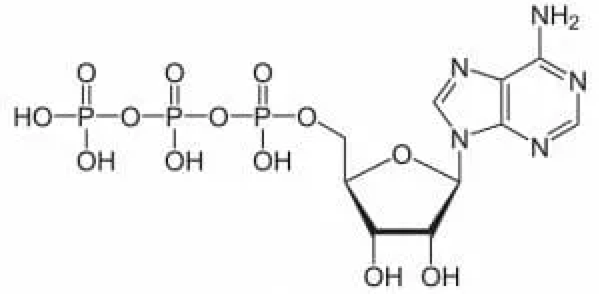
- Non-starch water insoluble polysaccharides are cellulose, linin, hemicellulose etc. These water insoluble polysaccharides are soluble in organic solvents e.g. methanol, ethanol



Cellular currency of energy (ATP):

Adenosine triphosphate, also known as ATP, is a molecule that carries energy within cells. It is the main energy currency of the cell, and it is an end product of the processes of photophosphorylation (adding a phosphate group to a molecule using energy from light), cellular respiration, and fermentation. All living things use ATP. In addition to being used as an energy source, it is also used in signal transduction pathways for cell communication and is incorporated into deoxyribonucleic acid (DNA) during DNA synthesis.

Structure of ATP:



This is a structural diagram of ATP. It is made up of the molecule adenosine (which itself is made up of adenine and a ribose sugar) and three phosphate groups. It is soluble in water and has a high energy content due to having two phosphoanhydride bonds connecting the three phosphate groups.

Functions of ATP: 1. Energy Source: ATP is the main carrier of energy that is used for all cellular activities. When ATP is hydrolyzed and converted to adenosine diphosphate (ADP), energy is released. The removal of one phosphate group releases 7.3 kilocalories per mole, or 30.6 kilojoules per mole, under standard conditions. This energy powers all reactions that take place inside the cell. ADP can also be converted back into ATP so that the energy is available for other cellular reactions.

ATP is produced through several different methods. Photophosphorylation is a method specific to plants and cyanobacteria. It is the creation of ATP from ADP using energy from sunlight, and occurs during photosynthesis. ATP is also formed from the process of cellular respiration in the mitochondria of a cell. This can be through aerobic respiration, which requires oxygen, or anaerobic respiration, which does not. Aerobic respiration produces ATP (along with carbon dioxide and water) from glucose and oxygen. Anaerobic respiration uses chemicals other than oxygen, and this process is primarily used by archaea and bacteria that live in anaerobic environments. Fermentation is another way of producing ATP that does not require oxygen; it is different from anaerobic respiration because it does not use an electron transport chain. Yeast and bacteria are examples of organisms that use fermentation to generate ATP.

2. Signal Transduction: ATP is a signaling molecule used for cell communication. Kinases, which are enzymes that phosphorylate molecules, use ATP as a source of phosphate groups. Kinases are important for signal transduction, which is how a physical or chemical signal is transmitted from receptors on the outside of the cell to the inside of the cell. Once the signal is inside the cell, the cell can respond appropriately. Cells may be given signals to grow, metabolize, differentiate into specific types, or even die.

3. DNA Synthesis: The nucleobase adenine is part of adenosine, a molecule that is formed from ATP and put directly into RNA. The other nucleobases in RNA, cytosine, guanine, and uracil, are similarly formed from CTP, GTP, and UTP. Adenine is also found in DNA, and its incorporation is very similar, except ATP is converted into the form deoxyadenosine triphosphate (dATP) before becoming part of a DNA strand.

CARBOHYDRATE METABOLISM

- Glucose is the major form of sugar moiety present in blood and other body fluids. The digestion of food carbohydrates, such as starch, sucrose, and lactose produces the monosaccharides glucose, fructose and galactose, which pass into the blood stream. The study of synthesis (Anabolism) and degradation (Catabolism) of biomolecules is biochemically termed as metabolism.

  Anabolism + Catabolism = *Metabolism*

  (Synthesis) (Degradation)

- Since glucose is the most important carbohydrate existing in physiological amounts in the body and is easily absorbed from the diet, the metabolism of carbohydrate resolves it self to the study of the metabolism of glucose and its main derivatives. The monosaccharides galactose and fructose are converted to glucose in the liver. All the monosaccharides are completely absorbed in the small intestine.
- The glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body and used for the production of energy. Normally carbohydrate metabolism supplies more than half of the energy requirements of the body. In fact the brain largely depends upon carbohydrate metabolism as a source of energy and quickly ceases to function properly when the blood glucose level falls much below normal.
- The major function of carbohydrate in metabolism is to serve as fuel and get oxidised to provide energy for other metabolic processes. The metabolic intermediates are used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

  1. Glycolysis 2. Tricarboxylic acid cycle

Glycogenesis:

- Glycogen is the major storage form of carbohydrate in animals similar to starch in plants.
- It is a homopolymer made up of repeated units of α- D glucose and each molecule is linked to another by 1→4 glycosidic bond which is a link connecting the 1st C atom of the active glucose residue to the 6th C atom of the approaching glucose molecule.
- Once there is a chain consisting of 8 to 10 glycosidic residues in the glycogen fragment, branching begins by 1→6 linkages.
- Glycogenesis is the process of glycogen synthesis, in which glucose molecules are added to chains of glycogen for storage.

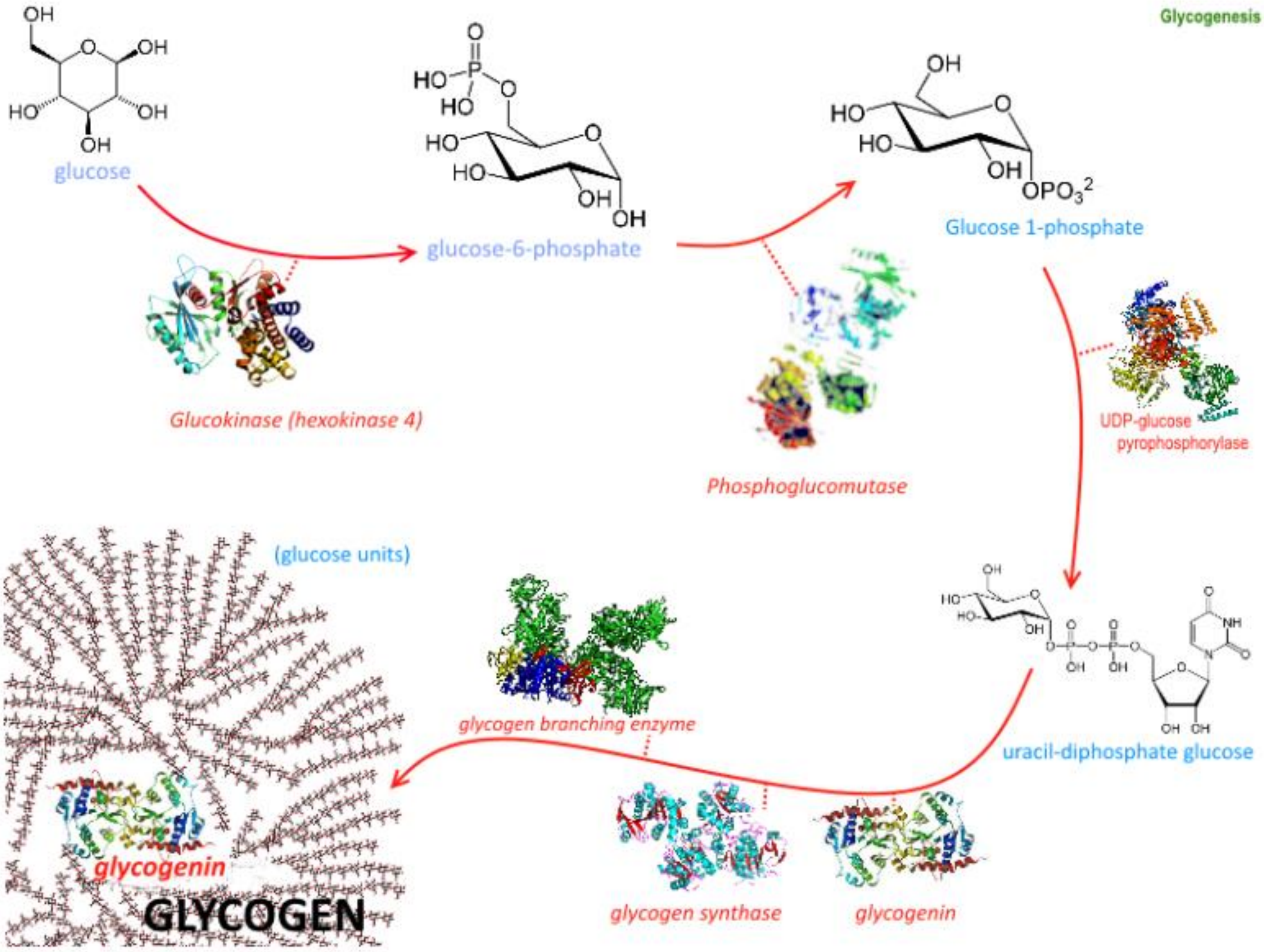
Location:

- Glycogenesis takes place in the cytoplasm of cells in muscle, liver, and adipose tissue.
- Substrate: UDP-glucose.
- Result : Changes glucose to glycogen

Steps Involved:

- Glucose is converted into glucose-6-phosphate by the action of glucokinase or hexokinase with conversion of ATP to ADP.
- Glucose-6-phosphate is converted into glucose-1-phosphate by the action of phosphoglucomutase, passing through the obligatory intermediate glucose-1,6-bisphosphate.

Glycogenesis



glucose

Glucokinase (hexokinase 4)

glucose-6-phosphate

Phosphoglucomutase

Glucose 1-phosphate

UDP-glucose pyrophosphorylase

uracil-diphosphate glucose

glycogen branching enzyme

glycogen synthase

glycogenin

(glucose units)

glycogenin

GLYCOGEN

- Glucose-1-phosphate is converted into UDP-glucose by the action of the enzyme UDP-glucose pyrophosphorylase. Pyrophosphate is formed, which is later hydrolysed by pyrophosphatase into two phosphate molecules.
- The enzyme glycogenin is needed to create initial short glycogen chains, which are then lengthened and branched by the other enzymes of glycogenesis.
- Glycogenin, a homodimer, has a tyrosine residue on each subunit that serves as the anchor for the reducing end of glycogen. Initially, about eight UDP-glucose molecules are added to each tyrosine residue by glycogenin, forming α (1→4) bonds.
- Once a chain of eight glucose monomers is formed, glycogen synthase binds to the growing glycogen chain and adds UDP-glucose to the 4-hydroxyl group of the glucosyl residue on the non-reducing end of the glycogen chain, forming more α(1→4) bonds in the process.
- Branches are made by glycogen branching enzyme (also known as amylo $\alpha(1:4)\rightarrow\alpha(1:6)$transglycosylase), which transfers the end of the chain onto an earlier part via α-1:6 glycosidic bond, forming branches, which further grow by addition of more α-1:4 glycosidic units.

Energy requirement:

- In the synthesis of glycogen, one ATP is required per glucose incorporated into the polymeric branched structure of glycogen.

Important Enzymes:

- **Glycogen synthase**: Adds glucose units to the existing chains in α-1,4 linkages.
- **Glucosyl (4:6) transferase :** Transfers seven-glucose-residue-long pieces from the nonreducing ends of the chains to create internal branches with α-1,6 linkages.

Stimulation:

- Insulin stimulates glycogenesis via dephosphorylation and thus activates glycogen synthase.
- The process is also activated during rest periods following the Cori cycle.

Inhibition:

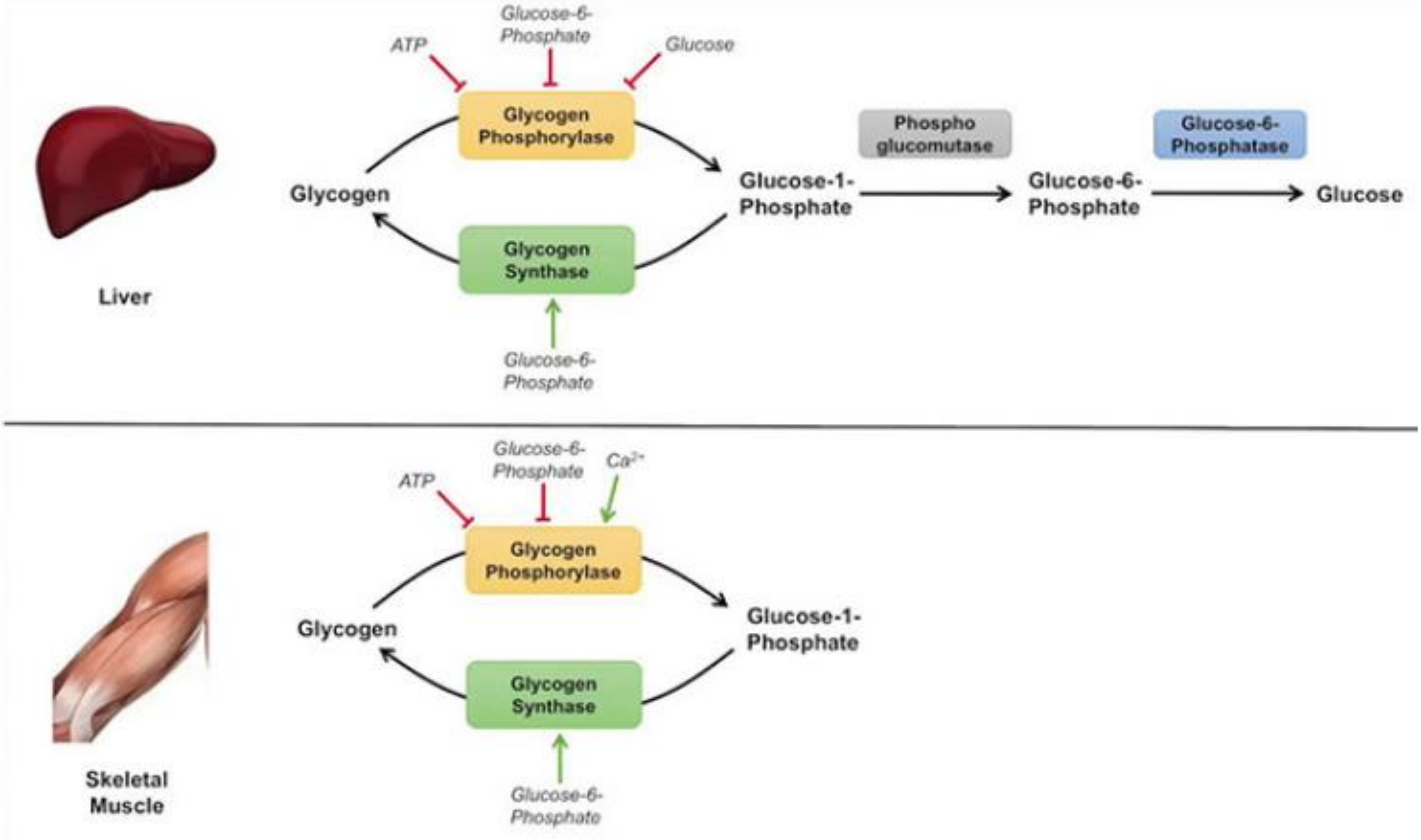
- Glucagon (liver) and epinephrine (liver and muscle) inhibit glycogenesis via the cAMP protein kinase A phosphorylation cascade, which results in phosphorylation and thus deactivation of glycogen synthase.

Significance:

- Glucose and its precursors are obtained through food. However under certain conditions they might not a reliable and continuous source of energy.
- The glycogenesis process is therefore a built in mechanism of the body which stores the excess carbohydrates we consume, in the form of glycogen which could be broken down to glucose when needed.

Glycogenolysis:

- Glycogenolysis is a process by which glycogen, the primary carbohydrate stored in the liver and muscle cells of animals, is broken down into glucose to provide immediate energy or to maintain blood glucose levels during the times of need.
- Glycogenolysis is thus the breakdown of glycogen (n) to glucose-1-phosphate and glycogen (n-1).



- Glycogen branches are catabolized by the sequential removal of glucose monomers via phosphorolysis, by the enzyme glycogen phosphorylase.

Location:

- Glygogenolysis takes place in the cytoplasm of cells in muscle, liver, and adipose tissue.
- **Result:** Glucose-1-phosphate is released from the non-reducing ends of glycogen chains.

Steps Involved:

- Glycogen phosphorylase cleaves the bond linking a terminal glucose residue to a glycogen branch by substitution of a phosphoryl group for the α-[1→4] linkage.
- Glucose-1-phosphate is converted to glucose-6-phosphate by the enzyme phosphoglucomutase.
- Glucose residues are phosphorolysed from branches of glycogen until four residues before a glucose that is branched with a α-[1→6] linkage.
- Glycogen de-branching enzyme then transfers three of the remaining four glucose units to the end of another glycogen branch.
- This exposes the α-[1→6] branching point, which is hydrolyzed by α[1→6] glucosidase, removing the final glucose residue of the branch as a molecule of glucose and eliminating the branch.
- This is the only case in which a glycogen metabolite is not glucose-1-phosphate. The glucose is subsequently phosphorylated to glucose-6-phosphate by hexokinase.

Important Enzymes:

- **Glycogen phosphorylase** breaks α-1,4 linkages and de-branching enzyme breaks α-1,6 linkages to release single units of glucose-1- phosphate.
- **Phosphoglucomutase** converts glucose- 1-phosphate to glucose-6-phosphate, which is then shuttled into the glycolytic pathway.

Stimulation:

- Glucagon (liver) and epinephrine (liver and muscle) stimulates glycogenolysis via the cAMP protein kinase A phosphorylation cascade, which results in the phosphorylation and thus activation of glycogen phosphorylase.

Inhibition:

- Insulin inhibits glycogenolysis via dephosphorylation and thus results in inactivation of glycogen phosphorylase.

Significance:

- Glycogenolysis plays an important role in the fight-or-flight response.
- It contributes to the regulation of glucose levels in the blood.
- The metabolism of glycogen polymers becomes important during fasting.

- In myocytes (muscle cells), glycogen degradation serves to provide an immediate source of glucose-6-phosphate for glycolysis, to provide energy for muscle contraction.
- In hepatocytes), the main purpose of the breakdown of glycogen is for the release of glucose into the bloodstream for uptake by other cells.

GLYCOLYSIS:

- Oxidation of glucose to pyruvate is called *glycolysis*. It was first described by ***Embden-Meyerho***f and ***Parnas***. Hence it is also called as ***Embden-Meyerh*** of pathway. Glycolysis occurs virtually in all tissues. Erythrocytes and nervous tissues derive the energy mainly from glycolysis. This pathway is unique in the sense that it can proceed in both aerobic (presence of O_2) and anaerobic (absence of O_2) conditions. All the enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.
- The overall equation for glycolysis from glucose to lactate is as follows:

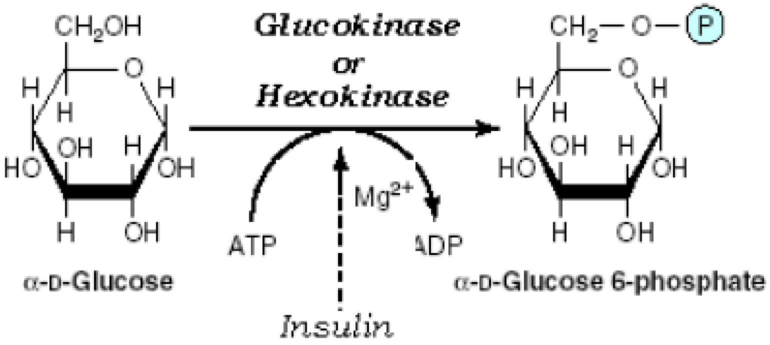
$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{L}(+) - \text{Lactate} + 2\text{ATP} + 2\text{H}_2\text{O}$$

Reactions of glycolytic pathway:

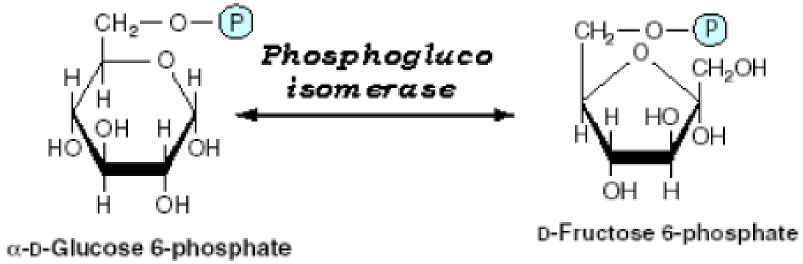
Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

Stage I: This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1, 6-diphosphate by donation of two phosphate groups from ATP.

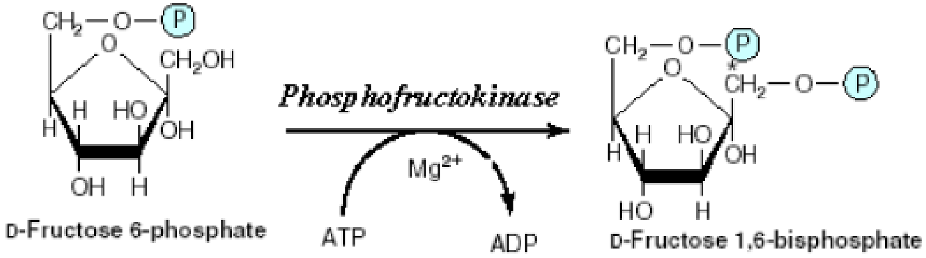
1. ***Uptake of glucose by cells and its phosphorylation:*** Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport. In other tissues ***insulin*** facilitates the uptake of glucose. Glucose is phosphorylated to form ***glucose 6-phosphate***. The enzyme involved in this reaction is *glucokinase or hexokinase*. This reaction is irreversible.



2. *Conversion of glucose 6-phosphate to fructose 6-phosphate:* Glucose 6-phosphate is converted to fructose 6-phosphate by the enzyme *phosphogluco isomerase*.

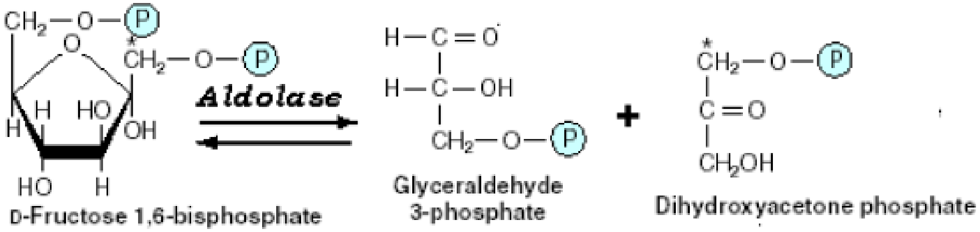


3. *Conversion of fructose 6-phosphate to fructose 1, 6 diphosphate:* Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme *phosphofructokinase* to produce fructose1, 6-diphosphate.



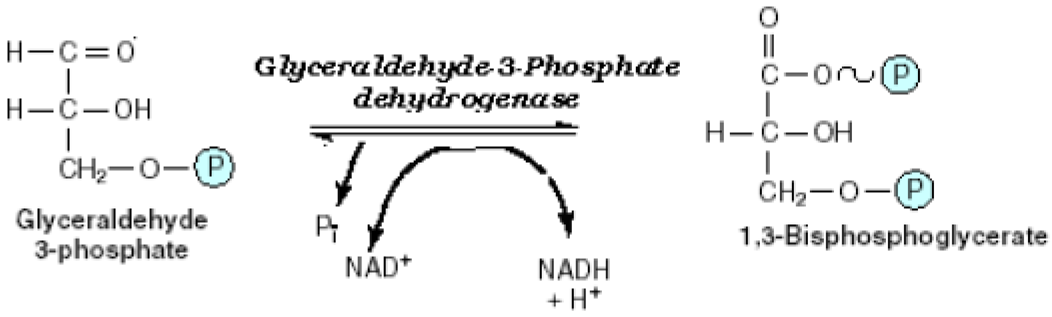
<u>Stage II</u>:

4. *Actual splitting of fructose 1, 6 diphosphate:* Fructose 1, 6 diphosphate is split by the enzyme *aldolase* into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one *ketotriose* - dihydroxy acetone phosphate. The reaction is reversible. There is neither expenditure of energy nor formation of ATP.

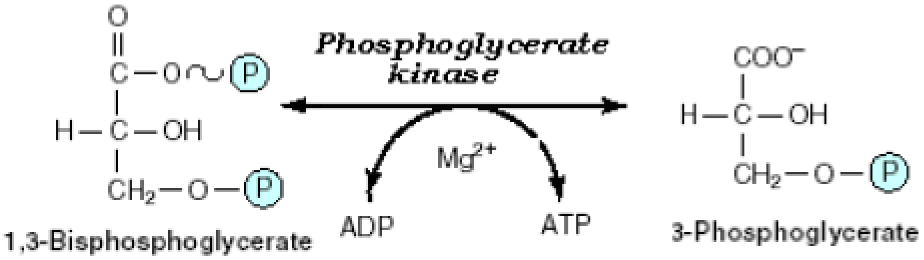


***Stage III*:** It is the energy yielding stage. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

6. ***Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate:*** Glycolysis proceeds by the oxidation of glyceraldehyde 3-phosphate to form 1, 3–bisphosphoglycerate. The reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphate dehydrogenas*.

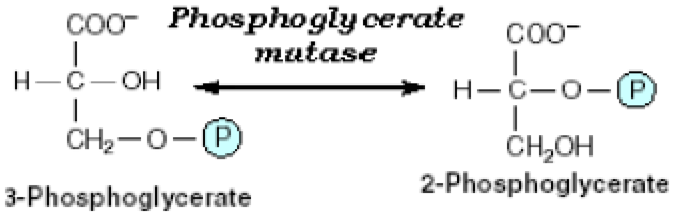


7. ***Conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate:*** The reaction is catalyzed by the enzyme *phosphoglycerate kinase*. The high energy phosphate bond at position-1 is transferred to ADP to form ATP molecule.

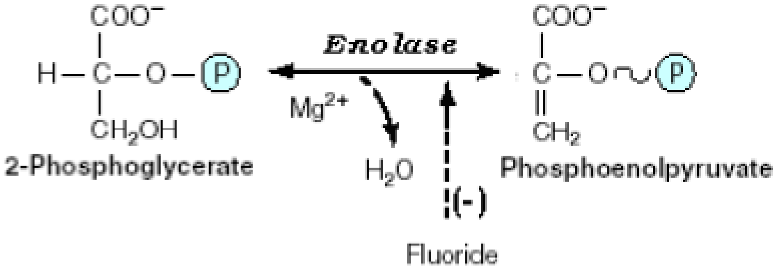


Stage IV: It is the recovery of the phosphate group from *3-phosphoglycerate*. The two molecules of *3-phosphoglycerate*, the end product of the previous stage, still retains the phosphate group, originally derived from ATP in Stage I.

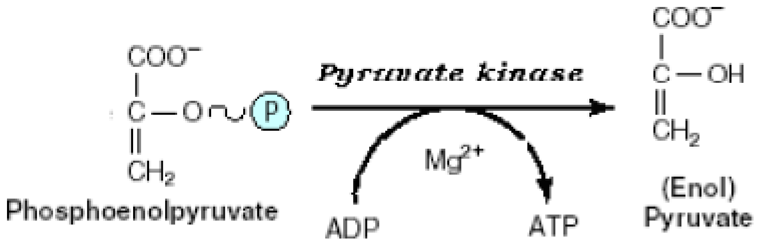
8. ***Conversion of 3-phosphoglycerate to 2-phosphoglycerate:*** *3-phosphoglycerate* formed by the above reaction is converted to 2-phosphoglycerate, catalyzed by the enzyme *phosphoglycerate mutase.*



9. ***Conversion of 2-phosphoglycerate to phosphoenol pyruvate:*** The reaction is catalyzed by the enzyme *enolase*, the enzyme requires the presence of either Mg^{2+} or Mn^{2+} ions for activity.



10. ***Conversion of phosphoenol pyruvate to pyruvate:*** *Phosphoenol pyruvate* is converted to *pyruvate*, the reaction is catalysed by the enzyme *pyruvate kinase*. The high energy phosphate group of phosphoenol pyruvate is directly transferred to ADP, producing ATP. The reaction is irreversible.

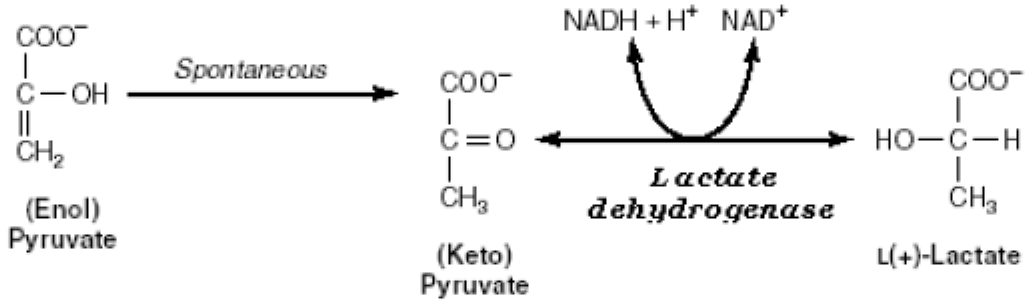


◆ *Summary of glycolysis:*

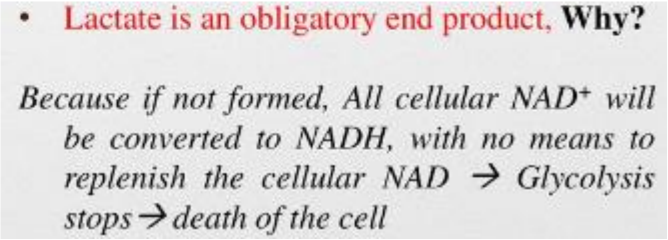
During glycolysis NAD$^+$ is reduced to NADH. At the same time, *glyceraldehyde 3-phosphate* is oxidized to *1, 3-bisphosphoglycerate*. To conserve the coenzyme NAD$^+$, NADH must be reoxidized. Under anaerobic conditions this is done when pyruvic acid is converted to lactic acid. In the presence of oxygen, NADH, can be oxidized to NAD$^+$ with the help of the respiratory enzymes.

◆ *Anaerobic phase:*

- In the absence of O_2, reoxidation of NADH at glyceraldehyde 3-phosphate dehydrogenase stage cannot take place in respiratory chain. But the cells have limited coenzyme. Hence to continue the glycolysis **NADH** must be reoxidized to **NAD$^+$**. This is achieved by reoxidation of NADH by conversionof pyruvate to lactate (without producing ATP).



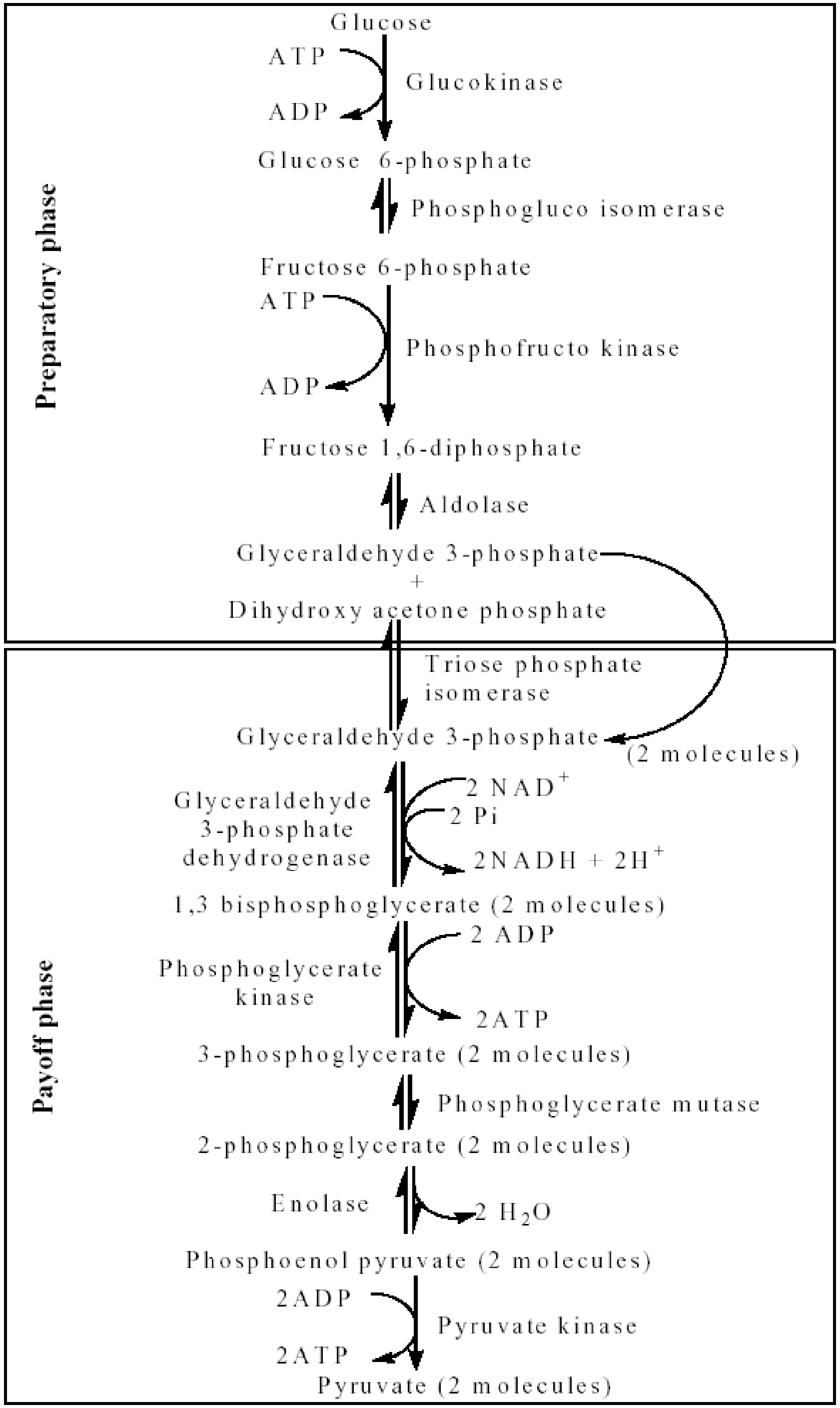
- It is to be noted that in the reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*, therefore, no ATP is produced.
- In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.



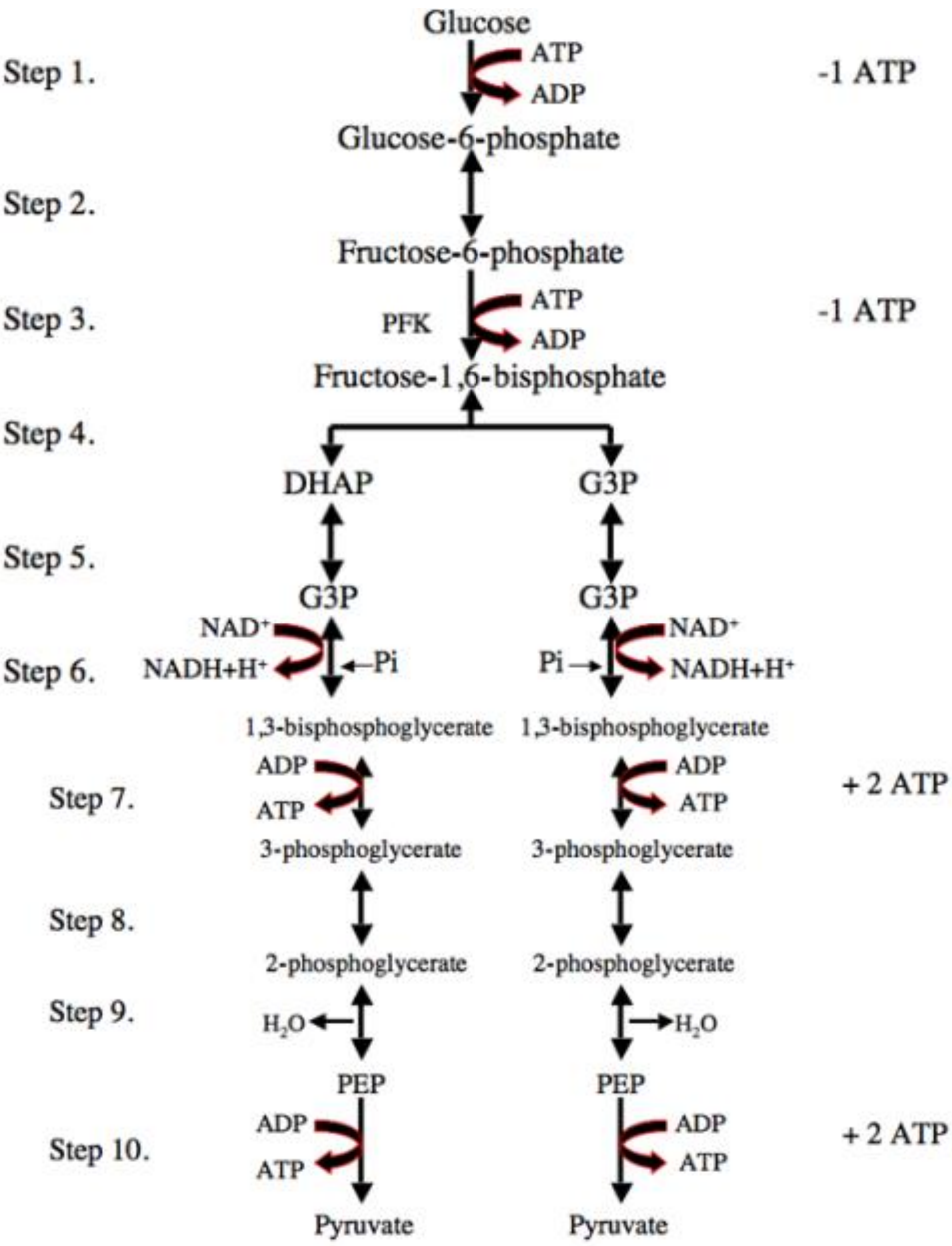
- Lactate is an obligatory end product, **Why?**

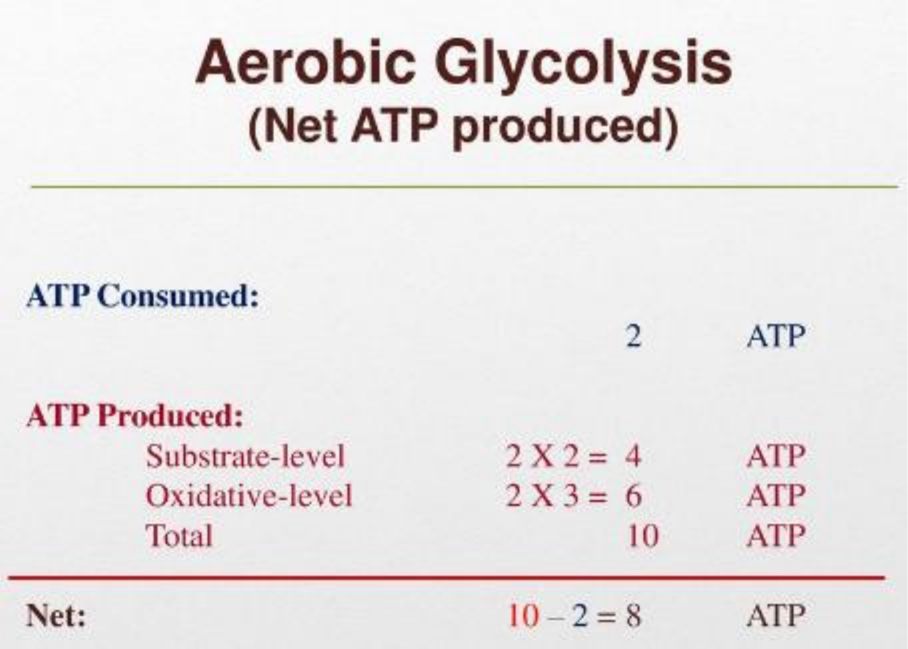
Because if not formed, All cellular NAD$^+$ will be converted to NADH, with no means to replenish the cellular NAD → Glycolysis stops → death of the cell

- **Schematic diagram of glycolytic pathway:**



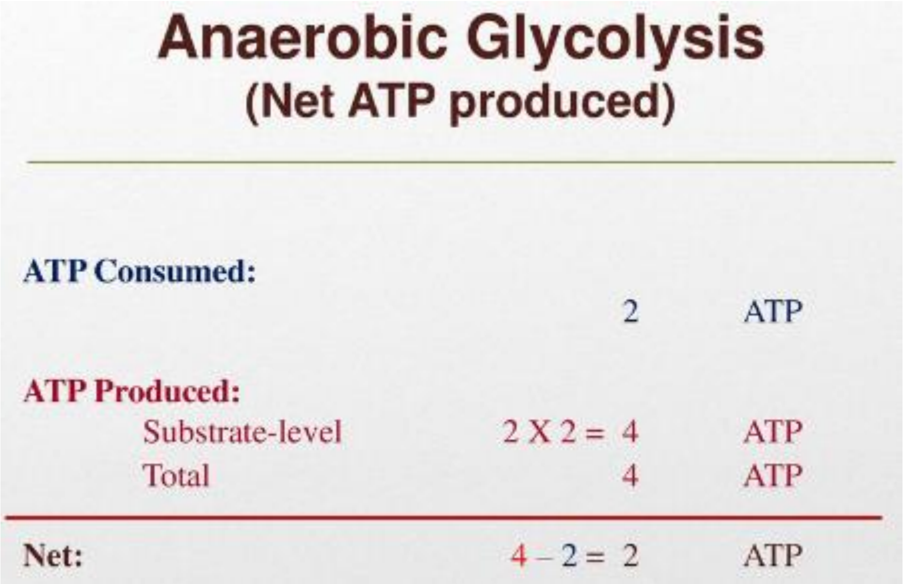
ATP Yield:





Aerobic Glycolysis
(Net ATP produced)

ATP Consumed:		
	2	ATP
ATP Produced:		
Substrate-level	2 X 2 = 4	ATP
Oxidative-level	2 X 3 = 6	ATP
Total	10	ATP
Net:	10 – 2 = 8	ATP

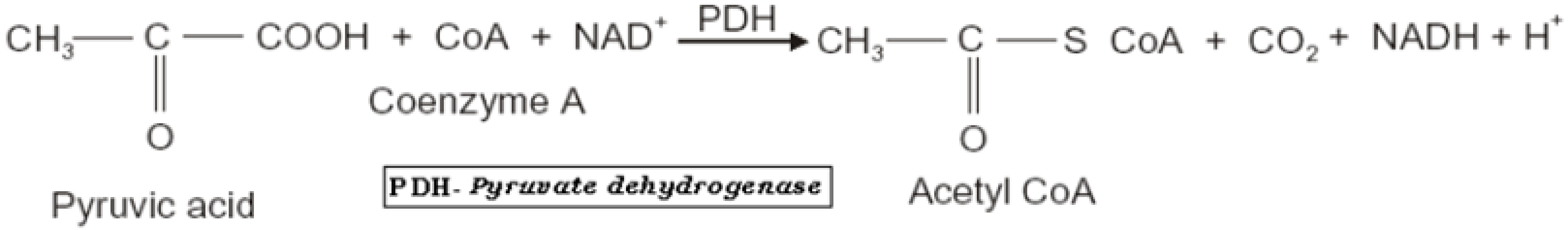


Anaerobic Glycolysis
(Net ATP produced)

ATP Consumed:		
	2	ATP
ATP Produced:		
Substrate-level	2 X 2 = 4	ATP
Total	4	ATP
Net:	4 – 2 = 2	ATP

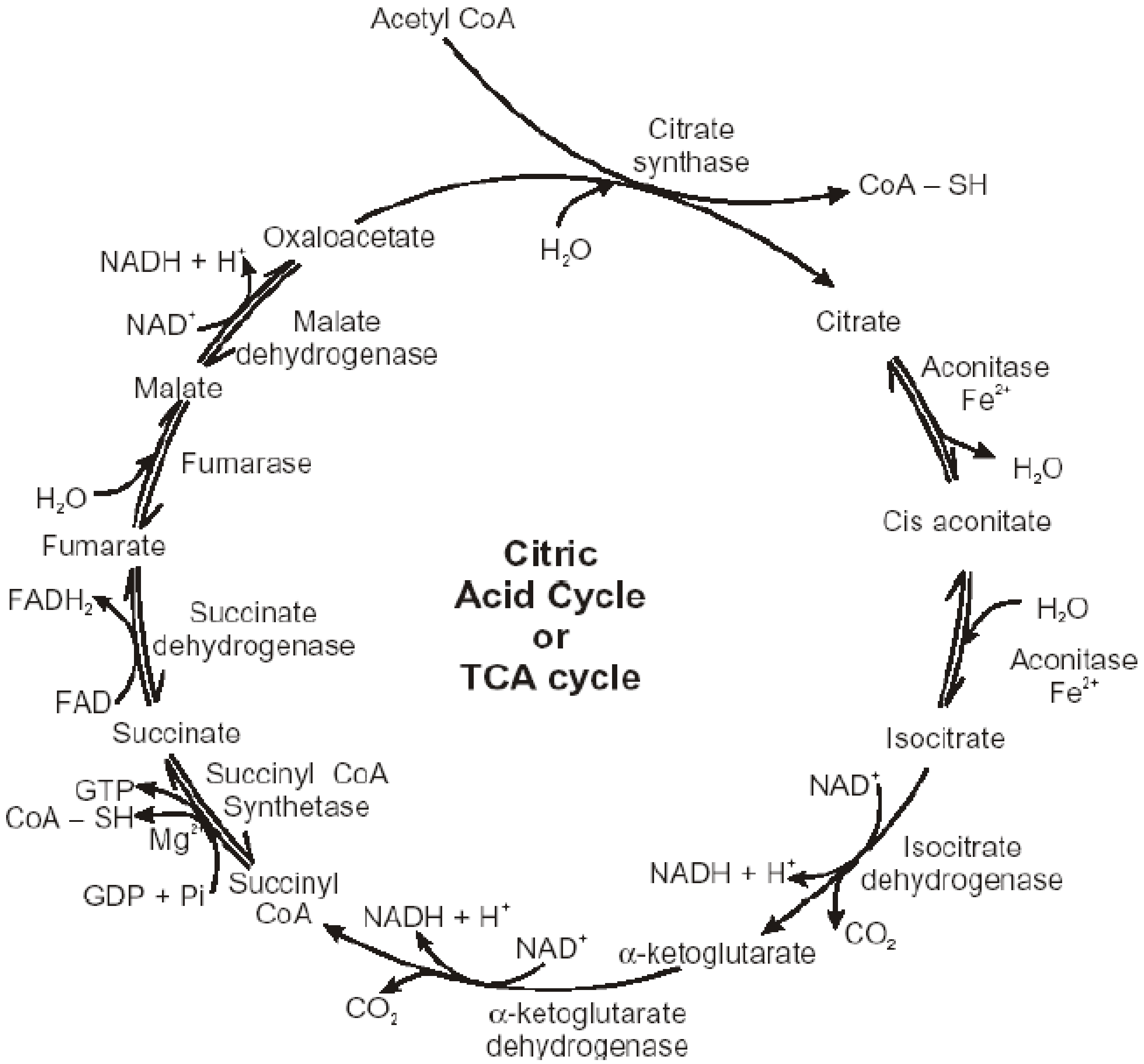
Tricarboxylic acid (TCA) / Krebs cycle:

- Named after English biochemist **Hans Krebs**, who studied it first.
- The **tricarboxylic acid (TCA) cycle,** also known as the Krebs or citric acid cycle, is the main source of energy for cells and an important part of aerobic respiration. The cycle harnesses the available chemical energy of **acetyl coenzyme A** (acetyl CoA) into the reducing power of nicotinamide adenine dinucleotide (NADH).
- The TCA cycle is part of the larger glucose metabolism whereby glucose is oxidized to form pyruvate, which is then oxidized and enters the TCA cycle as acetyl-CoA.
- Under aerobic conditions, in presence of multienzyme complex pyruvate dehydrogenase, the pyruvate is oxidatively decarboxylated to Aetyl CoA, before entering the TCA cycle. It occurs in the mitochondrila matrix and links Glycolysis with TCA cycle.



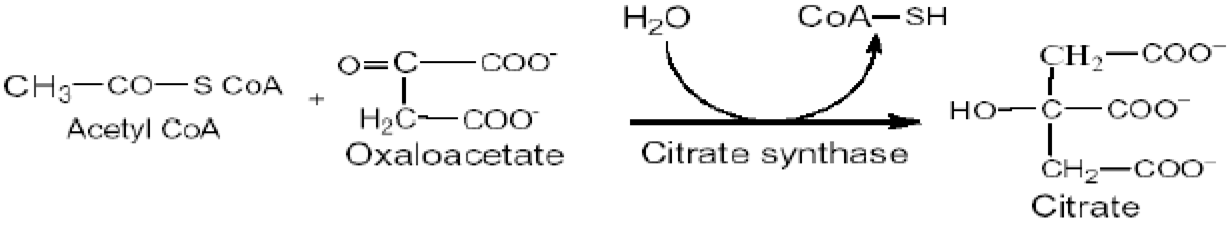
$$\underset{\text{Pyruvic acid}}{CH_3-\overset{\displaystyle O}{\overset{\|}{C}}-COOH} + \underset{\text{Coenzyme A}}{CoA} + NAD^+ \xrightarrow{PDH} \underset{\text{Acetyl CoA}}{CH_3-\overset{\displaystyle O}{\overset{\|}{C}}-S\ CoA} + CO_2 + NADH + H^+$$

Schematic Diagram of Krebs Cycle

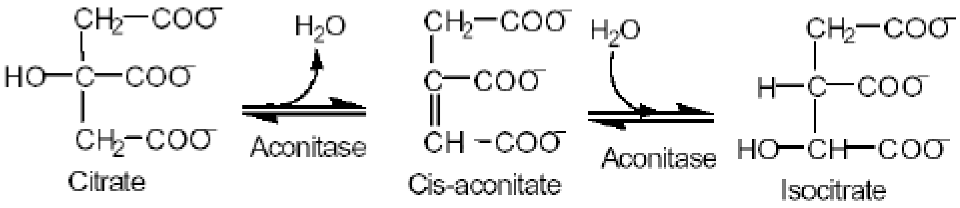


- **Reactions of the citric acid cycle**: There are eight steps in the cycle and the reactions are as follows.

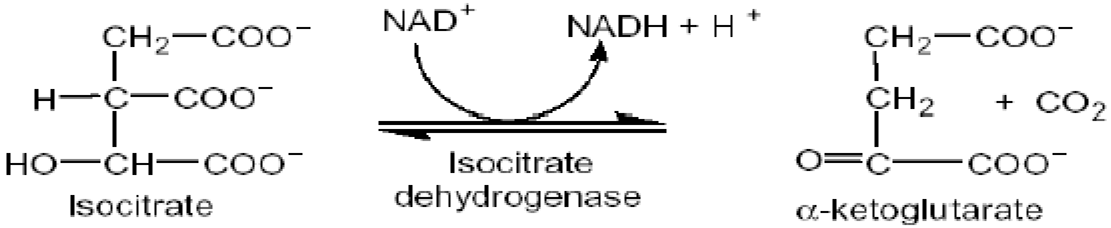
1. ***Formation of citrate:*** The first reaction of the cycle is the condensation of acetyl CoA with oxaloacetate to form citrate, catalyzed by *citrate synthase*. This is an irreversible reaction.



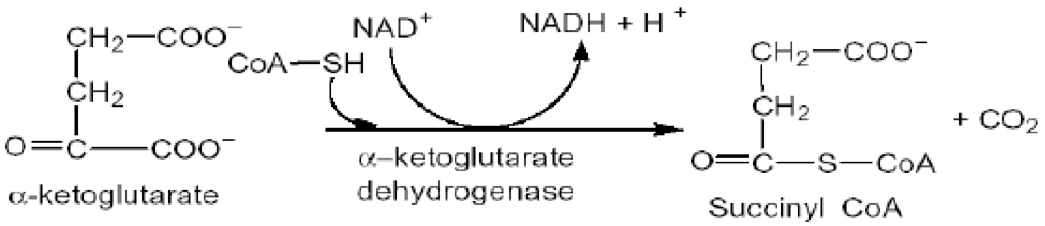
2. ***Formation of isocitrate via cis aconitate:*** The enzyme *aconitase* catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis aconitate.



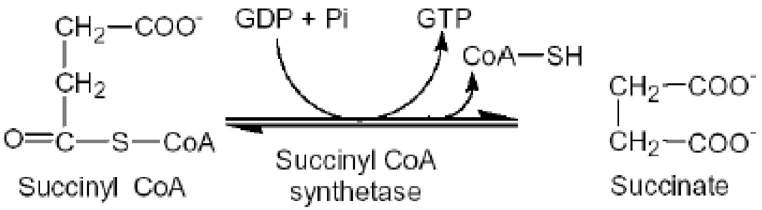
3. **Oxidation of isocitrate to α-ketoglutarate and CO_2**: In the next step, *isocitrate dehydrogenase* catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.



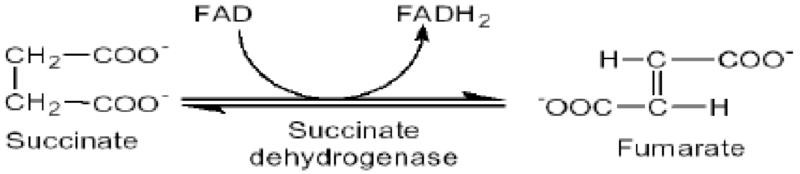
4. ***Oxidation of α-keto glutarate to succinyl CoA and CO_2:*** The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to succinyl CoA and CO2 by the action of the *α-ketoglutarate dehydrogenase* complex. The reaction is irreversible.



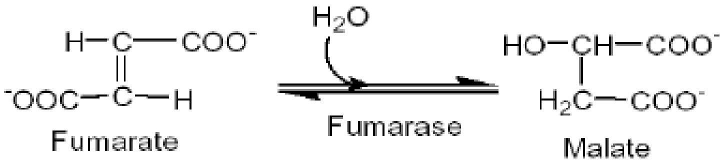
5. ***Conversion of succinyl CoA to succinate:*** The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation) and the enzyme that catalyzes this reversible reaction is called succinyl CoA synthetase or *succinic thiokinase*.



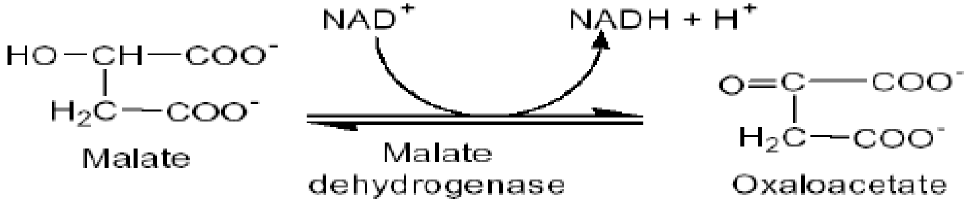
6. ***Oxidation of succinate to fumarate:*** The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme *succinate dehydrogenase*.



7. ***Hydration of fumarate to malate:*** The reversible hydration of fumarate to malate is catalyzed by *fumarase*.



8. ***Oxidation of malate to oxaloacetate:*** The last reaction of the citric acid cycle is, NAD linked *malate-dehydrogenase* which catalyses the oxidation of malate to oxaloacetate.



- **Energy yield from TCA cycle**: If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

Reaction	Number of ATP or reduced coenzymes	Number of ATP formed*
Glucose → glucose-6-phosphate	-1 ATP	-1
Fructose-6-phosphate → fructose-1,6-bisphosphate	-1 ATP	-1
2 Glyceraldehyde-3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	6
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	6
2 Isocitrate → α a-ketoglutarate	2 NADH	6
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	6
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 FADH2	4
2 Malate → 2 oxaloacetate	2 NADH	6
Total		38

* This is calculated as 3 ATP per NADH and 2 ATP per FADH$_2$. A negative value indicates consumption.

Alcoholic and Lactic acid fermentations : Making ATP Without Oxygen:

The cells of most living things make ATP from glucose through aerobic cellular respiration. But, there are processes, such as **fermentation,** of making ATP from glucose without oxygen.

Fermentation:

- The two most common types of fermentation are **alcoholic** and **lactic acid fermentation**.

- While alcoholic fermentation may be the most well-known (and is seen in many foods and beverages, such as wine, beer, fermented teas, bread and sausages), lactic acid fermentation is thought to be the oldest. Fermented foods, such as cheese, yogurt, kefir, sauerkraut and pickled foods have been found in almost every culture all over the world, going back thousands of years.

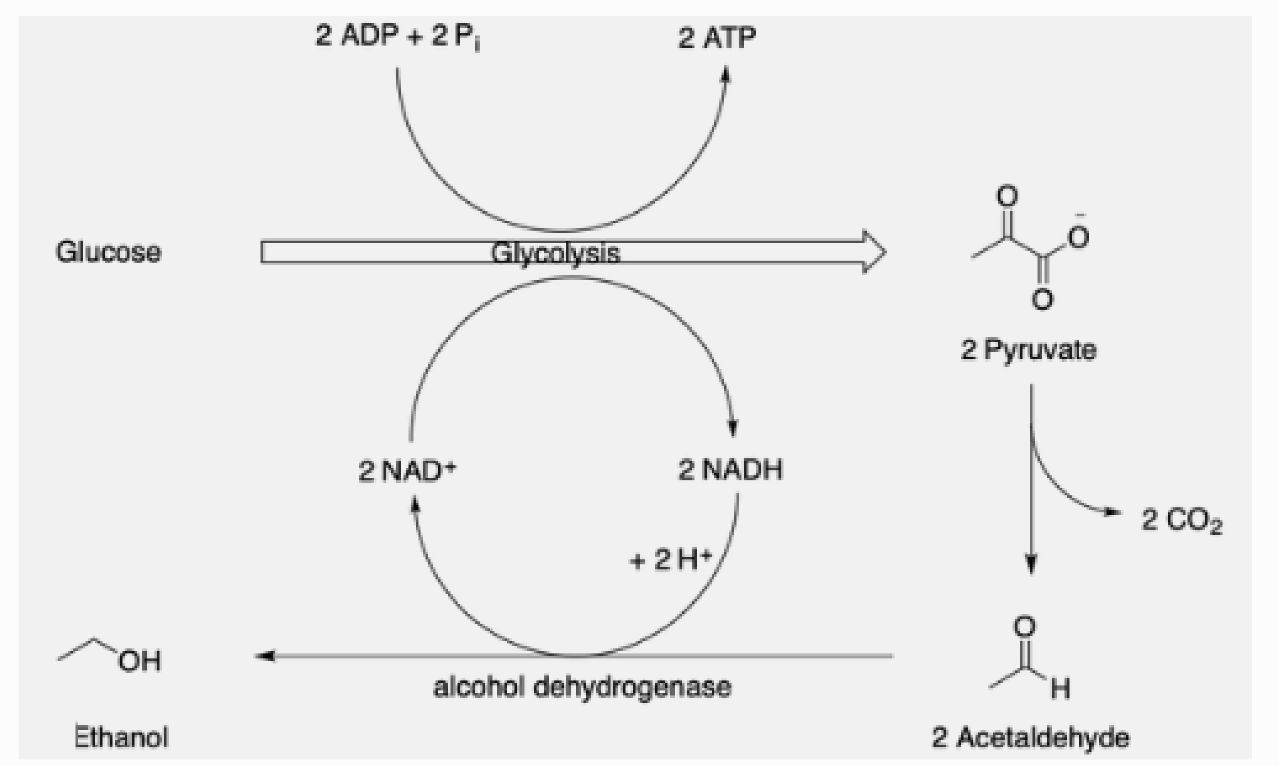
- Both lactic acid fermentation and alcoholic fermentation, both are oxidation-reduction reactions and involve glycolysis.

- **Alcohol fermentation** occurs in micro-organisms such as yeast and converts glucose to energy, which is released as carbon dioxide. The resultant by-product is called ethanol

- **Lactic acid fermentation** occurs in certain bacteria, yeast and muscle cells and converts glucose to energy. Its by-product is lactate. Lactic acid fermentation is also used in the food industry, which uses lactic acid bacteria.

Ethanol Fermentation:

Ethanol fermentation is defined as the biological process that turns sugar (glucose, fructose and sucrose) into ethanol, carbon dioxide and energy.
After the initial glycolysis step that converts one glucose molecule to two pyruvate molecules, the pyruvate molecules further break down into two acetaldehyde and two carbon dioxide molecules, a step catalyzed by pyruvate decarboxylase. Alcohol dehydrogenase then facilitates the conversion of the two acetaldehyde molecules to two ethanol molecules, utilizing the energy and hydrogen from NADH.

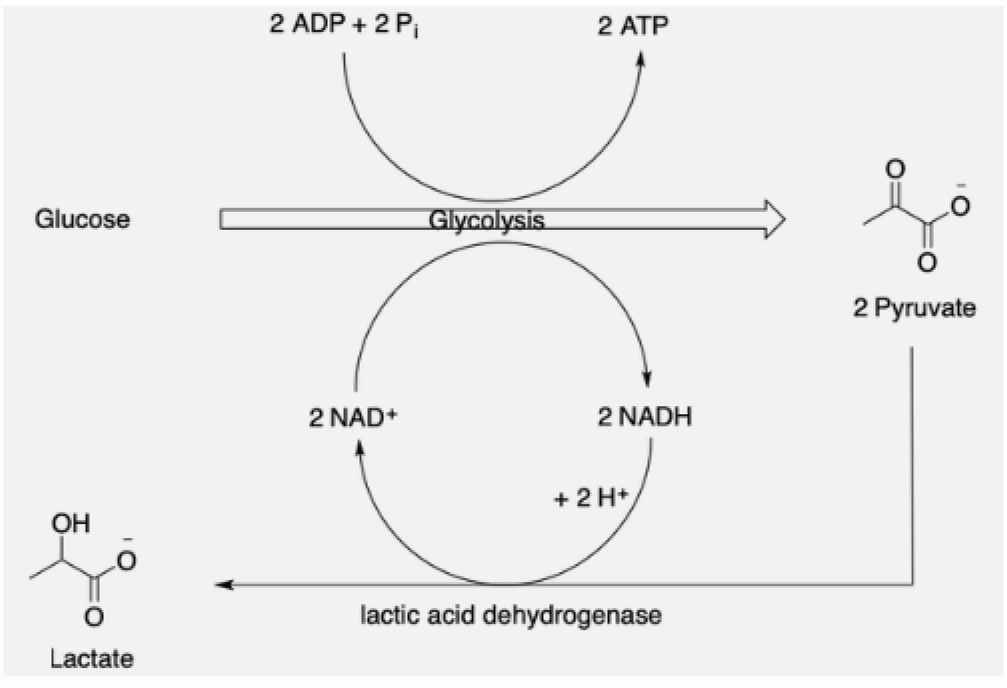


Lactic Acid Fermentation:

Lactic acid fermentation is another type of fermentation and is described as the metabolic process that transforms sugar into the metabolite lactate and energy. It is the only respiration process that does not produce a gas and occurs in some bacteria (such as *lactobacilli*) and muscle cells.

This type of fermentation converts the two molecules of pyruvate from glycolysis to two lactic acid molecules and regenerates the NAD^+ in the process, continuing the cycle. This redox reaction is catalyzed by lactic acid dehydrogenase.

Lactic acid bacteria can carry out either homolactic fermentation, where lactic acid is the major product, or heterolactic fermentation, where some lactate is further metabolized into ethanol, carbon dioxide and other byproducts



The Role of Yeast and Bacteria in Fermentation:

- Alcohol fermentation often involves *Saccharomyces cerevisiae*, (commonly known as baker’s yeast), which derives its name from sugar (saccharo), fungus (myces) and beer (cerevisiae). Yeast fermentation is essential to the processes of producing beer, wine, bread and biofuel. *S. cerevisiae* fermentation converts starch to simple sugars, which the yeast then consumes to create carbon dioxide and alcohol.

- *Lactobacillus* is the most common bacteria used in lactic acid fermentation. The sugar present in fruit samples naturally produces lactic acid, which lowers the pH, reducing the number of micro-organisms that can grow. This effectively preserves food.

- Lactic acid bacteria are found naturally in some foods and the carbohydrates already present in the food keep the reaction going. This is why cheeses that have “aged” for different amounts of time have different flavors.

Alcoholic vs. Lactic Acid Fermentation:

- *Alcohol fermentation* is a chemical reaction that uses yeast and sugar to produce energy, whereas, in lactic acid fermentation, an enzyme called lactate dehydrogenase prompts a reaction to start glycolysis, forming lactate in the process.

- lactic acid fermentation differs from ethyl alcohol fermentation in that lactic acid, rather than ethanol, is the resulting by-product.

Effects of Lactic Acid on the Body:

Muscle soreness experienced after exercise is caused by lactic acid buildup. With regular activity, the lungs can keep up with oxygen demands of the body, but during exercise, more energy is required. This leaves oxygen in short supply, so anaerobic respiration begins. This is a less efficient but faster process that results in the production of lactic acid.

Though often considered waste, lactic acid is then recycled through the liver where it is converted back to glucose. In some cases, a buildup of lactic acid can cause nausea, difficulty breathing and weakness. When the body does not have time to recover, uric acid crystals may build up on joints, causing pain known as gout.