Carbohydrates

Carbohydrates are one of the essential food ingredients , which we all require. Every one of us is quite familiar with the word glucose and cane sugar . They are simple carbohydrates and we consume carbohydrates in one form or every other meal we take. They are primary source of energy in the body and constitute an important part of any well balanced diet.
Bread, rice, potatoes, peas etc all contains carbohydrates the sweetening agents includes nothing but simple carbohydrates.

Biological Importance:

Carbohydrates are the most widespread organic compounds occurring in nature. they are present in almost all the plants and comprise about 80% of dry weight. The most important are cellulose-the chief structural material of plants, starches and sugars-sucrose and glucose. in animals and human beings glucose is an essential constituent of blood and takes part in various metabolic reactions carbohydrates occur in bound form in biologically important compounds like which is a key material in biological energy storage and transport system. ATP is called energy currency of the cell. carbohydrates are also present in nucleic acids which control the production of enzymes and transfer genetic information. Carbohydrates are major source of energy . Our body can mobilize carbohydrates more easily than fat , even though fats contain more energy on a gram for gram basis. Free glucose in the bloodstream is quickly depleted but we have stored glucose in readily accessible for as polymer glycogen. when we require energy glycogen breakdown into glucose in a quick response to need for energy. oligosaccharide play a key role in processes that take part on the surface of cells. polysaccharide such as cellulose are essential components of grass ,trees and other polysaccharide are major constituents of bacterial cell walls. starch is the principal food reserve of plant.

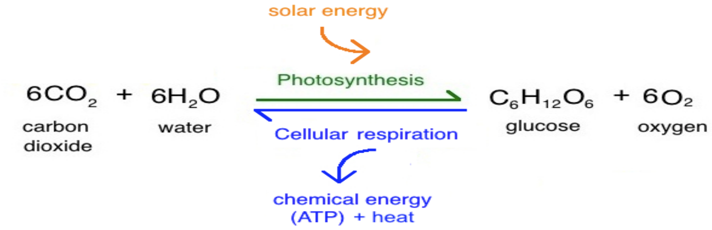
1. *Carbohydrates provides energy and regulation of blood glucose.*
2. *It will prevent the degradation of skeletal muscle and other tissues such as the heart, liver, and kidneys.*
3. *It prevents the breakdown of proteins for energy.*
4. *Carbohydrates also help with fat metabolism. If the body has enough energy for its immediate needs, it stores extra energy as fat.*
5. *Carbohydrates are an important component of many industries like textile, paper, lacquers and breweries.*
6. *Detoxification of physiological importance is carried out to some extent with carbohydrate derivatives.*
7. *Agar is polysaccharide used in culture media, laxative and food.*
8. *Carbohydrates form a part of genetic material like DNA and RNA in the form of deoxyribose and ribose sugars.*
9. *Hyaluronic acid found in between joints acts as synovial fluid and provides frictionless movement.*
10. *They help make up the body mass by being included in all the parts of the cell and tissues.*
11. *Adequate storage of hepatic glycogen helps in detoxifying a normal liver.*
12. *They form components of bio-molecules which have a key role in blood clotting, immunity, fertilization etc.*
13. *Carbohydrates is basically the main fibre of the diet or provide the bulk fibre for better digestion.*
14. *Carbohydrates help clear gut and prevent constipation.*
15. *Starch is the form the food is stored in plants.*

16. *It provides sweetness to foods.*
17. *Pectin and Hemicellulose are the structural carbohydrate in plant cell walls.*
18. *It plays important roles in cellular recognition processes.*

19. *Chitin forms the cell wall of fungi and the outer schelitone of insects.*
20. *Murine is a structural carbohydrate in bacterial cell wall.*

➡ Originally the name carbohydrate was given to all such compounds having the general formula $C_x(H_2O)_y$ i.e. , They were considered to be hydrates of carbon. now we know that carbohydrates are not simply hydrates of carbon but have a variety of other structural features. They are usually defined as Poly hydroxy aldehyde and ketones or substances that hydrolyse to yield Poly hydroxy aldehyde and ketones. Poly hydroxy aldehydes are also called aldoses and Poly hydroxy ketones are also called ketoses. Low molecular weight carbohydrates are also called as sugar or saccharides example glucose sucrose are simple sugars. Common sugars like glucose fructose and some less common sugars also occurs in the combined state with various hydroxy compounds. such derivatives are called glycosides and non-sugar component is called aglycone. When we have a sugar component (glucose) the compound is called glucoside, if fructose is present it is a fructoside and so on. Glycosides are widely distributed in plants and animals. Structurally, these compounds are related to simple methyl glucosides. When the sugar moiety forms an ether linkage with the aglycone. It is called *O*-glycoside and when there is a formation of a C-C bond, it is called *C*-glycoside.

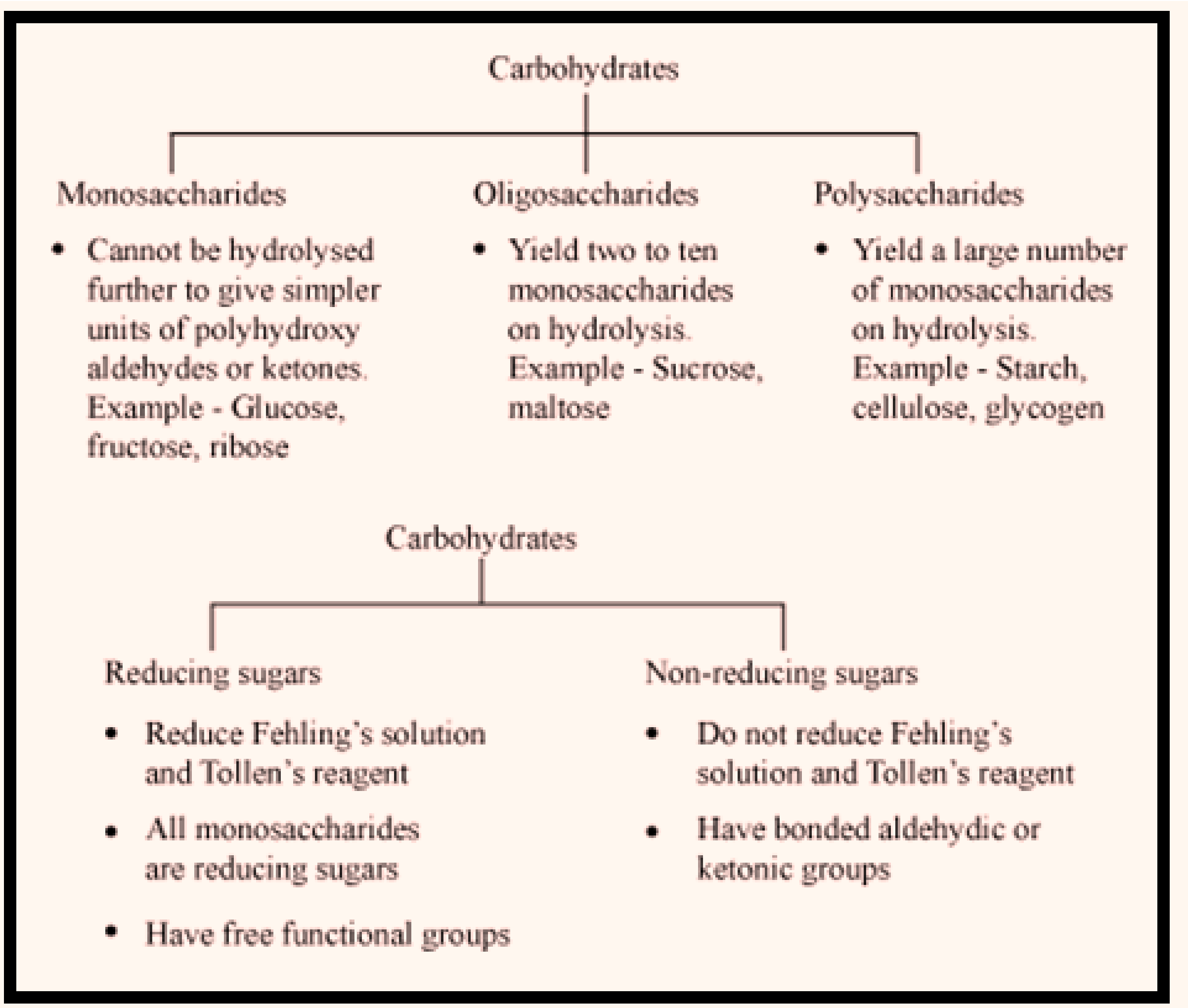
➡ Carbohydrates are produced in green plants as a result of photosynthesis. it involves chemical combination or fixation of carbon dioxide and water by utilization of light energy. in this process water is oxidised to oxygen and carbon dioxide is reduced. the overall equation for photosynthesis can be written as.



Besides providing energy, a major metabolic product of glucose is acetyl Coenzyme A, which acts as a starting point for synthesis of fats, fatty acids, amino acids and large number of other important compounds required by our body.

CLASSIFICATION OF CARBOHYDRATES

Carbohydrates are classified on the basis of number of aldose and/ or ketoses units produced upon hydrolysis.



Metabolism of Carbohydrates

Carbohydrates are organic molecules composed of carbon, hydrogen, and oxygen atoms. The family of carbohydrates includes both simple and complex sugars. Glucose and fructose are examples of simple sugars, and starch, glycogen, and cellulose are all examples of complex sugars. The complex sugars are also called **polysaccharides** and are made of multiple **monosaccharide** molecules. Polysaccharides serve as energy storage (e.g., starch and glycogen) and as structural components (e.g., chitin in insects and cellulose in plants).

During digestion, carbohydrates are broken down into simple, soluble sugars that can be transported across the intestinal wall into the circulatory system to be transported throughout the body. Carbohydrate digestion begins in the mouth with the action of **salivary amylase** on starches and ends with monosaccharides being absorbed across the epithelium of the small intestine. Once the absorbed monosaccharides are transported to the tissues, the process of **cellular respiration** begins

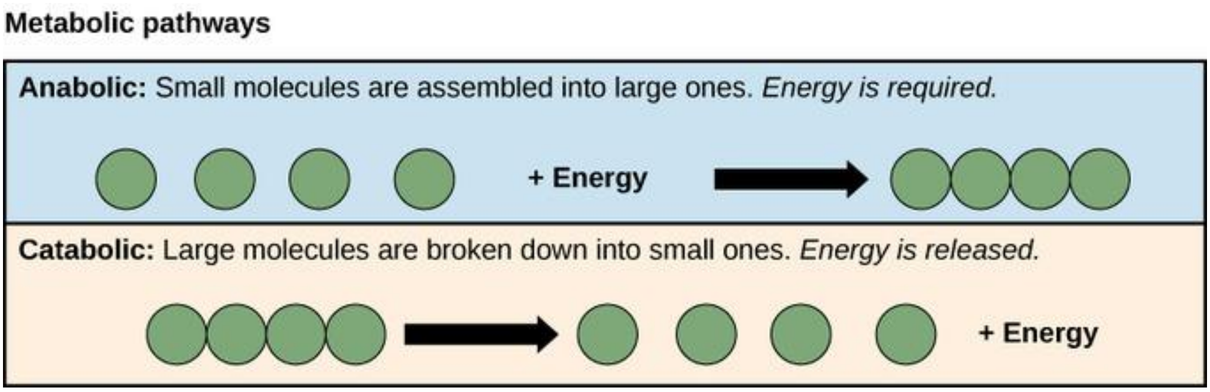
Metabolic pathways

Metabolic pathways and cycles are reaction chains where chemical products become the substrate for the next step. All substrates are chemically transformed in reactions that belong to either pathways (if the reactions are aligned in linear fashion) or metabolic cycles (if the moieties of the reactions are preserved). The term substrate oxidation is used for substrate degradation ultimately leading to CO_2 production.

- A metabolic pathway is a series of chemical reactions in a cell that build and breakdown molecules for cellular processes.
- Anabolic pathways synthesize molecules and require energy.
- Catabolic pathways break down molecules and produce energy.
- Because almost all metabolic reactions take place non-spontaneously, proteins called enzymes help facilitate those chemical reactions.

The processes of making and breaking down carbohydrate molecules illustrate two types of metabolic pathways. A metabolic pathway is a step-by-step series of interconnected biochemical reactions that convert a substrate molecule or molecules through a series of metabolic intermediates, eventually yielding a final product or products. For example, one metabolic pathway for carbohydrates breaks large molecules down into glucose. Another metabolic pathway might build glucose into large carbohydrate molecules for storage. The first of these processes requires energy and is referred to as anabolic. The second process produces energy and is referred to as catabolic. Consequently, metabolism is composed of these two opposite pathways:

1. Anabolism (building molecules)
2. Catabolism (breaking down molecules)



Anabolic Pathways

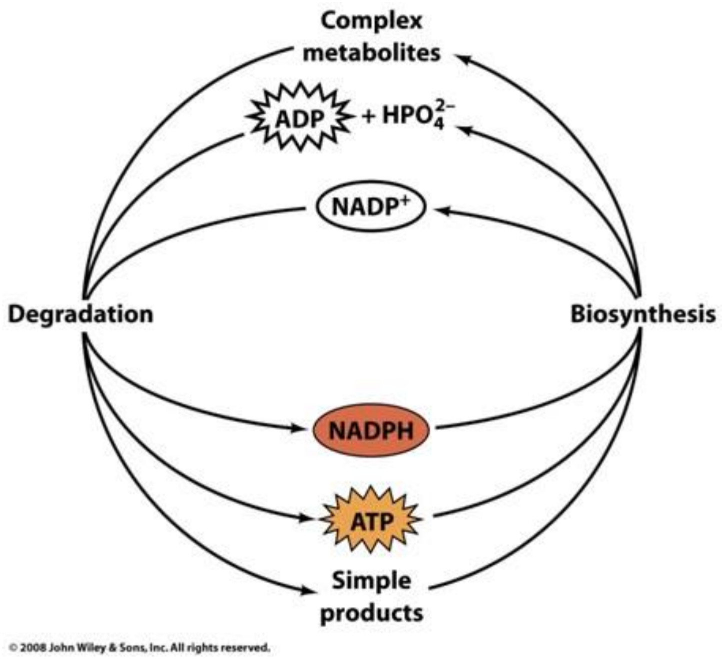
Anabolic pathways require an input of energy to synthesize complex molecules from simpler ones. One example of an anabolic pathway is the synthesis of sugar from CO_2. Other examples include the synthesis of large proteins from amino acid building blocks and the synthesis of new DNA strands from nucleic acid building blocks. These processes are critical to the life of the cell, take place constantly, and demand energy provided by ATP and other high-energy molecules like NADH (nicotinamide adenine dinucleotide) and NADPH.

Catabolic Pathways

Catabolic pathways involve the degradation of complex molecules into simpler ones, releasing the chemical energy stored in the bonds of those molecules. Some catabolic pathways can capture that energy to produce ATP, the molecule used to power all cellular processes. Other energy-storing molecules, such as lipids, are also broken down through similar catabolic reactions to release energy and make ATP.

Catabolism	Anabolism
Catabolism breaks down big complex molecules into smaller, easier to absorb molecules.	Anabolism builds molecules required for the body's functionality.
The process of catabolism releases energy.	Anabolic processes require energy.
Hormones involved in the processes are adrenaline, cytokine, glucagon, and cortisol.	Hormones involved in the process are estrogen, testosterone, growth hormones and insulin.
Examples of catabolic processes are proteins becoming amino acids, glycogen breaking down into glucose and triglycerides breaking up into fatty acids.	Examples include the formation of polypeptides from amino acids, glucose forming glycogen and fatty acids forming triglycerides.
In catabolism, potential energy is changed into kinetic energy.	In anabolism, kinetic energy is converted into potential energy.
It is required to perform different activities in living entities.	It is required for maintenance, growth, and storage.
Requires NADPH	Requires NAD+
Endergonic	Exergonic

The free energy released by catabolic processes is conserved through synthesis of ATP from ADP and phosphate or through reduction of coenzyme $NADP^+$ to NADPH.
ATP and NADPH are the major free energy sources for anabolic pathways.
Degradative metabolism is that converts diverse complex substances (carbohydrates, lipids and proteins) to common intermediates. These intermediates are then further metabolized in a central oxidative pathway that terminates in a few end products.
Biosynthesis carries out the opposite process, relatively few metabolites mainly pyruvate, acetyl coenzymeA and citric acid cycle intermediates serve as starting material for a host of varied biosynthetic products.



Metabolic pathways have following principal characteristics:

1. Metabolic pathways are irreversible.
2. If two metabolites are interconvertible, the synthetic route from first to second must differs from second to first.
3. Every metabolic pathway has exergonic first committed step.
4. All metabolic pathways are regulated, usually at the first committed step.
5. Metabolic pathways in eukaryotes occurs in specific subcellular compartments.

CoFactor

*A **cofactor** is a non-protein chemical compound or metallic ion that is required for an enzyme's activity as a catalyst (a catalyst is a substance that increases the rate of a chemical reaction). **Cofactors** can be considered "helper molecules" that assist in biochemical transformations.*

***Cofactor**, a component, other than the protein portion, of many enzymes. If the cofactor is removed from a complete enzyme (holoenzyme), the protein component (apoenzyme) no longer has catalytic activity. A cofactor that is firmly bound to the apoenzyme and cannot be removed without denaturing the latter is termed a prosthetic group; most such groups contain an atom of metal such as copper or iron. A cofactor that is bound loosely to the apoenzyme and can be readily separated from it is called a coenzyme. Coenzymes take part in the catalysed reaction, are modified during the reaction, and may require another enzyme-catalysed reaction for restoration to their original state.*

***A coenzyme** is an organic non-protein compound that binds with an enzyme to catalyze a reaction. Coenzymes are often broadly called cofactors, but they are chemically different. A coenzyme cannot function alone, but can be reused several times when paired with an enzyme*.

NAD^+, or nicotinamide adenine dinucleotide, is a critical coenzyme found in every cell in your body, and it's involved in hundreds of metabolic processes. But NAD^+ levels decline with age. NAD^+ has two general sets of reactions in the human body: helping turn nutrients into energy as

a key player in metabolism and working as a helper molecule for proteins that regulate other cellular functions.
NADH is the abbreviation for the naturally occurring biological substance, nicotinamide adenine dinucleotide hydride. The "H" stands for high-energy hydrogen and indicates that this substance is in the most biologically active form possible. Often referred to as coenzyme 1
NADH is also an important antioxidant; in fact, scientists acknowledge that NADH is the most powerful antioxidant to protect cells from damage by harmful substances

NAD	NADH
NAD is the most abundant coenzyme, which acts as the oxidizing-reducing agent inside the cell	NADH is the reduced form of NAD+, which is produced in the glycolysis and Krebs cycle
A coenzyme compound	Reduced form of NAD
Synthesized either by tryptophan pathway or vitamin B3 pathway	Synthesized in glycolysis and Krebs cycle
Naturally-occurring form of NAD inside the cell	Reduced form of NAD
Serves as an electron and hydrogen acceptor	Serves as an electron and hydrogen donor

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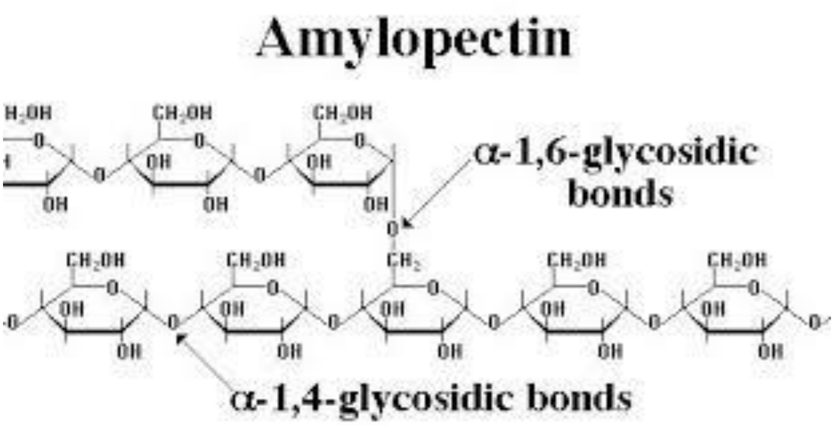


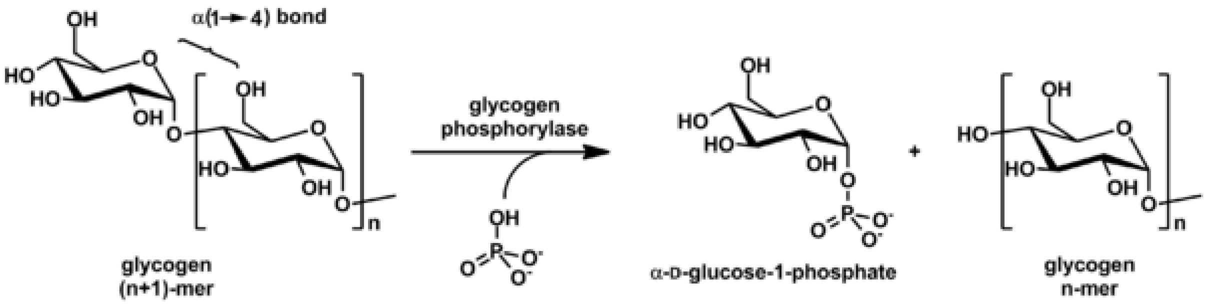
GLYCOLYSIS

In plants energy stored in the form of starches while glycogen is the storage polysaccharide present in animals . Glycogen is found in animal cells in granule similar to the starch granules in plant cells . Glycogen granules are observed in well fed liver and muscle cells.

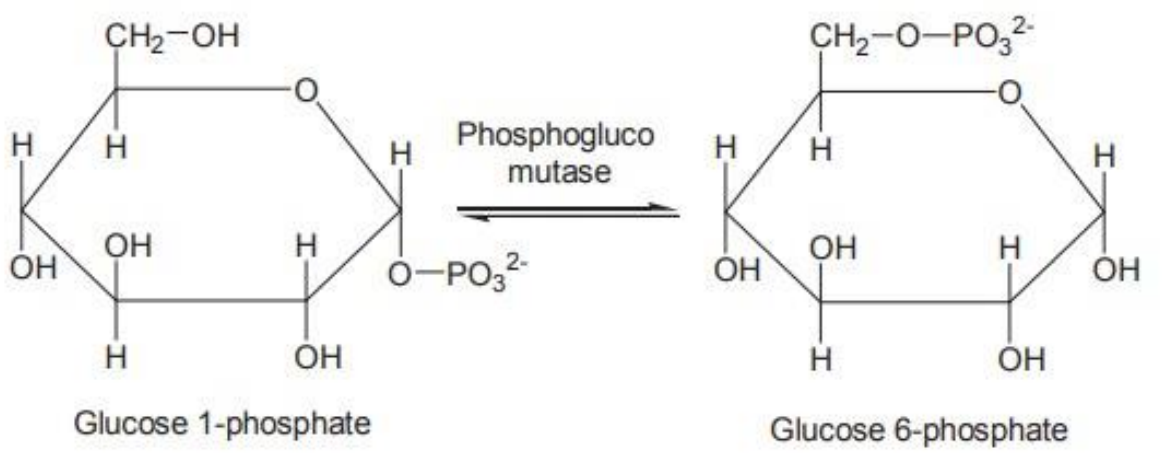
glycogen is the branch chain polymer of Alpha D glucose and in the respect it is similar to amylopectin fraction of starch . packed in glycogen consist of Alpha (1→6) linkages with Alpha (1→6) linkage at branch points however glycogen is more highly blanched than amylopectin . Branch point occurs about every 10 residue in glycogen and every 25 residue in amylopectin. in glycogen every chain length is 13 glucose residue there are 12 layers of branching.

When we digest a meal high in carbohydrates , we have a supply of glucose that exceeds our Immediate needs. therefore excess of glucose is stored in our body in the form of glycogen primarily in the liver and muscles. when we need energy, various degradative enzymes breakdown glycogen to smaller polysaccharides or free glucose units. in muscle glucose 6 phosphate obtained from glycogen breakdown, enters the glycolytic pathway directly rather than being hydrolysed glucose first. however liver glycogen breaks down to glucose-6-phosphate which is hydrolysed to give glucose. the release of glucose from liver by this breakdown of glycogen replenishes the supply of glucose in the blood. The conversion of glycogen to glucose-6 phosphate takes place in 3 steps:

1.In the first reaction each glucose residue clean from glycogen reacts with the inorganic phosphate to give glucose 6 phosphate. this cleavage reaction is phosphorolysis and not hydrolysis. the enzyme that catalyse this reaction is known as glycogen phosphorylase, which cleaves one glucose unit at a time from non-reducing end of a branch glycogen to produce glucose one phosphate and reminder of the glycogen molecule. this then enter the metabolic pathways of carbohydrate breakdown.



2.In the second reaction glucose one phosphate isomerase to give glucose-6phosphate by the enzyme phosphoglucomutase.



Complete breakdown of glycogen also requires de- branching enzymes that degrade alpha (1to6) Linkages and thereby expose additional 1to6 glycosidic linkages to be attacked by phosphorylase.

Glycolysis is the first stage of glucose metabolism. it is an anaerobic process and gives only small amount of energy in the form of only 2 molecules of ATP. we will see the complete aerobic oxidation of glucose to carbon dioxide and water via citric acid cycle yields high energy equivalent of 30-32 molecules of ATP.

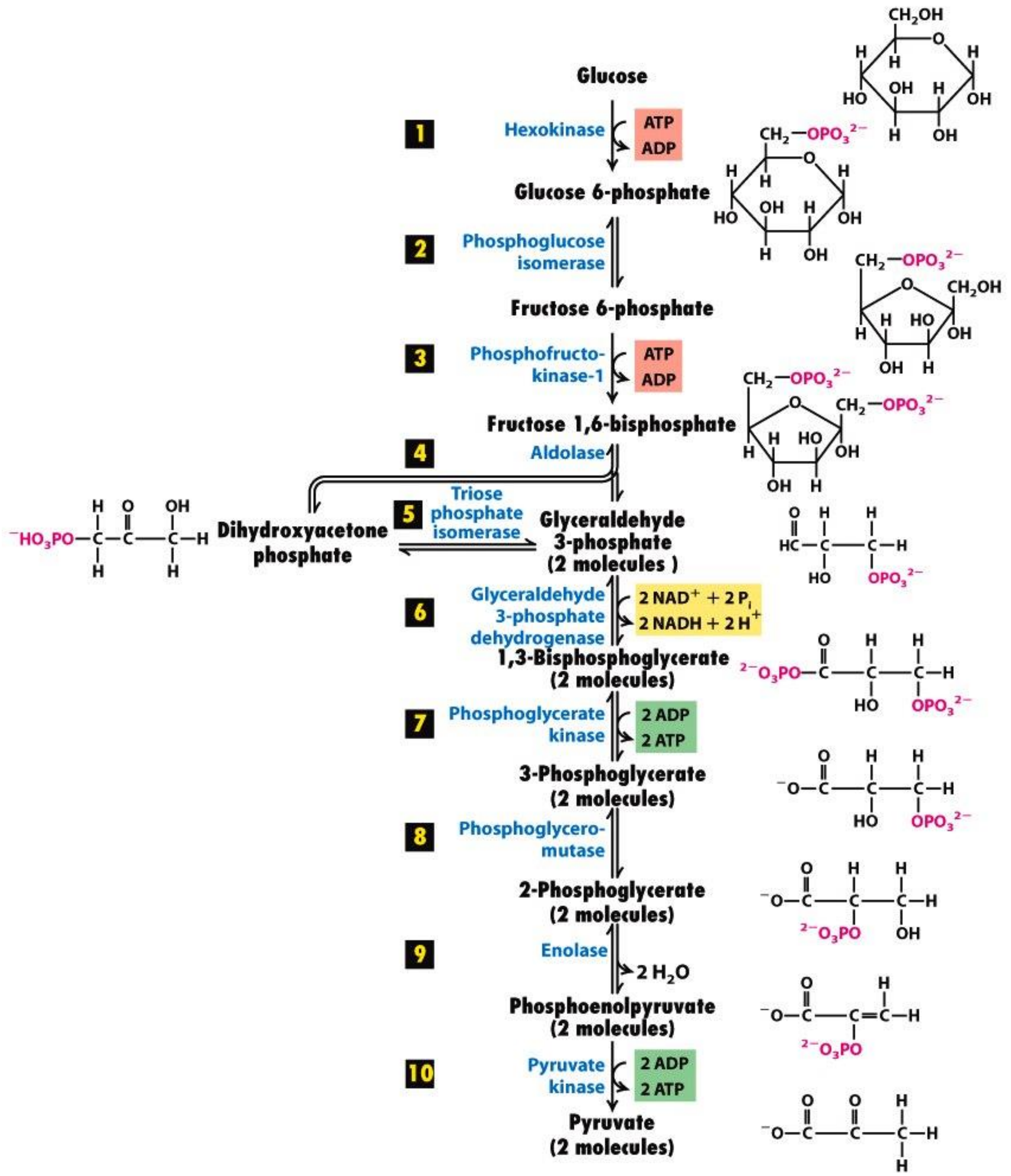


Figure 12-3
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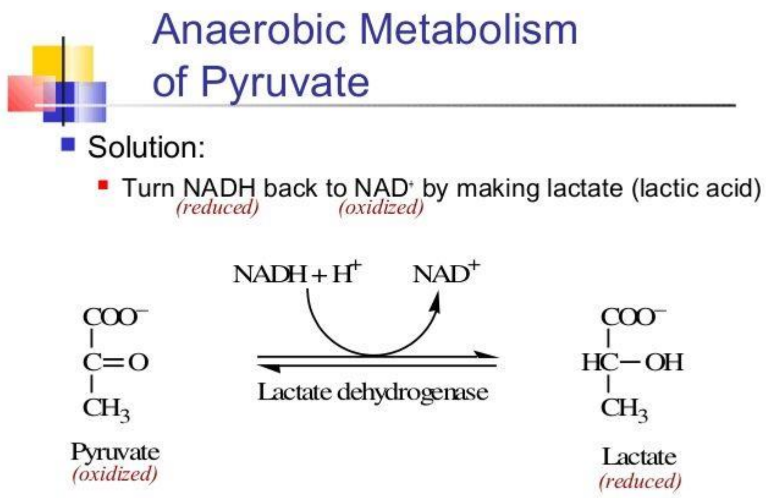
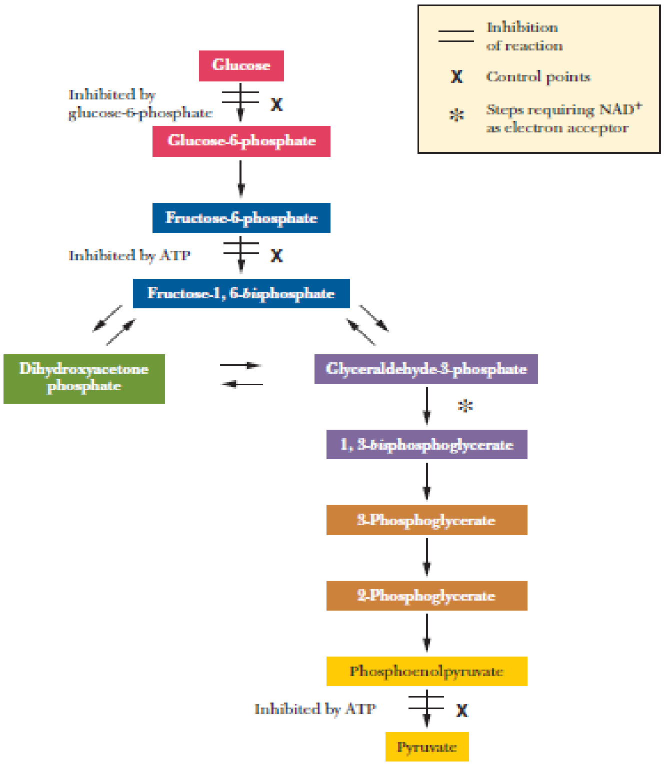
After the formation of glucose-6-phosphate, glycolysis and alcoholic fermentation follow a common pathway under anaerobic conditions until pyruvate is formed. In, glycolysis, pyruvate is converted to lactic acid, which will eventually be exported from the muscle to liver.
When pyruvate is formed, it can have one of the several fates:

Under aerobic conditions, pyruvate loses carbon dioxide and the remaining 2 carbon Atom become link to coenzyme A acetyl group to form acetyl-CoA which then enters the citric acid cycle. There are two fates of pyruvate in anaerobic metabolism.
in organisms capable of alcoholic fermentation pyruvate loses carbon dioxide producing acetaldehyde which in turn reduce to give ethanol.
in the second anaerobic metabolism lactate is produced especially in the muscles. This is anaerobic glycolysis. the conversion of glucose to pyruvate is simply called glycolysis.
Given below is the conversion of pyruvate to dictate in the muscle:
This is the final reaction of anaerobic glycolysis pyruvate is reduced to lactic in the presence of NADH. electric dehydrogenase is the enzyme that catalyses this reaction



the NADH produced from NAD+ by earlier oxidation of glyceraldehyde 3 phosphate is used up with no net change in the relative amounts of NADH and NAD+ in the cell this regeneration is required under anaerobic conditions in the cell so that NAD+ will you present for further glycolysis to take place.

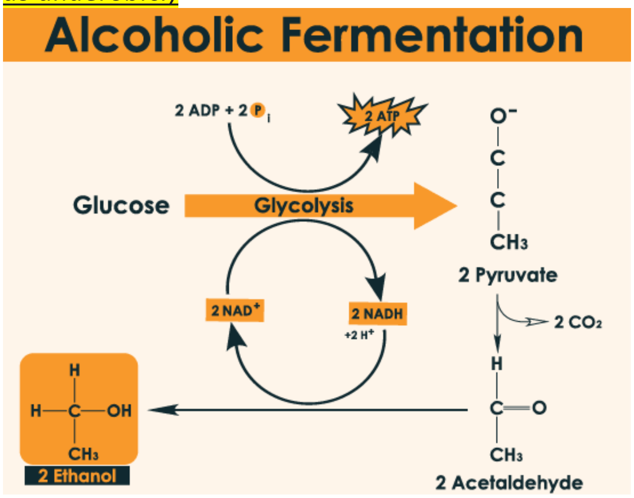
Control points in glycolysis
Pathways can be shut down if an organism has no immediate need for their products. This saves energy for the organism.
In glycolysis, there are three control points.
1.The first is reaction of glucose to glucose-6phosphate which is catalysed by hexokinase. This conversion is inhibited by glucose-6phosphate itself.
2.The second control point is the production of fructose-1,6-bisphosphate.This step is catalysed by phosphofructokinase and is inhibited by ATP
3.The last point is the conversion of phosphoenol pyruvate to pyruvate, catalysed by pyruvate kinase. This step is also inhibited by ATP



<u>Alcoholic Fermentation</u>

Ethanol fermentation, also called **alcoholic fermentation**, is a biological process which converts sugars such as glucose, fructose, and sucrose into cellular energy, producing **ethanol** and carbon dioxide as by-products.

<u>(Because yeast performs this conversion in absence of oxygen ethanolic fermentation is classified as anaerobic.)</u>



<u>Overall Pathway in Alcoholic Fermentation</u>

In alcoholic fermentation one molecule of glucose is converted to fructose-1,6-diphosphate. which eventually give rise to 2 molecules of pyruvate. pyruvate loses carbon dioxide producing acetaldehyde which in turn is reduced to give ethanol.

All these reaction can be divided into three phase.

Step 1. Glucose is phosphorylated to give glucose-6-phosphate

The enzyme that catalyse this reaction is hexokinase. this reaction uses ATP and gets converted into ADP. the term kinase is applied to the class of ATP dependent enzymes that transfer a phosphate group from ATP to a substrate. the substrate of hexokinase is not necessarily glucose; rather it can be any number of hexose such as glucose fructose and mannose. Mg^{2+} is cofactor.

*(A **cofactor** is a non-protein chemical compound or metallic ion that is required for an enzyme's activity as a catalyst (a catalyst is a substance that increases the rate of a chemical reaction). **Cofactors** can be considered "helper molecules" that assist in biochemical transformations).*

Step 2. Glucose-6-phosphate isomerises to give fructose-6-phospahte

Phosphoglucose isomerase is the enzyme that catalyse this reaction. The C-1 aldehyde group of glucose-6-phosphate is reduced to hydroxyl group and C-2 hydroxyl group is oxidized to give ketone of fructose-6-phosphate.

Step3. fructose 6 phosphate is further phosphorylated producing fructose-1,6-biphosphate.

this reaction takes place in the presence of ATP. phosphofructokinase is the enzyme and Mg^{2+} as a cofactor. after fructose-1,6-biphosphate is formed from original sugar, no other pathways are available and the molecule must undergo rest of the reactions of alcoholic fermentation this reaction is highly exothermic and irreversible.

Step 4. Fructose-1,6-bisphospahte is split into two 3-carbon fragments.

The cleavage reaction here is reverse of an aldol condensation. The enzyme that catalyses it is called aldolase. Fragments(Dihydroxy-acetone-phosphate, D-Glyceraldehyde-3-phospahte).

Step 5. The dihydroxyacetone phosphate is converted to glyceraldehyde-3-phosphate.

the enzyme that catalyses the reaction is triosephosphate isomerase. one molecule of glyceraldehyde has already been produced by aldolase reaction. we now have a second molecule of glyceraldehyde 3 phosphate produced in this reaction. the original molecule of glucose which contains 6 carbon atoms, has now been converted to 2 molecules of glyceraldehyde- 3-phosphate which contains 3 carbon atoms.

In the first 5 steps one molecule of glucose is split into two 3 carbon compounds that is D-glyceraldehyde-3-phosphate. Two molecules of ATP are required for these reactions.

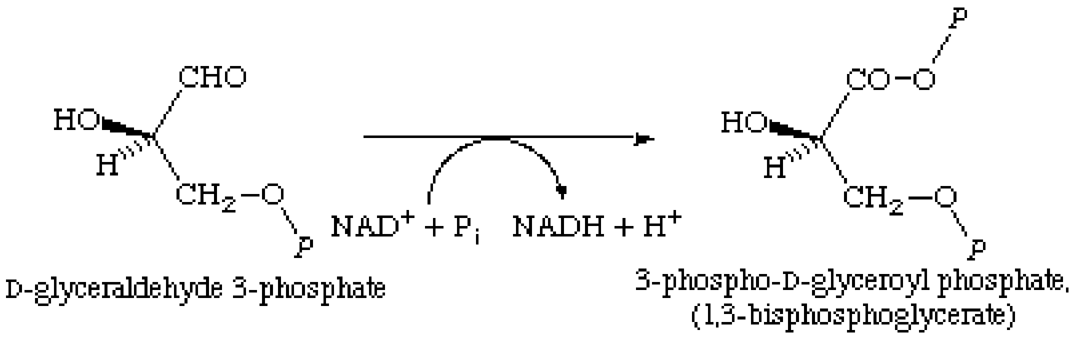
#*Up till now glucose has been converted to two molecules of glyceraldehyde 3 phosphate. we have not seen any oxidation reaction yet. in this phase of pathway, ATP is produced instead of being used*.

Step 6. The oxidation of glyceraldehyde 3 phosphate to 1,3-biphosphoglycerate.

Here NAD^+ is the coenzyme. The reaction involves the addition of a phosphate group to glyceraldehyde-3-phosphate as well as electron-transfer reaction from glyceraldehyde-3-phosphate as well as electron transfer reaction from glyceraldehyde-3-phosphate to NAD^+ i.e; NAD^+ is reduced to NAD.

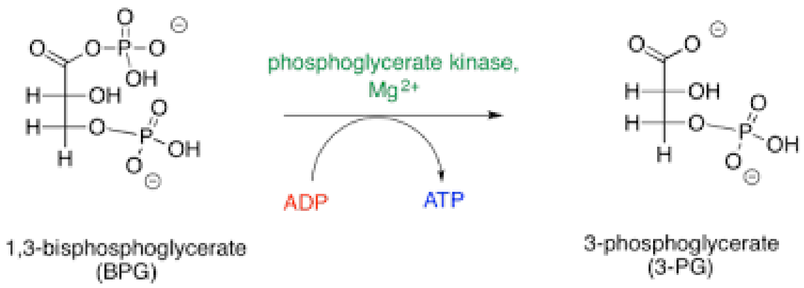
$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+$

The enzyme that catalyses this conversion is glyceraldehyde-3-phosphate dehydrogenase



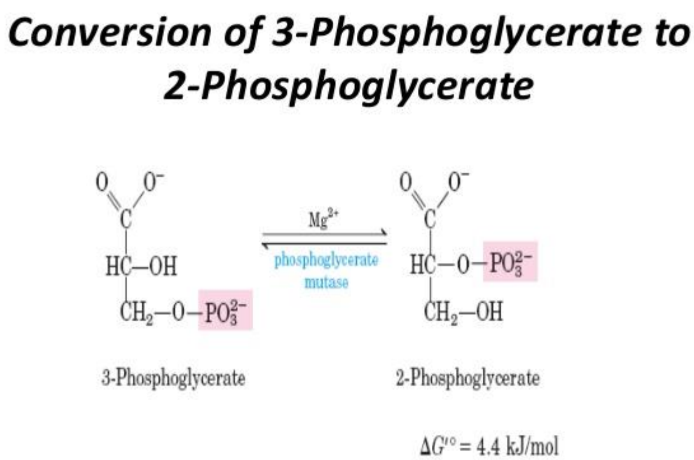
Step 7. In this step ATP is produced by phosphorylation of ADP and 3 phosphoglycerate is formed.

The enzyme that catalyses reactions called phosphoglycerate kinase



Step 8.The phosphate group is transferred from carbon 3 to carbon 2 of the glyceric acid backbone

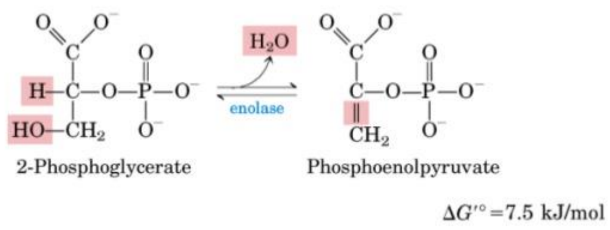
The enzyme that cut ties this reaction is phosphoglyceromutase.



Step 9. The 2-phosphoglycerate loses one molecule of water producing phosphoenol pyruvate

This reaction does not involve electron transfer , it is a dehydration reaction. enolase is the enzyme that catalyses the reaction, requires Mg^{2+} as a cofactor. the water molecule that is eliminated binds to Mg^{2+}in the course of reaction.

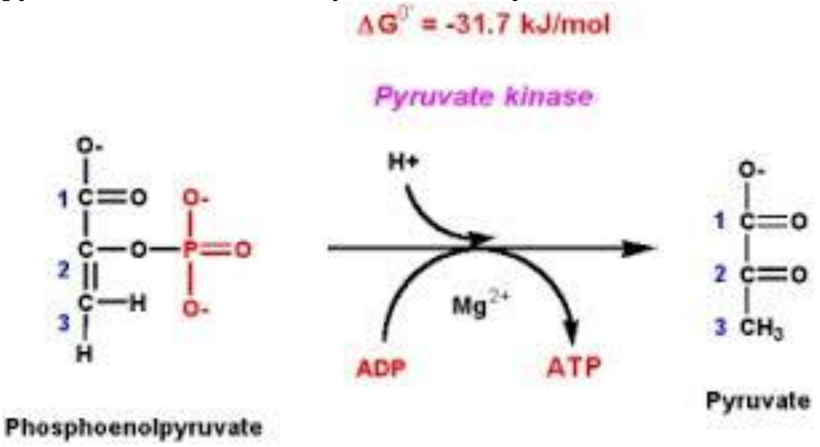
step 9: dehydration of 2-phosphoglycerate to phosphoenolpyruvate



- Enolase
- Reversible removal of water (a dehydration reaction).

Step 10. Phosphoenolpyruvate transfers it's phosphate group to ADP, producing pyruvate

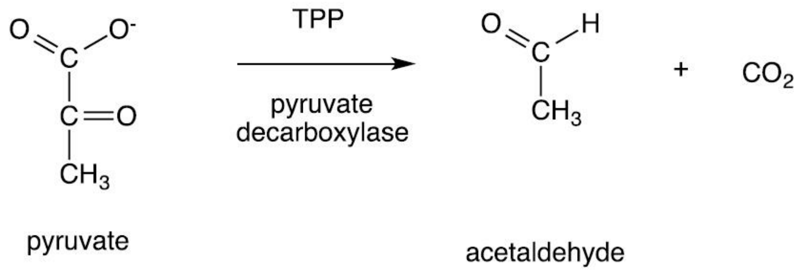
Here double bond shifts to oxygen on carbon 2 and hydrogen shifts to carbon 3.
pyruvate kinase is the enzyme that catalyse this reaction.



Thus in the second phase of alcoholic fermentation glyceraldehyde 3 phosphate is converted to pyruvate. these reactions yields 4 molecules of ATP two for each molecules of pyruvate produced.

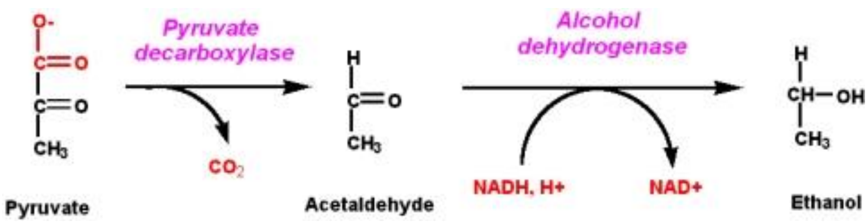
The conversion of pyruvate to acetaldehyde and then to ethanol

Pyruvate is decarboxylated to produce acetaldehyde. the enzyme that catalyse this reaction is pyruvate decarboxylase. this enzyme requires Mg^{2+} and co-factor thiamine pyrophosphate (TPP). Carbon dioxide splits off leaving a two carbon fragment covalently bonded to TPP. Thus acetaldehyde is produced. This step is irreversible.



The carbon dioxide produced is responsible for the bubbles in the beer and in sparkling wines.

Acetaldehyde is then oxidized to produce within all and at the same time one molecule of NADH is oxidized to NAD^+, for each molecule of ethanol produced.



This step provides recycling of NAD^+ and thus allows further anaerobic reactions.

The net reaction for alcoholic fermentation is

The conversion of glucose into ethanol is an example of ***alcoholic fermentation.***

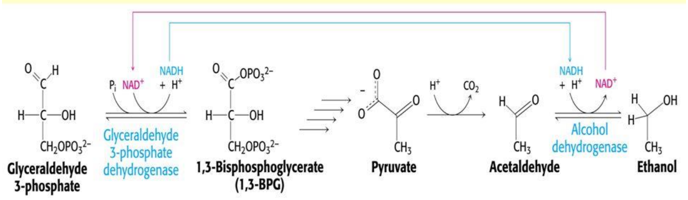
The net result of alcoholic fermentation is:

$Glucose + 2P_i + 2ADP + 2H^+ \rightarrow 2\ ethanol + 2CO_2 + 2ATP + 2H_2O$

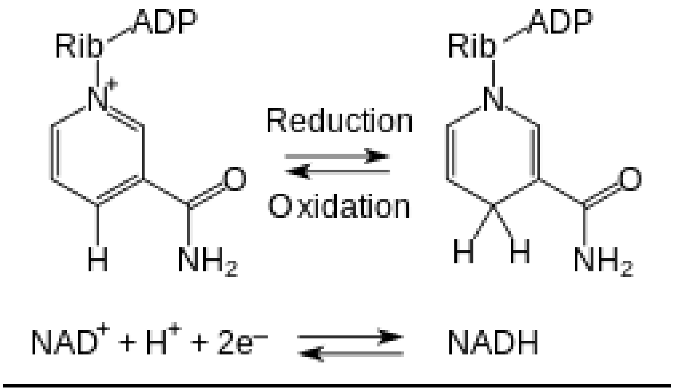
The ethanol formed in alcoholic fermentation provides a key ingredient for brewing and winemaking.

There is no net NADH formation in the conversion of glucose into ethanol.

NADH generated by the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of acetaldehyde to ethanol.



NAD^+ and NADH do not appear in net equation. It is essential that recycling of NADH to NAD^+ takes place at this step so that further anaerobic oxidation takes place. Alcohol dehydrogenase is the enzyme that catalyses the conversion of acetaldehyde to ethanol. It is NADH-linked dehydrogenase.



Coenzyme A (CoA, CoASH, or HSCoA) is a coenzyme, well known for it's role in the synthesis and oxidation of fatty acids, and the oxidation of pyruvate in the citric acid cycle. All genomes sequenced to date encode enzymes that use coenzyme A as a substrate, and around 4% of cellular enzymes use it, or a thioester form of it, as a substrate.

CITRIC ACID CYCLE

Glycolysis release only a very small amount of chemical energy which is available in the glucose molecule. Much more energy is released when glucose molecule is oxidised completely to CO_2 and water. This is aerobic metabolism. Organisms can obtain far more energy from nutrients by aerobic oxidation than anaerobic oxidation. We know that glycolysis produces only two molecules of ATP for each molecule of glucose metabolised. However, in complete aerobic oxidation each molecule of glucose produces about 30 to 32 molecules of ATP.

Three processes play roles in aerobic metabolism: the citric acid cycle, electron transport and oxidative phosphorylation.

Metabolism consists of catabolism which is the oxidative breakdown of nutrients and anabolism which is reductive synthesis of biomolecules. The citric acid cycle is amphibolic i.e., it plays a role in catabolism and anabolism. The citric acid cycle, as the hub of metabolic pathways, serves to connect the breakdown and synthesis of proteins, carbohydrates and lipids. Most of the metabolites of major nutrients can be fed into the citric acid cycle as acetyl-CoA and then oxidised to produce energy.

The citric acid cycle operates inside the mitochondrion which acts as its engine room. Here, metabolic fuels-glucose derived from carbohydrates, amino acids derived from protein and fatty acids from lipids-are fed into the cycle and will ultimately be oxidised to carbon dioxide and water. The energy is transferred to electron carriers and finally to the terminal electron acceptor-oxygen.

There are two other common names for the citric acid cycle. One is ***Krebs cycle*** and other name is ***tricarboxylic acid cycle***(or TCA cycle) because of acids with three carboxyl groups.

OVERALL PATHWAY OF THE CITRIC ACID CYCLE

The citric acid cycle takes place in mitochondria. Most of the enzymes of the citric acid cycle are present in the mitochondrial matrix.

Pyruvate produced by glycolysis is oxidized to one carbon dioxide molecule and one acetyl group which becomes linked to coenzyme A to give acetyl-CoA. It is then enters the citric acid cycle. Two more molecules of carbon dioxide produced for each molecule of acetyl-CoA that enters the cycle and electrons are transferred in the process. electronic accepter in all cases but one is NAD+, which is reduced to NADH. in one case electron acceptor is FAD (flavin adenine dinucleotide). this takes up two electrons and two hydrogen ions to produce $FADH_2$. The electrons are passed from NADH and $FADH_2$ to several stages and final acceptor is oxygen with water as the product.

There are 8 step in the citric acid cycle, each catalysed by a different enzyme. Four of the 8 steps -steps 3, 6 , 6 and 8- are oxidation steps.

The oxidizing agent is NAD+ in all except step 6, in which it is FAD. In step 5 , a molecule of GDP (guanosine diphosphate) is phosphorylated to produce GTP.

in the first reaction of the cycle, the two carbon acetyl group condenses with four carbon oxaloacetate to produce six carbon citrate ion. in the next few steps citrate isomerises and then oxidative decarboxylation takes place to give Alpha-keto-glutarate, which again is oxidatively decarboxylated to produce four-carbon compound succinate. the cycle is completed by regeneration of oxaloacetate from succinate via the fumarate in several steps.

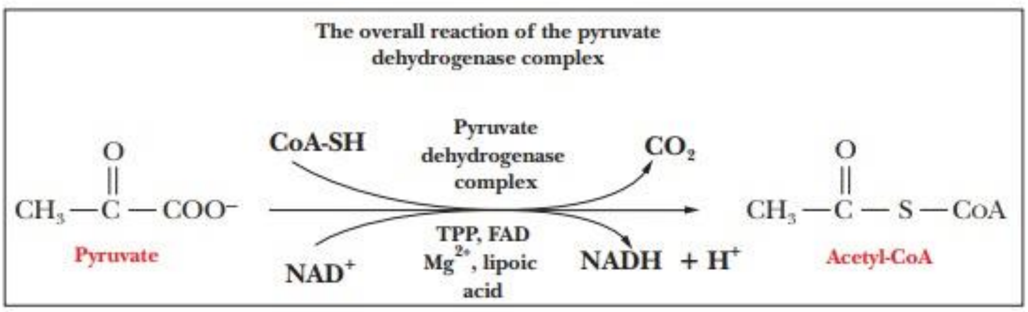
<u>Conversion of pyruvate to acetyl-CoA</u>

Pyruvate is generated from glycolysis under anaerobic conditions. It moves from the cytosol in the mitochondrion via specific transporter. Here pyruvate is converted to carbon dioxide and the acetyl portion of acetyl-CoA. An oxidation reaction precedes the transfer of acetyl group to the CoA.

The whole process involves several enzymes, all of which are part of pyruvate dehydrogenase complex. the overall reaction is

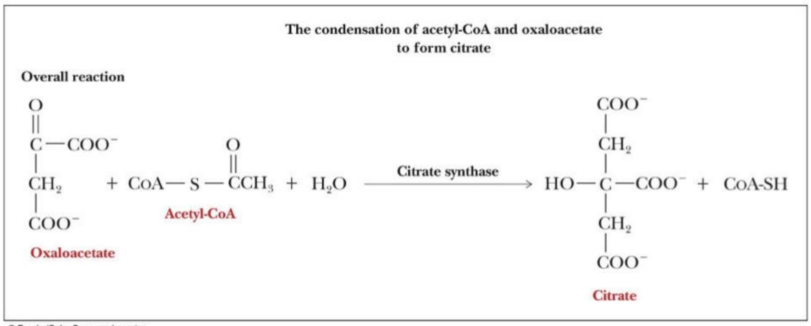
Pyruvate + CoA-SH + NAD^+ → Acetyl-CoA + CO_2+ H^+ +NADH

There is -SH group at one end of CoA, it is frequently shown in equations as CoA-SH. The reaction is catalysed by four cofactors, TPP, FAD, Mg^{2+} and lipoic acid.



<u>Step 1. Formation of Citrate:</u>

This is the first step of citric acid cycle. it involves reaction of acetyl-CoA and oxaloacetate to form citrate and CoA-SH. it is a condensation reaction because a new carbon-carbon bond is formed. reactions catalysed by enzyme citrate synthase.



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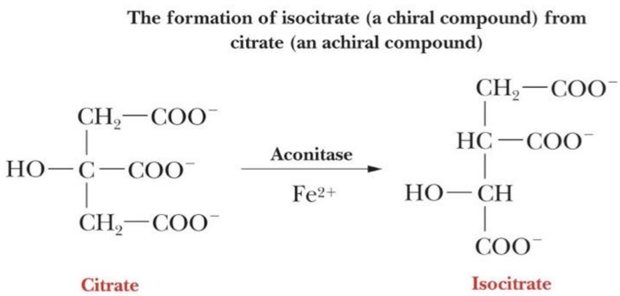
Step 2. Isomerisation of Citrate to Isocitrate:

This reaction is catalysed by aconitase. the enzyme requires Fe^{2+} as cofactor. the enzyme is able to select one end of the citrate molecule in preference to the other.



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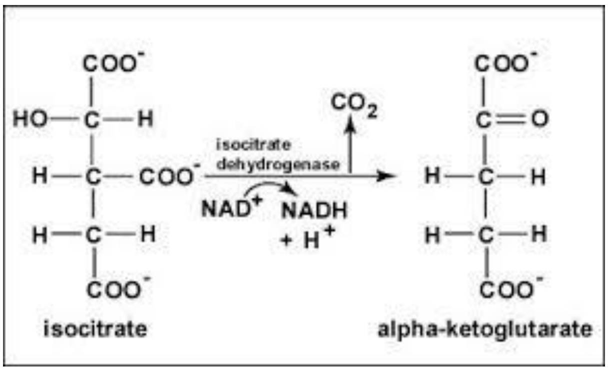
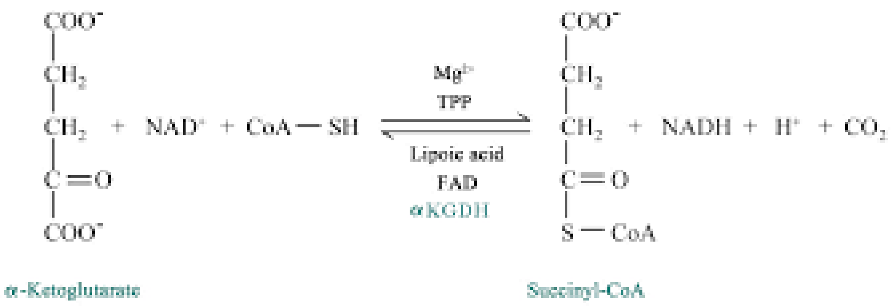
Step 3. Formation of α- ketoglutarate and CO_2 (First Oxidation Step)

This is the oxidative decarboxylation of isocitrate to Alpha ketoglutarate and carbon dioxide. the enzyme catalysis this reaction is isocitrate dehydrogenase. this is the first reaction in which NADH is produced.



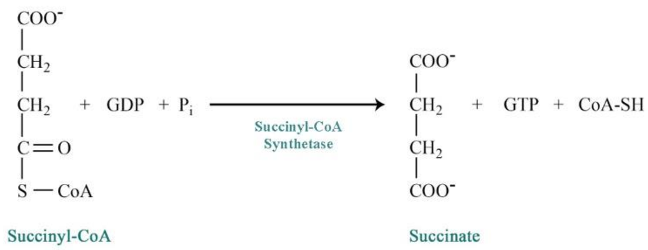
Step 4. Formation of Succinyl-CoA and CO_2 (Second oxidation step)

This reaction occurs in several stages and is catalysed by enzyme called α-ketoglutarate dehydrogenase complex. The co-factors used are TPP, FAD, Mg^{2+} and lipoic acid.



Step 5. Formation of Succinate

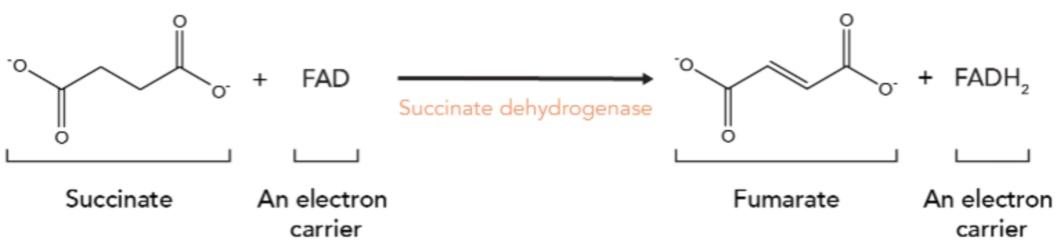
In this step succinyl-CoA is hydrolysed to produce succinate and CoA-SH. the reaction is catalysed by enzyme succinyl-CoA synthetase. the accompanying reaction is phosphorylation of GDP to GTP. the energy required for phosphorylation of GDP to GTP is provided by the hydrolysis of succinyl-CoA to produce succinate and CoA.



Step 6. Formation of Fumarate (Oxidation Step)

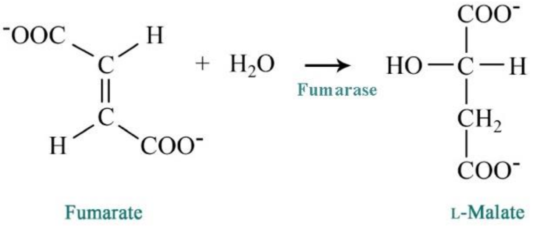
Succinate is oxidized to fumarate, reaction is catalyzed by enzyme succinate dehydrogenase. in this reaction, FAD is the Co enzyme which is reduce to $FADH_2$.

The figure below depicts a reaction that occurs in the TCA cycle. This reaction is catalyzed by succinate dehydrogenase.



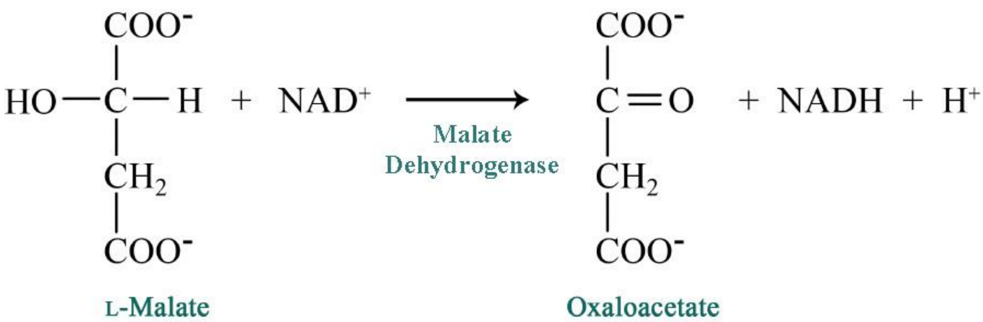
Step 7. Formation of L-Malate

This reactions catalyzed by enzymes fumarase. water is added across the double bond of fumarate in a hydration reaction to give malate. here the reaction is stereospecific. it gives only L-Malate.



Step 8. Regeneration of Oxaloacetate (Final Oxidation Step)

This is the final step in which malate is oxidized to oxaloacetate. This reaction catalysed by maleate dehydrogenase. Another molecule of NAD+ is reduced to NADH. the oxaloacetate produced can then react with another molecule of Acetyl-CoA to start another round of cycle.



The oxidation of pyruvate by the pyruvate dehydrogenase complex and the citric acid results in the production of 3 molecules of CO2. during this. One molecule of GDP is phosphorylated to GTP, one molecule of FAD is reduced to FADH2 and four molecules of NAD+ are reduced to NADH. Three molecules of life NADH come from the citric acid cycle and one comes from the reaction of the pyruvate dehydrogenase complex.

From pyruvate dehydrogenase complex

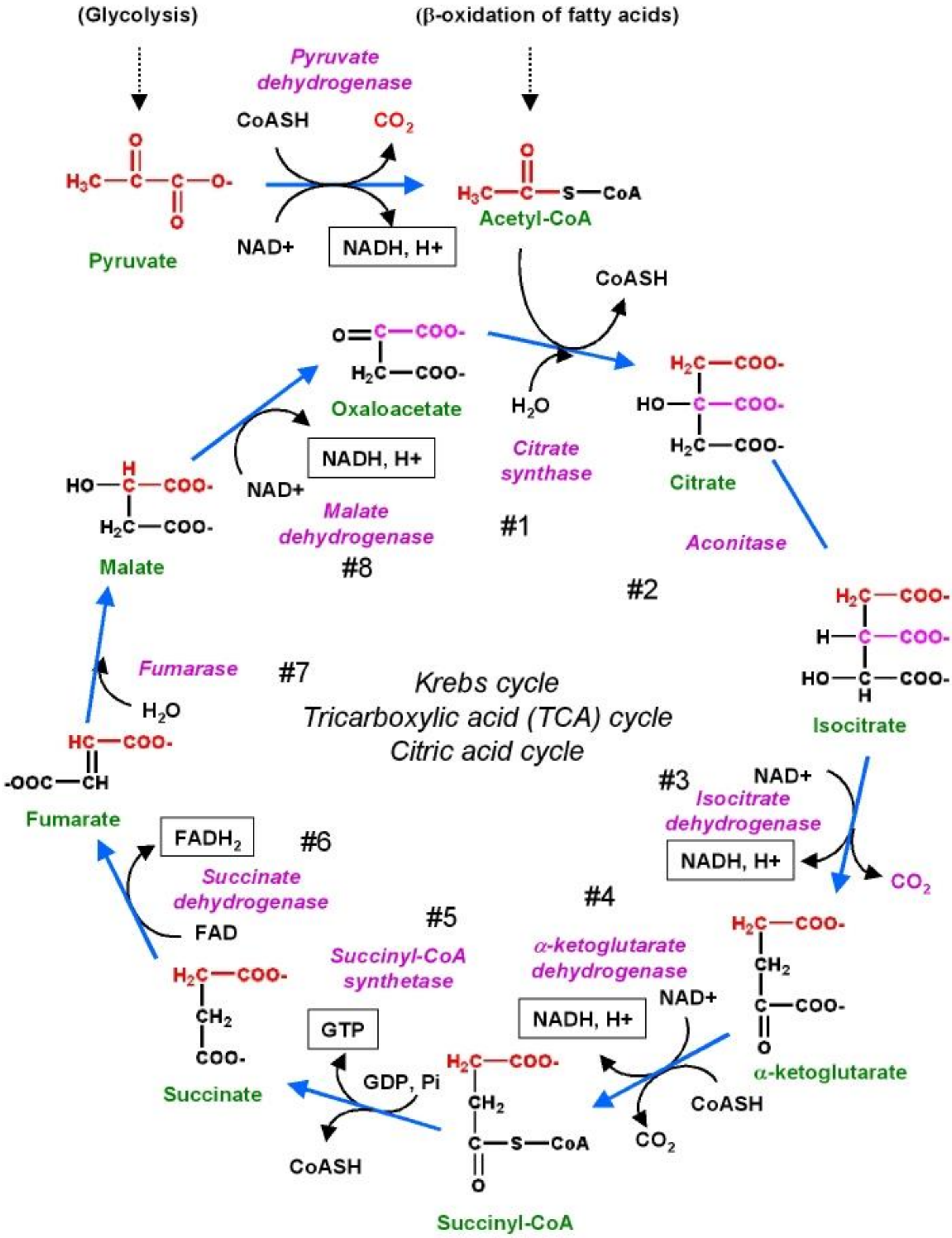
Pyruvate + CoA-SH + NAD^+ → Acetyl-CoA + CO_2+ H^+ +NADH

From citric acid cycle

Net Effect of the Citric Acid Cycle

Acetyl-CoA + $3NAD^+$ + FAD + GDP + P_i + 2 H_2O →
$2CO_2$ +3NADH + $FADH_2$ + GTP + CoA + $3H^+$

- carbons of acetyl groups in acetyl-CoA are oxidized to CO_2
- electrons from this process reduce NAD^+ and FAD
- one GTP is formed per cycle, this can be converted to ATP
- intermediates in the cycle are not depleted



(Glycolysis)
(β-oxidation of fatty acids)
Pyruvate dehydrogenase
CoASH
CO2
NAD+
NADH, H+
Pyruvate
Acetyl-CoA
CoASH
H2O
Citrate synthase
#1
Oxaloacetate
NADH, H+
NAD+
Malate dehydrogenase
#8
Malate
Citrate
Aconitase
#2
Isocitrate
Fumarase
#7
Krebs cycle
Tricarboxylic acid (TCA) cycle
Citric acid cycle
Fumarate
FADH2
#6
Succinate dehydrogenase
FAD
#3
NAD+
Isocitrate dehydrogenase
NADH, H+
CO2
#4
#5
α-ketoglutarate dehydrogenase
Succinyl-CoA synthetase
GTP
NADH, H+
NAD+
α-ketoglutarate
CoASH
CO2
GDP, Pi
CoASH
Succinate
Succinyl-CoA