Respiratory substrate. The substrates which are broken down in respiration for the release of energy may be carbohydrates, fats or proteins. Proteins are used up as respiratory substrate only when carbohydrates and fats are not available. Blackman proposed that respiration in which carbohydrates are used as respiratory substrate are called *floating respiration* and if proteins are used, *protoplasmic respiration.*

Fats are used as respiratory substrates after their hydrolysis to fatty acids and glycerol by *lipase* and their subsequent conversion to hexose sugars. Proteins serve as substrates after their breakdown to amino acids by proteolytic enzymes. As regards carbohydrates, not only simple hexose sugars like glucose and fructose but complex disaccharides particularly sucrose and polysaccharides such as starch, inulin and hemicelluloses are also used as respiratory substrates.

During respiration, the complex substrates are broken down into simpler ones and finally CO_2 is liberated and water is formed. During oxidation of respiratory substrate, certain amount of energy is released. Part of this energy is trapped in the form of energy rich compounds such as ATP while the remaining part is lost in the form of heat. The energy trapped in ATP molecules can be used in various ways (both for physical and chemical requirements). Here, the typical example of respiratory substrate, *i.e.* carbohydrate has been discussed. Oxidation of fats and proteins are given separately.

All complex carbohydrates are firstly converted into hexose (glucose or fructose) before actually entering into the respiratory process (Fig. 16.1). The oxidation of glucose to CO_2 and water consists of two distinguishable phases:

(*i*) Glycolysis, and

(*ii*) Krebs cycle.

In glycolysis glucose is converted into pyruvic acid. Steps of glycolytic pathway are common to all kinds of respiration, hence may be called common respiratory

metabolism. The fate of pyruvic acid, however, depends on the presence or the absence of oxygen. In presence of oxygen, the final degradation products are carbon dioxide and water (Krebs cycle) while in absence of oxygen ethyl alcohol and carbon dioxide in fermentation and lactic acid in lactic acid formation are formed.

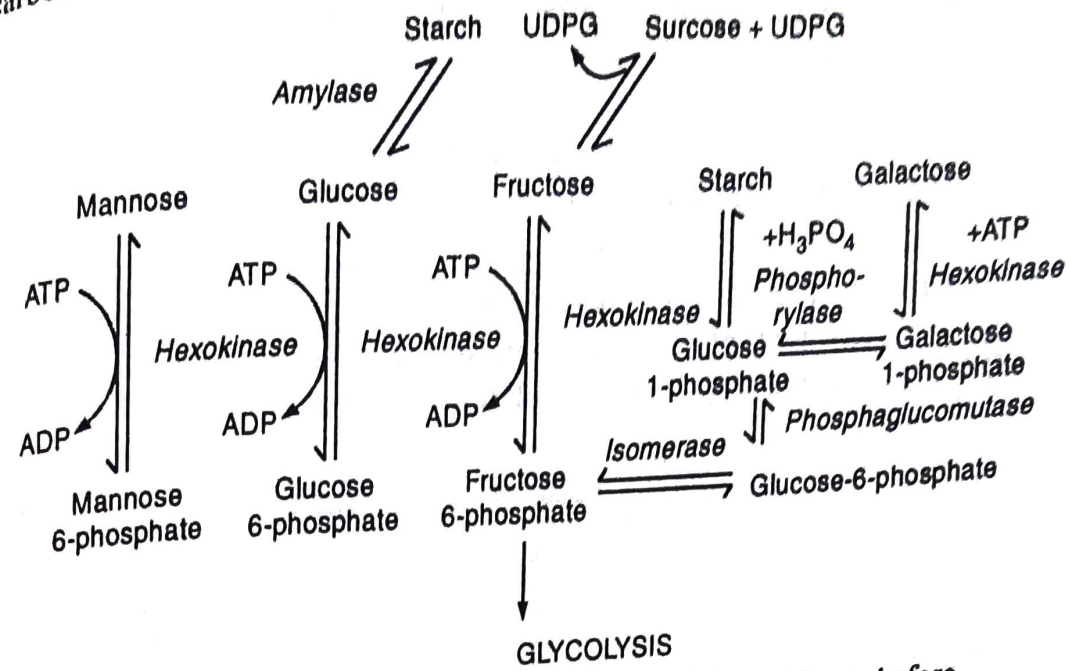


Fig. 16.1. Schematic conversion of complex carbohydrates before entering into Glycolysis.

Glycolysis, fermentation, anaerobic respiration and lactic acid formation processes occur freely in the cytoplasm while Krebs cycle occurs in the matrix of mitochondria in the eukaryotic cells and on the surface of mesosomes in prokaryotic cells. Enzymes of glycolysis are found in the soluble portion of cytoplasm, called *cytosol.* These remain active throughout the life time and are required again and again. Such enzymes are called *constitutive enzymes.*

GLYCOLYSIS

(EMP pathway = Embden Meyerhof Paranas pathway, Common respiratory pathway, Cytoplasmic respiration).

The course of stepwise degradation from glucose to pyruvic acid is termed as *glycolysis.* After the name of its tracers, the glycolytic pathway is also known as Embden Meyerhof Paranas pathway (EMP pathway).

Glycolysis can be broadly represented as follows:

$$\underset{\text{(Hexose)}}{C_6H_{12}O_6} \longrightarrow \underset{\text{(Pyruvate)}}{2C_3H_4O_3} + 4H$$

It thus states that a molecule of glucose which is a 6-carbon compound is broken down into two molecules of pyruvic acid which is a 3-carbon compound through a large number of stepwise closely integrated reactions. It occurs in the following three important phases.

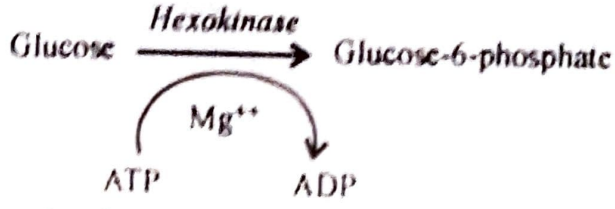
In the **first phase** of glycolysis, the glucose molecule is phosphorylated with the introduction of two phosphate groups into its structure. For this phase two molecules of ATP are needed.

The **second phase** involves the breaking up of 6-carbon compound Fructose 1,6-diphosphate into two molecules of 3-carbon compounds, *i.e.*, 3-Phosphoglyceraldehyde and Dihydroxyacetone phosphate. These two 3-carbon compounds are interconvertible.

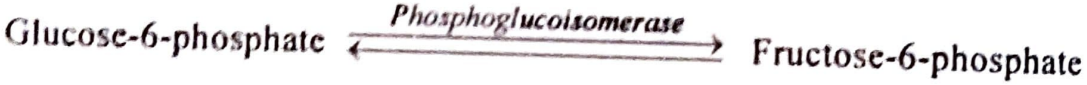
The **third phase** involves degradation of 3-PGAld into pyruvic acid with the production of four molecules of ATP. As in the phosphorylation of glucose during the first phase where two molecules of ATP have already been used up, there is a net gain of only two molecules of ATP during glycolytic reactions.

The various steps of glycolysis are detailed as follows.

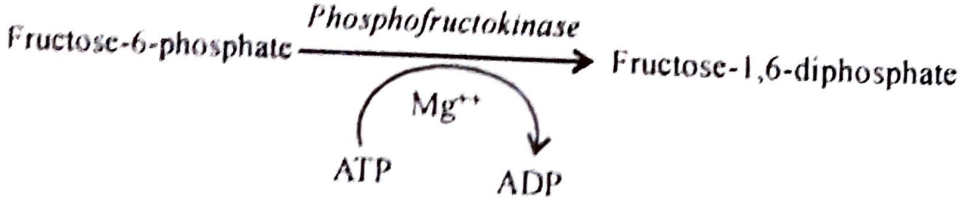
(1) First of all in presence of the enzyme *hexokinase* and with the help of one ATP molecule, the sixth carbon position of glucose molecule is phosphorylated and glucose is converted into Glucose-6-phosphate. ATP is, however, converted into ADP. The reaction may be represented as follows.



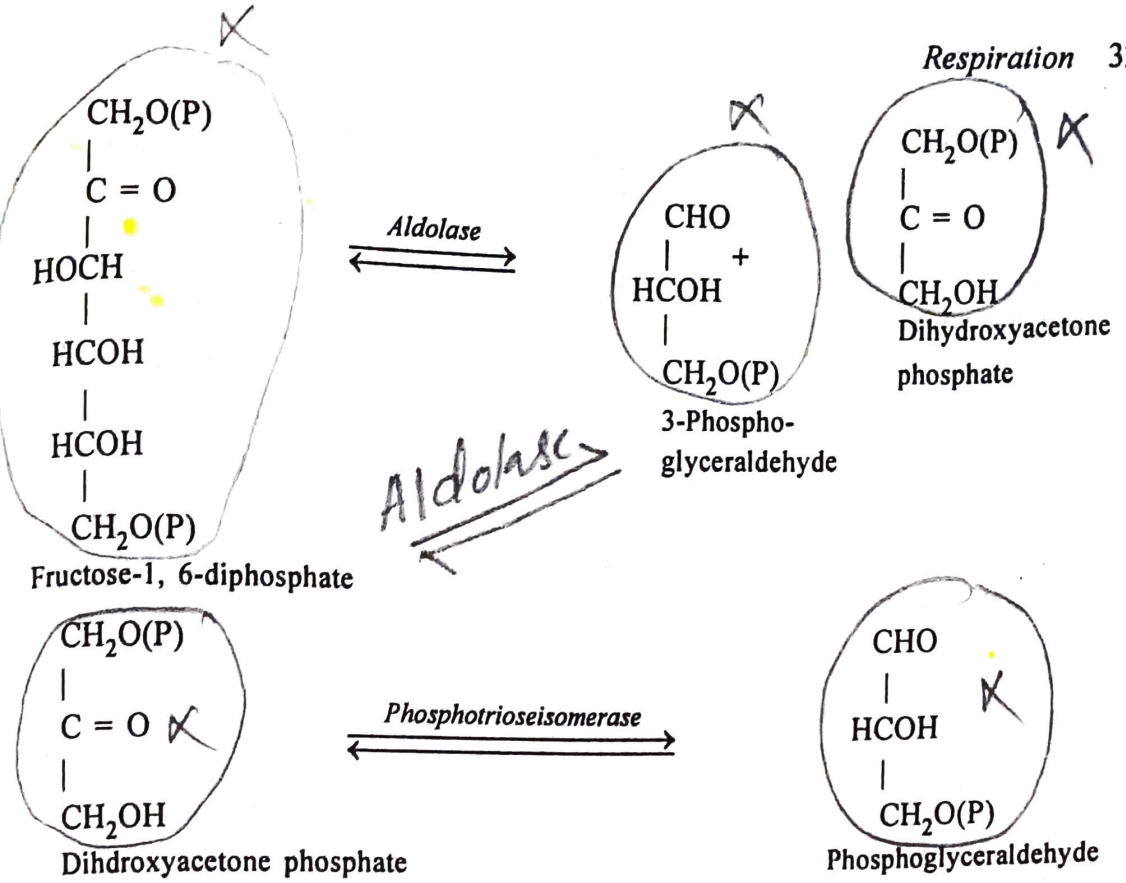
(2) Next reaction involves the isomerisation and conversion of Glucose-6-phosphate into Fructose-6-phosphate. The conversion is catalysed by the enzyme *phosphoglucoisomerase*.



(3) Now the first carbon of Fructose-6-phosphate also gets phosphorylated with the help of another ATP molecule in the presence of the enzyme *phospho-fructokinase* and is converted into fructose-1,6-diphosphate. Magnesium ions are needed for enzymatic activity of kinase.

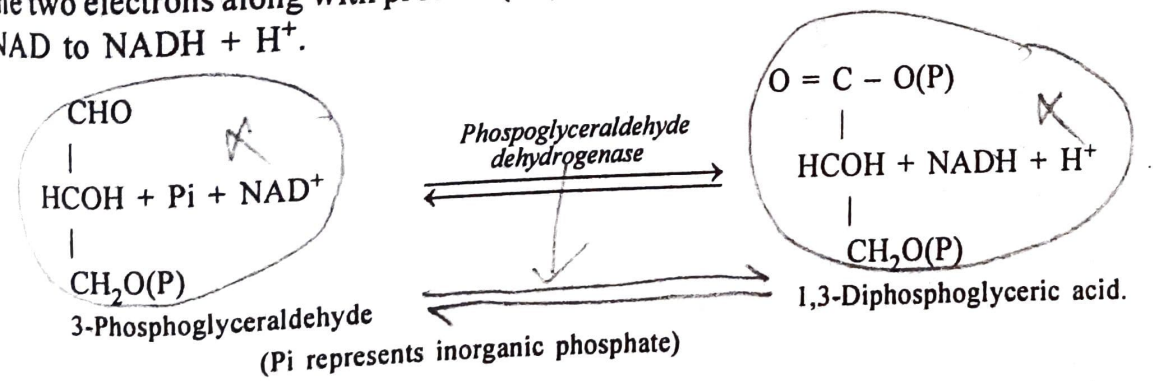


(4) This two-fold phosphorylation of hexose permits its break-up. Fructose 1, 6-diphosphate breaks into two molecules of 3-carbon compounds in presence of the enzyme *aldolase*. The two 3-carbon compounds formed are 3-Phosphoglyceraldehyde and Dihydroxyacetone phosphate. These two compounds are interconvertible and an equilibrium is maintained between them. The interconversion of 3-Phosphoglyceraldehyde and Dihydroxyacetone phosphate is catalysed by the enzyme *phosphotrioseisomerase*.



Here (P) refers to phosphate, *i.e.*, PO_3H_2

(5) The next step is the oxidation of 3-phosphoglyceraldehyde and the attachments f inorganic phosphate H_3PO_4 to the molecule forming 1, 3-Diphosphoglyceric cid. The 3-phosphoglyceraldehyde molecule is oxidised with the release of two lectrons and two protons (H^+). The two steps of the reaction are coupled in the ense that the energy supplied by one step (oxidation of 3-Phosphoglyceraldehyde) utilised by the other step (formation of organic linkage between inorganic phosphate nd oxidised 3-Phosphoglyceraldehyde in the C_1 position to produce 1, 3- iphosphoglyceric acid). The two steps in fact serve to trap most of the energy berated in oxidation which otherwise would simply be dissipated as heat. This nergy is however recovered as ATP in the next step. The two steps of the above eaction are catalysed by the enzyme *phosphoglyceraldehyde dehydrogenase* and ne two electrons along with protons (H^+) released are however used up in reducing NAD to NADH + H^+.

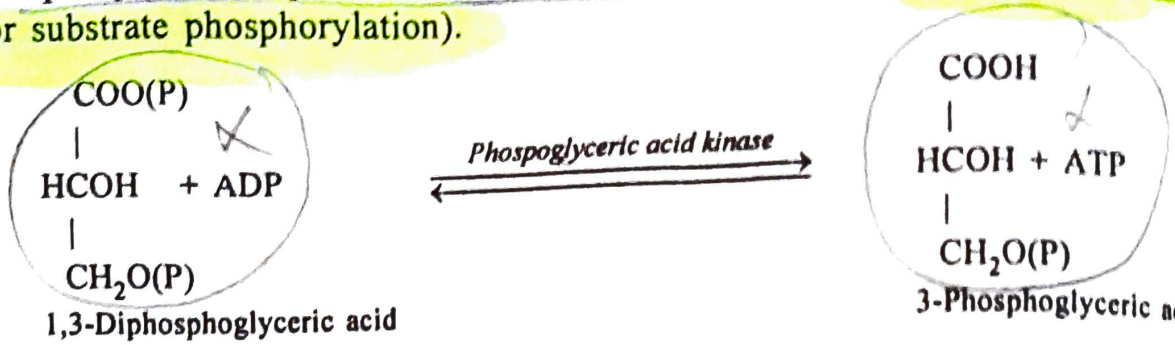


(Pi represents inorganic phosphate)

(6) With the conversion of the 3phosphoglyceraldehyde to 1,3-diphospho-glyceric acid a shift in the balance is affected and to maintain it more of Dihydroxyacetone phosphate is converted into 3-Phosphoglyceraldehyde.

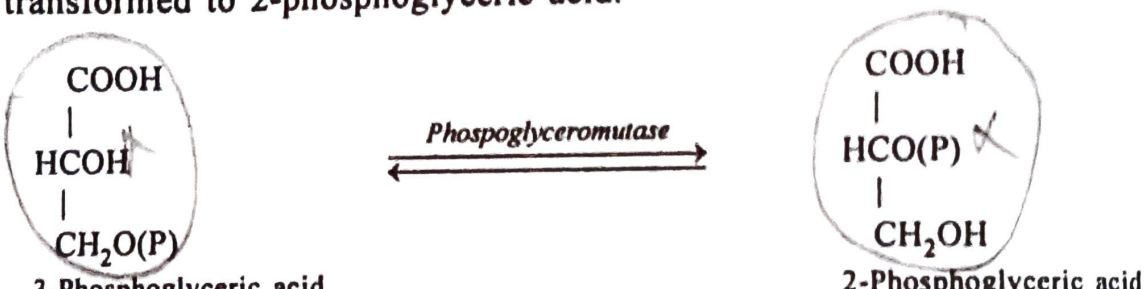
The incorporation of Pi (inorganic phosphate) in the formation of 1,3-Diphosphoglyceric acid along with the energy released in the oxidation of 3-Phosphoglyceraldehyde is important because in the next step this phosphate attaches

itself with ADP to produce ATP where 1, 3-Diphosphoglyceric acid is converted into 3-Phosphoglyceric acid in presence of the enzyme *phosphoglyceric acid kinase*. This kind of reaction in which a phosphate group is transferred from another already phosphorylated compound to ADP to form ATP is called **transphosphorylation** (or substrate phosphorylation).



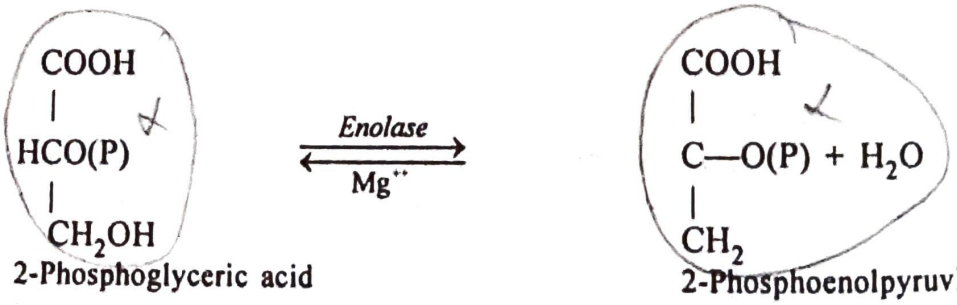
1,3-Diphosphoglyceric acid — 3-Phosphoglyceric acid

(7) In presence of the enzyme *phosphoglyceromutase*, 3-Phosphoglyceric acid is transformed to 2-phosphoglyceric acid.



3-Phosphoglyceric acid — 2-Phosphoglyceric acid

(8) In the next step catalysed by the enzyme *Enolase* one molecule of water is eliminated from 2-Phosphoglyceric acid and it is converted into 2-Phosphoenolpyruvic acid.



2-Phosphoglyceric acid — 2-Phosphoenolpyruvic acid

(9) The removal of water from 2-Phosphoglyceric acid alters its molecular structure and changes its internal distribution in such a way that a much greater part of the molecule energy is concentrated in the region of the phosphate group. When this phosphate group is taken over by ADP in the conversion of phosphoenolpyruvic acid to pyruvic acid in the presence of the enzyme *pyruvic acid kinase*, a considerable part of energy is conserved as ATP (For details see Fig. 16.3). This kind of reaction is also called *transphosphorylation reaction*.

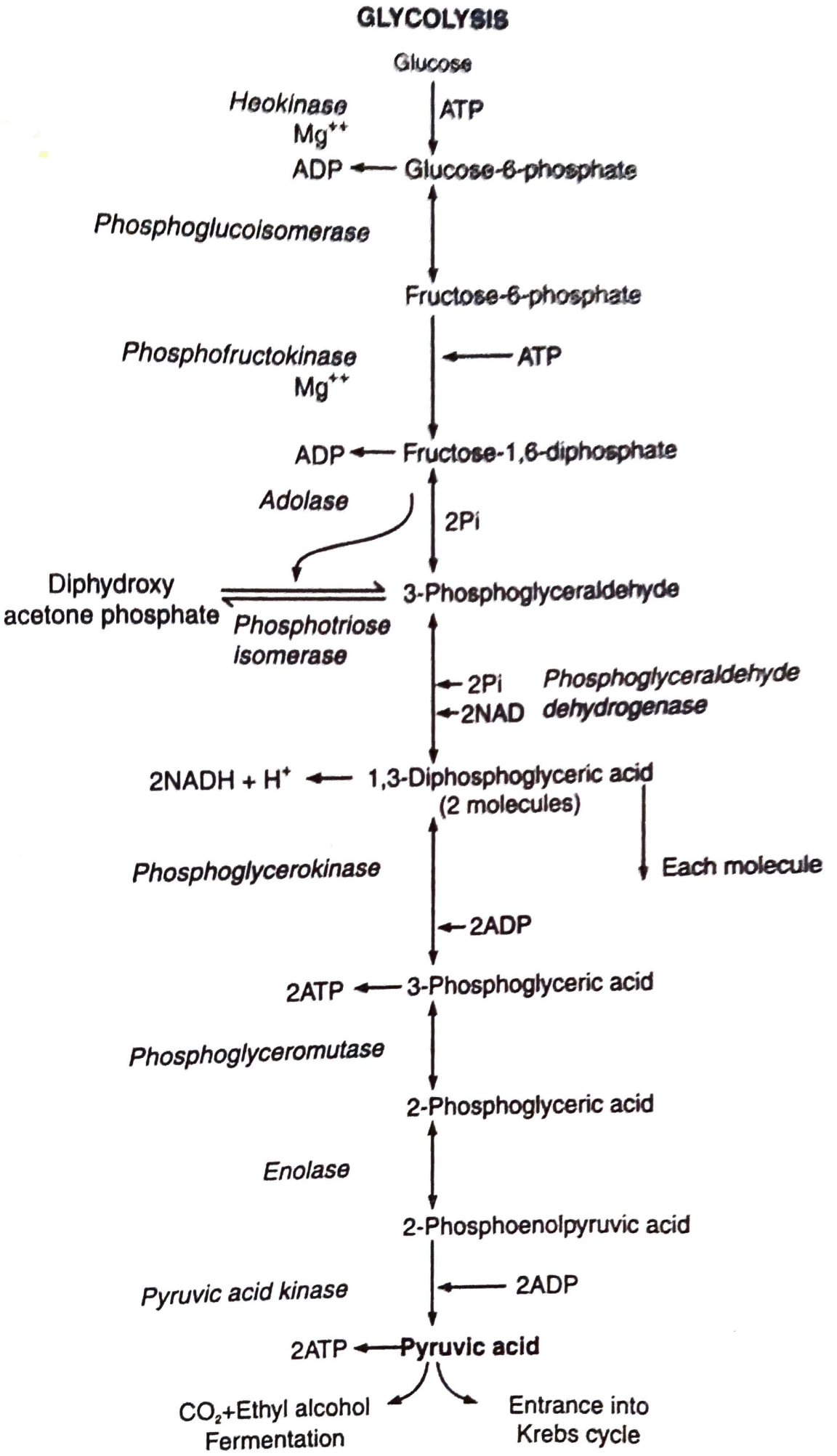


Fig. 16.2. Schematic representation of glycolysis.

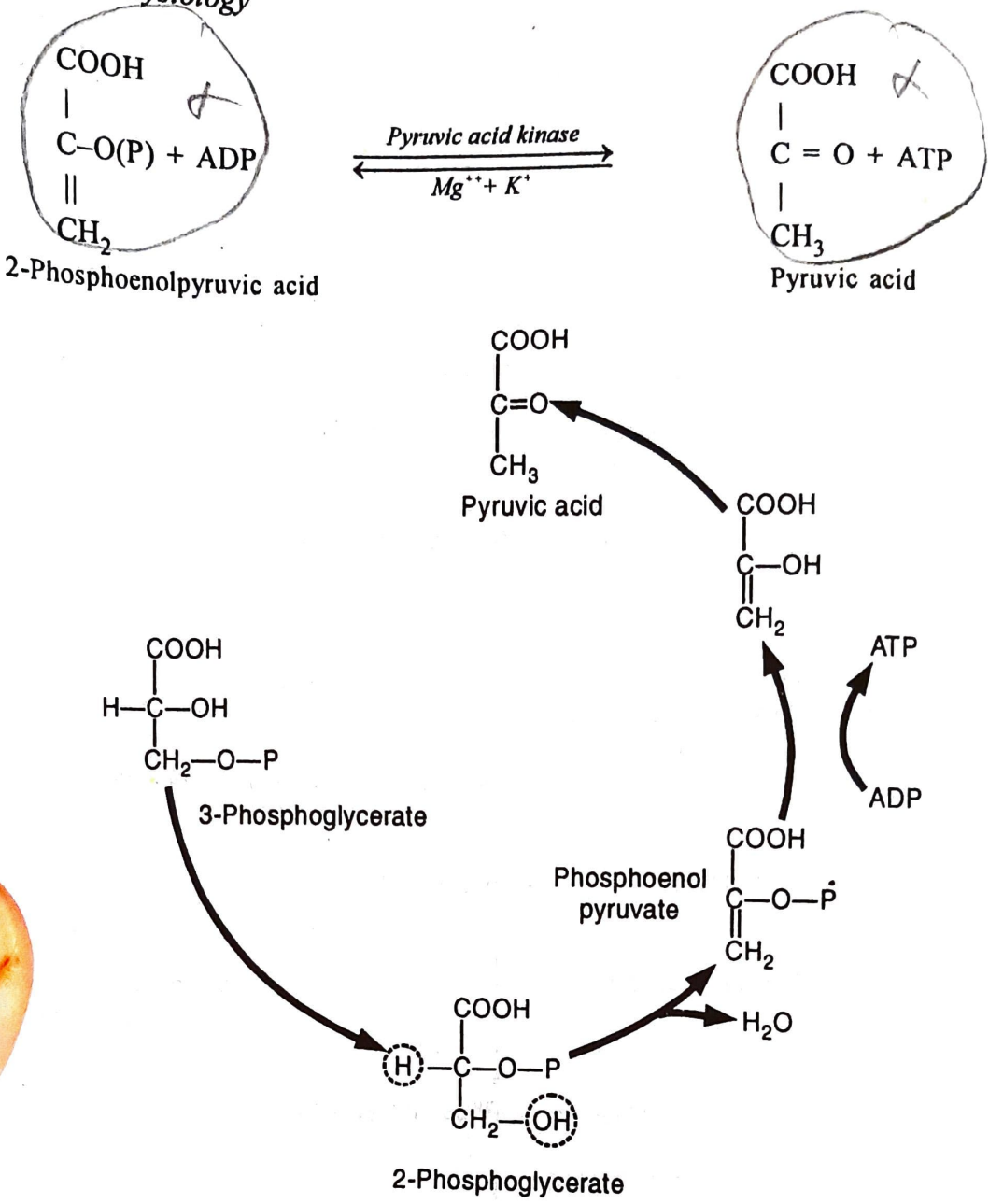


Fig. 16.3. Figure shows conversion of phosphoenolpyruvic acid to pyruvic acid and synthesis of ATP during conversion. 2-Phosphoglyceric acid (2-Phosphoglycerate) molecule makes chemical energy available through the formation of double bond due to liberation of a water molecule. Carbon atom-2 of phosphoenolpyruvic acid receives increased concentration of electrons which causes a shift in the internal energy of the molecule more toward the phosphate group. When this charged phosphate group is donated to ADP it forms ATP.

Phosphorylation in Glycolysis (Transphosphorylation)

With the production of Pyruvic acid the glycolysis comes to an end. Now if ATP molecules used up and produced during glycolysis are calculated, it is found that during the whole sequence of reactions, two molecules of ATP were used up—*one* when Glucose was phosphorylated and converted into Glucose-6-phosphate and the *other* when Fructose-6-phosphate was phosphorylated and converted into Fructose-1, 6-diphosphate but four molecules of ATP are produced. Two ATP molecules are produced during conversion of 1, 3-Diphosphoglyceric acid into 3-Phosphoglyceric acid and another two during conversion of phosphoenolpyruvic acid into Pyruvic

acid. This means that in the sequence of reactions of glycolysis there is a net gain of two molecules of ATP. These steps may be summarised as follows:

Glucose + 2ATP ⟶ Fructose-1,6-diphosphate + 2ADP

2ATP molecules are used

2(1,3-Diphosphoglyceric acid) + 2ADP ⟶ 2(3-Phosphoglyceric acid) + 2ATP

2(2-Phosphoenolpyruvic acid) + 2ADP ⟶ 2 Pyruvic acid + 2ATP

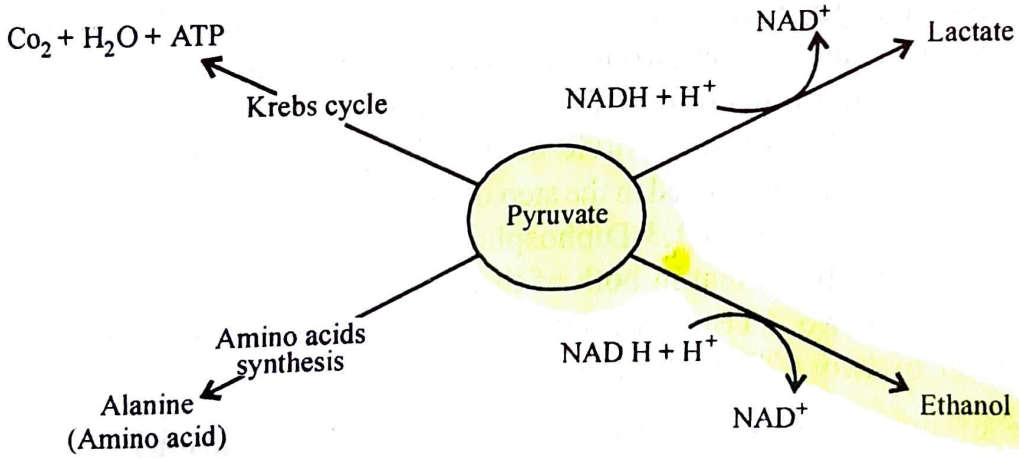
4ATP molecules produced

(Since 2ATP are used, there is a net gain of 2ATP only.)

However, two $NADH_2$ molecules are also produced in the process (see Fig. 16.2) and from each of its molecule 3 ATP molecules are produced. As a result 6 more ATP molecules are formed, though these are from ETS chain. In eukaryotes, $NADH_2$ from glycolysis enters one step late in ETS (due to loss of energy or utilisation of one ATP in transfer), hence it yields only 2 ATP molecules. $NADH_2$ generated during glycolysis remains in the cytoplasm as it cannot enter the mitochondria. However, its reducing power is transferred to ETS of mitochondria through **shuttle system** (*described in the chapter* Translocation of Solutes). But in this transfer, there is a loss of energy equivalent to one ATP molecule. In prokaryotes, 3ATP molecules per $NADH_2$ molecule are formed.

BREAKDOWN OF PYRUVIC ACID

As far as the fate of pyruvic acid is concerned it can be metabolised through four different paths leading to different products (as shown here in figure).



(1) Alcoholic Fermentation (Anaerobic Respiration)

In alcoholic fermentation pyruvic acid is converted into Ethyl alcohol and carbon dioxide. It involves two steps—in the first step, in the presence of the enzyme *pyruvic acid decarboxylase* Pyruvic acid is converted to Acetaldehyde with the production of one molecule of carbon dioxide.

$$\text{Pyruvic acid} \xrightarrow{\textit{Pyruvic acid decarboxylase}} \text{Acetaldehyde} + CO_2$$

And in the second step in the presence of *alcohol dehydrogenase* and $NADH_2$ Acetaldehyde is reduced to Ethyl alcohol.

$$\text{Acetaldehyde} + NADH + H^+ \xrightarrow{\textit{Alcohol dehydrogenase}} \text{Ethyl alcohol} + NAD$$

The utilisation of pyruvate by fermentation is very common in yeast cells and many plant tissues. The process of alcoholic fermentation stops after some time even when yeasts and respiratory substrate are present. The process stops when the concentration of alcohol exceeds 12-15 per cent, thereafter it becomes toxic to the growth of yeast.

Alcoholic fermentation is a type of anaerobic respiration, *i.e.* the process takes place in absence of oxygen. However, it differs from all the other types of anaerobic respiration of beings (*i*) extracellular, (*ii*) in presence of *zymase* or (*iii*) by participation of micro-organisms.

(2) Fate of Pyruvic Acid to Alanine

When needed, Pyruvic acid can be utilised to synthetize amino acids. The synthesis of Alanine from Pyruvic acid involves a number of biosynthetic transamination reactions and a detailed discussion is not possible here, however, one example can be

$$\text{Pyruvic acid} + \text{Gultamate} \longrightarrow \text{Alanine} + \alpha\text{-Ketoglutarate}$$

(3) Breakdown of Pyruvic Acid to Lactic Acid

Though the breakdown of Pyruvic acid to lactic acid is not common in higher plants, it is very common in animal tissues and muscle glycolysis is a perfect example of it. In this case in presence of the enzyme *lactic acid dehydrogenase* and $NADH_2$, Pyruvic acid is metabolised to lactic acid.

$$\text{Pyruvic acid} + \text{NADH} + \text{H+} \xrightarrow{\textit{Lactic acid dehydrogenase}} \text{Lactic acid} + \text{NAD}$$

Uptil now it is given how Glucose is broken down to Ethyl alcohol and carbon dioxide or Lactic acid levels through Pyruvic acid with the consumption of two ATP molecules and production of four ATP molecules. Two $NADH_2$ molecules required for the reduction of Pyruvic acid to Ethyl alcohol and carbon dioxide or to Lactic acid are also produced in the step of glycolytic reaction where 3-Phosphoglyceraldehyde is oxidised to 1,3-Diphosphoglyceric acid. A look on the balance of ATP molecules shows that in both of these pathways only four ATP molecules are produced and two ATP molecules are consumed meaning thereby that there is a net gain of two ATP molecules.

(4) Fate of Pyruvate to CO_2 and H_2O (Aerobic Respiration)

Further breakdown of Pyruvate is of considerable importance and significance from the standpoint of energy production. In the absence of oxygen it may be degraded to Ethyl alcohol and CO_2 or Lactic acid or Alanine but in the presence of oxygen the usual fate of Pyruvate is to degrade up to CO_2 and H_2O level through Krebs cycle and respiratory chain in which energy is trapped in the form of ATP molecules. These steps take place inside the mitochondria, *i.e.* Pyruvic acid enters the mitochondria. Mitochondria have all the enzymes necessary for complete oxidation of pyruvic acid to CO_2 and H_2O. All enzymes of TCA cycle are found in the mitochondrial matrix. A summary representation of the scheme can be.

$$\text{Pyruvate} \xrightarrow{\textit{Krebs cycle}} CO_2 + \text{electrons} + \text{protons}$$

$$\text{Pi} + \text{ADP} + \text{electrons} + \text{protons} + \text{oxygen} \xrightarrow{\textit{Respiratory chain}} \text{Water} + \text{ATP}$$

Formation of Acetyl CoA

The oxidative decarboxylation of Pyruvate into Acetyl CoA involves the presence of at least five essential cofactors and a complex enzyme. The cofactors involved are Mg ions, thiamine pyrophosphate (TPP), NAD^+, Coenzyme A(CoA) and Lipoic acid. Various steps in the oxidative decarboxylation of Pyruvic acid to Acetyl CoA are summarised as follows and in Fig. 16.4.

$$\text{Pyruvic acid} + \text{TPP} \longrightarrow \text{TPP complex} + CO_2$$

$$\text{TPP complex} + \text{Lipoic acid (oxidised form)} \longrightarrow \text{Acetyl lipoic acid complex} + \text{TPP}$$

$$\text{Acetyl lipoic acid complex} + \text{CoA} \longrightarrow \text{Acetyl CoA} + \text{Lipoic acid (reduced form)}$$

$$\text{Lipoic acid (reduced form)} + \text{NAD} \longrightarrow \text{Lipoic acid (oxidised form)} + \text{NADH} + H^+ \ (NADH_2)$$

The summary representation of the reactions can be

$$\text{Pyruvate} + \text{CoA} + NAD^+ \longrightarrow \text{Acetyl CoA} + CO_2 + \text{NADH} + H^+$$

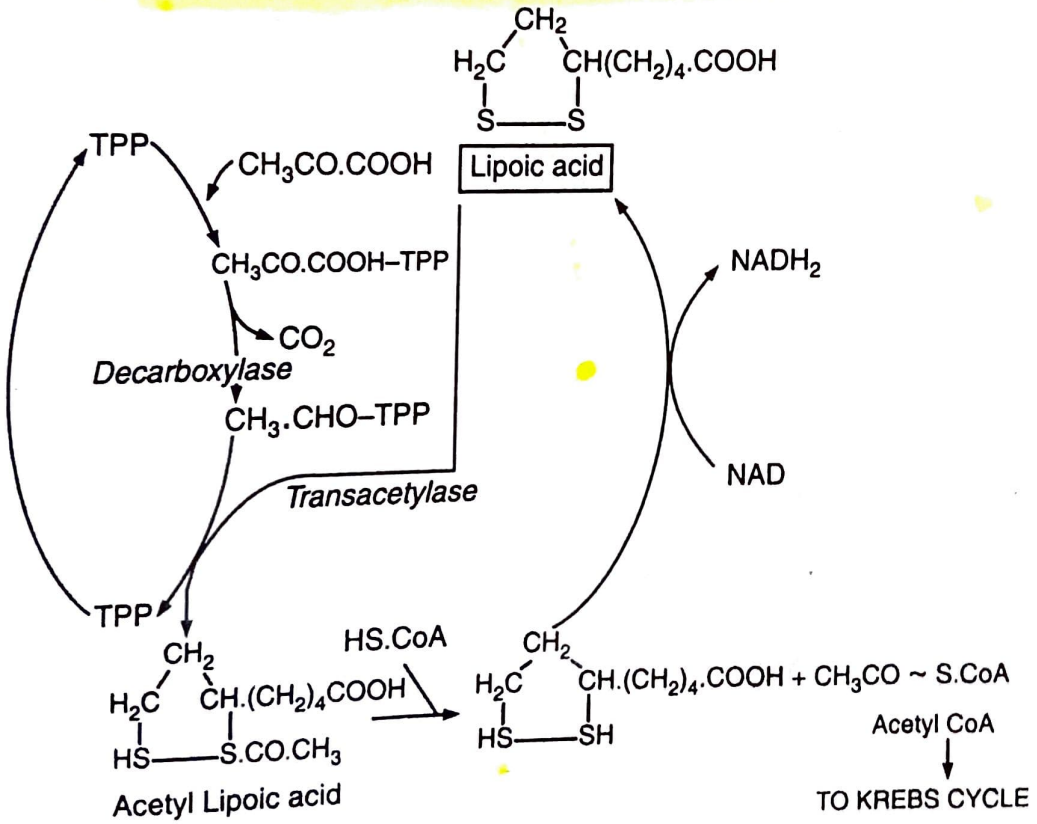


Fig. 16.4. Formation of acetyl CoA from pyruvic acid.

KREBS CYCLE

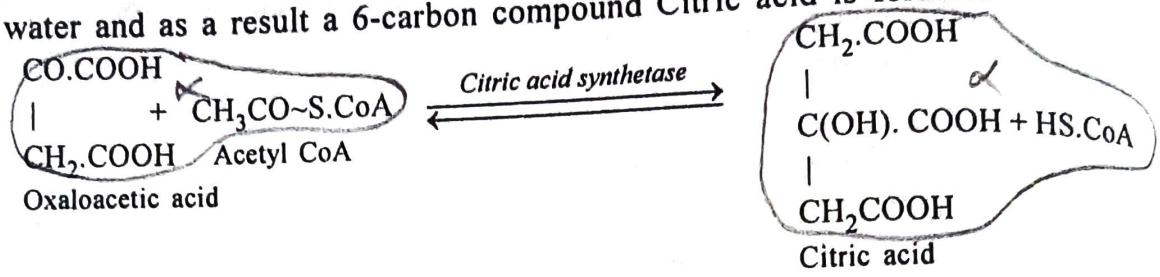
(Named *after* H.A. Kreb)

(*Tricarboxylic acid cycle, TCA cycle, Organic acid cycle, Mitochondrial respiration, Oxidation of pyruvate, Citric acid cycle*)

The cycle is found in mitochondrial matrix and consists of following. Some important steps.

(1) The 2-carbon compound Acetyl CoA is the connecting link between EMP pathway and Krebs cycle. The reactions of EMP pathway occur in the cytoplasm

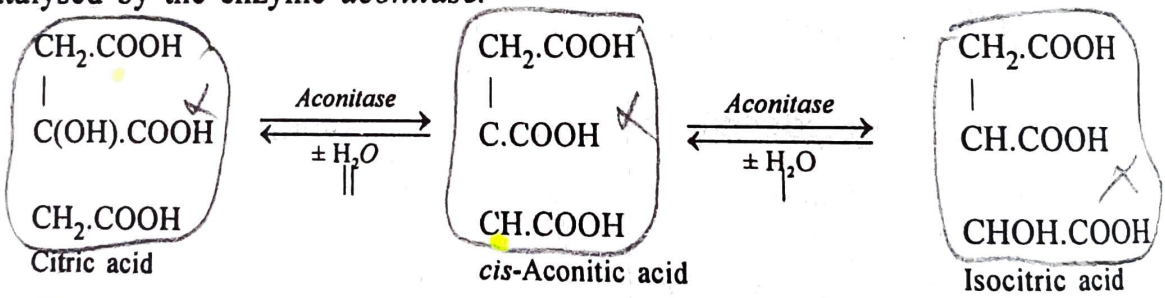
and the respiratory substrate enters inside the mitochondria for Krebs cycle in the form of pyruvic acid. Pyruvic acid is first converted into Acetyl CoA. Acetyl CoA reacts with a 4-carbon compound oxaloacetic acid with the use of one molecule of water and as a result a 6-carbon compound Citric acid is formed.



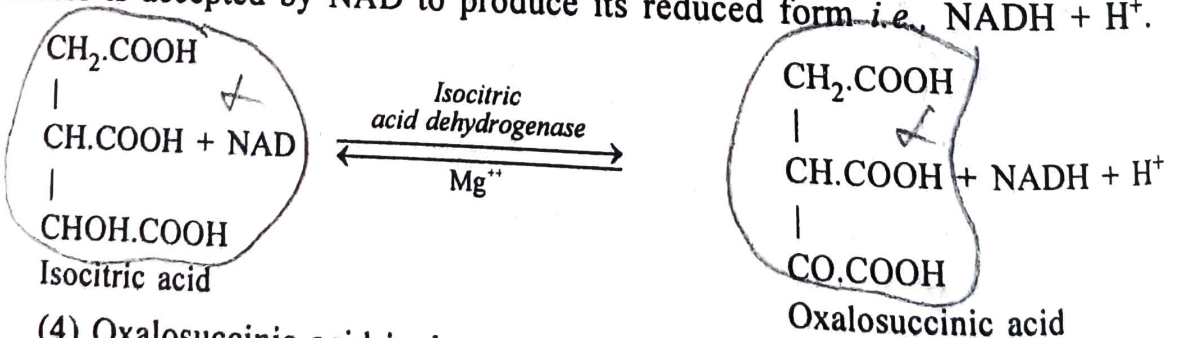
The CoA becomes once again available for the breakdown of Pyruvate to Acetyl CoA. At this stage CoA has a controlling influence on respiration. When intermediates of TCA cycle are used in other processes, the oxaloacetic acid is made available by an anaplerotic reaction catalysed by *PEP carboxylase* found in cytosol.

$$\text{Phosphoenol pyruvate} + HCO_3^- \longrightarrow \text{Oxaloacetate} + H_2PO_3^-$$

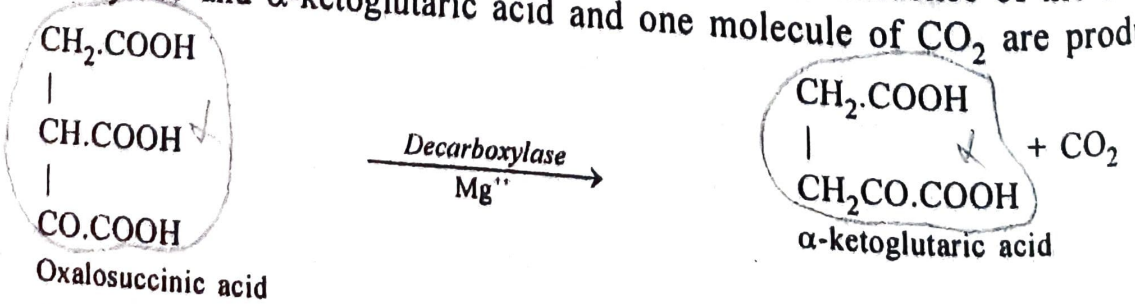
(2) In the next step Citric acid loses one molecule of water to form *Cis*–aconitic acid which acts as an intermediate in the isomerisation of Citric acid to Isocitric acid after taking back the molecule of water. Both the reactions of this step are catalysed by the enzyme *aconitase.*



(3) Now under the influence of *Isocitric acid dehydrogenase,* the dehydrogenation of Isocitric acid to Oxalosuccinic acid occurs and the hydrogen released in the process is accepted by NAD to produce its reduced form *i.e.,* NADH + H^+.



(4) Oxalosuccinic acid is decarboxylised under the influence of the enzyme *decarboxylase* and α-ketoglutaric acid and one molecule of CO_2 are produced



(5) The next step involves the oxidation of α-ketoglutaric acid which take place in two steps.

(*i*) *First step.* In the first step, α-ketoglutaric acid is converted into Succinyl CoA and the reaction is catalysed by the enzyme α-*ketoglutaric acid dehydrogenase* which requires TPP, Mg^{++}, NAD, FAD, lipoic acid and coenzyme A(CoA) as cofactors. The step is analogous to that of oxidation of pyruvic acid to Acetyl CoA. In this step one molecule of NAD is also reduced to $NADH_2$ and one molecule of CO_2 is liberated.

$CH_2.COOH$ | $CH_2CO.COOH$ (α-ketoglutaric acid) + HS.CoA + NAD^+ —(*α-ketoglutaric acid dehydrogenase*; Mg^{++}, TPP, lipoic acid, FAD)→ $CH_2.COOH$ | $CH_2.CO \simeq S.CoA$ (Succinyl CoA) + $NADH_2$ + CO_2

(*ii*) *Second step.* In the second step with the use of one molecule of H_2O Succinyl CoA is hydrolysed to Succinic acid regenerating CoA for further use in the cycle. A phosphorylating enzyme is involved in the formation of Succinic acid. Succinyl CoA loses CoA in a reaction in which Guanosine diphosphate (GDP) reacts with the inorganic phosphate to form Guanosine triphosphate (GTP) by a process known as substrate phosphorylation. This GTP can enzymatically react with ADP to produce ATP.

$CH_2.COOH$ | $CH_2.CO \simeq S.CoA$ (Succinyl CoA) + GDP + Pi ⇌ (*Succinyl thiokinase*) $CH_2.COOH$ | $CH_2.COOH$ (Succinic acid) + GTP + HS.CoA

$$GTP + ADP \rightleftharpoons GDP + ATP$$

(6) The Succinic acid is oxidised to Fumaric acid. The enzyme involved is *succinic acid dehydrogenase.* This is the only reaction of Krebs cycle which does not involve the utilisation of Co I or II (NAD or NADP) the place of which is taken by Flavin adenine dinucleotide (FAD) of the enzyme *succinic acid dehydrogenase.* FAD takes up the two hydrogen ions and the two electrons from Succinic acid and gets reduced.

$CH_2.COOH$ | $CH_2.COOH$ (Succinic acid) + FAD ⇌ (*Succinic acid dehydrogenase*) CH.COOH | CH.COOH (Fumaric acid) + $FADH_2$

Now with the addition of one molecule of water in the presence of the enzyme *fumarase,* Fumaric acid is converted into Malic acid.

CH.COOH | CH.COOH (Fumaric acid) + H_2O ⇌ (*Fumarase*) CHOH.COOH | CH_2COOH (Malic acid)

(7) In the last oxidation step under the influence of the enzyme *malic acid dehydrogenase,* Malic acid is converted back to Oxaloacetic acid from which the cycle started.

CHOH.COOH | $CH_2.COOH$ (Malic acid) + NAD ⇌ (*Malic acid dehydrogenase*) CO.COOH | $CH_2.COOH$ (Oxaloacetic acid) + $NADH_2$

Reactions of Krebs cycle are summarised in Fig.16.5 (dark lines).