Respiration:

Introduction:

We know that during photosynthesis, light energy is converted into chemical energy, and is stored in carbohydrate molecules, such as glucose and starch. Organisms make use of such energy for their activities by oxidising these high energy food molecules into simple low energy molecules, i.e., carbon dioxide and water. The reactions involved in process of oxidation are known as respiration. The compounds that are oxidised during process of respiration are called respiratory substrates.

Technically, Respiration is defined as follows:

This is a process by which living cells break down complex high energy food molecules into simple low energy molecules, i.e., CO_2 and H_2O, releasing the energy trapped within the chemical bonds. The energy released during oxidation of energy rich compounds is made available for activities of cells through an intermediate compound called adenosine triphosphate (ATP).

During process of respiration, the whole of energy contained in respiratory substrates is not released all at a time. It is released slowly in several steps of reactions controlled by different enzymes.

Respiration takes place in all types of living cells, and generally called cellular respiration. During the process of respiration oxygen is utilised, and CO_2 water and energy are released as products. The released energy is utilised in various energy-requiring activities of the organisms, and the carbon dioxide released during respiration is used for biosynthesis of other molecules in the cell.

As we know, important life processes, such as synthesis of proteins, fats and carbohydrates, require a certain expenditure of energy. Where does this energy come from, how is it stored, and how is it made available to the living cell, are some of the questions, which are to be answered by process of respiration.

The reaction that occurs in common respiration of glucose may be summed up as follows:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{enzymes}} 6CO_2 + 6H_2O + \text{Energy}$$

(2870 kJ or 686 kcal)

Here, 686 kcal or 2870 kJ of energy is liberated per molecule of glucose. One kcal is equal to 1000 calories. This means that one molecule of glucose on complete oxidation yields 686 kcal (kilocalories) of energy, (i.e., 686, 000 calories).

The main facts associated with respiration are:

a. Consumption of atmospheric oxygen.

b. Oxidation and decomposition of a portion of the stored food resulting in a loss of dry weight as seen in the seeds germinating in dark.

c. Liberation of carbon dioxide and a small quantity of water (the volume of CO_2 liberated is equal to volume of O_2 consumed).

d. Release of energy by breakdown of organic food, (such as carbohydrates).

Respiratory Substrates:

Respiratory substrates are those organic substances which are oxidised during respiration. They are high energy compounds and are called respiratory substrates. They may be carbohydrates, fats and proteins. Carbohydrates, such as glucose, fructose (hexoses), sucrose (disaccharide) or starch, inulin, hemicellulose (polysaccharide), etc., are main respiratory substrates.

The most common respiratory substrate is glucose. It is a hexose monosaccharide. Another related compound is fructose. Glucose is formed from storage carbohydrates like starch in most plants and glycogen in animals and fungi.

In rare circumstances, when carbohydrate reserves are exhausted, fats and proteins also serve as respiratory substrates.

Fats are used as respiratory substrates by a variety of organisms as they contain more energy than carbohydrates. Fats are used as respiratory substrates by a number of organisms because they contain more

energy as compared to carbohydrates. However, fats are not directly used in respiration. Instead they are first broken down to intermediates common to glucose oxidation, viz., acetyl CoA, glyceraldehyde phosphate. Proteins are used in respiration only rarely, as during germination of protein rich seeds and spores. Proteins are hydrolysed to form amino acids from which organic acids are produced through deamination. In animals, excess amino acids are regularly delaminated to produce organic acids. Organic acids enter Krebs Cycle, e.g., aspartic acid, glutamic acid.
At other times, proteins are employed as respiratory substrates under starvation conditions only when carbohydrates and fats become unavailable.
Respiration involving proteins as respiratory substrate is called protoplasmic respiration as compared to floating respiration which uses carbohydrates and fats. Protoplasmic respiration cannot be continued for long as it depletes protoplasm of structural and functional proteins as well as liberates toxic ammonia.

Respiratory Quotient:
Respiratory quotient (RQ) or respiratory ratio is defined as the ratio of the volume of CO_2 released to the volume of O_2 consumed in respiration per unit time.

$$RQ = \frac{\text{Volume of } CO_2 \text{ releasd}}{\text{Volume of } O_2 \text{ consumed}} \text{ per unit time.}$$

The measurement of RQ gives a clue about the type of food being oxidised by the cell at a given time.
The RQ value of carbohydrates is one, for proteins and lipids less than one, for organic acids more than one, for succulents it is zero and for anaerobic respiration it is infinite.

Photorespiration:
Krotkov (1963) introduced the term photorespiration. It is defined as the process of respiration taking place in green plant, only in the presence of light. During this process-CO_2 is evolved but no ATPs are produced. It is normally observed in plants like pea, sunflower, wheat, barley, etc., in which C_3-cycle operates. It is absent in tropical grasses (maize, sugarcane, etc.) and in some of the dicots like Amaranthus and Artiplex.
Site of Photorespiration:
It occurs only in green parts of the plant and involves three organelles viz.; chloroplast, mitochondria and peroxisomes.

Types of Respiration:
There are two main types of respiration:
(i) Aerobic, and
(ii) Anaerobic.
(i) Aerobic Respiration:
This type of respiration leads to a complete oxidation of stored food (organic substances) in the presence of oxygen, and releases carbon dioxide, water and a large amount of energy present in respiratory substrate. Such type of respiration is generally found in higher organisms.
This is a general term covering a vast series of chemical reactions of very complicated nature, by means of which the chemical energy of foods is converted into some easily available or ready to use form, usually adenosine triphosphate (ATP) in the presence of oxygen. The energy contained in the food is made available for work in many ways, but the most common method is via glycolysis and the Krebs cycle. Any food can be used for the production of energy, but glucose, the basic product of photosynthesis is used by most of the living cells for this purpose. Glucose is oxidised and broken in its components, i.e., CO_2 and water with the release of energy which is stored in the form of ATP.
The overall equation is:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow[\text{oxidation}]{\text{enzymes}} 6CO_2 + 6H_2O + \text{energy}$$
(2870 kJ) or (686 k cal)

The series of reaction taking place in the decomposition of glucose are divided in two phases. The Phase I, is known as glycolysis, and takes place in the cytosol of the cell. In this phase neither oxygen is used nor are the CO_2 and water produced. Therefore this step is common in anaerobic as well as aerobic respiration.
The second phase is known as Krebs cycle, in which oxygen is utilised, energy is produced with the formation of water and CO_2. This phase takes place in the mitochondria and not in cytosol.

(ii) Anaerobic respiration:
This type of respiration occurs in complete absence of oxygen. In the absence of free oxygen, many tissues of higher plants, seeds in storage, fleshy fruits, and succulent plants, such as cacti temporarily take to a kind of respiration, called anaerobic respiration. Anaerobic respiration, i.e., respiration without air is the only method of respiration in many kinds of fungi and bacteria. This type of anaerobic respiration is called as the fermentation. It differs from anaeorobic respiration in that the substrate is present outside the cell in a soluble form.
This results in incomplete oxidation of stored food and formation of carbon dioxide and ethyl alcohol, and sometimes also various organic acids, such as malic, citric, oxalic, tartaric, etc. Very little energy is released by this process to maintain activity of protoplasm.
The equation is as follows:
$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + \text{Energy}$$
(247 kJ) or (28 k cal)

Depending upon the nature of the end product, fermentation is of following types: (*details provided for understanding, however only two lines writing of each point is sufficient, reactions not required for this part i.e., the different types of fermentation)
1. Alcoholic Fermentation:
In the year 1897 Buchner showed that the yeast extract contained an enzyme complex which can catalyse the conversion of sugar into alcohol and CO_2. This extract was named as zymase. The process of alcoholic fermentation can be explained as follows:
The pyruvic acid produced during glycolysis is converted into acetaldehyde and CO_2 by the enzyme carboxylase which is believed to be one of the components of zymase complex. Acetaldehyde is reduced by another enzyme alcohol dehydrogenase, belonging to the same complex, into ethyl alcohol. The hydrogen atoms are donated by $NADH_2$ produced during glycolysis. It thus regenerates NAD for smooth operation of glycolysis.
The above steps can be represented in the form of following equations:

(i) $\text{Pyruvic acid} \xrightarrow{\text{Carboxylase}} \text{Acetaldehyde} + CO_2$

(ii) $\text{Acetaldehyde} + NADH_2 \xrightarrow{\text{Alcohol dehydrogenase}} \text{Ethyl alcohol} + NAD$

The overall reaction is, therefore :

$$\text{Pyruvic acid} \xrightarrow{\text{Zymase}} \text{Ethyl alcohol} + CO_2 + 2ATPs$$

Alcoholic fermentation may occur in almost any dilute sugar solution which is inoculated with yeast or is left exposed to air. It is of great economic importance and is applied on industrial scale for production of alcoholic beverages and in bakeries.
It is worth mentioning here that pure zymase is incapable of carrying out fermentation. It requires the presence of phosphate ions for proper activity. Secondly, starch cannot be fermented by yeast as it does not contain amylase.
2. Lactic Acid Fermentation:

Certain bacteria like *Lactobacillus lactici* convert the milk sugar (lactose) into lactic acid. It causes souring of the milk.

This fermentation is of two types:

(i) Homofermentive:

It results into production of only one product, i.e. lactic acid molecules. It takes place in muscles during oxygen stress.

$$\text{Pyruvic acid} + NADH_2 \xrightarrow{\text{Lactic acid dehydrogenase}} \text{Lactic acid}$$

(ii) Heterofermentive:

In addition to lactic acid, ethyl alcohol and CO_2 are also produced. It takes place in microbes. Thus it results in formation of more than one product.

$$\underset{\text{Pyruvic acid}}{2CH_3COCOOH} \longrightarrow \underset{\text{Lactic acid}}{C_3H_6O_3} + \underset{\text{Ethyl alcohol}}{C_2H_5OH} + CO_2$$

3. Butyric Add Fermentation:

It takes place in butter and produces butyric acid which causes its rancidity.

4. Acetic Acid Fermentation:

It is brought about by bacteria like *Acetobacter aceti* and *A. xylinum* which oxidise ethyl alcohol into acetic acid. This type of fermentation is commercially used in the production of vinegar.

Mechanism of Respiration:

There are two major phases of respiration:

(i) Glycolysis, and

(ii) Krebs cycle.

During process of respiration, carbohydrates are converted into pyruvic acid through a series of enzymatic reactions. This series of reactions is known as glycolysis which takes place in cytosol.

Now, pyruvic acid enters mitochondria, where several enzymes catalyse the reactions, and pyruvic acid finally converts into CO_2 and water. This series of enzymatic reactions is known as Krebs cycle (after name of its discoverer Sir Hans Adolf Krebs (1900-1981), awarded Nobel Prize in 1953), or tricarboxylic acid (TCA) or citric acid cycle.

Glycolysis: Glycolysis is a term used to describe the sequential series of reactions present in a wide variety of tissues that starts with a hexose sugar (usually glucose) and ends with pyruvic acid. This term has originated from Greek words, glycos = sugar and lysis = splitting.

The scheme of glycolysis was discovered by three German Scientists, Gustav Embden, Otto Meyerhof and J. Parnas, and therefore, referred as EMP pathway, after the abbreviation of their last names.

Glycolysis is the first stage in the breakdown of glucose and is common to all organisms. This means, glycolysis is common to both aerobic and anaerobic modes of respiration. In anaerobic organisms, this is only process in respiration. Glycolysis occurs in cytoplasm of cells. During this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid.

In plants, glucose is derived from sucrose, which is the end product of photosynthetic carbon reactions (also known as dark reactions) or from storage carbohydrates.

Sucrose is converted into glucose and fructose by the enzyme invertase. Now, these two monosaccharides (i.e., glucose and fructose) enter glycolysis or EMP pathway.

The main steps of glycolytic pathway are as follows:

Glycolysis is carried out in following different steps:

a. Phosphorylation of Sugar (i.e., First Phosphorylation):

Glucose and fructose are phosphorylated to give rise to glucose-6-phosphate and fructose-6-phosphate, respectively, by the activity of enzyme hexokinase, in presence of ATR The phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Isomerisation takes place with the help of enzyme phosphohexose isomerase.
Further steps of metabolism of glucose and fructose are quite similar.
Equations are as follows:

$$\text{Glucose (6C)} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

Now isomerisation occurs :

$$\text{Glucose-6-phosphate} \underset{\text{isomerase } Mg^{2+}}{\overset{\text{phosphohexose}}{\rightleftharpoons}} \text{Fructose-6-phosphate}$$

b. Phosphorylation of Fructose-6-Phosphate (i.e., Second Phosphorylation):
Now, fructose-6-phosphate is phosphorylated and fructose-1, 6-bisphosphate produced by the action of enzyme phosphofructokinase in presence of ATP.

$$\text{Fructose-6-phosphate} + \text{ATP} \xrightarrow[Mg^{2+}]{\text{phosphofructokinase}} \text{Fructose-1, 6-bisphosphate} + \text{ADP}$$

c. Splitting:
Now, fructose- 1, 6-bisphosphate splits into two molecules of triose phosphate, i.e., 3-phosphoglyceraldehyde (PGAL) and dihydroxyacetone phosphate (Di HAP), which are interconvertible.

$$\text{Fructose-1, 6-bisphosphate} \overset{\text{aldolase}}{\rightleftharpoons} \text{3-phosphoglyceraldehyde (PGAL)} + \text{dihydroxyacetone phosphate (Di HAP).}$$

d. Oxidative
Dehydrogenation:
After formation of 3-phosphoglycerldehyde (PGAL), the glycolytic pathway enters the energy conserving phase. Here, it is oxidized to a carboxylic acid, i.e., 1,3-bisphosphoglycerate, and NAD is reduced to NADH.

$$\text{3-phosphoglyceraldehyde} + \text{NAD} \underset{\text{dehydrogenase}}{\overset{\text{glyceraldehyde phosphate}}{\rightleftharpoons}} \text{1, 3-bisphosphoglycerate} + \text{NADH} + \text{H}$$

e. Formation of ATP:
In next step of glycolysis, 3-phosphoglycerate is formed from 1, 3-bisphosphoglycerate by enzymatic activity of phosphoglycerate kinase, and ATP is generated during this process. Direct synthesis of ATP from intermediate metabolites is called substrate level phosphorylsation.

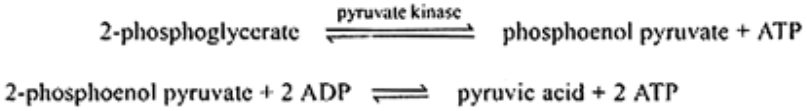
$$\text{1, 3-bisphosphoglycerate} + \text{ADP} \underset{\text{kinase} + Mg^{2+}}{\overset{\text{phosphoglycerate}}{\rightleftharpoons}} \text{3-phosphoglycerate} + \text{ATP}$$

This type of formation of ATP, where a phosphate group is directly transferred from a substrate to ADP to form ATP, is different from the ATP produced by ATP synthesis during oxidative phosphorylation in mitochondria or in chloroplasts (During photophosphorylation in photosynthesis).
f. Isomerisation:
In next step 3-phosphoglycerate converts into its isomer 2-phosphoglycerate by catalytic activity of enzyme phosphoglyceromutase.

$$\text{3-phosphoglycerate} \underset{\text{glyceromutase} + Mg^{2+}}{\overset{\text{phospho-}}{\rightleftharpoons}} \text{2-phosphoglycerate}$$

g. Dehydration:

In subsequent step 2-phosphoglycerate converts into phosphoenol pyruvate (PEP) in the presence of enzyme pyruvate kinase and liberates ATP.



Glycolysis pathway or EMP pathway:

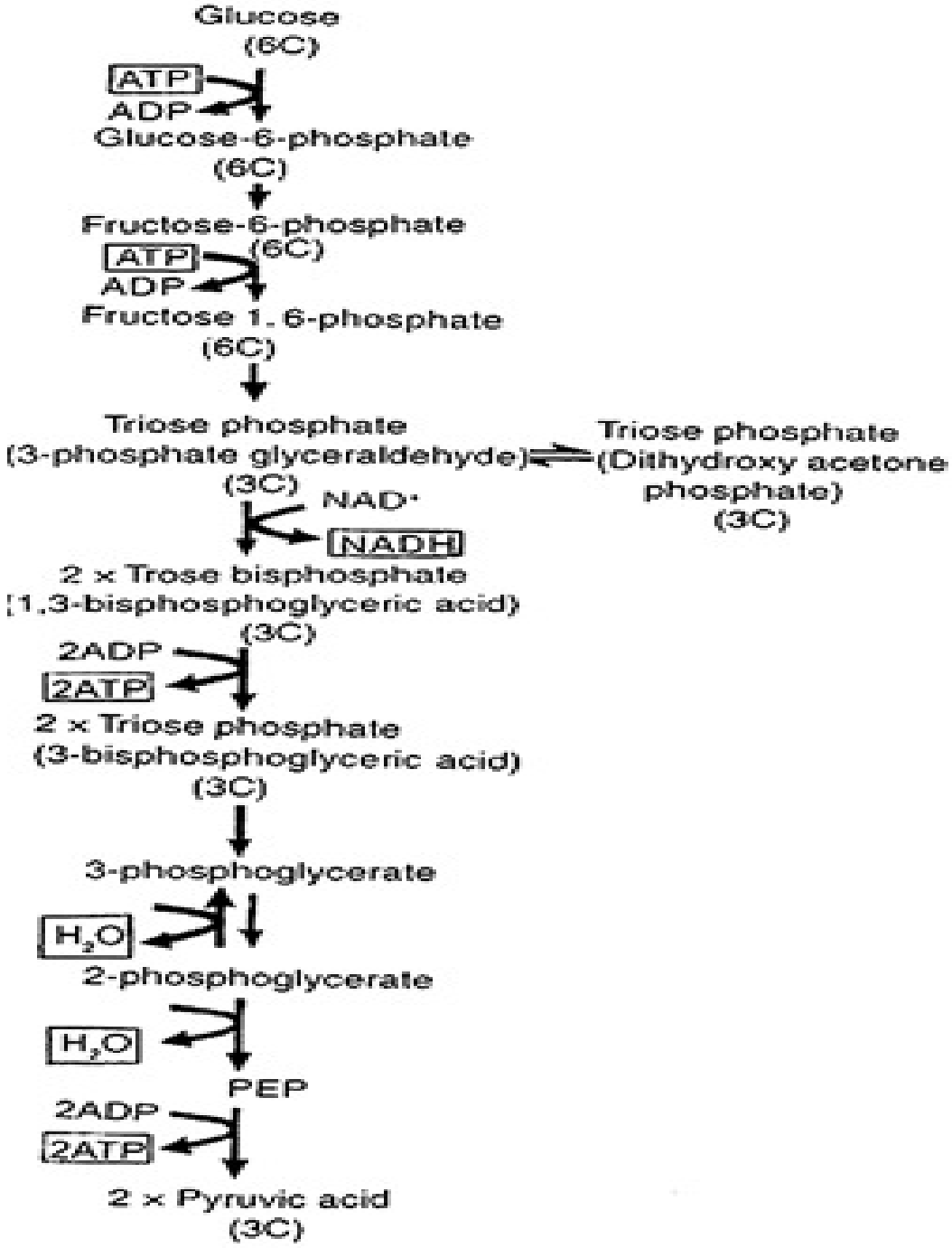


Fig. 4.2. Main steps of glycolysis or EMP pathway.

h. Generation and Utilisation of ATP during Glycolysis:
During glycolytic pathway, the molecules of ATP are produced as follows:
(i) Direct transfer of phosphate to ATP.
(ii) Oxidation of NADH prod uced during glycolytic pathway to NAD^+.
In the end of glycolysis net gain of ATP:
Number of ATP molecules consumed:
(i) Glucose → Glucose-6-phosphate = 1 ATP
(ii) Fructose-6-P → Fructose-1, 6-diphosphate = 1 ATP]
Total number of ATP utilized=21+1 = 2 ATPs
Number of ATP Molecules Produced:
Due to substrate level phosphorylation:
(i) 1, 3-diPGA → 3-PGA =1 ATP × 2 = 2 ATPs
(ii) PEPA → Pyruvic acid = 1 ATP × 2 = 2 ATPs
(iii) Due to oxidation of $NADH_2$ (Produced in the conversion of 3-PGA into 1, 3-diPGA) in Electron transport system, i.e., Oxidative phosphorylation) = 3 ATP × 2 = 6 ATPs
Total number of ATPs produced = 10 ATPs
Net Gain of ATPs at ETS level (only under aerobic conditions) = (10 ATPs – 2 ATPs) = 8 ATPs

Oxidative Decarboxylation of Pyruvic Acid:
(Aerobic Oxidation of Pyruvic Acid)
Now, pyruvic acid generated in cytoplasm through glycolysis is transferred to mitochondria. This is initiation of second phase of respiration. As soon as, pyruvic acid enters the mitochondria, one of the three carbon atoms of pyruvic acid is oxidised to carbon dioxide in a reaction called oxidative decarboxylation.
Here, pyruvate is first decarboxylated, and thereafter oxidised by enzyme pyruvate dehydrogenase. (This enzyme is made up of a decarboxylase, lipoic acid, TPP, transacetylase and Mg^{+2}.)
Acetyl Co-A acts as substrate entrant for Krebs cycle. This enzyme complex includes three enzymes (viz. Pyruvic acid decarboxylase, lipoyl transacetylase and lipoyl dehydrogenase) and six co-factors (viz., coenzyme 'A', thiamine pyrophosphate, NAD, Lipoic acid, FAD and Mg^{++}-ion).)
The acetyl CoA, thus produced, now enters the Krebs cycle where it acts as the substrate for the cycle. It acts as the connecting link between glycolysis and Krebs cycle. It is also known as the active acetate. It was discovered by Lohman.
The equation is as follows:

$$\text{Pyruvate} + \text{NAD+} + \text{Co-A} \xrightarrow[\text{dehydrogenase}]{\text{pyruvate}} \text{Acetyl Co-A} + \text{NADH} + H^- + CO_2^-$$

Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Acetyl Co-A can enter into mitochondria while pyruvate cannot.

Krebs Cycle:
Sir Hans Adolf Krebs, discovered role of pyruvate in conversion of glucose hydrogens into fumarate. He discovered, in 1937, tricarboxylic acid cycle (i.e., TCA cycle), also known as Citric acid cycle or Krebs cycle. Citric acid cycle occurs in matrix of mitochondria. This cycle involves two decarboxylations and four dehydrogenations.
Various steps of these reactions are as follows:
The starting point of Krebs cycle is entrance of acetyl Co-A into a reaction to form citric acid. Krebs elucidated this cycle, and explained how pyruvate is broken down to $CO_2$and H_2O with an uptake of oxygen and release of energy, which is stored as ATP. For this pioneer work Krebs was awarded Nobel Prize in 1953.

In the first reaction of Krebs cycle, one molecule of acetyl Co-A combines with 4-carbon oxaloacetic acid (OAA); with the result 6-carbon citric acid is produced, and Co-A is released. This reaction is catalysed by enzyme citrate synthase.

$$\underset{(4C)}{\text{Acetyl Co-A + oxaloacetic acid}} \xrightarrow[\text{enzyme}]{\text{citrate synthase}} \underset{(6C)}{\text{Citric acid + Co-A}}$$

Now, citrate (citric acid) is isomerised to isocitrate (isocitric acid).

$$\text{Citric acid} \xrightarrow[\text{enzyme}]{\text{aconitase}} \text{Cis-aconitic acid} + H_2O$$

$$\text{Cis-aconitic acid} + H_2O \xrightarrow[\text{enzyme}]{\text{aconitase}} \underset{\text{(Isocitrate)}}{\text{Isocitric acid}}$$

Cis-aconitic acid is converted into isocitric acid with the addition of water in the presence of iron containing enzyme aconitase.

isocitric acid then follows through a number of steps which are as follows which ultimately leads to formation of CO_2 and water and other products through which the cycles continues.

$$\underset{\text{(Isocitrate)}}{\text{Isocitric acid + NAD}^+} \xrightarrow[\text{dehydrogenase}]{\text{isocitrate}} \underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid + NADH + H}^+}$$

$$\underset{\text{(oxalosuccinate)}}{\text{Oxalosuccinic acid}} \xrightarrow[\text{decarboxylase}]{\text{oxalosuccinic}} \text{α-Ketoglutaric acid} + CO_2$$

$$\text{α-ketoglutaric acid + Co-A + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{α- ketoglutaric}} \text{Succinyl Co-A + NADH + H}^+ + CO_2$$

$$\text{Succinyl Co-A} + H_2O + \text{GDP} + \text{iP} \xrightarrow[\text{thiokinase}]{\text{succinic}} \text{Succinic acid + Co-A + GTP}$$

$$\text{GTP + ADP} \longrightarrow \text{GDP + ATP (substrate phosphorylation)}$$

$$\text{Succinic acid + FAD} \xrightarrow[\text{dehydrogenase}]{\text{Succinic}} \text{Fumaric acid} + FADH_2$$

$$\text{Fumaric acid} + H_2O \xrightarrow[\text{enzyme}]{\text{Fumarase}} \text{Malic acid}$$

$$\text{Malic acid + NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{Malic}} \text{Oxaloacetic acid (OAA) + NADH + H}^+$$

During citric acid cycle (Krebs cycle) 3 molecules of NAD^+ and one molecule of FAD (Flavin Adenine Dinucleotide) are reduced to produce NADH and $FADH_2$, respectively.
During citric acid cycle NADH and FADH, are produced. Now, they are linked with electron transport system (ETS) and produce ATP by oxidative phosphorylation.

This may be summarised in following equation:

$$\text{Pyruvic acid} + 4NAD^+ + FAD + 2H_2O + ADP + iP$$
$$\downarrow \text{ mitochondrial matrix}$$
$$3CO_2 + 4NADH + 4H^+ + FADH_2 + ATP$$

In the end of Krebs cycle, glucose molecule is completely oxidised. From one glucose molecule, two pyruvic acid molecules are formed. After oxidation of one pyruvic acid molecule, three CO_2 molecules are released. Thus, in all 6 molecules of CO_2 are released.

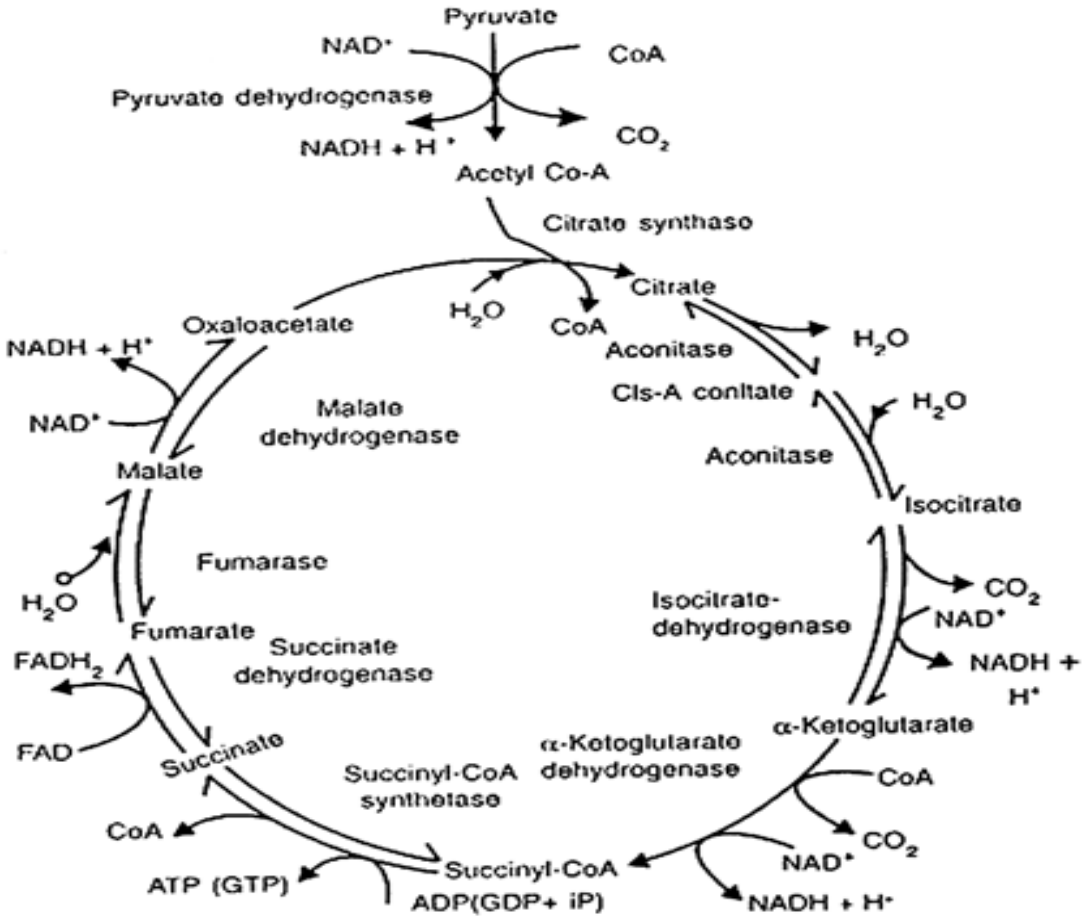


Fig. 4.3. Krebs cycle or citric acid cycle.

Electron Transport System (ETS):

By the end of Krebs cycle, glucose molecule oxidises completely, but the energy does not release till NADH and $FADH_2$ oxidise through electron transport system (ETS). The metabolic pathway through which electron passes from one carrier to another, is called electron transport system (ETS). The electron transport system is also known as electron transport chain or mitochondrial respiratory chain.

The electron transport system consists of a series of coenzymes and cytochromes that take part in passage of electrons from a chemical to its ultimate acceptor. The passage of electrons from one-enzyme or cytochrome to the next takes place with a loss of energy at each step. Electron transport system is operative in the inner mitochondrial membrane.

The electron carriers include flavins, iron sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins.

Electron transport system in mitochondria consists of four complexes which are found in bases of stalked particles in the inner mitochondrial membrane, and also ubiquinone (UQ) or coenzyme Q and cytochrome c which are not bound to stalked particles but act as mobile electron carriers between the complexes.

Complex-I:

Consists of NADH-dehydrogenase or NADH-Q reductase which contains a flavoprotein FMN (flavin mononucleotide) and is associated with iron-sulphur (Fe-S) proteins. This complex is responsible for passing electrons (also protons) from mitochondrial NADH to ubiquinone (UQ), located within inner mitochondrial membrane.

$$NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$$

$$FMNH_2 + 2Fe^{3+}\,S \longrightarrow FMN + 2Fe^{2+}\,S + 2H^+$$

$$2Fe^{2+}\,S + Q + 2H^+ \longrightarrow 2Fe^{3+}\,S + QH_2$$

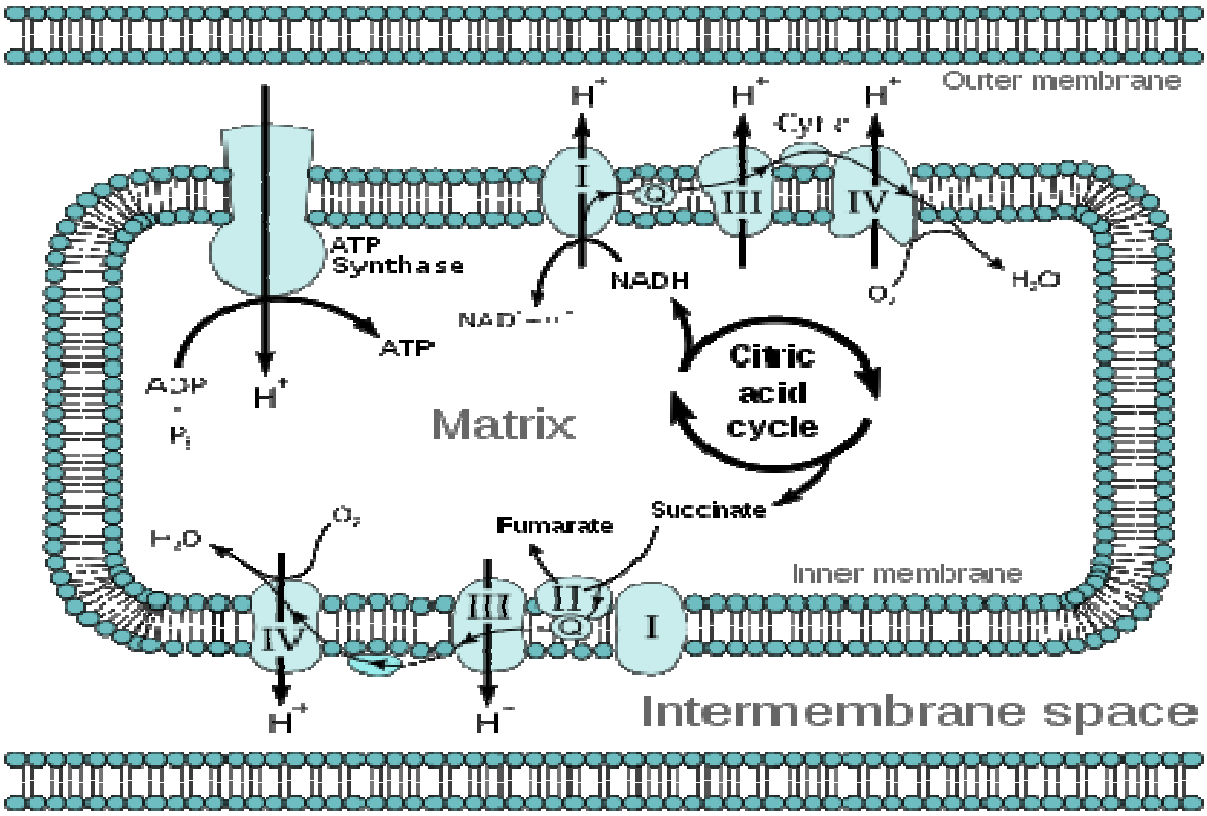
Complex-II:

Consists of succinate dehydrogenase which contains a flavoprotein FAD (flavin adenine dinucleotide) in its prosthetic group and is associated with non heme iron-sulphur (Fe S) proteins.

This complex receives electrons (also protons) from succinic acid (which is oxidised in Krebs cycle to form fumaric acid) and passes them to ubiquinone (UQ). Ubiquinone also receives reducing equivalents via $FADH_2$ that is generated during oxidation of succinate, through the activity of energy succinate dehydrogenase, in Krebs cycle.

$$FADH_2 + 2Fe^{3+}\ S \longrightarrow 2Fe^{2} + S + 2H^{+} + FAD$$

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$



Complex-III:

Consists of ubiquinol, cytochrome c and cytochrome bc_1 .The reduced ubiquinone is called ubiquinol. Here ubiquinol is oxidised with the transfer of electrons to cytochrome c via cytochrome bc_1. Cytochrome c is a small protein attached to outer surface of the inner mitochondrial membrane and acts as a mobile carrier for transfer of electrons between complex III and complex IV.

This complex is called QH_2-cytochrome c reductase complex. This bears three components, i.e., cytochrome b, non-heme iron sulphur (Fe – S), and cytochrome c_1. Coenzyme Q is also involved between Fe-S and cytochrome c_1.

The equations are as follows:

$$QH_2 + 2Fe^{3+}\ cy\ b \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ b$$

$$2Fe^{2+}\ cy\ b + 2Fe^{3+}\ S \longrightarrow 2Fe^{3+}\ cy\ b + 2Fe^{2+}\ S$$

$$2Fe^{2+}\ S + Q + 2H^{+} \longrightarrow 2Fe^{3+}\ S + QH_2$$

$$QH_2 + 2Fe^{3+}\ cy\ c_1 \longrightarrow Q + 2H^{+} + 2Fe^{2+}\ cy\ c_1$$

Now, cytochrome c, transfers electrons to cy c. Like coenzyme Q, cy c is also mobile carrier of electrons.

Complex-IV:
Is known as cytochrome c oxidase complex. This contains cytochromes a and a_3, along with two copper centres. This complex receives electrons from cytochrome c and passes them to 1/2 O. Two protons are needed and Hp molecule is formed (terminal oxidation). Here, O_2 is ultimate acceptor of electrons. It combines with protons to form metabolic water or respiratory water.

$$2\ \underset{(Fe^{++})}{\text{Ferrocytochrome}} + 2H^+ + 1/2\ O_2 \rightleftharpoons 2\ \underset{(Fe^{+++})}{\text{ferricytochrome } c} + H_2O$$

In short: Working of ETS (Electron Transport System) in Respiration of Plants:
1. The pairs of hydrogen atoms removed from the substrates in various oxidation steps of glycolysis and Krebs cycle are accepted by NAD or FAD. It results into formation of $NADH_2$ or $FADH_2$.
2. $NADH_2$ is reoxidised by NADH-dehydrogenase (complex I). The hydrogen atoms are accepted by its coenzyme FAD which gets reduced to $FADH_2$. The energy released during oxidation of $NADH_2$ is utilised for joining ADP and inorganic phosphate to synthesize one molecule of ATP.
3. $FADH_2$ passes its pair of hydrogen atoms to coenzyme Q which gets reduced into coenzyme QH_2 and $FADH_2$ gets reoxidised to FAD (complex II).
4. The oxidation of $CoQH_2$ is brought about by the transfer of electrons ($2e^-$) to the ferric iron (Fe^{+++}) of the cytochromes while the protons ($2H^+$) are expelled out in the perimitochondrial space (complex III).
5. From CoQ, the electrons are accepted first of all by the Fe of cytochrome 'b' from where they pass sequentially through cytochrome 'C_1' 'c' 'a' and 'a_3', causing them to be reduced and oxidised alternately. Cytochromes are conjugated proteins having haem prosthetic group. The haem group facilitates the passage of electrons by means of its Fe^{+3} atoms which becomes reversibly reduced and oxidised by accepting and donating electron according to the scheme- $Fe^{+3} \xrightarrow{+e^-} Fe^{+2} \xrightarrow{-e^-} Fe^{+3}$ (complex Iv)
6. During transfer of electrons from cytochrome 'b' to cytochrome 'c' and from cytochrome 'a' to cytochrome 'a_3', one molecule each of ATP is generated by phosphorylation of ATP.
7. The electrons are finally passed on to one atom of oxygen which results into its activation. The activated oxygen reacts with the $2H^+$ ions (made available from perimitochondrial space) to form water.
In this way oxidation of $NADH_2$ via ETS provides three molecules of ATP. But $FADH_2$ on oxidation by ETS, yields only two ATP molecules.

Complex-V: (site for Oxidative Phosphorylation:)
When electrons are transferred from one carrier to next carrier via complexes 1 to IV in electron transport system (ETS), they are coupled to ATP synthase enzyme complex for production of ATP from ADP and inorganic phosphate (iP).
Here, number of ATP molecules synthesised during ETS, depends on nature of electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, and one molecule of $FADH_2$ gives rise to 2 molecules of ATP. ATP synthase complex is called complex V.
During transportation of electrons, hydrogen atoms split into protons and electrons. The electrons are carried by cytochromes. Before last stage, where hydrogen atom is accepted by oxygen to form water, the electrons again recombine with their protons. Oxygen acts as final hydrogen acceptor.

The whole process, where oxygen effectively allows the production of ATP by phosphorylation of ADP, is called oxidative phosphorylation. In other words, synthesis of ATP is called phosphorylation, and as it takes place in presence of oxygen, it is called oxidative phosphorylation.

$$2H \rightleftharpoons 2H^+ + 2e^-$$

$$NAD^+ + H^+ + H^+ + e^- + e^- \longrightarrow NADH + H^+$$

The enzyme required for synthesis of ATP, is called ATP synthase. This is located in F_1, or head piece of F_0 – F_1 or elementary particles. ATP synthase enzyme becomes active in ATP formation, where there is a proton gradient saving higher concentration of H_2.
ATP synthase, also known as complex V consists of two major components, i.e., F_1, and F_0. The F_1 headpiece is a peripheral membrane protein complex and contains the site for ATP from ADP and inorganic phosphate (iP).

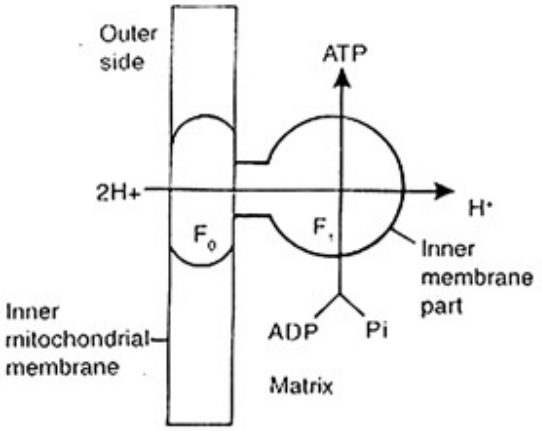


Fig. 4.5. Synthesis of ATP by inner membrane particles of mitochondrion.

Whereas, F_0 is an integral membrane mitochondrial-protein complex which forms the channel through which protons cross the inner membrane. The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP.
Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules whereas a similar oxidation of $FADH_2$ produces 2 ATP molecules.

Net gain of ATP:
Each NADH + H^+ produces 3 ATP molecules, while $FADH_2$ forms only 2 ATP molecules at the end of reaction.

$$NADH + H^+ + 1/2\ O_2 + 3\ ADP + 3\ iP \longrightarrow NAD + 3\ ATP + H_2O$$

$$FADH_2 + 1/2\ O_2 + 2\ ADP + 2\ iP \longrightarrow FAD + 2\ ATP + H_2O$$

Energetics of Krebs cycle:

Step	Reducing Equivalent	No. of ATPs Produced
1. Pyruvic acid → Acetyl coA	$NADH_2$	2 × 3 = 6
2. Isocitric acid → Oxalosuccinic acid	$NADH_2$	2 × 3 = 6
3. α-Ketoglutaric acid → Succinyl CoA	$NADH_2$	2 × 3 = 6
4. Succinic acid → Fumaric acid	$FADH_2$	2 × 2 = 4
5. Malic acid → Oxaloacetic acid	$NADH_2$	2 × 3 = 6
6. Succinyl CoA → Succinic acid	substrate level phosphorylation (Via GTP)	2 × 1 = 2
Total number of ATP from 2 molecules of pyruvic acid	→	30 ATPs

Thus, total gain of ATP in aerobic respiration is as follows:

Glycolysis ⟶ 8 ATP

Pyruvic acid ⟶ Acetyl Co-A ⟶ 6 ATP

Krebs cycle ⟶ 24 ATP

Total ⟶ 38 ATP

$$C_6H_{12}O_6 + 6O_2 + 8H_2O + 38\ (ADP + H_3PO_4) \longrightarrow 6CO_2 + 14H_2O + 38\ (ATP + H_2O).$$

Complete oxidation of glucose to CO_2 and water shows that there is a net gain of 38 ATP.
However, in most eukaryotic cells, 2 molecules of ATP are required for transport of NADH produced in glycolysis into mitochondrion for further oxidation, and therefore, net gain of ATP is 36 molecules.

Significance of Krebs Cycle:

a. During Krebs cycle, carbon skeletons are obtained for use in growth and maintenance of the cell.
b. Many intermediate compounds are formed which are used in synthesis of other biomolecules, such as amino acids, nucleotides, chlorophyll, cytochromes and fats.
c. During this pathway amino acids are synthesised from α-ketoglutaric acid, pyruvic acid and oxaloacetic acid.
d. Here succinyl Co-A acts as starting molecule for synthesis of chlorophyll.
e. Krebs cycle is major pathway for generation of ATP molecules, which make energy currency of the cell.
f. Energy is released from glucose, and is used in various biochemical reactions.
g. Phenol, anthocyanin, etc., are produced from acetyl Co-A, whereas fatty acids are formed from glycerol.
h. Glutamic acid is formed from α-ketoglutaric acid; aspartic acid from oxaloacetic acid, and alanine from aspartic acid.
i. Amino acids are used in synthesis of proteins, nucleic acids, purines and pyrimidines.
j. Succinyl Co-A carries synthesis of pyrrole compounds of chlorophyll, cytochrome and phytochrome.
k. Krebs cycle is directly related to nitrogen metabolism, α-ketoglutaric acid, an intermediate of Krebs cycle is first acceptor molecule of NH_3 forming an amino acid, the glutamic acid. From glutamic acid various transamination reactions begin to form different amino acids which ultimately condense to form proteins.
l. Krebs cycle is also intimately related with fat metabolism. Dihydroxyacetone phosphate produced in glycolysis may be converted into glycerol via glycerol-3-phosphate and vice versa. After β-oxidation, fatty acids give rise to active 2-C units, the acetyl Co-A which enters the Krebs cycle.

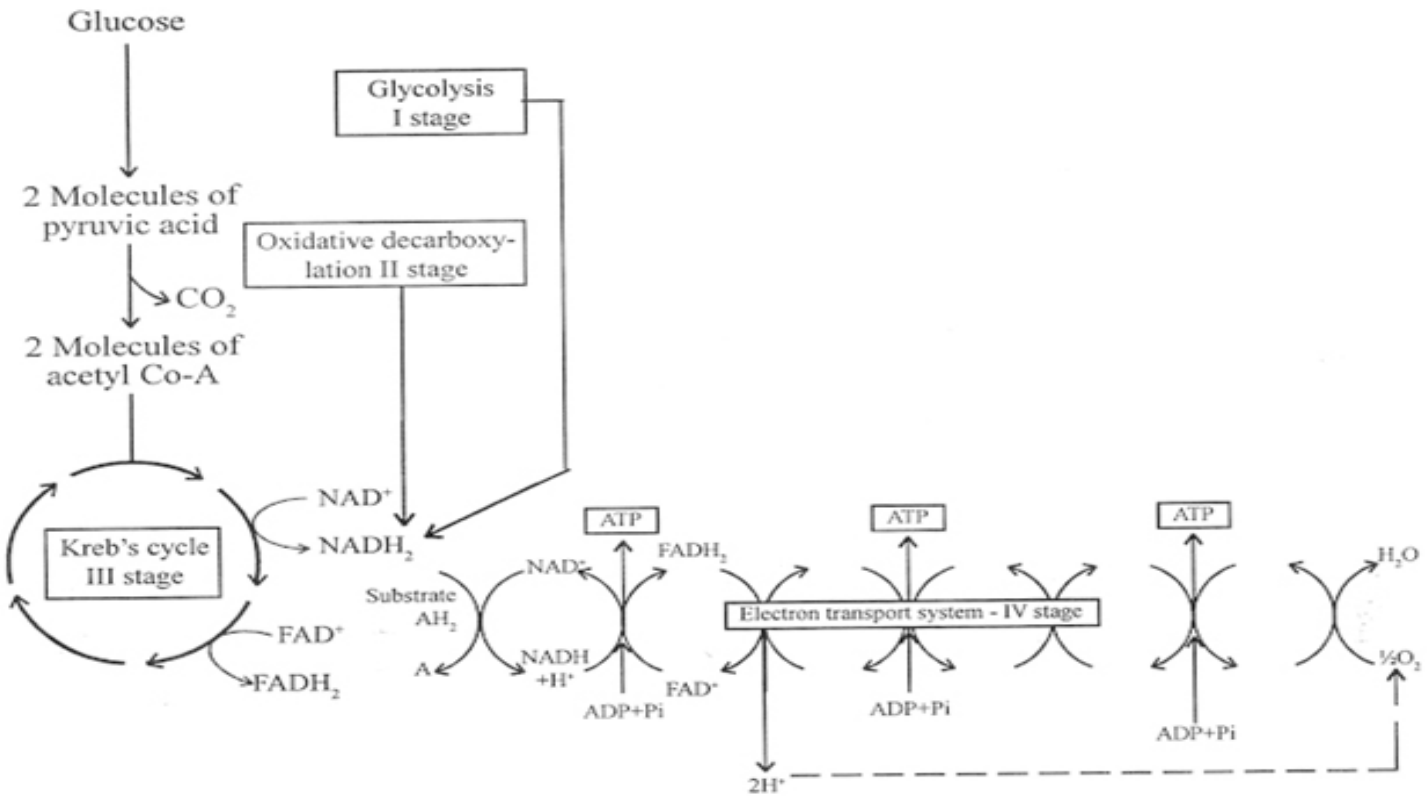
Percent Efficiency of Respiration:

If a molecule of glucose is completely oxidised in air it produces 686,000 calories, while as a result of complete oxidation of glucose molecule inside an aerobically respiring cell, 38 molecules of ATP are produced.

For synthesis of one ATP from ADP and Pi, 7300 calories are required therefore the amount of energy which can be conserved by phosphorylation can be calculated as follows:

$$\frac{38 \times 7300 \text{ calories}}{686000 \text{ calories}} \times 100 = 40.44\%$$

ENTIRE RESPIRATORY PROCESS IN PLANTS INCLUDES :



SITE OF DIFFERENT PROCESSES OF RESPIRATION OCCURING ARE AS FOLLOWS :