

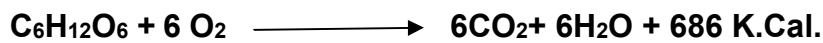
## RESPIRATION

### 15.1 INTRODUCTION

The word respiration is derived from the Latin word '*respirare*' (literally, to breathe). Aerobic (oxygen requiring) respiration is common to nearly all eukaryotic organisms. It is a biological process by which reduced organic compounds are mobilized and subsequently oxidized in a controlled manner. During respiration, free energy is released and transiently stored in a compound, ATP, which can be readily utilized for the maintenance and development of plants.

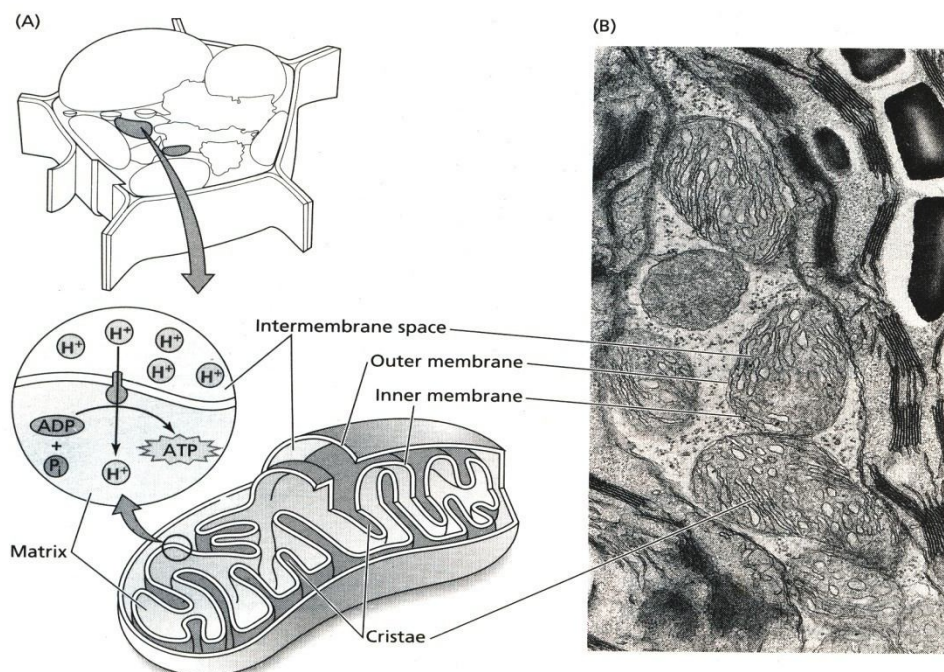
Respiration can be defined as '**an oxidation-reduction process**' in which respirable substrates such as Carbohydrates, proteins, fats etc. are oxidized to CO<sub>2</sub> and O<sub>2</sub> absorbed is reduced to water, with the release of chemical energy stored in the bonds of the complex molecules (ATP).

The overall respiration process may be represented as



### Mitochondria and respiration:

Various enzymes of oxidation of pyruvic acid (TCA Cycle) and electron transport chain (which is a series of oxidation-reduction reactions) are located in matrix of Mitochondria. The mitochondrion is made of double unit membrane. The inner layer of this membrane is deeply folded inward to form the cristae (transverse membranes) that lie more or less cross wise in the mitochondrion. The membranes appear to have small knob like structures about 70 Å in diameter called F<sub>1</sub> – ATP ase attached to their inner surface by stalks about 30 Å long called F<sub>o</sub> particles. These structures are concerned with the synthesis of ATP, the cells energy mobilization compound.



\*Figure. is from reference 6

## **15.2 SIGNIFICANCE OF RESPIRATION:**

Respiration is an important process because

1. It releases energy which is consumed in various metabolic processes essential for plant life and activates cell division.
2. It brings about the formation of other necessary compounds participating as important cell constituents.
3. It converts insoluble food into soluble form.
4. It liberates CO<sub>2</sub> and plays a part actively in maintaining the balance of carbon cycle in nature.
5. It converts stored energy (potential energy) into usable form (kinetic energy).

**Respiration is complex process which includes:**

- i. Absorption of oxygen.
- ii. Conversion of carbohydrate (complex) to CO<sub>2</sub> and water (simpler substances) i.e., oxidation of food.
- iii. Release of energy.
- iv. Formation of intermediate products playing different roles in metabolism
- v. Loss of weight in plants as a result of oxidation.

## **15.3 Out line of Respiratory Metabolism:**

The breakdown of a respiratory substrate in respiration, proceed through a series of reactions each catalyzed by a specific enzyme. The sequence of reactions is generally referred to as respiratory metabolism. Which Includes the following steps.

### **STEP I : Glycolysis:**

This step operates in the cytoplasm and is common to both aerobic and anaerobic respiration. Pyruvic acid (CH<sub>3</sub>COCOOH) – a key respiratory metabolite – is formed from **oxidation of glucose** (starting point for respiratory metabolism in plants) in a series of reactions collectively called as Glycolysis.

### **Oxidative Pentose Phosphate Pathway**

It is another sequence of reactions where in glucose molecule after its phosphorylation as Glucose – 6 – Phosphate is directly oxidized to phosphogluconic acid and then to ribulose – 5 – phosphate. Electrons released in between these two steps reduce NADP to NADPH<sub>2</sub>, each molecule of which after passing through ETS system produces 3 molecules of ATP like in glycolysis. This is another way of breakdown of glucose and production of energy.

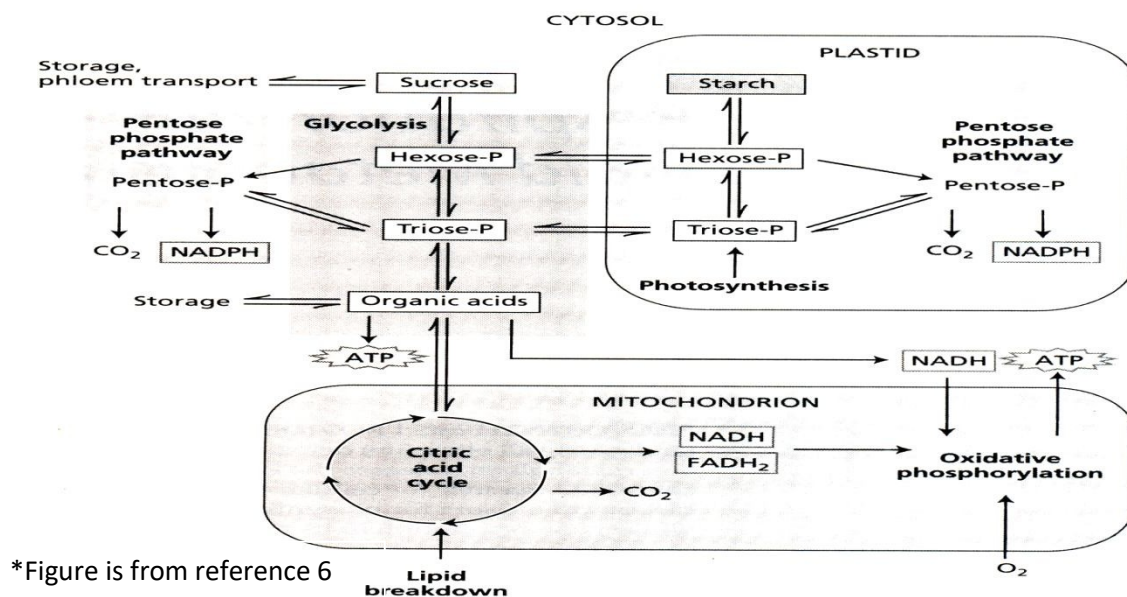
### **STEP-II: Tricarboxylic Acid Cycle (TCA cycle/Krebs Cycle/ Citric Acid Cycle)**

Pyruvic acid produced by glycolysis is oxidized in a sequence of reactions referred to as tricarboxylic acid cycle. Several intermediates of the tri carboxylic acid cycle under go decarboxylations so that CO<sub>2</sub> is produced. Other

intermediates are oxidized through the agency of the co – enzyme  $\text{NAD}^+$  and hence  $\text{NADH}$  (reducing power) is generated in the process.

### STEP III: Respiratory chain / Electron Transport Chain

$\text{NADH}$  is oxidized via, the respiratory chain, so that  $\text{NAD}^+$  is regenerated. Electron Transport Chain transfers electrons from  $\text{NADH}$  – produced during glycolysis, the pentose phosphate pathway and the TCA cycle – to Oxygen.  **$\text{O}_2$  is the final electron acceptor** in the respiratory chain and is reduced to water. This electron transfer releases a large amount of free energy much of which is conserved through the synthesis of ATP from ADP and  $\text{P}_i$  catalyzed by the enzyme ATP Synthase. Collectively the redox reactions of the electron transport chain and the synthesis of ATP are called **Oxidative Phosphorylation**. (synthesis of ATP in light reaction of photosynthesis is called as photophosphorylation).

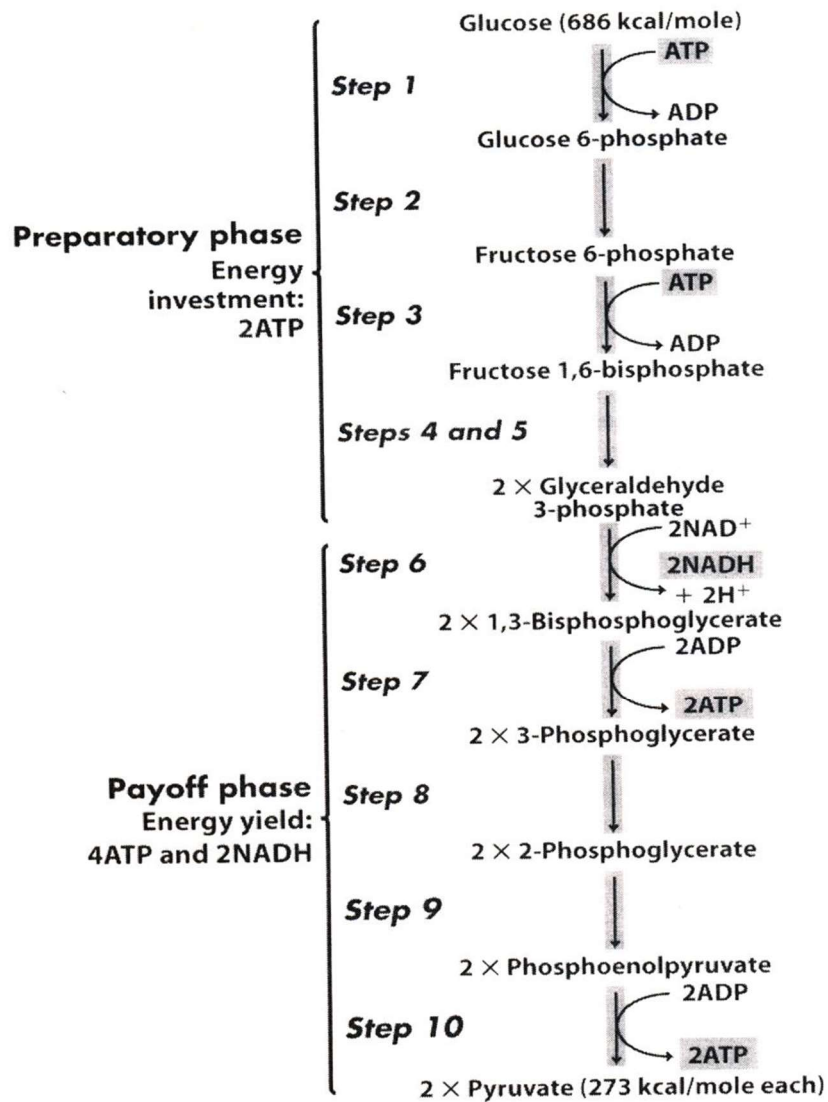


\*Figure is from reference 6

### 15.4 GLYCOLYSIS:

The reactions of glycolysis process may be divided into two phases: **Preparatory phase** and **Oxidative phase**. Preparatory phase consists of 4 steps and oxidative phase of 5 steps. During preparatory phase glucose is converted into fructose – 1,6 diphosphate and during oxidative phase fructose 1,6 diphosphate split into two three carbon compounds which are converted to **pyruvic acid**.

# Glycolysis



## REACTIONS OF GLYCOLYSIS

### Preparatory phase

In this step glucose is phosphorylated with ATP in the presence of the enzyme hexokinase to produce glucose-6-phosphate and ADP. Then glucose 6-phosphate is converted to its isomer fructose-6-phosphate in the presence of phosphoglucose isomerase enzyme. Fructose 6-phosphate in the presence of ATP molecule and the enzyme phosphohexokinase forms fructose 1,6-diphosphate and ADP.

### Oxidative phase

In this step splitting of fructose 1,6-diphosphate into two three-carbon compounds – 3-phosphoglyceraldehyde and dihydroxyacetone phosphate in the presence of enzyme **aldolase** occurs. These two sugars are interconverted through the enzyme phosphotriose isomerase.

If 3 – Phosphoglycerldehyde is depleted, additional amounts will be formed by the isomerization of dihydroxyacetone phosphate, continuing with glycolysis, 3-phosphoglycerldehyde is converted to 1,3 – *diphosphoglycerate*. This reaction involves the incorporation or addition of inorganic phosphate to first carbon of 3 phosphoglycerldehyde and the reduction of NAD<sup>+</sup> to NADH and is catalyzed by the enzyme *phosphoglycerldehyde dehydrogenase*.

The consumption of incorgnic phosphate in the oxidation of 3 – phosphoglycerldehyde is important to the plant because this phosphate is involved in the synthesis of ATP in the next reaction of the glycolytic sequence. In the presence of ADP and the enzyme *phosphoglycero kinase*, 1,3-diphosphoglycerate is converted to 3 - *phospho-glycerate* and ATP. The process of ATP formation by the transfer of phosphate from one of the intermediates of the pathway (in this case; 1,3 – diphosphoglycerate ) to ADP is termed as **substrate level phosphorylation** . This process represents the major way in which ATP is generated from bond energy under anaerobic conditions and is particularly important to fermentation.

The 3 – Phosphoglycerate that is formed in the above reaction is transformed to 2 – *phosphoglycerate* by the activity of the enzyme *Phosphoglyceromutase*. The elimination of the elements of water (dehydration) from 2 – Phosphoglycerate in the presence of enolase results in the formation of *phosphoenolpyruvate*. In the presence of ADP and pyruvate kinase, phosphoenolpyruvate is transferred to ADP to form ATP, another example of substrate level Phosphorylation,

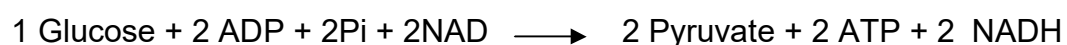
#### 5.4.1 Significance of Glycolysis:

The EMP pathway( or glycolysis) , which is also referred to as the *hexose diphosphate pathway*, is the chief pathway in which glucose or intermediates are converted to pyruvate (Pyruvic acid). It involves the inter conversion of sugars and transfer of phosphate groups and the ultimate conversion of one six – carbon compound to two three – carbon molecules. It is an anaerobic pathway in which some NADH and ATP is generated. The ATP is generated by substrate level phosphorylation. The overall reaction sequence for the EMP pathway is based on the following:-

Overall Reaction Sequence



Balanced Reaction

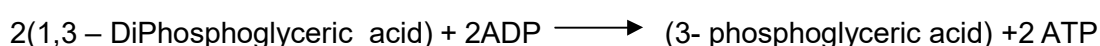


In the first phase, the conversion of glucose to fructose – 1,6 – diphosphate, there is no energy gain. Indeed, two ATP molecules are consumed for every glucose molecule phosphorylated. However, in the second phase, the conversion of fructose – 1,6 – diphosphate to two molecules of pyruvate, four ATP molecules are formed – two for each triose split off from fructose – 1,6 – diphosphate. If we consider the complete EMP pathway, the conversion of one molecule of glucose to two molecules of pyruvate results in a net gain of two ATP and two NADH molecules.

In the sequence of reactions of glycolysis there is a net gain of two molecules of ATP. These steps may be summarized as follows:



**2 ATP molecules are used**



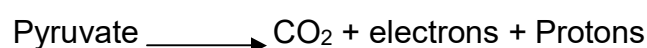
**4 ATP molecules produced**

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

However, Two NADH<sub>2</sub> molecules are also produced in the process and from its each molecule 3 ATP molecules are produced. As a result 6 more ATP molecules are formed, though these are from ETS chain. In eukaryotes, NADH<sub>2</sub> from Glycolysis enters one step late in ETS (due to loss of energy or utilization of one ATP in transfer), hence it yields only 2 ATP molecules, but in prokaryotes, it yields 3 ATP molecules.

### **15.5 FATE OF PYRUVATE TO CO<sub>2</sub> AND H<sub>2</sub>O: (Aerobic Respiration)**

Breakdown of Pyruvate is of considerable importance and significance from the stand point of energy production. In the absence of oxygen it may be degraded to Ethyl alcohol and CO<sub>2</sub> or Lactic acid or Alanine but in presence of Oxygen the usual fate of Pyruvate is to degrade up to CO<sub>2</sub> and H<sub>2</sub>O level through Krebs Cycle and respiratory chain in which energy is trapped in the form of ATP molecules. Mitochondria have all the enzymes necessary for complete oxidation of pyruvic acid to CO<sub>2</sub> and H<sub>2</sub>O. All enzymes of TCA cycle are found in the mitochondrial matrix. A summary representation of the scheme can be **Krebs Cycle**



**Respiratory Chain** (electron transport chain)



## 15.6 FORMATION OF ACETYL COA

Pyruvic acid cannot directly enter in to the krebs cycle and therefore converts in to Acetyl Co-A. The Oxidative decarboxylation of Pyruavte into Acetyl CoA involves the presence of at least five essential co factors and a complex enzyme. The Cofactors involved are Mg ions, thiamine pyrophosphate (TPP),  $\text{NAD}^+$ , Co enzyme A (CoA) and Lipoic Acid.

## 15.7 KREBS CYCLE (CITRIC ACID CYCLE. TRICARBOXYLIC ACID CYCLE)

The first reaction of the krebs cycle is the condensation of acetyl CoA with oxaloacetate to form citric acid and release CoA. The result of this reaction, catalyzed by condensing enzyme, is that a four carbon dicarboxylic acid is converted to a six carbon tri carboxylic acid.(Fig. )

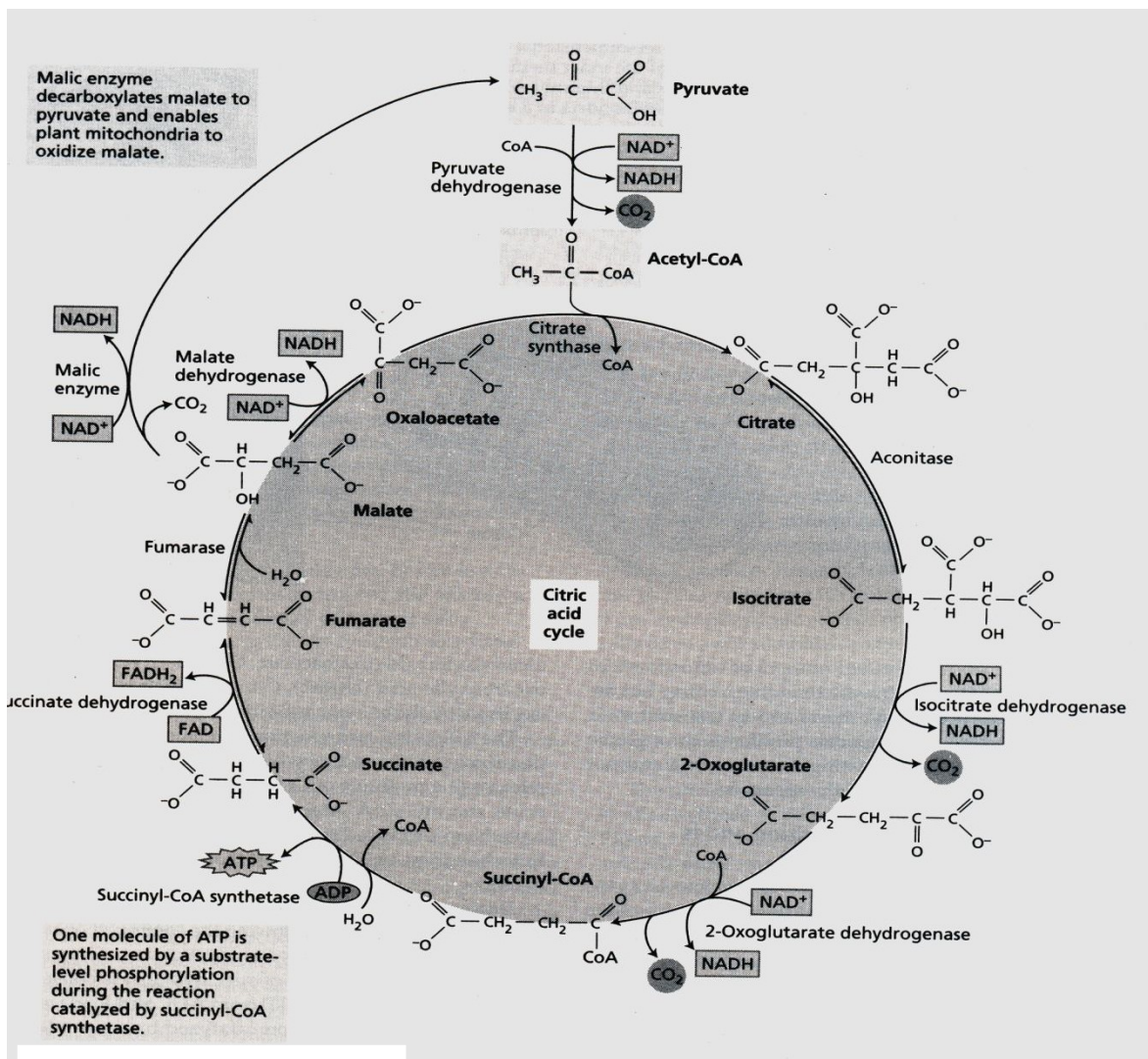


Figure is from reference 6

Through a series of reactions involving four oxidation steps and three molecules of  $\text{H}_2\text{O}$  (one utilized in the condensation reaction), oxaloacetic acid is regenerated from citric acid. In the process two molecules of  $\text{CO}_2$  and 8 H atoms are released. We should note that these acids and other organic acids of the Krebs cycle are displayed in the ionized form ( $\text{R} - \text{COO}^-$ ) and are labeled accordingly (i.e., Citrate, oxaloacetate, and so on.)

Acetyl CoA is the connecting link between glycolysis , and the Krebs Cycle (*citric acid cycle or tricarboxylic acid cycle*) so named because of the cyclic manner in which the starting compound, *oxaloacetate* is regenerated. The cycle is named after an English biochemist Krebs who played a major role in its discovery.

### **15.8 SIGNIFICANCE OF TCA CYCLE:**

In the four oxidation steps four pairs of H<sup>+</sup> ions and four pairs of electrons are removed from the intermediates of the cycle. Three of these pairs of H<sup>+</sup> ions and electrons are utilized in the reduction of pyridine nucleotides (NAD). The one remaining pair of H<sup>+</sup> ions and electrons is taken up in the reduction of the FAD prosthetic group of succinic dehydrogenase.

The NADH and FADH produced by the reactions of the Krebs cycle represent reducing power that can be harnessed in the presence of oxygen to produce ATP. The production of ATP is accomplished by a series of oxidation-reduction reactions involving chemicals that comprise the electron transport system (ETS). The ETS is intimately associated with the mitochondrial membranes and the Krebs cycle apparatus.

By means of the Krebs cycle, and the *electron transport system*, pyruvate is oxidized to CO<sub>2</sub> and H<sub>2</sub>O. Thus the complete oxidation of glucose to CO<sub>2</sub> and H<sub>2</sub>O may occur through glycolysis, the Krebs Cycle, and the electron transport system. Through its association with the electron transport system, the oxidations of the krebs cycle can account for the formation of 24 ATP molecules. Thus the krebs cycle is far more efficient in the release of energy than are either glycolysis or fermentation. The reactions of the krebs cycle and the electron transport system require the presence of oxygen and are confined to the mitochondria.

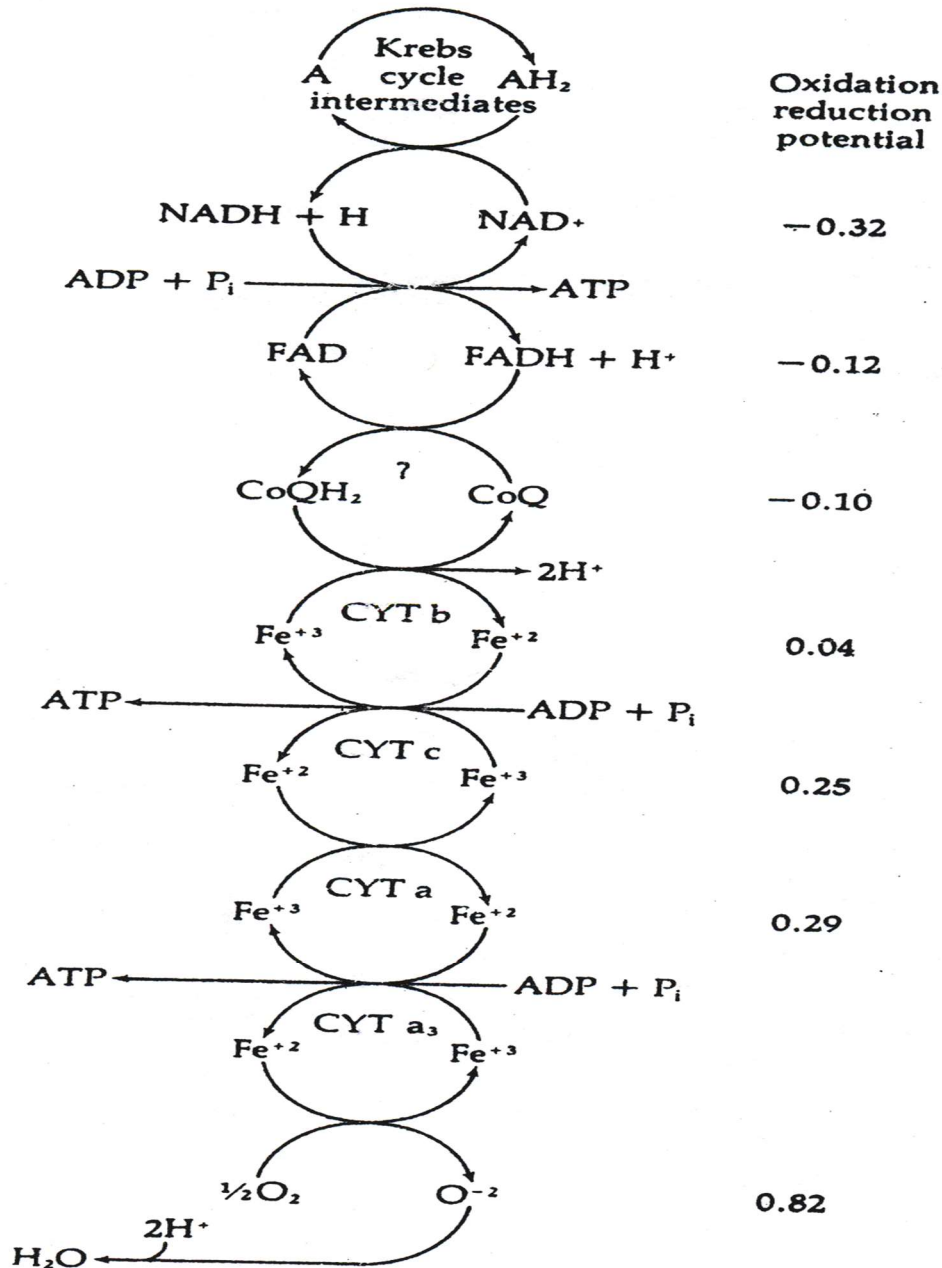
### **15.9 ELECTRON TRANSPORT SYSTEM AND PHOSPHORYLATION:**

For aerobic organisms it is essential that the enzymes and reduction products of the Krebs cycle be associated with the Electron Transport System. Through this association, the reduced pyrimidine nucleotide NADH, FADH, and (rarely) NADPH are reoxidized. The energy released from these oxidations is utilized in the synthesis of ATP. The synthesis of ATP via electron flow through the ETS, with oxygen as the terminal electron acceptor, is known as *oxidative phosphorylation* and takes place in mitochondria.

In essence, the ETS is a chain of carriers consisting of nicotinamide adenine dinucleotide (NAD) flavin nucleotides (FAD, sometimes FMN,) Co enzyme Q (CoQ) , and the cytochromes (cyt b, c, a, a<sub>3</sub>) and non-heme iron proteins also seem to be involved but their role is not known exactly.



Most important to the living plant is the fact that each step in the system is characterized by a decrease in the energy level. In other words, the carriers presumably operate in order of an increasing tendency to undergo reduction (reducing potential becomes increasingly positive from NADH through cytochrome a<sub>3</sub>).



The electron will flow from a higher to lower energy level. Thus with each step in the system the energy level of the electron is lowered and the energy difference is transformed into phosphate bond energy by the conversion of ADP to ATP. Hydrogen ions are released in the oxidation of reduced co enzyme Q (CoQ).

These hydrogen ions may play an important role in ATP production. Only the electrons are passed along the series of cytochromes.

### **15.9 SIGNIFICANCE OF ELECTRON TRANSPORT CHAIN:**

A further study will show that for every pair of electrons passed along this system, three ATP are formed. The synthesis of ATP occurs in the oxidation of NADH, in the oxidation two cytochrome b's and in the oxidation of two cytochrome a's. At their lowest energy level, the electrons are passed to oxygen from reduced cytochrome a<sub>3</sub> thereby activating the oxygen. In this state, oxygen will accept free hydrogen ions to form water.

### **15.10 ENERGY BALANCE SHEET OF RESPIRATORY METABOLISM:**

If we now consider the complete degradation of a molecule of glucose, first to two pyruvic acid molecules via the EMP pathway and then to the two acetyl CoA molecules through the oxygen-requiring Krebs cycle to CO<sub>2</sub> and water, it is possible to have thirty-eight ATP molecules generated.

Pathway	NADH (3ATP)	FADH (2ATP)	ATP	Total ATP
EMP (yield)	2 (6)	0	2	8
Pyruvic acid to Acetyl CoA	2 (6)	0	0	6
Krebs Cycle (yield)	6 (18)	2 (4)	2	24
Total ATP	10 X 3 = 30	2X 2 = 4	4	38 ATP

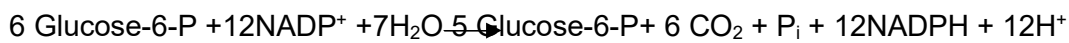
### **15.11 THE PENTOSE PHOSPHATE PATHWAY PRODUCES NADPH AND**

#### **BIOSYNTHETIC INTERMEDIATES:**

The glycolytic pathway is not the only route available for the oxidation of sugars in plant cells. Sharing common metabolites, the **oxidative pentose phosphate pathway** (also known as the **hexose monophosphate shunt**) can also accomplish this task. The reactions are carried out by soluble enzymes present in the cytosol and in plastids. Generally, the pathway in plastids predominates over the cytosolic pathway.

The First two reactions of this pathway involve the Oxidative events that convert the six – carbon glucose – 6 – phosphate to a five – carbon sugar, ribulose 5 – phosphate, with loss of a CO<sub>2</sub> molecule and generation of two molecules of NADPH (not NADH). The remaining reactions of the pathway convert ribulose 5 phosphate to the glycolytic intermediates glyceraldehyde,3- phosphate and fructose-6- phosphate. Because glucose 6 phosphate can be regenerated from

glyceraldehyde- 3- phosphate and fructose- 6- phosphate by glycolytic enzymes, for six turns of the cycle we can write the reactions as follows:



The net result is the complete oxidation of one glucose – 6- phosphate molecule to CO<sub>2</sub> with the concomitant synthesis of 12 NADPH molecules.

Studies of the release of <sup>14</sup>CO<sub>2</sub> from isotopic ally labeled glucose indicate that glycolysis is the moiré dominant breakdown pathway, accounting for 80 to 95% of the total carbon flux in most plant tissues. However the pentose phosphate pathway does contribute to the flux and developmental studies indicate that its contribution increases as plant cells develop from a meristematic to more differentiated state.

**The oxidative pentose phosphate pathway plays several roles in plant metabolism:**

- The Product of two oxidative steps is NADPH, and this NADPH is thought to drive reductive steps associated with various biosynthetic reactions that occur in the cytosol such as **lipid biosynthesis** and **nitrogen assimilation**.
- Some of the reducing power generated by this pathway may **contribute to cellular energy metabolism**; that is, electrons from NADPH may end up reducing O<sub>2</sub> and generating ATP.
- The pathway produces ribose 5 phosphate, a precursor of the ribose and deoxyribose needed in the **synthesis of RNA and DNA** respectively.
- Another intermediate in this pathway, the four carbon erythrose – 4 – phosphate, combines with PEP in the initial reaction that **produces plant phenolic compounds** including the aromatic amino acids and the precursors of lignin, flavonoids and phytoalexins.
- During the early stages of greening, before leaf tissues become fully photoautotrophic, the oxidative pentose phosphate pathway is thought to be **involved in generating Calvin Cycle intermediates**.

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