

## BOTANY

COURSE: M.Sc. II Sem. / B.Sc. III Part /IMB II Part  
BIOCHEMISTRY

## RESPIRATION

Respiration is a catabolic process in which foods (Carbohydrate, protein, fat) are chemically burnt /oxidised to produce energy (ATP), carbon dioxide and water. Since, it involves both breakdown of food as well as synthesis of energy (ATP) and other compounds, it is referred to be an amphibolic (both catabolic & anabolic) process. The term respiration was initially used for breathing in animals but was subsequently extended to include the biochemical reactions by which organic substances like carbohydrates, fats and proteins are broken down to release CO<sub>2</sub>, water and energy in all the living organisms.

In respiration, the high energy compounds are oxidised which are called as respiratory substrates. The most ready substrate in respiration is glucose (carbohydrate). Fats and proteins are also respiratory substrates but are next to carbohydrates. Glucose occupies the central position in metabolism. It is stored in the form of high molecular weight polymer such as starch in plants and glycogen in animals. Both types of polysaccharides occur in bacteria. These compounds release glucose to meet the energy demand.

Respiration is one of the prime vital processes, universally present in all the living cells. In aerobes, respiration takes place utilising O<sub>2</sub>, but in anaerobes O<sub>2</sub> is not required. Anaerobic respiration is found in Yeasts and some other microorganisms. The energy produced in the aerobic respiration is more than that of anaerobic one. The biochemical reactions of respiration can be written as:

**Aerobic--**  $C_6H_{12}O_6 + 6 O_2 = 6 CO_2 + 6 H_2O + \text{energy (673 K cal.)}$

**Anaerobic--**  $C_6H_{12}O_6 \text{ -----} \rightarrow 2 C_2H_5OH + 2 CO_2 + \text{energy (28 K cal.)}$

### Difference between combustion and respiration:

Burning of coal and other fuels (combustion) is also an oxidative process but it differs from respiration. Both the processes involve oxidation/chemical burning. Combustion is non enzymatic and unregulated process such as burning of fuels and firewood. There is no ATP production. The energy is released as heat & light in uncontrolled manner. Contrary to this, respiration is an enzyme mediated and well regulated biochemical process, which is a prime character of living cells. The energy is stored in the form of bio molecule (ATP), which releases energy as per cell requirements. Whole process of respiration includes following biochemical steps:

**Glycolysis** – Glycolysis is the first step of glucose breakdown in respiration which does not require oxygen. Glucose is oxidised to pyruvate in this process. It is ten step enzyme mediated process in which one molecule of glucose gives 2 molecules of pyruvate molecules, with the net gain of 2ATP and 2molecules of reduced *Co-factor (NADH)* at the

end of the process. It takes place in the cytosol and is found both in aerobic and anaerobic organisms. Though, it is energy producing process, initially it consumes two ATP molecules per glucose molecule utilised, but produces four ATP molecules during further reactions.

### ***Krebs' Cycle/Citric Acid Cycle /Tricarboxylic Acid Cycle -***

Pyruvate formed after glycolysis enters into mitochondria. Its entry is catalysed by the enzyme *Pyruvate translocase*. In mitochondria pyruvate undergoes oxidative decarboxylation and produces an important intermediate compound *Acetyl-CoA*. *Acetyl-CoA* follows a cyclic path to generate reduced *cofactors* such as *NADH+ H<sup>+</sup>*, *FADH<sub>2</sub>*, GTP/ATP and CO<sub>2</sub>. This cycle is referred to as Krebs' cycle. This cycle is the major source of formation of ATP generating compounds (*NADH*, *FADH<sub>2</sub>* & GTP) in aerobic respiration. In this cycle, *acetyl- CoA* obtained after oxidative decarboxylation of pyruvate combines with oxaloacetate to produce many organic acids and other compounds of metabolic interest.

**3. Electron Transport System** - This is a sequential chain of redox reactions operating in the mitochondrial inner membrane. This process involves oxidation of reduced Cofactors ***NADH & FADH<sub>2</sub>*** (electron & proton donor) by protein complexes arranged in sequence with increasing order of their ***Reduction potentials***. Electrons flow from reduced cofactors through these protein carriers. This transfer of electrons continues through electron carriers (*Cytochromes*) arranged in many protein complexes which finally reduces to O<sub>2</sub> (ultimate electron acceptor). Since, ATP is produced in this process (oxidative phosphorylation) in mitochondria; mitochondria are also called as ***power house*** of the cell.

## **GLYCOLYSIS/ Embden Meyerhof Parnas (EMP) pathway**

Gr. glykys: sweet, sugar; lysis: breakdown

This pathway is a series of reactions of breakdown of glucose in respiration without involvement of oxygen. This is common to both in aerobic and anaerobic respiration. It is named as EMP pathway after its discoverer G. Embden, O. Meyerhof & J K Parnas. This includes total ten steps of biochemical reactions catalysed by different enzymes. Step 1, 3 and step 10 reactions are irreversible, rest are reversible. This is due to thermodynamic infeasibility in the above mentioned three steps. Enzymes catalysing glycolysis, are present in the cytosol, hence the site for glycolysis is cytosol.

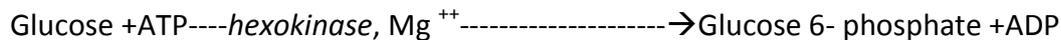
Breakdown of glucose through glycolysis can be written as:



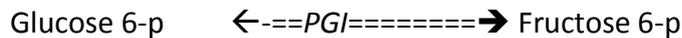
This takes place in the following steps:-

### **A. Preparatory phase (ATP consuming phase)**

1. In the first step of reaction glucose is phosphorylated by ATP catalysed by the enzyme *Hexokinase* in presence of  $Mg^{++}$ .



2. Glucose 6 phosphate is converted to Fructose 6 phosphate by the enzyme *Phosphohoglucoisomerase (PGI)*



3. Fructose 6-p is phosphorylated by another ATP producing Fr. 1, 6- bi phosphate, catalysed by the enz. *Phosphofructokinase (PFK)* in presence of  $Mg^{++}$ .



4. FR.1,6- BP is splitted to one molecule each of glyceraldehyde 3-Phosphate (Gly3P) and dihydroxyacetone phosphate (DHAP) catalysed by the enz. *aldolase*.



5. Gly 3-P & DHAP are inter-convertible to each other catalysed by the enz. *Triose phosphate isomerase (TPI)*. The reaction is reversible and only Gly 3P is used in further conversion. DHAP readily converts to Gly-3P to continue further reactions.

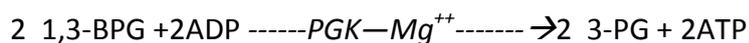


### B. Pay-off phase (ATP generating phase)

6. Each Gly 3-P is acted upon by *Gly 3-P dehydrogenase* along with addition of inorganic phosphate (Pi) to produce 1, 3-biphosphoglycerate. This reaction is a combination of two reactions (a) oxidation of glyceraldehydes to glycerate, by  $NAD^+$  (b) joining of an orthophosphate with the carboxylic group to form acyl-phosphate. Two molecules of Gly3-P proceed to get oxidised. It is to be noted that, of the two molecules of Gly3-P, one molecule comes from the reversal of DHAP.



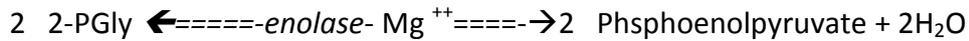
7. Two molecules of 1,3-BPG are converted to two mol. of 3-Phoshoglycerate (3-PG) by the enz. *3P-glyceric kinase (PGK)* in presence of  $Mg^{++}$



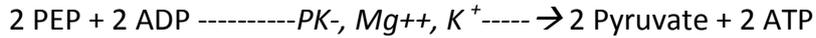
8. Two molecules of 3-PGlycerate are converted to two mol. of 2-PGlycerate (2-PG) by the enz. *Phosphoglycerate mutase (PGM)*



9. Two molecules of 2-PG are dehydrated to two mol. of Phosphoenolpyruvate (PEP) by the enz. *enolase*



10. Two mol. of PEP are acted upon by enz. *Pyruvate kinase (PK)* in presence of  $\text{Mg}^{++}$  to produce 2 molecules each of ATP and Pyruvate



This should be noted that 2 Pyruvate molecules are produced from each glucose molecule oxidised. Splitting of Fructose 1, 6-biP gives one molecule each of Gly 3-P & DHAP (STEP4). DHAP is also converted to Gly-3P (step 5) .

The overall balance sheet of energy in glycolysis shows a net gain of 2 ATP

ATP consumed- per unit glucose molecule utilised -----	2
ATP produced -----	2X2=4 molecules (step 7 & 10)
Net gain of ATP per glucose mol. utilised ----	2

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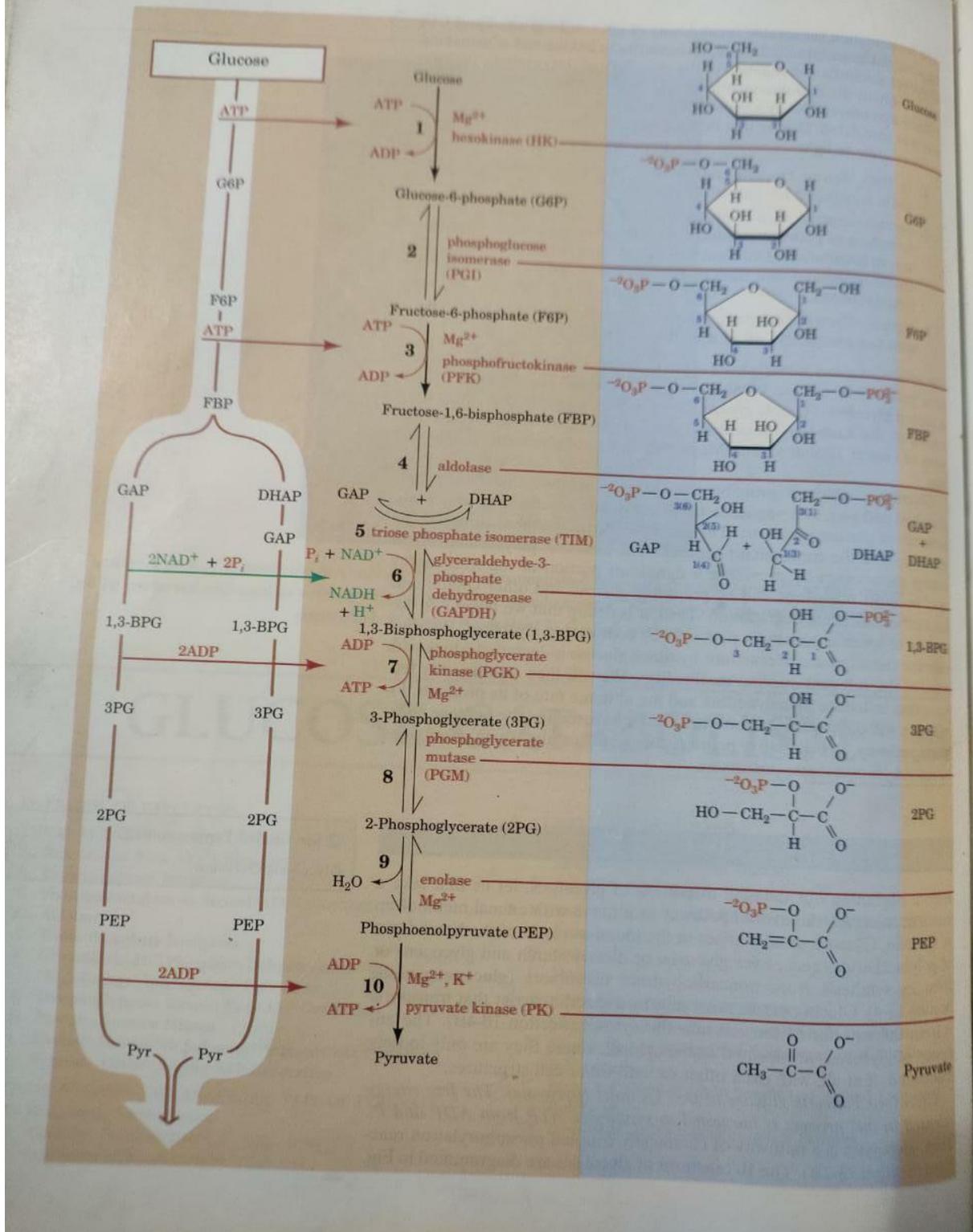
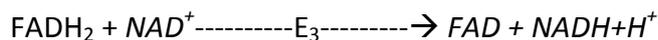
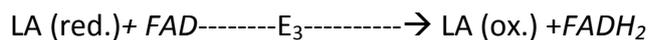


Fig. Glycolysis (courtesy: Fundamentals of Biochemistry, Voet, Voet & Pratt)

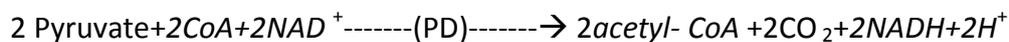
## Fate of Pyruvate after glycolysis—

In eukaryotic aerobic respiration, pyruvate enters into mitochondria and undergoes oxidative decarboxylation mediated by an enz. *Pyruvate dehydrogenase (PD)*. It is a multienzyme complex having combination of three different enzymes namely (*PD*; *E<sub>1</sub>*), *Dihydrolipoyl transacetylase (E<sub>2</sub>; DLT)* & *Dihydrolipoyl dehydrogenase (E<sub>3</sub>; DLD)* resulting to decarboxylation & dehydrogenation of Pyruvate. This enzyme is located in the mitochondrial matrix. In prokaryotes such as bacteria, mitochondria are absent and PD is located in the cytosol.

Oxidative decarboxylation of pyruvate requires **five Co-factors--Thiamine pyrophosphate (TPP), Lipoamide (LA), acetyl-CoA, FAD & NAD<sup>+</sup>**. Following are different steps of reaction:-



Since, two molecules of pyruvate are produced from one molecule of glucose, the overall reaction of oxidative decarboxylation of pyruvate can be summed up as :



(CoA—Coenzyme A)

In anaerobic respiration, as found in Yeasts, pyruvate is converted to ethyl alcohol & CO<sub>2</sub>. However, Yeasts are facultative anaerobes and respire aerobically but under anaerobic condition they respire anaerobically.

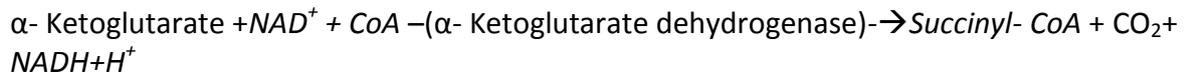


This reaction is used in production of ethyl alcohol by yeasts. The process is called as alcoholic fermentation and has industrial importance. This is used in alcohol producing industries using yeasts to ferment with suga. CO<sub>2</sub> produced in the reaction leavens bread which is important for bakeries. Another product of anaerobic respiration is Lactic acid, which is produced by *Lactobacilli*.

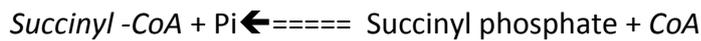
Lactic acid bacteria (*Lactobacilli*) convert Pyruvate to Lactate by the following reaction (Lactic acid fermentation) :



4 Enz.  $\alpha$ -ketoglutarate dehydrogenase catalyses the oxidative decarboxylation of  $\alpha$ -Ketoglutarate to produce Succinyl- CoA , NADH & CO<sub>2</sub>



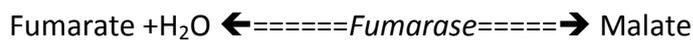
5 The next reaction is catalysed by the enz. Succinate thiokinase which generates one GTP equivalent to high energy compound ATP and Succinate.



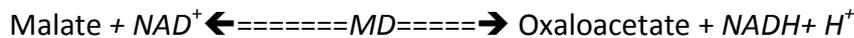
6 Succinate is acted upon by the enz. Succinate dehydrogenase (SD) which converts it to Fumarate



7 Fumarate is hydrated by the enz. Fumarase to produce Malate



8 Malate is oxidised (dehydrogenated ) by the enz. Malate dehydrogenase (MD) to produce Oxaloacetate, the last reaction of this cycle. It's cycle because the reaction ends where from it begins (Oxaloacetate)



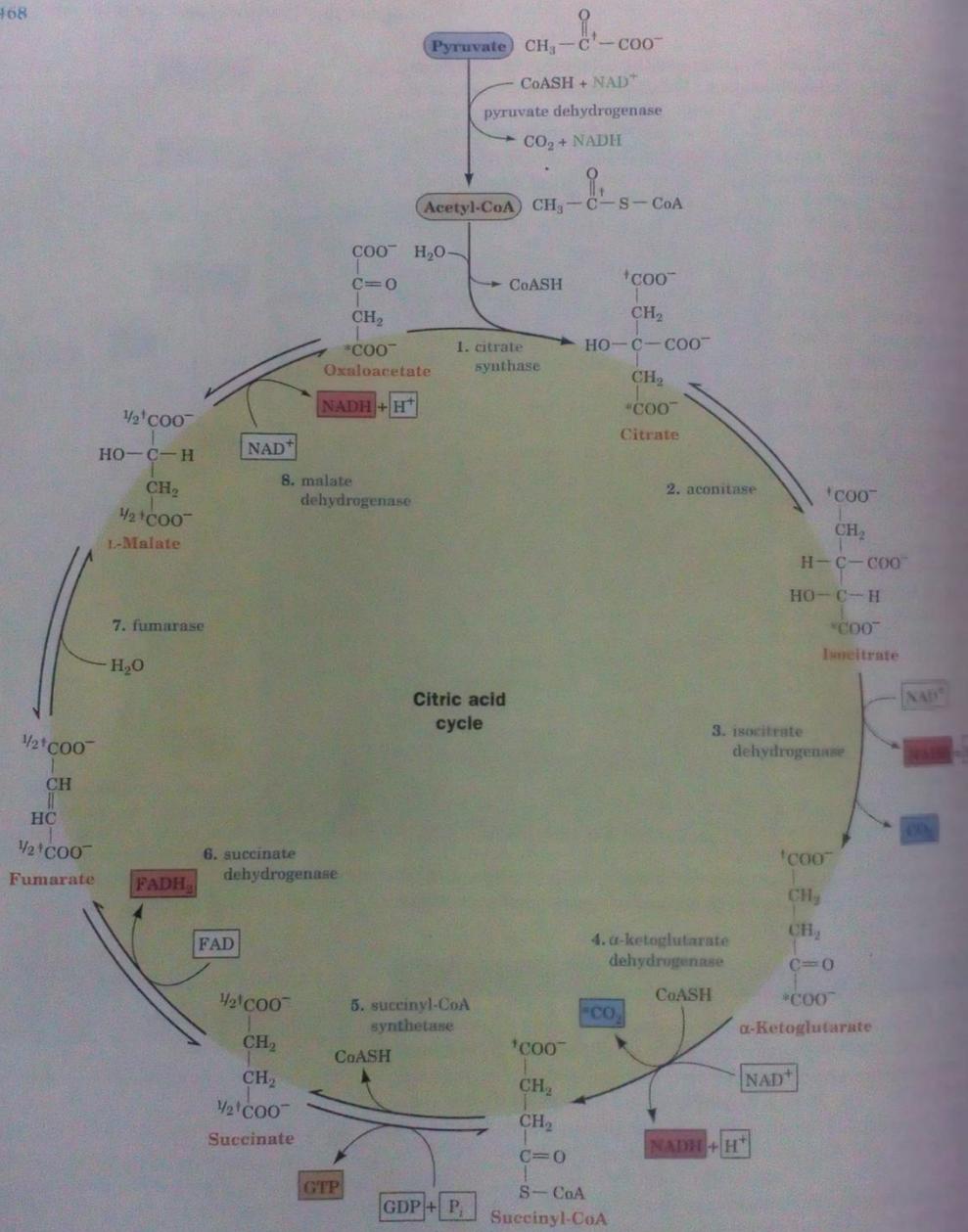
Energy balance sheet:

Reaction step	Reduced Co-factors	ATP molecules produced
Steps -1- Pyruvate $\rightarrow$ acetyl- CoA	2 molecules NADH <sub>2</sub>	2x3=6
Citric acid cycle energy output:-		
2-Isocitrate $\rightarrow$ oxalosuccinate	2 mol. NADH <sub>2</sub>	2x3=6
3. $\alpha$ -Ketoglutarate $\rightarrow$ Succinyl -CoA	2 mol. NADH <sub>2</sub>	2x3=6
4-Succinate $\rightarrow$ Fumarate	2mol. FADH <sub>2</sub>	2x2=4
5-Malate $\rightarrow$ Oxaloacetate	2mol. NADH <sub>2</sub>	2x3=6
GTP produced-- Succinyl-CoA $\rightarrow$ Succinate	1 ATP	2x1=2

Note: 1 NADH<sub>2</sub> produces 3 ATP while 1 FADH<sub>2</sub> produces 2 ATP mol. during oxidative phosphorylation (electron transport system).

ATP molecules produced in aerobic respiration from 1 glucose mol :-

Glycolysis	8
Pyruvate $\rightarrow$ acetyl- CoA	6
Krebs' cycle	24
Total	38



**Figure 16-2. Key to Metabolism.** The reactions of the citric acid cycle. The reactants and products of this catalytic cycle are boxed. The pyruvate → acetyl-CoA reaction (top) supplies the cycle's substrate via carbohydrate metabolism but is not considered to be part of the cycle. An isotopic label at C4 of oxaloacetate (\*)

becomes C1 of α-ketoglutarate and is released as CO<sub>2</sub> in Reaction 4. An isotopic label at C1 of acetyl-CoA (+) becomes C2 of α-ketoglutarate and is scrambled in Reaction 5 between C2 and C4 of succinate (1/2+)

Citric acid cycle .(Fig. Courtesy ; Fundamentals of Biochemistry,Voet,Voet &Pratt.)

**Significance of Krebs' cycle:** Krebs' cycle bears multiple importance in the cellular metabolism. It holds central position in the metabolism. In respiration, it produces most of the energy from glucose molecule. Out of 38 molecules of ATP produced from one mol. of glucose, 24 ATP molecules are contributed from this cycle. Besides this, many other compounds required for the synthesis of amino acids, are intermediates of Krebs' cycle. Amino acids are used in the synthesis of nucleic acids, proteins. Many organic acids formed in this cycle are precursors of many amino acids such as :

$\alpha$  ketoglutarate  $\rightarrow$  glutamate

oxaloacetate  $\rightarrow$  aspartate

many amino acids are derived from these preformed amino acids by transamination reactions.

It is directly related to the nitrogen metabolism.  $\alpha$  ketoglutarate is the first acceptor molecule of  $\text{NH}_3$ , forming amino acid glutamic acid. Amino acids are building blocks of proteins; hence citric acid cycle is directly related with the cellular proteins and enzyme system.

Glutamic acid is the first formed amino acid from  $\alpha$  ketoglutarate, which is part of entry of nitrogen in the form of  $\text{NH}_3$  in the metabolic pool after nitrogen fixation in  $\text{N}_2$  fixing plants

*Succinyl-CoA* formed in the cycle acts as precursor of chlorophyll, cytochromes and phytochromes.

Krebs' cycle is also intimately related with the synthesis of anthocyanin and phenols. *Acetyl-CoA*, which enters into Krebs' cycle, is also important for the synthesis of anthocyanin and phenols.

## Mitochondrial Electron Transport System

Respiratory electron transport system is the sequence of events leading to the synthesis of ATP in the mitochondria and reduction of oxygen to water. It is universally found in all the aerobic organisms. In this process reduced cofactors produced during glycolysis, oxidative decarboxylation of pyruvate and in Krebs' cycle, donate electrons to electron receiving centres arranged in series in the inner membrane of mitochondria.. Reduced Cofactors (*NADH & FADH<sub>2</sub>*) are oxidised by the adjacent protein complex (I). Transfer of electrons continues till it reaches to the last receiver  $\text{O}_2$ , reducing it to  $\text{H}_2\text{O}$ . Electron transfer from *NADH and FADH<sub>2</sub>* to  $\text{O}_2$  takes place through a series of electron (e) carriers arranged sequentially in the increasing order of their reduction potentials ( $E^0$ ) along the inner membrane of mitochondria. These electron carriers include five protein complexes. These contain flavin mononucleotide (*FMN*-complex I), Flavin adenine dinucleotide (*FAD*-complex II), *Ubiquinone/CoQ* and cytochromes (complex III & IV). Transport of electrons is accompanied with proton ( $\text{H}^+$ ) transport from matrix to inter membranous space of mitochondria. The reduced coenzyme *FMNH<sub>2</sub>* and *FADH<sub>2</sub>* transfer one electron at a time in the subsequent steps in complex III & IV containing cytochromes. Cytochromes are iron

containing proteins in which Fe exists in two forms, which continuously gets reduced ( $\text{Fe}^{2+}$ ) and oxidised ( $\text{Fe}^{3+}$ ). Different protein complexes are grouped as:

- Complex I NADH : CoenzymeQ Oxidoreductase
- II Succinate: CoenzymeQ Oxidoreductase
- III Coenzyme Q: Cytochrome c Oxidoreductase/ Cytochrome b c<sub>1</sub> complex
- IV Cytochrome c Oxidase
- V ATP Synthase / F<sub>1</sub>F<sub>0</sub>-ATP ase

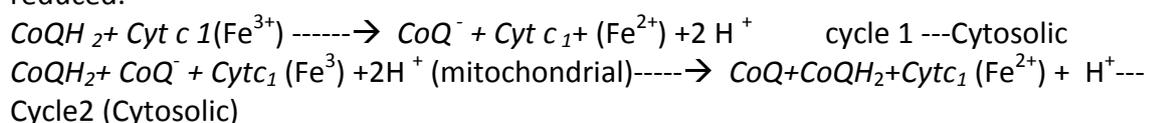
Transfer of electrons from one complex to another is guided by their respective reduction potentials ( $E^{\circ}$ ). Flow of electrons takes place from complexes having lesser reduction potential ( $E^{\circ}$ ) to those having greater  $E^{\circ}$  in the increasing direction. *NADH* produced in previous reactions bears  $E^{\circ}$  -0.315 V &  $\text{O}_2$  has  $E^{\circ}$  0.815, therefore electron transfer takes place from *NADH* to  $\text{O}_2$  through different protein complexes. This is associated with release of energy which depends on the difference of  $E^{\circ}$  of the two redox groups. Difference of  $E^{\circ}$  between *NADH* and  $\text{O}_2$  ( $E^{\circ}_{\text{acceptor}} - E^{\circ}_{\text{donor}}$ ) is 1.130 V. The amount of free energy difference is sufficient to produce three ATP molecules which are conserved in the system. Transfer of electrons through the protein complexes in the inner membrane of mitochondria is associated with transfer of  $\text{H}^+$  which gets transferred from mitochondrial matrix to Inter membranous space (IMS). IMS lies between the inner and outer membranes of the mitochondria.

The sequence of electron transport can be understood by the following steps :

**Complex I (NADH : CoQ Oxidoreductase)**- This is the largest protein among all the complexes involved in ETS which receives 2 electrons (e) from each *NADH*, which get transferred to Flavin mononucleotide (*FMN*). *FMN* contains 2 Fe-2S & 4Fe-4S clusters which participate in electron transfer. *FMN* gets reduced after receiving electrons. The electrons transit to CoQ takes place and as a result *FMN* gets oxidised while CoQ gets reduced to *CoQH*<sub>2</sub>. Protons are also translocated from matrix to IMS along with the electrons.

**Complex II (Succinate : CoQ Oxidoreductase)** –This complex is also called as Succinate dehydrogenase. This complex contains FAD with three Fe-S centres to facilitate transfer of electrons. The sequence of electron transfer from succinate to CoQ involves reduction of *FAD* to *FADH*<sub>2</sub>. This is followed by transfer of electrons to CoQ, Two- one electron transfer takes place from *FADH*<sub>2</sub> to the series of three Fe-S clusters. As a result of which CoQ gets reduced to *CoQH*<sub>2</sub>. In this sequence, Succinate is oxidised to Fumarate.

**Complex III –( CoQ : Cytochrome c oxidoreductase )** - This complex consists of a 2 Fe-2S centre, *Cytochrome b* and *Cytochrome c*<sub>1</sub>. *Cyt b* here exists in two forms *Cyt b<sub>h</sub>* & *Cyt b<sub>l</sub>*. This complex functions to allow one molecule of *CoQH*<sub>2</sub>, a two electron carrier to reduce two molecules of *Cytochrome c*, which can accommodate only one electron at a time. *Cyt c* gets reduced.



*Cytochrome c* is a mobile, peripheral membrane protein which shuttles between complex III & IV. It transfers one electron at a time from *Cyt c*<sub>1</sub>(complex III) to *Cu<sub>A</sub>* (complex IV).

**Complex IV -( Cytochrome c Oxidase)** - This is the last complex associated with ETS. This contains *Cytochromes a* and  $a_3$  and reduction factors  $Cu_A$  and  $Cu_B$ . This receives electrons from *Cytochrome c*, (a mobile protein). Electrons flow through  $Cyt a, a_3, Cu_A$  and  $Cu_B$ . This transfers electrons to  $O_2$  which gets reduced to water. In this transfer of electrons protons are translocated from reduced cofactors to the inter membranous space.

**Complex V – ATP synthase/ $F_1$ - $F_0$  ATPase** – This complex is a multi subunit protein of  $180 \text{ \AA}$  size which has two major units  $F_0$  ( $50 \text{ \AA}$ ) and  $F_1$  ( $50 \text{ \AA}$ ). The former  $F_0$  is a water insoluble transmembrane proton channel having eight subunits while the later  $F_1$  is a water soluble peripheral protein consisting of five types of protein subunits. This subunit is the site of ATP generation. The electron micrograph shows that the  $F_1$  faces the matrix but  $F_0$  is embedded in the inner membrane.  $F_1$  and  $F_0$  are connected with a stalk ( $50 \text{ \AA}$ ). The protein subunits of  $F_1$  undergoes conformational changes. They receive ADP and  $P_i$ , bind them and release ATP.

### **Chemiosmotic theory and Proton motive force :**

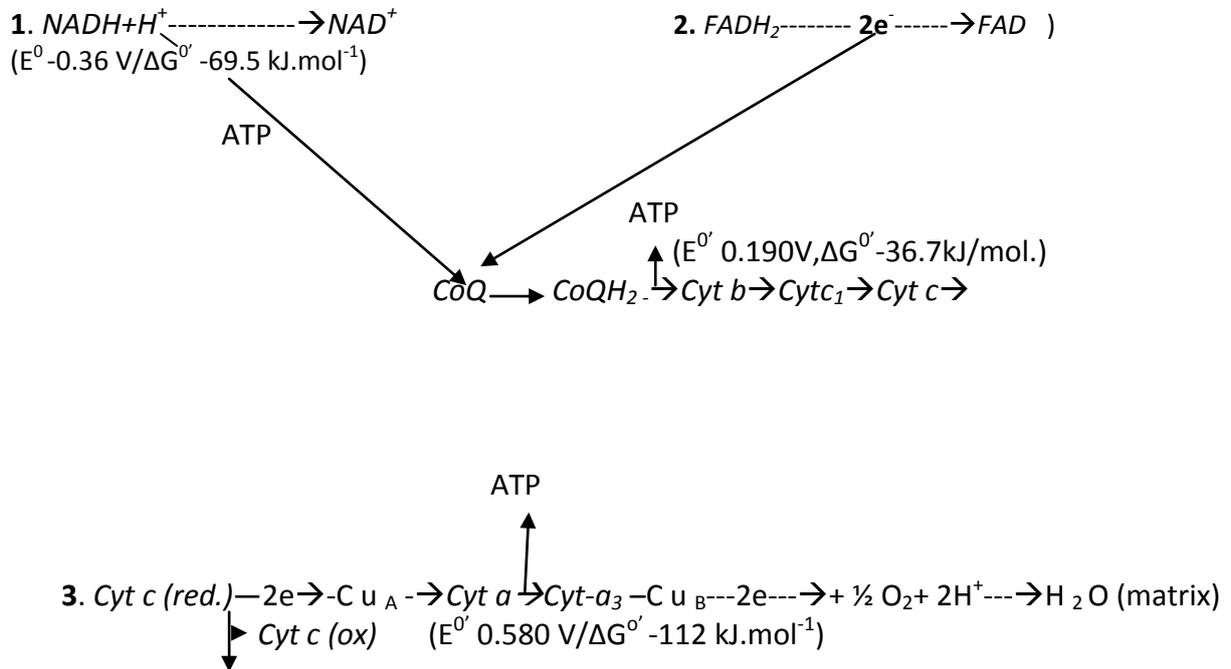
Chemiosmotic theory was given by Peter Mitchell (1961) to explain the ATP generation mechanism due to the *proton motive force (pmf)*, developed between the inter membranous space and matrix of mitochondria. Since protons/ $H^+$  are translocated from the matrix to the IMS along with electrons flow, the concentration of  $H^+$  becomes greater than that in matrix, an electrochemical  $H^+$  concentration gradient develops between the mitochondrial matrix and inter membranous space. There develops a force, called as *Proton Motive Force (pmf)*. The electrochemical potential of this gradient is harnessed to synthesize ATP. (Oxidative Phosphorylation) As a result of accumulation of  $H^+$  in the IMS,  $H^+$  tends to move to matrix through the  $H^+$  transport channel present in the  $F_1$ - $F_0$  ATPase (complex V). Hence  $F_1$  stalk of the  $F_1 - F_0$  complex is named as  $H^+$  transport channel. This process is responsible to drive the force to generate ATP in the *ATP Synthase* (complex V). This theory is supported by many experimental evidences and nature of mitochondrial inner membrane.

1. The inner membrane is impermeable to  $H^+$ ,  $OH^-$ ,  $K^+$ ,  $Cl^-$  and their free movement /diffusion would discharge the electrochemical gradient. Oxidation of NADH and  $FADH_2$  is synchronized with ATP production due to  $H^+$  transport & electrochemical potential gradient.
2. Many compounds (chemical uncouplers) which increase the membrane permeability to protons are also able to dissipate the electrochemical potential developed there. As a result of this, ATP synthesis is inhibited.

### **Oxidative phosphorylation :**

Mitochondria are the site of oxidative phosphorylation was discovered by Kennedy and Lehninger (1948). In eukaryotic aerobic respiration, ATP is generated from ADP and  $P_i$  as a consequence of electron transport in mitochondria. This is referred to as oxidative phosphorylation. Since, the phosphorylation occurs during oxidation of reduced cofactors *NADH &  $FADH_2$*  by  $O_2$ , this is named as oxidative phosphorylation. ATP formation requires energy which is made available by the

redox reactions during the electron transport. The energy conservation in the form of ATP is linked with electron transport and is referred to as chemical coupling. During phosphorylation the required amount of energy to bind ADP & Pi, is made available by the free energy produced at following steps:



*FADH*<sub>2</sub> also transfers electrons to *CoQ* parallelly, but the free energy ( $\Delta G^0$ ) for electron transfer from succinate to *CoQ* is insufficient to generate ATP synthesis. This is the reason that **only two** ATP molecules are produced from *FADH*<sub>2</sub>

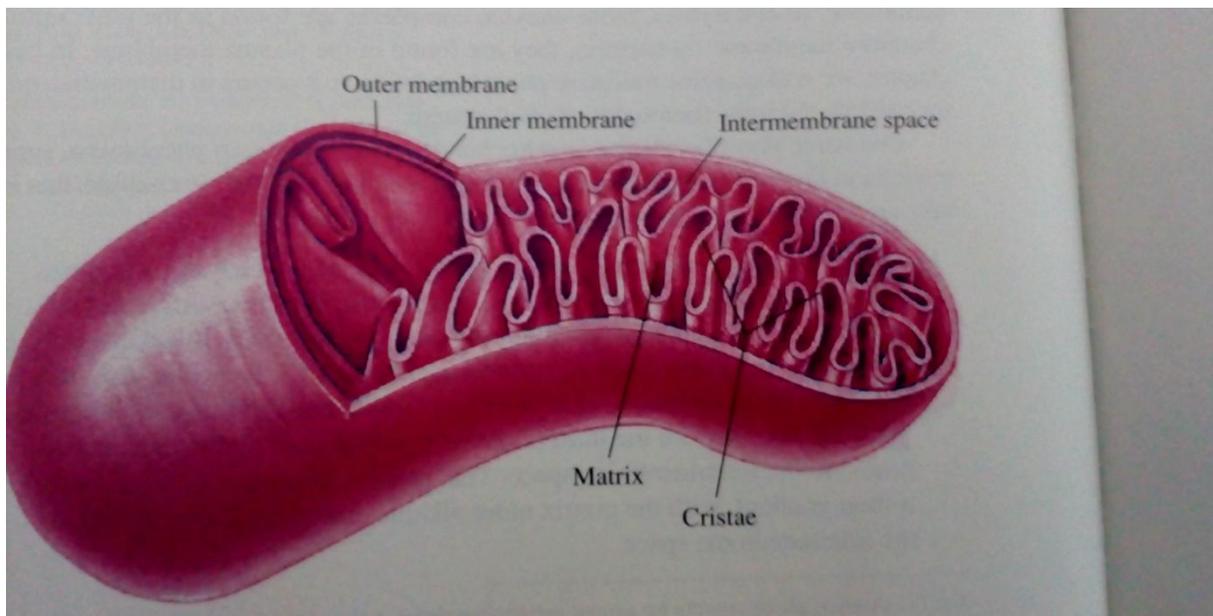


Fig. Showing external and internal structure of mitochondrion. (Courtesy; Principles of Biochemistry, Horton, Moran, Ochs, Rawn & Scrimgeour. Publ. Prentice –Hall IN, Inc.)

**Electron transport and Oxidative phosphorylation in Bacteria :**

In bacteria, mitochondria are absent, the electron transport system is located in the plasma membrane and the protons are translocated from the cytosol to the outside of plasma membrane. Like mitochondrial system, in aerobic bacteria electrons flow from CoQ through cytochrome based oxidoreductases to O<sub>2</sub> which is reduced to H<sub>2</sub>O.

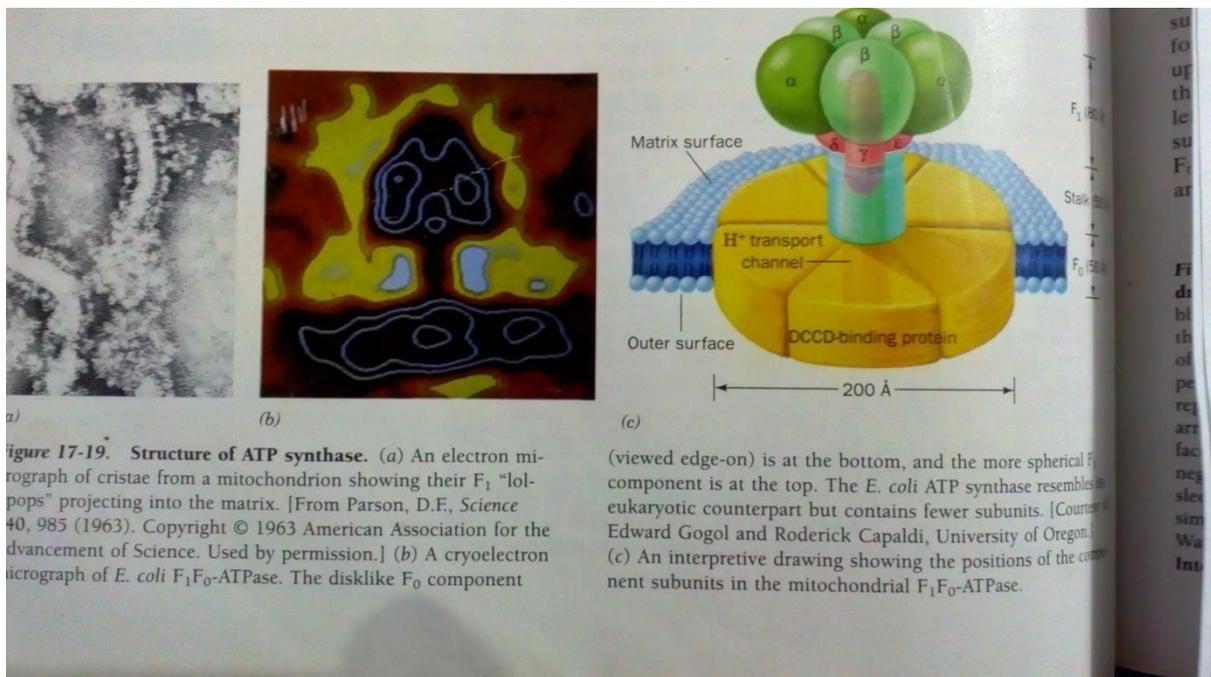


Fig. ATP synthase/ F<sub>1</sub>-F<sub>0</sub> ATP ase (Courtesy ; Fundamentals of Biochemistry, Voet, Voet & Pratt. Publ. John Wiley & sons, Inc.)

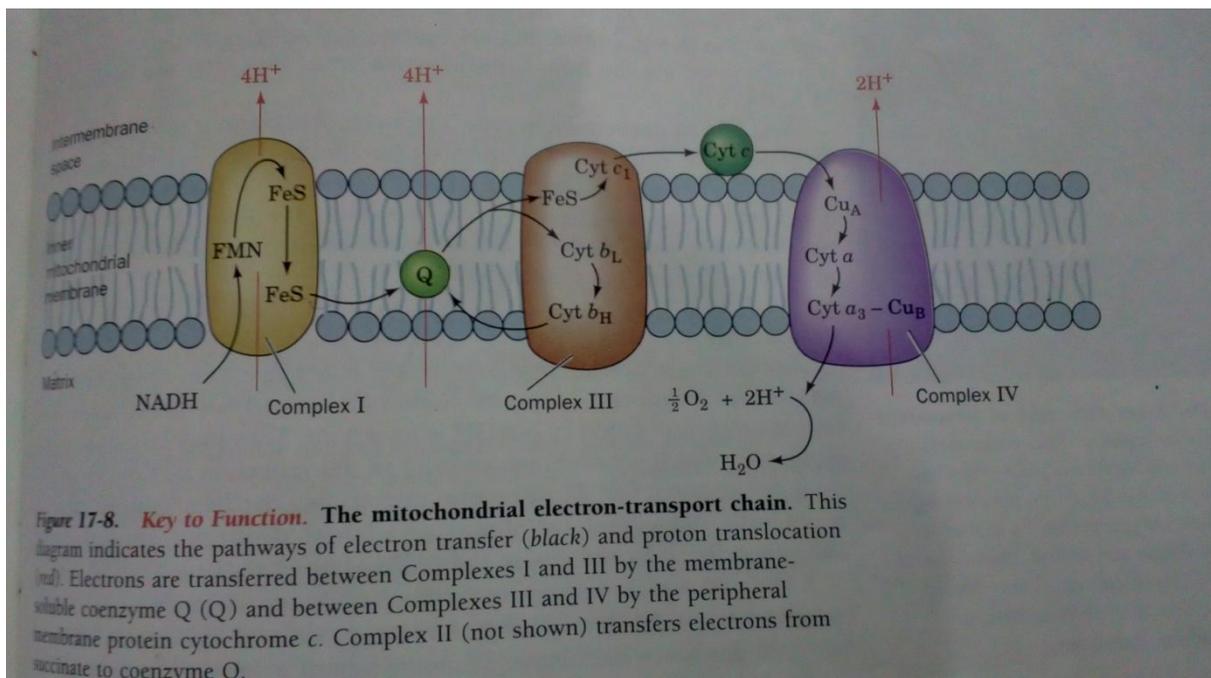


Fig. showing mitochondrial electron transport system. (Courtesy; Fundamentals of Biochemistry, Voet, Voet & Pratt. Publ. John Wiley & sons, Inc.)

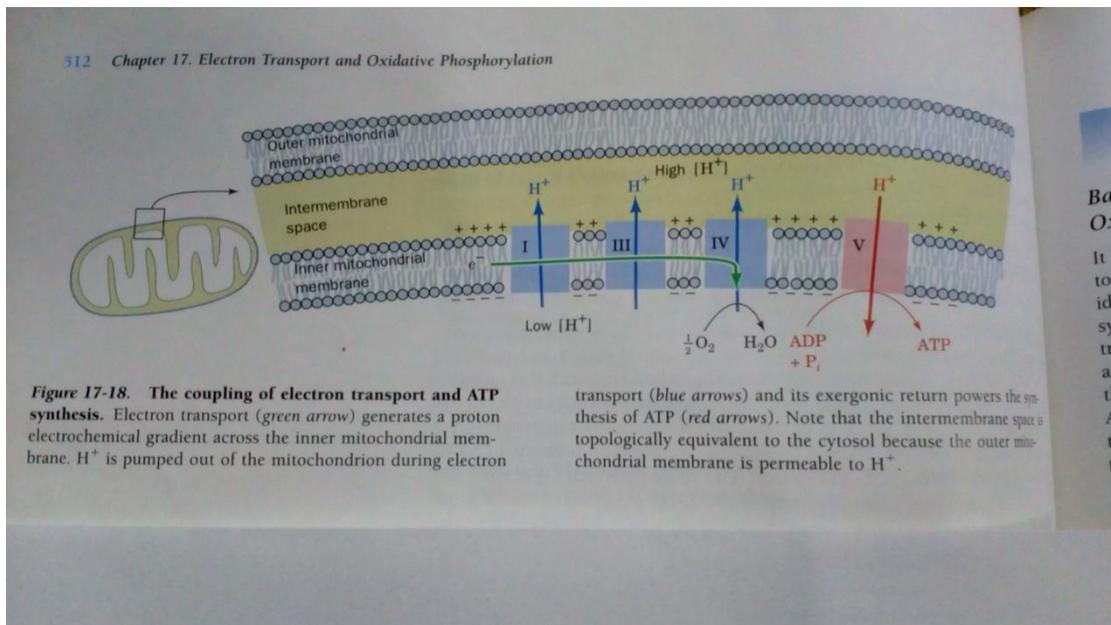


Fig. showing coupling of electron transport with oxidative phosphorylation. (Courtesy; Fundamentals of Biochemistry, Voet, Voet & Pratt.)