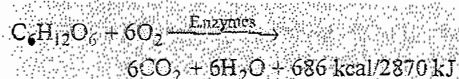
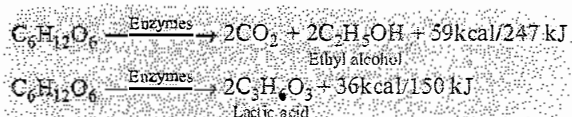


## RESPIRATION IN PLANTS

- Respiration is an important life process which involves liberation of energy from food. It has two phases. First phase is gaseous exchange between environment and organism through body surface or special respiratory organs. Second phase is cellular respiration.
- **Cellular respiration** is an energy releasing, enzymatic catabolic process which involves a step wise oxidative breakdown of food substances inside living cells. The most actively respiring regions are growing regions like floral and vegetative buds, germinating seedlings, stem and root apices. Cellular respiration can be of two types: **aerobic** and **anaerobic**.
- **Aerobic respiration** is that type of respiration in which organic food is completely oxidised with the help of oxygen (as terminal oxidant) into carbon dioxide and water. It takes place inside the cell's mitochondria.



- **Anaerobic respiration** is a type of respiration where oxygen is not used as an oxidant and the organic food is broken down incompletely to liberate energy due to breaking of bonds between various types of atoms. The common products of anaerobic respiration are  $\text{CO}_2$ , ethyl alcohol and lactic acid.



- **Respiratory substrates** are those organic substances which can be catabolised to liberate energy inside the living cells. The most common respiratory substrate is glucose, a hexose monosaccharide. Fats are used as respiratory substrates by a number of organisms because they contain more energy as compared to carbohydrates. Proteins are used in respiration only rarely, such as during germination of protein rich seeds and spores.
- Plants require oxygen for respiration and release carbon dioxide. For this gaseous exchange, they unlike animals, have no specialised organs. It occurs through stomata and lenticels.

### Reasons for absence of respiratory organs in plants

- Each part of plant takes care of its own gas exchange needs. There is little transport of gases from one part to another
- They do not require much demands for gas exchange.
- Leaves are well adapted to take care of their own needs of gases.
- Loose parenchyma cells in leaves, stem and roots provide interconnecting network of air spaces for quick gas exchange.
- Thus most cells of a plant have atleast one part of their surface in contact with air.

### MECHANISM OF CELLULAR RESPIRATION

- First step of cellular respiration is glycolysis which is common to both aerobic and anaerobic respiration. Later on, the processes are different. In anaerobic respiration glycolysis is followed by fermentation while in aerobic respiration Krebs' cycle is entered.

#### Glycolysis

- Glycolysis was discovered by three German scientists Embden, Meyerhof and Paranas, so also called **EMP pathway**. Glycolysis occurs in cytoplasm. It is present in all cells. In this one molecule of glucose is reduced to **two molecules of pyruvate**. During glycolysis, there is no production of  $\text{CO}_2$  molecules as there is no decarboxylation. It is the hub of carbohydrate metabolism where all the sugars ultimately converted to glucose, are broken down to provide energy and intermediates for other metabolic pathway.
- Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g., erythrocytes, cornea, lens, etc.

#### Steps involved in glycolysis

- Glucose is phosphorylated to **glucose-6-phosphate** by ATP in the presence of enzyme hexokinase (Meyerhof, 1927) or glucokinase and  $\text{Mg}^{2+}$ .
- Glucose-6-phosphate is changed to its isomer **fructose-6-phosphate** with the help of enzyme phosphohexose isomerase.
- Fructose 6-phosphate is further **phosphorylated** by means of ATP in presence of enzyme phosphofructokinase and  $\text{Mg}^{2+}$ . The product is **fructose-1, 6-diphosphate** (or bisphosphate). The irreversible phosphorylation reaction catalyzed by phosphofructokinase is the **rate limiting step** in glycolysis. It is controlled by the concentrations of substrates, ATP and fructose-6-phosphate.
- Fructose 1, 6-diphosphate **splits up** enzymatically to form one molecule each of 3-carbon compounds, **glyceraldehyde 3-phosphate** or 3 phosphoglyceraldehyde (PGAL) and **dihydroxyacetone 3-phosphate** (DHAP) by the enzyme aldolase. The latter undergoes isomerization and changed to glyceraldehyde 3-phosphate by enzyme triose phosphate isomerase.
- In the presence of enzyme glyceraldehyde 3-phosphate dehydrogenase, glyceraldehyde 3-phosphate loses hydrogen to NAD to form NADH and accepts inorganic phosphate to form **1, 3-diphosphoglycerate**.
- One of the two phosphates of diphosphoglycerate is linked by high energy bond which can synthesise ATP and form **3-phosphoglycerate** in the presence of the enzyme phosphoglycerate kinase. The direct synthesis of ATP from metabolites is called **substrate level phosphorylation**.
- 3-phosphoglycerate is changed to its isomer **2-phosphoglycerate** by enzyme phosphoglyceromutase which transfers the phosphate from third to second carbon.

- Through the agency of enzyme enolase, 2-phosphoglycerate is converted to **phosphoenolpyruvate (PEP)**. A molecule of water is removed in the process so, called **dehydration**. The enzyme enolase is dependent on the presence of either  $Mn^{2+}$  or  $Mg^{2+}$ .
- The high energy phosphate of 2-phosphoenolpyruvate is transferred to ADP by the enzyme pyruvate kinase to generate (at this stage), 2 molecules of ATP per molecule of the glucose oxidized. The molecular form resulting from the loss of the high energy phosphate is pyruvic acid or pyruvate.

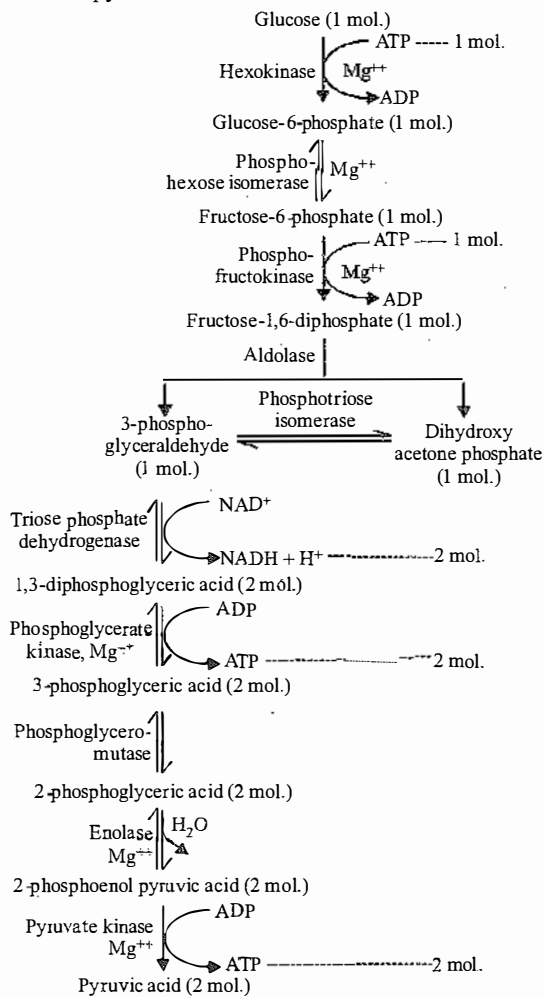


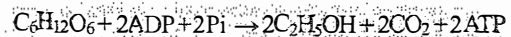
Fig.: Glycolysis or EMP-pathway.

- Since 2 ATPs are used, there is a net gain of 2 ATPs only. Net gain of ATP = 4ATP - 2ATP = 2ATP. Two molecules of NADH are formed at the time of oxidation of glyceraldehyde 3-phosphate to 1, 3- diphosphoglycerate.
- The net reaction is as follows:  

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{H}_3\text{PO}_4 \rightarrow 2\text{Pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP}$$
- Each NADH is equivalent to 3ATP. Thus total 10 ATP molecules are produced but as two ATP are consumed in early steps of glycolysis thus net gain in glycolysis is of 8 ATP.

## Anaerobic respiration

- In microorganisms, the term anaerobic respiration is replaced by **fermentation** (Cruickshank, 1897). Fermentation is also defined as anaerobic breakdown of carbohydrates and other organic compounds to form alcohol and organic acids with the help of microorganisms or their enzymes. Different methods of fermentation can be distinguished on the basis of formation of their products.
- **Alcoholic fermentation** is involved in brewing-industry for producing beverages like beer, rum, whisky etc. It is done by brewing yeast *Saccharomyces cerevisiae*.
  - In it pyruvic acid obtained from glycolysis is decarboxylated in the presence of an enzyme pyruvate decarboxylase, co-enzyme thiamine pyrophosphate (TPP) and  $Zn^{2+}$  as cofactor. It produces acetaldehyde.
  - Acetaldehyde accepts 2H-atoms from  $\text{NADH}_2$  in the presence of enzyme ethanol dehydrogenase and changes into ethanol. So in alcoholic fermentation, one glucose molecule produces two molecules of ethyl alcohol.
  - Ethanol is lost so large amount of energy goes waste.
  - Accumulation of alcohol formed by fermentation in a culture of yeast stops multiplication of yeast cells and may even lead to the death of cells.



- **Lactic acid fermentation** is used in milk-industry and in the muscles of the vertebrates. In the milk-industry it occurs in the presence of lactic acid bacteria, *Lactobacillus*. In this, pyruvic acid acts as H-acceptor and receives two H-atoms from  $\text{NADH}_2$  and changes into lactic acid. It occurs in the presence of enzyme lactate dehydrogenase which operates in the presence of FMN as coenzyme and  $Zn^{2+}$  as cofactor. So one glucose molecule produces two molecules of lactic acid. Lactic acid is excreted out so large amount of energy of glucose is lost in the lactic acid. **No  $\text{CO}_2$  is produced.**
- **Mixed acid fermentation** is a characteristic of members of family enterobacteriaceae. Mixed acid fermentation results in the formation of lactic acid, ethanol, formic acid and succinic acid.

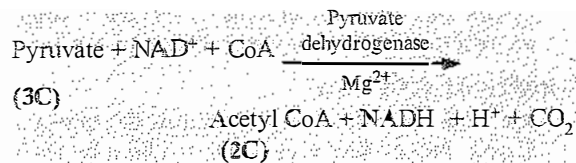
## Aerobic respiration

- Aerobic respiration is the more common form of cellular respiration. It takes place inside the cell's **mitochondria**. In aerobic respiration **oxygen** serves as the final electron acceptor, accepting electrons that ultimately come from the energy rich organic compounds. Glucose as a common energy source for cell is used in cellular respiration. **Under aerobic condition glucose metabolism after glycolysis takes place in two stages Krebs' cycle and terminal oxidation.** Pyruvate produced in glycolysis must be oxidised first to enter Krebs' cycle.

## Pyruvate oxidation

- The next step after glycolysis is the citric acid cycle or Krebs' cycle Pyruvate, the end product of glycolysis enters mitochondrial matrix through a specific transport

protein. It is decarboxylated oxidatively to produce CO<sub>2</sub> and NADH. The product combines with sulphur containing coenzyme A to form acetyl CoA or activated acetate. The reaction occurs in the presence of an enzyme complex pyruvate dehydrogenase made up of pyruvate decarboxylase whose prosthetic group is coenzyme thiamine pyrophosphate (TPP); dihydrolipoyl transacetylase (prosthetic group is lipoic acid) and dihydrolipoyl dehydrogenase (prosthetic group is flavin adenine dinucleotide FAD) and Mg<sup>2+</sup>.



- It is called **gateway step** or **link reaction** because acetyl CoA acts as a connecting link between glycolysis and Krebs' cycle. This formation of acetyl CoA is the intermediate step and results in the production of 2 molecules of CO<sub>2</sub> and 2 molecules of NADH<sub>2</sub>.

**Krebs' cycle**

- The citric acid cycle or TCA cycle was proposed by **Hans Adolf Krebs** in **1937** based on the studies of oxygen consumption in pigeon breast muscle. The cycle is named in his honour (Nobel Prize for Physiology and Medicine in 1954). Citric acid cycle essentially involves the oxidation of acetyl coenzyme A (CoA) to carbon dioxide (CO<sub>2</sub>) and water (H<sub>2</sub>O). Krebs' cycle operates in the **mitochondrial matrix** (the power house of cell) because it contains complex mixture of soluble enzymes that catalyse the respiration of pyruvic acid and other small molecules. The cycle consists of 10 enzymatic steps, four of which are dehydrogenations. These steps are discussed hereafter.

- Condensation** – Acetyl CoA (2-carbon compound) combines with oxaloacetate (4-carbon compound) in the presence of condensing enzyme citrate synthase to form a tricarboxylic 6-carbon compound called **citric acid** or **citrate**. It is the first product of Krebs' cycle. CoA is liberated.
- Dehydration** – Citrate undergoes reorganization in the presence of aconitase forming *cis* – aconitate and water.
- Hydration I** – *Cis*-aconitate is converted into **isocitrate** with the addition of water in the presence of iron containing enzyme aconitase.

- Dehydrogenation I (Oxidation)** – Isocitrate is dehydrogenated to **oxalosuccinate** in the presence of enzyme isocitrate dehydrogenase and Mn<sup>2+</sup>. NADH<sub>2</sub> is produced.
- Decarboxylation I** – Oxalosuccinate (unstable) is decarboxylated to form **α-ketoglutarate** through the enzyme decarboxylase. This step releases one CO<sub>2</sub>.
- Dehydrogenation II and Decarboxylation II** – α-ketoglutarate is both dehydrogenated (with the help of NAD<sup>+</sup>) and decarboxylated by an enzyme complex α-ketoglutarate dehydrogenase. The enzyme complex contains TPP and lipoic acid. The product combines with CoA to form **succinyl CoA**.
- Substrate level phosphorylation of GDP** – To continue the cycle, succinyl CoA is converted to **succinic acid** by the enzyme succinyl thiokinase or succinyl CoA synthetase. This reaction requires GDP (Guanosine diphosphate). In the presence of inorganic phosphate, the high energy bond of succinyl CoA is transferred to GDP converting

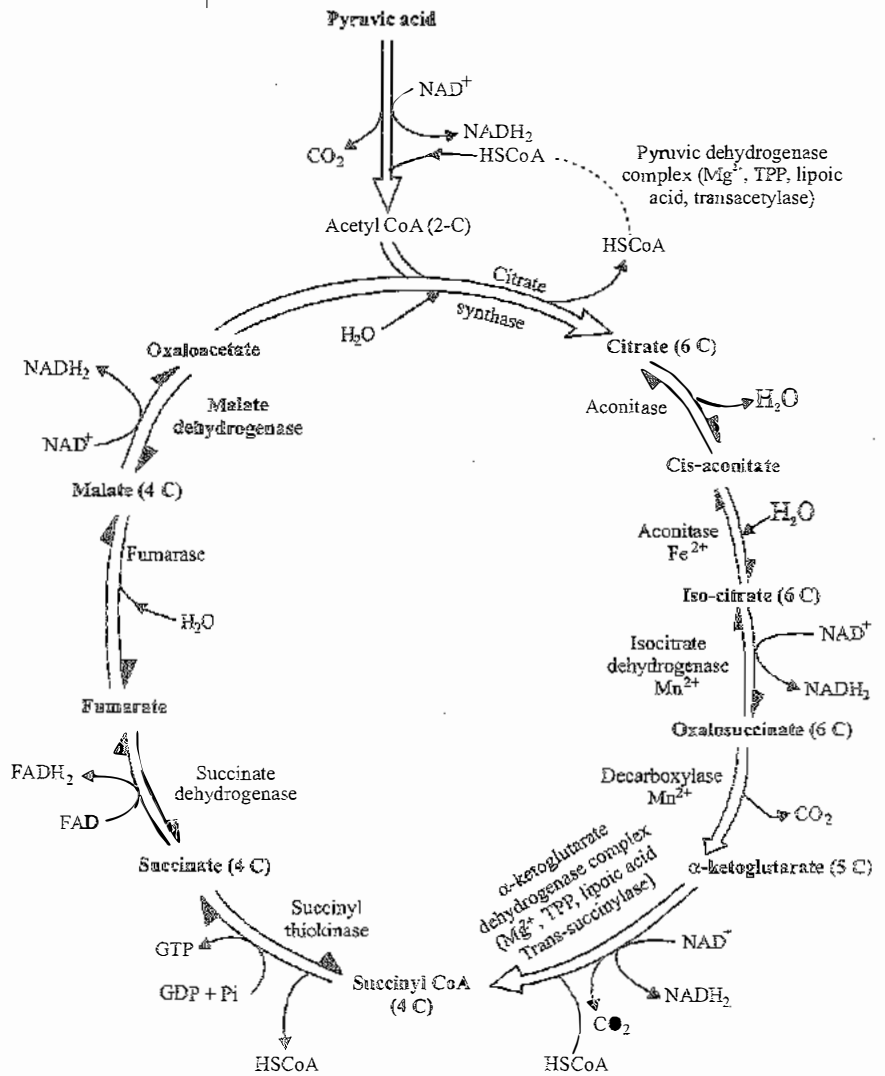
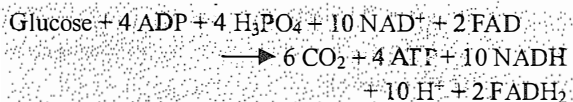


Fig.: Schematic representation of Krebs' cycle

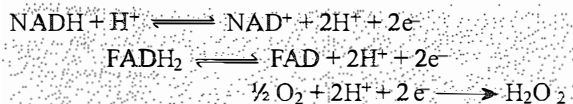
it to GTP. This is the only step in the citric acid cycle where a high energy phosphate bond is generated at the substrate level.

- By the action of **phosphokinase** ATP may be formed from GTP. A nucleoside diphosphokinase catalyses phosphate transfer from GTP to ADP.
- **Dehydrogenation III** – Succinic acid is metabolized to **fumaric acid** by dehydrogenation catalysed by succinic dehydrogenase. It is the only dehydrogenation reaction in the citric acid cycle which involves the direct transfer of hydrogen from the substrate to FAD without the participation of NAD. Since NAD is bypassed, one of the sites of ATP formation is eliminated during the electron transport. Hence this dehydrogenation yields only two ATP molecules per molecule of substrate rather than the common three molecules of ATP for the entire system.
- **Hydration II** – A molecule of water gets added to fumarate to form malate. The enzyme is called fumarase.
- **Dehydrogenation IV** – Malate is dehydrogenated or oxidised through the agency of malate dehydrogenase to produce oxaloacetate. Hydrogen is accepted by  $\text{NAD}^+$ .
- Oxaloacetate picks up another molecule of activated acetate to repeat the cycle, as two molecules of acetyl-CoA are formed from one molecule of glucose. The whole reaction can be summarised as:



### Terminal oxidation

- It is culmination of energy-yielding metabolism in aerobic organisms. All oxidative steps in the degradation of carbohydrates, fats and amino acids converge at this final stage of cellular respiration in which the energy of oxidation drives the synthesis of ATP.



- Terminal oxidation consists of two processes – electron transport chain and oxidative phosphorylation.

### ELECTRON TRANSPORT CHAIN

- The inner mitochondrial membrane has groups of several proton ( $\text{H}^+$ ) and electron ( $\text{e}^-$ ) acceptors. These groups are arranged in a specific series called **electron transport chain (ETC)** or **electron transport system (ETS)**.
- The electron transport chain is comprised of **four complexes** and two **mobile electron carriers** i.e. **coenzyme Q** - a non-protein part of the chain (except this all the members of the chain are proteins) and **cytochrome c**.
- **Complex I** or **NADH-Q-reductase** (largest complex) consists of flavoproteins of NADH dehydrogenase (FPN) of which **FMN** is the prosthetic group. Combined with the flavoprotein is non-heme iron of NADH dehydrogenase. This complex spans inner mitochondrial membrane and is able to translocate protons across it from matrix side to outer (cytosol) side.

- **Complex II** or **succinate-Q-reductase** consists of flavoprotein of succinate dehydrogenase, of which **FAD** is the prosthetic group. Combined with the flavoprotein is non-heme iron of succinate dehydrogenase.
- **Complex III** or **QH<sub>2</sub>-cytochrome-c-reductase** consists of cytochrome *b* and cytochrome *c*<sub>1</sub>. Associated with cytochrome *b* is non-heme iron of complex III. Between complexes II and III is the mobile carrier – coenzyme Q (CoQ) or ubiquinone (UQ).
- **Complex IV** or **cytochrome-c-oxidase** consists of cytochrome *a* and cytochrome *a*<sub>3</sub> and bound copper that are required for this complex reaction to occur.

### Transfer of electrons

- Electrons from NADH produced in the mitochondrial matrix during Krebs' cycle are oxidised by an **NADH dehydrogenase (complex I)**, and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via **FADH<sub>2</sub> (complex II)** that is generated during oxidation of **succinate** in the Krebs' cycle.
- The reduced ubiquinone (QH<sub>2</sub>) serves as a mobile carrier of electrons and protons. It passes electrons to complex III, which passes them to another mobile **connecting link**, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to  $\text{O}_2$ .
- Electron flow through complexes I, III and IV is accompanied by proton flow from the matrix to the intermembrane space.

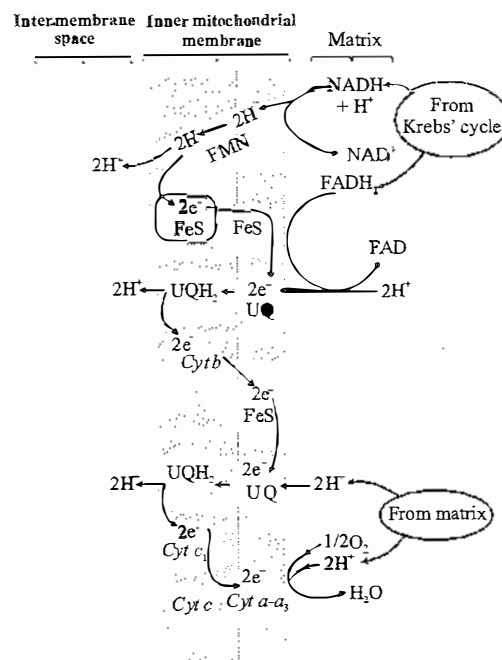


Fig.: Electron transport system (ETS).

### Shuttle system

- The **NADH** formed during glycolysis is present in the **cytoplasm**. It must reach the inner membrane of mitochondria where ETS is located so that ATP may be produced.
- The inner mitochondrial membrane is impermeable to NADH. A special electron carrier system, called the **shuttle**

system, located in the mitochondrial membrane picks up the electrons from the hydrogen of NADH present in the cytoplasm, transfers them across the mitochondrial membranes, and delivers them to the electron carriers inside the mitochondrion.

- There are two distinct shuttle systems:
  - **Malate – aspartate shuttle** : When this electron shuttle occurs, transfer of electrons from NADH<sub>2</sub> in cytoplasm occurs to NAD inside the mitochondria.
  - **Glycerol – phosphate shuttle** : In this shuttle transfer of electrons from NADH<sub>2</sub> in cytoplasm occurs to FAD inside mitochondria.

**Oxidative phosphorylation**

- Oxidative phosphorylation is the synthesis of energy rich ATP molecules with the help of energy liberated during oxidation of reduced coenzymes (NADH, FADH<sub>2</sub>) produced in respiration. The enzyme required for this synthesis called ATP synthase or mitochondrial ATPase (multi-polypeptide complex).
- It consists of three parts :
  - The **F<sub>1</sub> particle**, which is in the head piece of the projection observed on the matrix side of the mitochondrial crests. F<sub>1</sub> contains five associated subunits.
  - The **F<sub>0</sub> complex** localised in the membrane bilayer and containing the proton translocating mechanism. It is composed of 3-4 distinct polypeptides and one proteolipid.
  - A protein stalk that connects the two.
- The electron transport system of mitochondria is coupled at three points with the phosphorylation system. The protons (H<sup>+</sup>) originating from electron transfer are translocated by the respiratory chain across the membrane from the M (matrix) side to the C (cytosol) side or outer side.
- Transport of the electrons from NADH over ETC helps in pushing three pairs of protons to the outer chamber while two pairs of protons are sent outwardly during electron flow from FADH<sub>2</sub> (as the latter donates its electrons further down to the ETC).

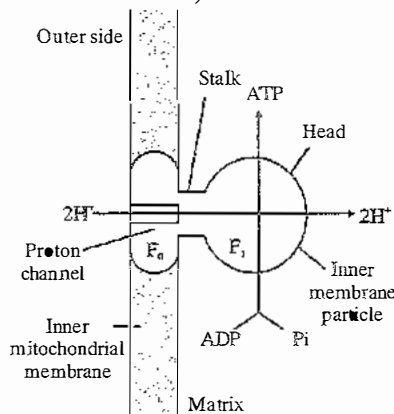


Fig.: ATP synthesis by F<sub>0</sub>-F<sub>1</sub> particle.

- According to the **chemiosmotic hypothesis** of Peter Mitchell, this translocation creates a pH difference and a membrane potential. Both constitute the **proton-motive force** that tends to move H<sup>+</sup> from the C side back to the

M side of the membrane. Since the inner mitochondrial membrane is highly impermeable to H<sup>+</sup> ions, these can only reach the M side through the “proton channel“ of the ATPase. When H<sup>+</sup> moves from the C side to the M side, the F<sub>1</sub>-ATPase, operating in reverse, catalyzes ATP synthesis. The flow of protons through the F<sub>0</sub> channel induces F<sub>1</sub> particle to function as ATP-synthase.

- The energy of the proton gradient is used in attaching a phosphate radical to ADP by high-energy bond. This produces ATP. **Oxidation of one molecule of NADH<sub>2</sub> produces 3 ATP molecules while a similar oxidation of FADH<sub>2</sub> forms 2ATP molecules.** The transfer of energy from electron flow to electrochemical proton gradient and thence to the phosphate bonds of ATP is called **oxidative phosphorylation**. The ETS is, therefore, also known as **oxidative phosphorylation pathway**.

**Table : Balance sheet of ATP during complete oxidation of one molecule of glucose.**

Stage	ATP by substrate level phosphorylation	Formation of NADH/ FADH <sub>2</sub>	ATP through ETS in mitochondria
Glycolysis	2	2 NADH	2 × 3 = 6
Formation of Acetyl-CoA	-	2 NADH	2 × 3 = 6
Krebs' cycle	2	2 FADH <sub>2</sub> 6 NADH	2 × 2 = 4 6 × 3 = 18
	4		34 (or 32)

Total net gain of ATP = 36 or 38 depending upon type of aerobic respiration.

**AMPHIBOLIC PATHWAY**

- Amphibolic pathway (Gk. *amphi*-both, *bole*-throw) is the one which is used for **both breakdown** (catabolism) and **build up** (anabolism) reactions. Krebs' cycle is amphibolic in nature.
- It is a common pathway of oxidative breakdown of carbohydrates, fatty acids, and amino acids. Thus here it performs catabolic functions.
- Krebs' cycle also provides a number of intermediates which are used in different anabolic pathways to form important biomolecules like glutamic acid, aspartic acid, etc., hence this cycle is **amphibolic in nature**.
- Acetyl CoA provides 2-carbon compounds for the synthesis of fatty acids, cutin, aromatic compounds and isoprenoids for forming phytol chain of chlorophyll, carotenoids, steroids, terpenes, gibberellins, etc.

**RESPIRATORY QUOTIENT**

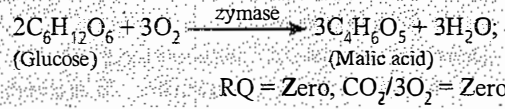
- Respiratory quotient (RQ) is the ratio of volume of carbon dioxide evolved to the volume of oxygen consumed in respiration per unit time per unit weight at standard temperature and pressure.

$$RQ = \frac{\text{Volume of CO}_2 \text{ evolved}}{\text{Volume of O}_2 \text{ consumed}}$$

- The instrument used to measure respiratory quotient and rate of respiration is called **respirometer**.

- Respiratory quotient is equal to unity if carbohydrates are respiratory substrate and the respiration is aerobic.  
 $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ ;  $RQ = 6CO_2/6O_2 = 1$
- RQ is about 0.7 for most of the common fats. It occurs during germination of fatty seeds.  
 $C_{57}H_{104}O_6 + 80O_2 \rightarrow 57CO_2 + 52H_2O$   
 (Triolein)  $RQ = 57CO_2/80O_2 = 0.71$   
 $2C_{51}H_{98}O_6 + 145O_2 \rightarrow 102CO_2 + 98H_2O$   
 (Tripalmitin)  $RQ = \frac{102CO_2}{145O_2} = 0.7$
- RQ is about 0.9 in case of proteins, peptones, etc.
- Succulents such as *Opuntia* do not evolve carbon dioxide during night (when their stomata are open) as the same is

used in carbon fixation. They also change carbohydrates to organic acids which utilise oxygen but do not evolve carbon dioxide.



- RQ slightly more than unity is found when organic acids are broken down as respiratory substrates under aerobic conditions,  
*e.g.*,  $C_4H_6O_5 + 3O_2 \rightarrow 4CO_2 + 3H_2O$ ;  
 $RQ = 4CO_2/3O_2 = 1.3$  (Malic acid)
- In anaerobic respiration there is no consumption of oxygen. Therefore, respiratory quotient is infinity.

