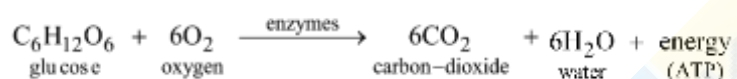


RESPIRATION IN PLANTS

INTRODUCTION

- Various cellular activities in living organisms like absorption, transport, muscle-contraction, locomotion, nerve-impulse conduction, reproduction, growth, development, seed germination or breathing require energy.
- All the energy required for 'life' processes in all living organisms comes from the oxidation of organic molecules.
- Only green plants and cyanobacteria (blue-green algae) can prepare their own food by the process of photosynthesis. In green plants, only cells containing chloroplasts carry out photosynthesis. Even in green plants all other organs, tissues and cells that are non-green, need food for oxidation.
- Animals obtain their food from plants directly (herbivores) or indirectly (carnivores). Saprophytes like fungi are dependent on dead and decaying matter for obtaining energy.
- **Cellular respiration** is an enzyme controlled process of biological oxidation of food materials in a living cell, using molecular O₂, producing CO₂ and H₂O and releasing energy in gradual steps and storing it in biologically useful forms, generally ATP.
- So respiration is catabolic, exothermic and oxidative process.



- Most of the respiration processes occur in mitochondria.
- **Respiratory substrates** are compounds that are oxidised during the process of respiration. Usually, carbohydrates are oxidised to release energy but proteins, fats and even organic acids can be used as respiratory substances in some plants, under certain conditions.
- Energy trapped in ATP is utilised in various energy requiring processes of organisms, and the carbon compounds produced during respiration are used as precursors for biosynthesis of other molecules in the cell.

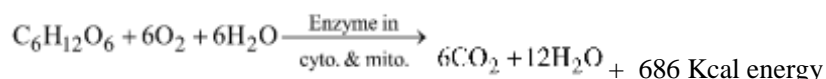
DO PLANTS BREATHE?

- Plants require O₂ for respiration to occur and they also give out CO₂. Hence, plants have systems in place that ensure the availability of O₂. Plants, unlike animals, have no specialized organs for gaseous exchange but they have stomata and lenticels for this purpose.
- Plants get along without respiratory organs because each plant part takes care of its own gas exchange. There is little transport of gases from one part to another.
- Roots, stems and leaves respire at rates far lower than animals do. Only during photosynthesis, large volumes of gases are exchanged and, each leaf is well adapted to take care of its own needs during these periods.

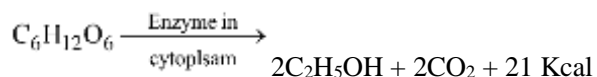
TYPES OF RESPIRATION

On the basis of the availability of oxygen and the complete or incomplete oxidation of respiratory substrate, it is of two types :

- **Aerobic respiration** : When O₂ is utilized during the process of respiration it is called aerobic respiration. In this process, there is complete oxidation of food and entire carbon is released as CO₂ and large amount of energy is released.



- **Anaerobic respiration** : When there is no utilisation of O₂ during respiration, then food substances are incompletely oxidized and produce alcohol or organic acids and most of the energy is lost in the form of heat.

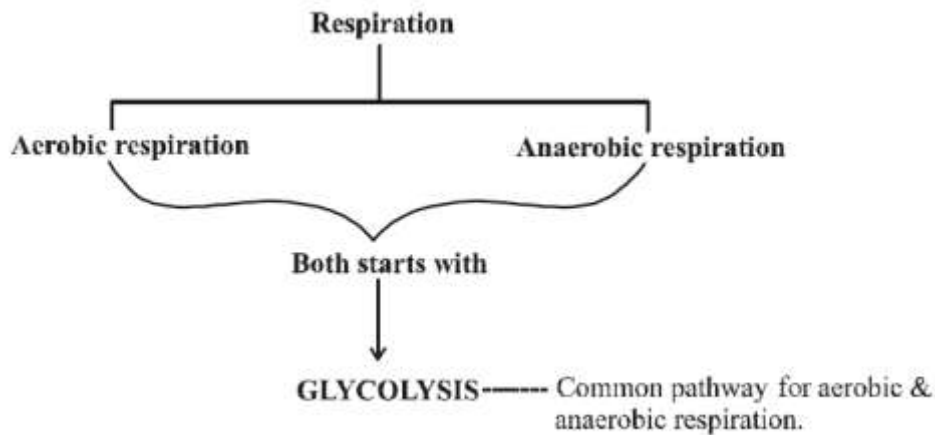


Organisms can be grouped into the following four classes on the basis of their respiratory habit

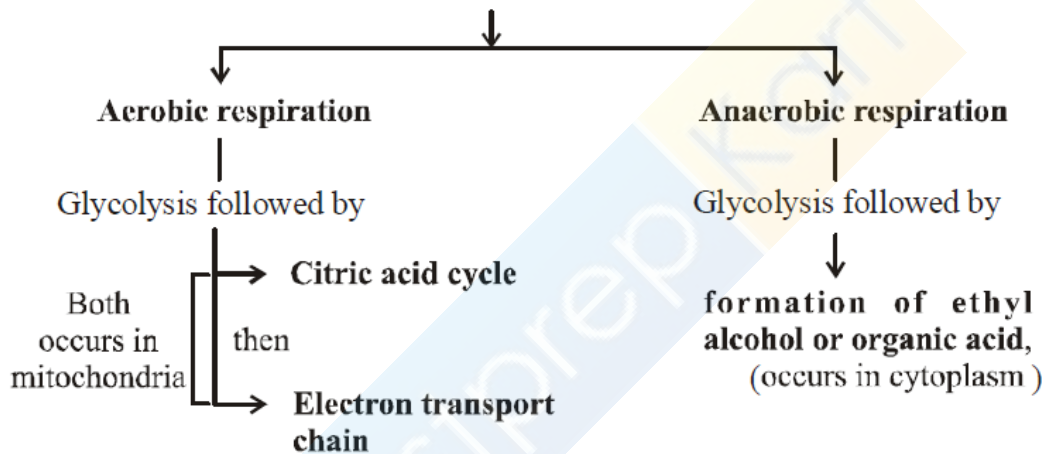
- **Obligate aerobes** : These organisms can respire only in the presence of oxygen. Thus, oxygen is essential for their survival (e.g., bacterium *Bacillus subtilis*).
- **Facultative anaerobes** : Such organisms usually respire aerobically (i.e., in the presence of oxygen) but under certain conditions may also respire anaerobically (e.g., Yeast, parasites of the alimentary canal).
- **Obligate anaerobes** : These organisms normally respire anaerobically. Such organisms are in fact killed in the presence of substantial amounts of oxygen (e.g., *Clostridium botulinum* and *C. tetani*).
- **Facultative aerobes** : These are primarily anaerobic organisms but under certain conditions may also respire aerobically (e.g., yeast).

Table : Differences between Aerobic and Anaerobic respiration

| S. No. | Aerobic respiration | Anaerobic respiration |
|--------|---|---|
| 1. | It takes place in presence of oxygen. | It does not require oxygen. |
| 2. | It always releases carbon dioxide. | It may or may not release carbon dioxide. |
| 3. | It provides much more energy (38 ATP molecules). | It provides less energy (just 2 ATP molecule). |
| 4. | It occurs both in cytoplasm (glycolysis) & in the mitochondria (Kreb's cycle & electron transport chain). | It takes place in the cytoplasm, certain tissues and cells of higher animals. |
| 5. | Examples - In most plants and animals. | Examples – In anaerobic bacteria, yeasts, muscles and parasitic worms like, <i>Ascaris</i> , <i>Fasciola</i> , <i>Taenia</i> and germinating seeds. |



- Occurs in cytoplasm.
- In this, glucose is partially oxidized into two molecules of pyruvic acids.

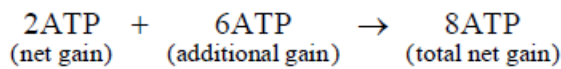


Flow Chart : Types & Mechanism of cellular respiration.

GLYCOLYSIS

- All living organisms retain the enzymatic machinery to partially oxidise glucose without the help of oxygen. This breakdown of glucose to pyruvic acid is called **glycolysis**.
- The scheme of glycolysis was given by Gustav Embden, Otto Meyerhof and J. Parnas, and is often referred to as the **EMP pathway**.
- In anaerobic organisms, it is the only process of respiration.
- Glycolysis involves a series of ten biochemical reactions in cytoplasm.
- In plants, glucose is derived from sucrose, which is the end product of photosynthesis, or from storage carbohydrates. Sucrose is converted into glucose and fructose by the enzyme, invertase, and these two monosaccharides readily enter the glycolytic pathway.
- In glycolysis, neither consumption of oxygen nor liberation of CO₂ takes place.
- In glycolysis, 1 glucose, produces 2 molecules of pyruvic acid (3C).
- In glycolysis, four molecules of ATP are formed by two ways:
 - Direct / substrate phosphorylation of ADP to ATP.
 - Another ATP is synthesized during the conversion of PEP to pyruvic acid.

- During aerobic respiration (when oxygen is available) each NADH₂ forms 3 ATP and H₂O through electron transport system of mitochondria. In this way during aerobic respiration there is additional gain of 6 ATP in glycolysis



- Glycolysis is also known as **oxidative anabolism** or **catabolic resynthesis**, because it is linked with anabolism of fats and amino acids. An intermediate **phosphoglyceraldehyde** (PGAL) is used for the synthesis of glycerol which later forms fats or lipids. PGA is used for synthesis of amino acids like serine, glycine, cystine. Alanine forms from pyruvate.

- **Phosphofructokinase** is an allosteric enzyme. The phosphorylation of fructose-6-phosphate is the most important regulation reaction of glycolysis.

- Phosphofructokinase has multiple allosteric modulators. It's activity is inhibited by ATP (-ve modulator) and stimulated by ADP & AMP (+ve modulator).

- The end product of glycolysis are 2 molecules of pyruvic acid, NADH + H⁺, H₂O and ATPs.

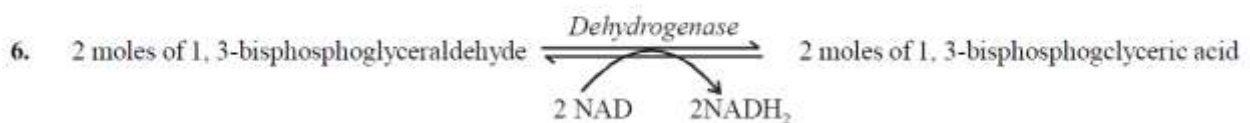
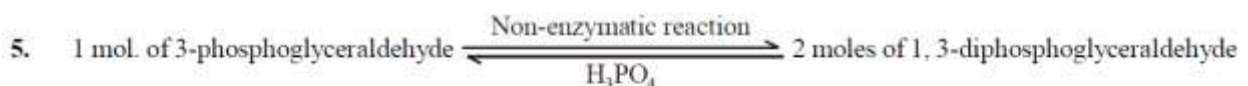
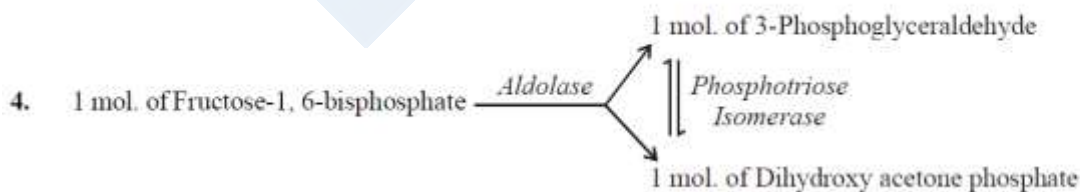
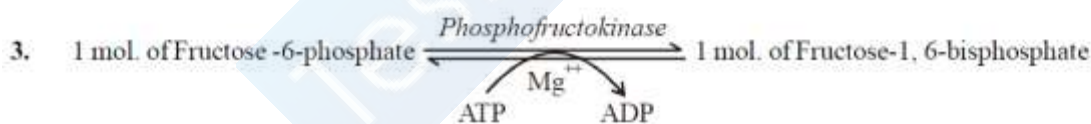
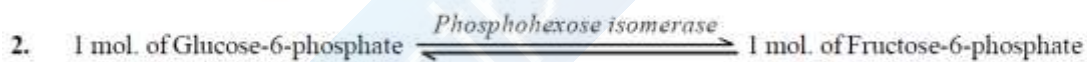
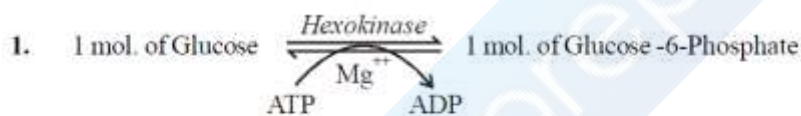
- Further oxidation of pyruvic acid and NADH₂ after glycolysis in mitochondria requires oxygen.

- Pyruvic acid is the key product of glycolysis. The metabolic fate of pyruvate depends on the cellular need.

- Further fate of pyruvic acid depends upon the availability of O₂ and one of the given three routes is followed

- Lactic acid fermentation
- Aerobic respiration
- Alcoholic fermentation

BIO-CHEMICAL REACTIONS OF GLYCOLYSIS



[Iodoacetate inhibits this reaction]

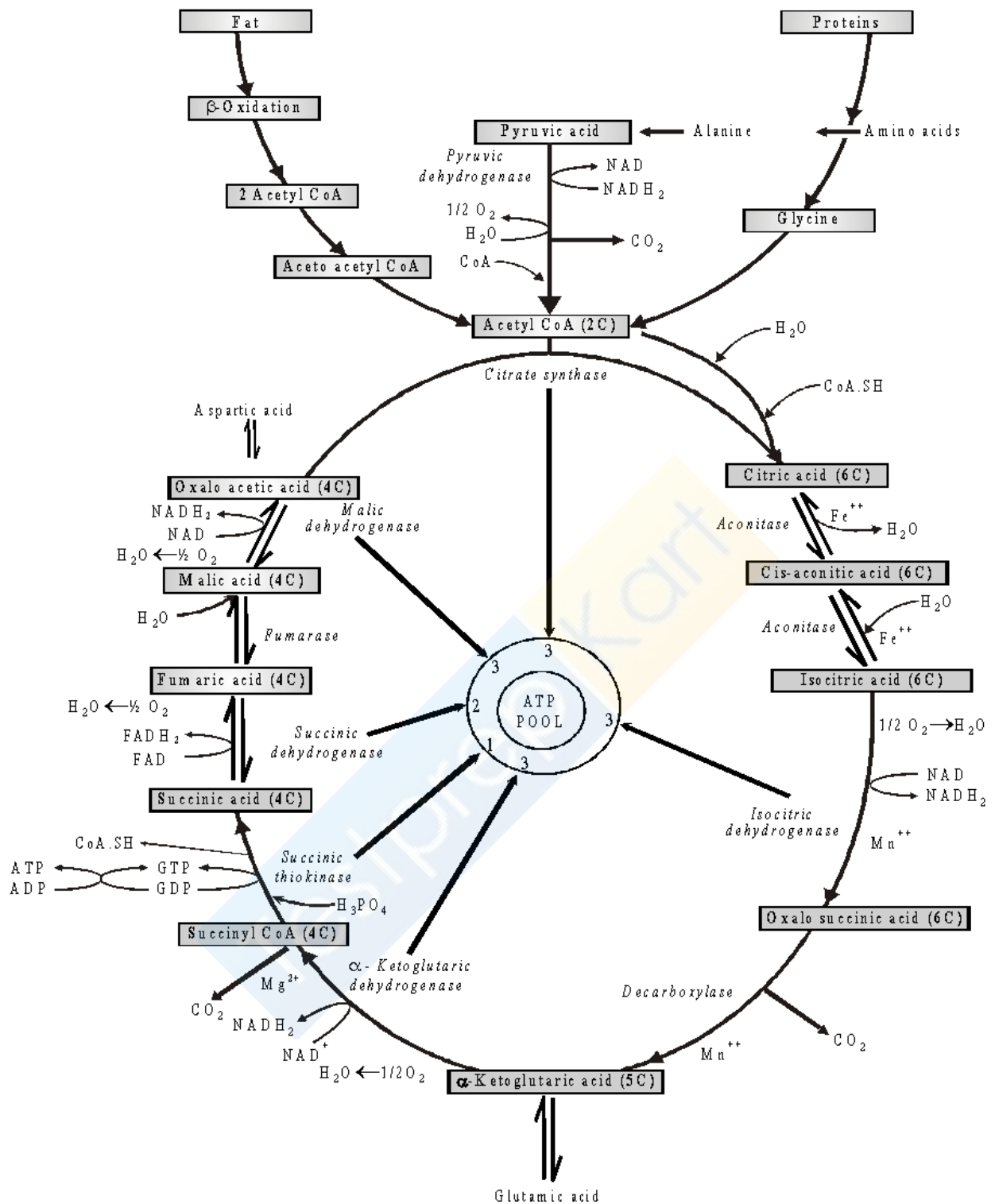
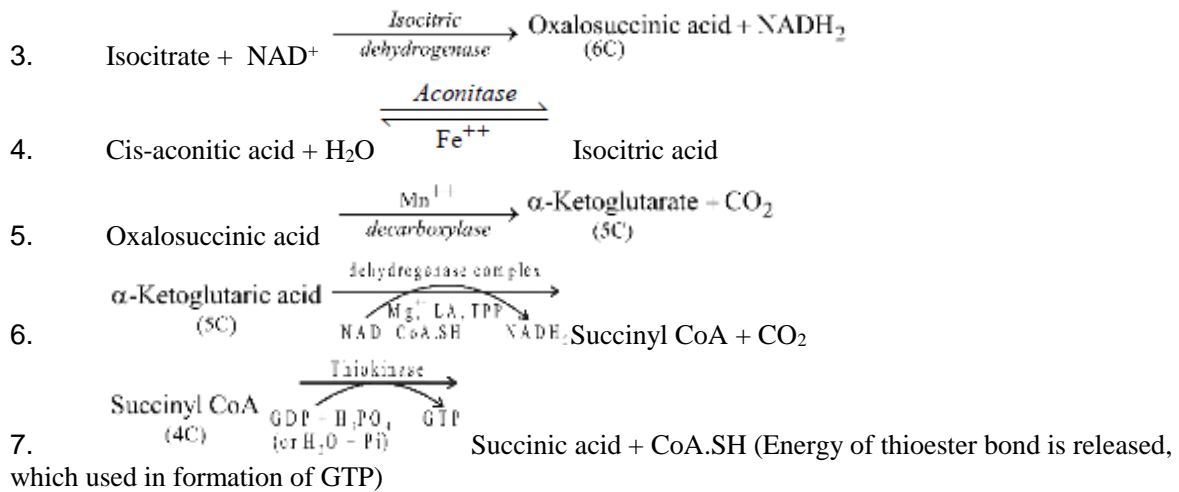


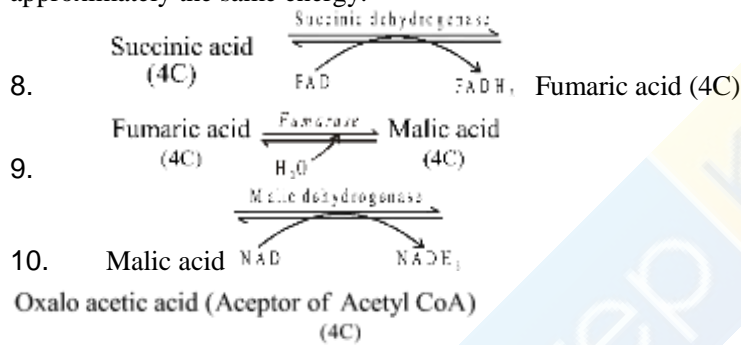
Fig. : Diagrammatic representation of oxidative decarboxylation of pyruvic acid and different chemical reactions in Krebs's cycle starting from Acetyl CoA

BIO-CHEMICAL REACTIONS IN KREBS CYCLE

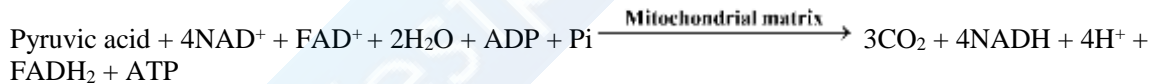
- $$\text{Acetyl CoA (2C)} + \text{OAA (Oxaloacetic acid) (4C)} + \text{H}_2\text{O} \xrightarrow[\text{Synthase}]{\text{Citrate}} \text{Citric acid} + \text{CoA.SH}$$
- $$\text{Citric acid} \xrightleftharpoons[\text{Fe}^{++}]{\text{Aconitase}} \text{Cis-aconitic acid (6C)} + \text{H}_2\text{O}$$



The GTP formed in reaction 7, reacts with ADP to form ATP and GDP, as GTP and ATP have approximately the same energy.



The summary equation for this phase of respiration may be written as follows :



Because of the decomposition of one molecule of glucose, 2 molecules of Acetyl CoA are formed. So, due to decomposition of 1 molecule of glucose, the cycle runs two times.

Total energy production in TCA cycle

| | |
|--|--------|
| 6 NAD.2H (NAD.2H \longrightarrow 3ATP) | 18 ATP |
| 2 GTP (GTP \longrightarrow 1ATP) | 2 ATP |
| 2 FAD.H ₂ (FAD.H ₂ \longrightarrow 2ATP) | 4 ATP |
| | 24 ATP |

DIFFERENCES BETWEEN GLYCOLYSIS AND KREB'S CYCLE

| S. No. | Glycolysis | Kreb's cycle |
|--------|--|---|
| 1. | It takes place in the cytoplasm. | It takes place in the matrix of mitochondria. |
| 2. | It occurs in aerobic as well as anaerobic respiration. | It occurs in aerobic respiration only. |
| 3. | It is a linear pathway. | It is a cyclic pathway. |
| 4. | It oxidizes glucose partly, producing pyruvate. | It completely oxidises acetyl coenzyme A. |
| 5. | It consumes 2 ATP molecules. | It does not consume ATP. |
| 6. | It generates 2 ATP molecules from 1 glucose molecule. | It generates 2 GTP/ATP molecules from 2 succinyl coenzyme A molecules |
| 7. | It yields 2 NADH per glucose molecule. | It yields 6 NADH molecules and 2 FADH ₂ molecules from 2 acetyl coenzyme A molecules. |
| 8. | It does not produce CO ₂ . | It produces CO ₂ . |
| 9. | All enzyme catalysing glycolytic reactions are dissolved in cytosol. | Enzymes of Kreb's cycle reactions are located both in the inner mitochondrial membrane and in the matrix. |

ELECTRON TRANSPORT SYSTEM (ETS) AND OXIDATIVE PHOSPHORYLATION

- The metabolic pathway through which the electrons pass from one carrier to another, is called the electron transport system and it is present in the inner mitochondrial membrane.
- The system consists of a series of precisely arranged nine electron carriers (coenzyme) in the inner membrane of the mitochondrion. These nine electron-carriers function in a specific sequence: Nicotinamide adenine dinucleotide (NAD), Flavin mononucleotide (FMN), Flavin adenine dinucleotide (FAD), Co-enzyme-Q or ubiquinone, Cytochrome-b, Cytochrome-c₁, Cytochrome-c, Cytochrome-a and Cytochrome-a₃.
- The ETC is comprised of four complexes and two mobile carriers i.e. coenzyme Q, a non protein part of the chain
- **Complex I** : Consists of flavoproteins of NADH dehydrogenase (FP_N).
- **Complex II** : Consists of flavoproteins of succinic dehydrogenase.
- Between complexes II and III, is the mobile carrier-coenzyme Q (CoQ) or ubiquinone (UQ).
- **Complexes III** : Consists of cytochrome b and cytochrome c₁. Associated with cytochrome b is the non-haeme iron of complex III (Fe NH_R).
- **Complex IV** : Consists of cytochrome a and cytochrome a₃ and bound copper that are required for this complex reaction to occur.
- The electrons either follow the pathway of complexes I, III and IV or II, III and IV.
- Electrons from NADH produced in the mitochondrial matrix during citric acid cycle are oxidized by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane.
- Ubiquinone also receives reducing equivalents via FADH₂ generated during the oxidation of succinate by succinate dehydrogenase (complex II).
- The reduced **ubiquinone**, called **ubiquinol**, is then oxidized by transfer of electrons to cytochrome c, cytochrome bc₁ – complex (complex III).
- Cytochrome c acts as a mobile carrier between complex III and complex IV.
- Complex IV refers to cytochrome c oxidase complex containing cytochromes a and a₃ and two copper centres.
- When the electrons pass from one carrier to another carrier via complex I to IV in the electron transport chain, they are coupled to ATP synthase (complex V) for the formation of ATP from ADP and Pi.
- Oxygen functions as the terminal acceptor of electrons and is reduced to water along with the hydrogen atoms. It drives the whole process by removing hydrogen from the system.
- In respiration, energy of oxidation-reduction is utilized for the production of proton gradient.

- Higher proton concentration in the outer chamber causes the protons to pass inwardly into the matrix or inner chamber through the inner membrane.
- The energy of the proton gradient is used in attaching a phosphate radicle to ADP by high energy bond. So the process is called **oxidative phosphorylation**.
- Oxidation of one molecule of NADH_2 produces 3 ATP molecules while a similar oxidation of FADH_2 forms 2 ATP molecules.

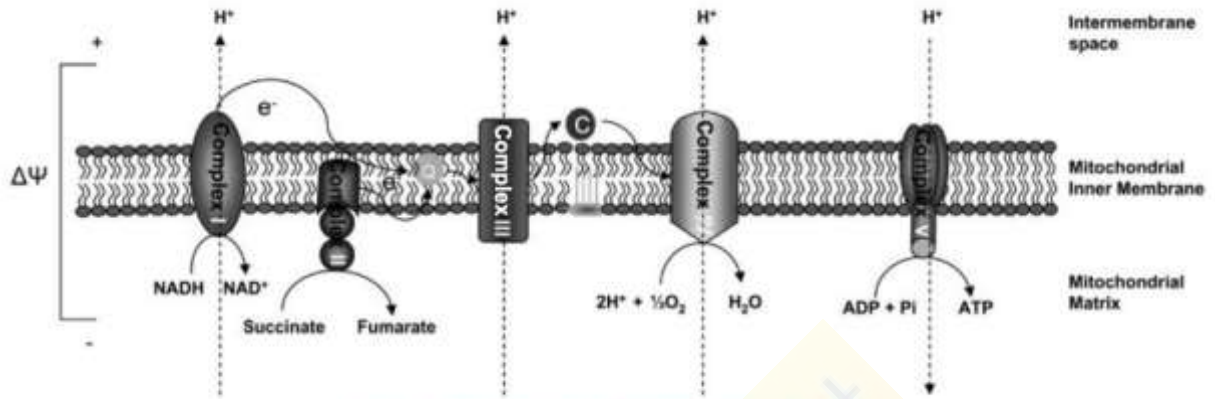


Fig. : Mitochondrial electron transport system (ETS)

- **ATP synthase** (complex V) helps in ATP synthesis. It consists of two major components F_1 and F_0 . F_1 (head piece) is a peripheral membrane protein complex and contains the site for ATP synthesis while F_0 is an integral membrane protein complex that forms a channel through which protons cross the inner membrane. For each ATP produced, $2H^+$ passes through F_0 from the intermembrane space to the matrix down the electrochemical proton gradient.

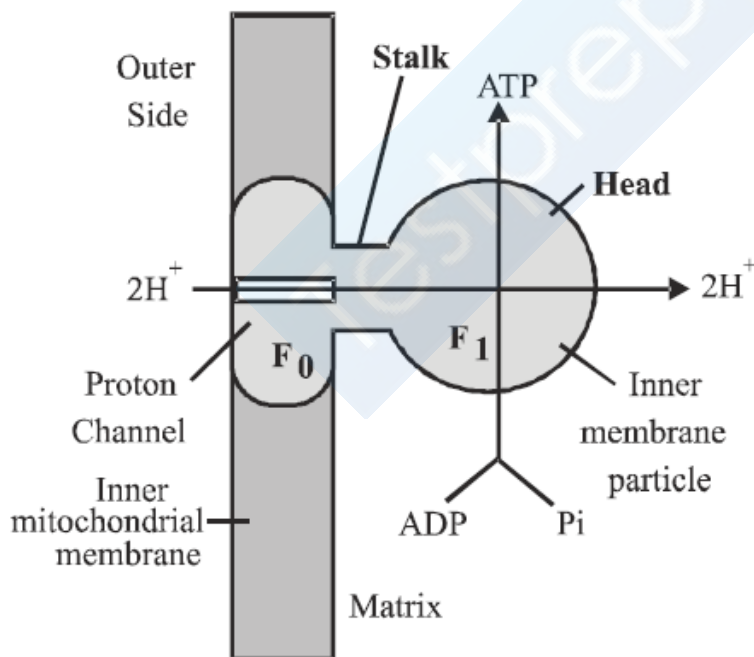


Fig. : ATP synthesis by F_0-F_1 particle

| Stages | ATP produce by substrate phosphorylation | Formation of NADH / FADH | ATP produce through ETS in Mitochondria |
|--|--|---|---|
| Glycolysis in cytoplasm | 2 | 2NADH (one NADH on oxidation) through ETS form 3 or 2 ATP depending upon shuttle system | $2 \times 3 = 6$ |
| Formation of Acetyl ~ CoA in matrix of mitochondria | – | 2 NADH | $2 \times 3 = 6$ |
| Krebs cycle | 2 | 2 FADH ₂ 6 NADH | $2 \times 2 = 4$ $6 \times 3 = 18$ |
| | 4 | | 34 (or 32) |
| Total net gain of ATP = 36 or 38 depending upon type of aerobic respiration. | | | |

RESPIRATORY CHAIN INHIBITORS

- **Rotenone** : It checks flow of electrons from NADH / FADH₂ to CoQ.
- **Antimycin A** : Transfer of electrons from Cyt b to Cyt c₁ is prevented.
- **Cyanide** : It prevents flow of electrons from Cyt a₃ to oxygen.
- **Dinitrophenol (2, 4-DNP)** : It prevents synthesis of ATP from ADP because it directs electrons from coQ to Q₂.

ROLE OF SHUTTLE SYSTEM IN ENERGY PRODUCTION

Glycolysis occurs in the cytoplasm outside the mitochondrion in which 2NADH₂ molecules are produced but ETC is located along the inner mitochondrial membrane, so NADH₂ of glycolysis must enter inside the mitochondrion to release energy. But the inner mitochondrial membrane is impermeable to NADH₂. In mitochondrial membrane, there are 2 shuttle-systems, each formed of carrier-molecules. These shuttle system are - **malate aspartate system** and **glycerol phosphate shuttle system**.

- **Malate-Aspartate shuttle** : When this electron shuttle operates, transfer of electrons takes place from NADPH₂ (in cytoplasm) to NAD inside the mitochondria. This is more efficient and results in production of 38 ATP molecules.

- **Glycerol-Phosphate shuttle** : In this shuttle, electrons are transferred from NADH₂ (in cytoplasm) to FAD (inside mitochondria). It results in production of 36 ATP molecules. It is less efficient and results in the reduction of FAD inside the mitochondrion.

GLYOXYLATE CYCLE

- Discovered by Kornberg & Krebs, during germination of fatty seeds.
- This cycle converts fats into sugars, so it is an example of gluconeogenesis in plants.
- Glyoxylate cycle occurs in glyoxysome, cytosol and mitochondria.

RESPIRATORY BALANCE SHEET

The calculations of net gain of ATP, for every glucose molecule oxidized, is made on certain assumptions that are as follows :

- There is a sequential, orderly pathway functioning, with one substrate forming the next with glycolysis, TCA cycle and ETS pathway following one after another.
- The NADH synthesized in glycolysis is transferred into the mitochondria and undergoes oxidative phosphorylation.
- Hence, there can be net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose.

Table : Differences between Aerobic Respiration and Fermentation

| S. No. | Aerobic respiration | Fermentation |
|--------|--|--|
| 1. | Complete oxidation of organic substances in the presence of oxygen, and releases CO ₂ , H ₂ O and a large amount of energy (in the form of ATP). | Incomplete oxidation of glucose is achieved under anaerobic conditions where pyruvic acid is converted into CO ₂ and ethanol and some bacteria produce lactic acid from pyruvic acid. |
| 2. | There can be a net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose. | There is a net gain of only two molecules of ATP for each molecule of glucose degraded to pyruvic acid. |
| 3. | Oxidation of NADH to NAD ⁺ is vigorous. | Oxidation of NADH to NAD ⁺ is quite slow. |
| 4. | Molecular oxygen is the ultimate electron acceptor for biological oxidation. | The final electron acceptors are organic compounds. |

AMPHIBOLIC PATHWAY

- Respiration involves the breakdown of organic compounds (glucose, pyruvate, acetyl co-A), so it has been considered as a catabolic process.

- Many amino-acids (α -ketoglutarate etc.) and fatty acids precursors are formed, so it is also an anabolic process.
- As it constitutes both catabolic and anabolic process, it is known as an amphibolic process.

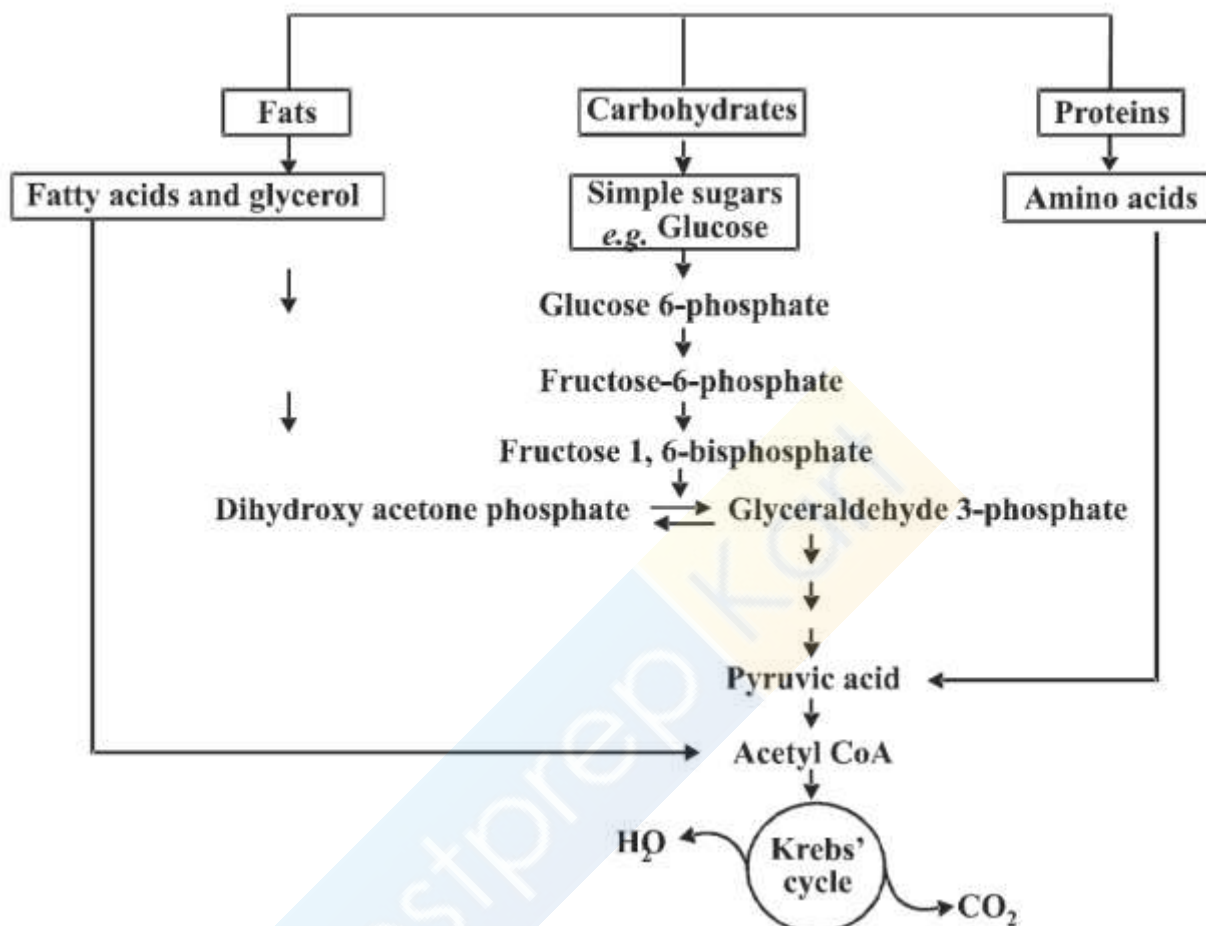


Fig. : Inter-relationship among metabolic pathways showing respiration mediated breakdown of different organic molecules to CO_2 and H_2O

RESPIRATORY QUOTIENT (R.Q.)

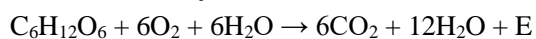
The ratio of the volume of CO_2 released to the volume of O_2 taken in during respiration is called Respiratory Quotient (R.Q.)

$$\text{R.Q.} = \frac{\text{Volume of } \text{CO}_2 \text{ evolved}}{\text{Volume of } \text{O}_2 \text{ absorbed}}$$

Value of R.Q. depends upon the nature of respiratory substrate used, amount of CO_2 present in respiratory substrate, extent to which substrate is broken down, inter-conversion of one substrate, into another in the cell.

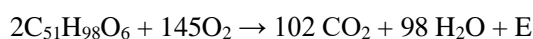
It is measured by **Ganong's respirometer**.

- **Carbohydrates**



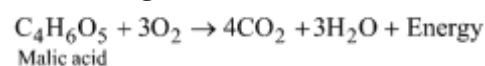
$$\text{RQ} = \frac{\text{CO}_2}{\text{O}_2} = \frac{6}{6} = 1 \text{ (Unity)}$$

- **Fat/Oil**



$$RQ = \frac{CO_2}{O_2} = \frac{102}{145} = 0.7 \text{ (less than unity)}$$

- **Organic acids**



$$RQ = \frac{CO_2}{O_2} = \frac{4}{3} = 1.33 \text{ (more than unity)}$$

Oxalic acid, R.Q. = 4

Citric acid, R.Q. = 1.3

- **Incomplete oxidation of carbohydrates** (in the respiration of succulents i.e., Bryophyllum, Opuntia)

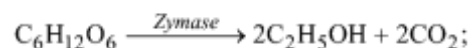


$$RQ = \frac{CO_2}{O_2} = \frac{0}{3} = 0$$

- **Proteins**

R.Q. = 0.8 or 0.9 or < 1

- **Respiration in the absence of O₂** (anaerobic respiration)



$$RQ = \frac{CO_2}{O_2} = \frac{2}{0} = \infty$$

FACTORS AFFECTING THE RATE OF RESPIRATION

- **Temperature :**

- Optimum temperature for respiration is between 20-35°C. Maximum temperature is around 45°C.

- At low temperature, respiration is low due to inactivation of enzymes (refrigerator preserves food) while at very high temperature enzymes get denatured. Temperature coefficient $Q_{10} = 2$ to 2.5 for respiration.

- **Oxygen :** The inhibition of anaerobic respiration by increase in concentration of O₂ is called as Pasteur's effect.

- **CO₂ :** If CO₂ concentration increases, then the rate of respiration decreases in plants because stomata get closed.

- **Salts :** If a plant is transferred from water to salt solution, its respiration increases, this is known as salt respiration because absorption of ions requires metabolic energy.

- **Hormones :** IAA, GA and cytokinin increase the respiration rate.

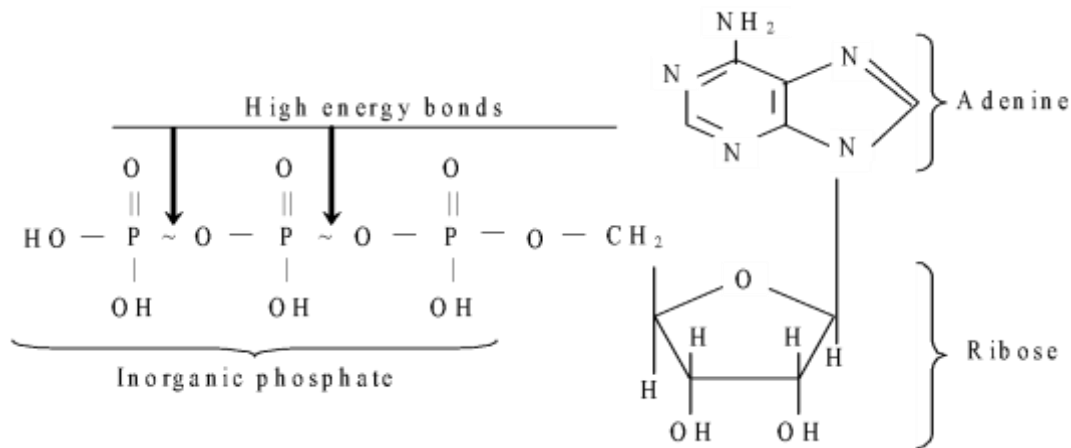
The rapid increase in the rate of respiration during ripening of fruits and senescence of leaves and plant organs is called as "climacteric respiration". This rate decreases after sometime. It is due to production of ethylene hormone.

- **Light :** Rate of respiration increases with increase in light intensity. Light controls the stomatal opening and influence temperature and also produces respiratory substrates.

- **Injury, disease & wounds :** Respiration increases due to injury, wounding & infection.

- **Age :** Rate of respiration is more in young cells. Rate of respiration at meristem apex is high.

STRUCTURE OF ATP



Adenosine triphosphate $\xrightarrow{\text{hydrolysis}}$ Adenosine diphosphate (ADP) + Pi + 7.3Kcal....

Adenosine diphosphate $\xrightarrow{\text{hydrolysis}}$ Adenosine monophosphate (AMP) + Pi + 7.3Kcal.

- Nicotinamide adenine dinucleotide phosphate / Nicotinamide adenine dinucleotide (NADP/NAD)** : It is called universal hydrogen acceptor, produced during aerobic respiration (glycolysis+Kreb's cycle) and also in anaerobic respiration, works as a coenzyme in ATP generation via electron transport system. NADP has one additional phosphate.
- NAD plays a crucial role in dehydrogenation processes. Some dehydrogenases do not work with NAD, but react with NADP (Nicotinamide adenine dinucleotide phosphate). Nicotinamide is a vitamin of B group.
- Initially NAD and NADP both function as hydrogen acceptors. Later H^+ ions and electrons (e^-) from these are transported through a chain of carriers and after being released at the end of a chain, react with O_2 and form H_2O . During the release of 2 electrons from $2H^+$ atoms from NAD_2H and their reaction with O_2 to form water, 3 ATP molecules are synthesized from NAD or NADP.