RESPIRATION

- It is a **catabolic** and **exergonic** process.
- The bonds between C – C of carbohydrates, fatty acids, aminoacids and other organic acids are broken to release considerable amount of energy.
- The cell organelle involved in aerobic respiration is **Mitochondrion.**
- Its inner membrane is highly invaginated to form **cristae**. It is selectively permeable and energy transducing membrane.
- Inner membrane is permeable to **water, CO_2 and O_2**. Its permeability to **protons is very low** or absent. It is significant for ATP synthesis. Electron transport system is operated in this membrane.
- The gap between inner and outer membrane is called as inter membrane space.
- The stalked particles on the inner membrane are called as **F_0 – F_1** complex. These are centres of Oxidative phosphorylation.
- Matrix has about **70%** of respiratory enzymes.
- The energy released in respiration is liberated in the form of **heat** or trapped in **ATP**. ATP is known as energy currency. [$C_{10}H_{16}N_5O_{13}P_3$ – ATP]
- Whenever energy is required, it is released from ATP upon hydrolysis. ATP moves freely in the cell.
- ATP is a nucleotide containing a **Ribose sugar, Adenine** and **three inorganic phosphates** named as **α, β** and **γ**. The bond between β and γ phosphates is high energy bond, which releases **7.6 k.cal** upon hydrolysis.
- During respiration the sugars are modified to form other substances required for cell structure, growth and development.
- Respiration is of two types known as **Aerobic** and **Anaerobic** respirations.

Mechanism of Aerobic respiration

- It is defined as complete oxidation of a molecule of glucose into CO_2 and H_2O and liberation of more energy by utilising O_2 and H_2O.
- It can be expressed as $C_6H_{12}O_6 + 6\ H_2O + 6\ O_2 \rightarrow 6\ CO_2 + 12\ H_2O + 686$ K.cal.
- It has four stages known as **Glycolysis, conversion of Pyruvic acid to Acetyl Co. A, Krebs cycle and Electron transport system** coupled to **Oxidative phosphorylation.**
- In Glycolysis, glucose is converted to Pyruvic acid in the cytosol during which a small amount of ATP is synthesised by **substrate level phosphorylation.**
- In the second stage Pyruvic acid is oxidatively decarboxylated to Acetyl Co.A in mitochondrial **matrix**.
- In the third stage, Acetyl Co.A is oxidised to CO_2 and H_2O in mitochondrial matrix. In this stage there is generation of more **$NADH_2$** and **$FADH_2$.**
- In the fourth stage the $NADH_2$ and $FADH_2$ of 1^{st}, 2^{nd} and 3^{rd} stages is oxidised in the inner membrane of mitochondrion to form **ATP** in accordance to **Mitchel's** Chemiosmotic theory in the oxysomes.

Glycolysis

- The term glycolysis is derived from a Greek word, which means **sweet splitting.**
- It is also known as **Hexose diphosphate pathway.**
- The biochemical reactions of this pathway were traced by **Gustav Embden**, **Andrew Mayerhoff** and **Jacob Paranas**. Hence it is also known as **EMP** pathway.
- It is operated in the cytosol. It utilises neither oxygen nor liberates CO_2.
- It is conversion of a molecule of glucose into 2 molecules of Pyruvic acid through a series of **10** bio-chemical reactions.
- It is **common** for both aerobic and anaerobic respirations.
- The net gain of chemical energy of this stage is **2 $NADH_2$** and **2 ATP.**

Reactions

1. **Phosphorylation**

- The enzyme **hexokinase** phosphorylates a molecule of glucose to Glucose-6-phosphate by utilising a molecule of ATP.
- It is represented as: Glucose + ATP $\leftrightarrow$ Glucose – 6 – Phosphate + ADP

2. **Isomerisation**

- **Phosphohexose isomerase** converts Glucose – 6 – phosphate to Fructose – 6 – phosphate.

3. **Phosphorylation**

- The enzyme **phospho fructokinase** phosphorylates Fructose – 6 – phosphate to Fructose – 1,6 – bisphosphate by utilising another molecule of ATP.

4. **Cleavage**

- **Aldolase** cleaves FBP into a molecule of Glyceraldehyde – 3 – phosphate and a molecule of Dihydroxyacetone phosphate.

5. **Isomerisation**

- Among the two trioses only GAP can be directly oxidised in further glycolytic reactions. DHAP cannot be oxidised. Hence DHAP is converted to its isomeric form GAP by the action of **triose phosphate isomerase.**

Oxidation

- 2 molecules of GAP is oxidised and phosphorylated to 1, 3 – bisphospho glyceric acid during which 2 inorganic phosphates are used and 2 $NADH_2$ are formed. It is catalysed by dehydrogenise.
- It is the only oxidation reaction of Glycolysis.

7. **Dephosphorylation**

- The enzyme **phosphoglycerate kinase** dephosphorylates 1,3 – BPGA to 3 – phospho glyceric acid.
- In the reaction there is formation of 2 ATP from 2 ADP. This is the **first substrate level phosphorylation** reaction of respiration.

8. **Intramolecular shift**

- **Phosphoglycerate mutase** converts 3 – PGA to 2 – phospho glyceric acid by shifting the position of phosphate from 3^{rd} carbon to 2^{nd} carbon.

9. **Dehydration**

- The enzyme **enolase** removes water molecule from 2 – PGA to form Phospho Enol Pyruvic acid.

10. **Dephosphorylation**

- 2 molecules of PEP is dephosphorylated to Pyruvic acid by the action of **pyruvic kinase** during which 2 ATP are formed from 2 ADP.
- It is the **second substrate level phosphorylation** reaction of respiration.
- When one molecule of Glucose is involved in glycolysis, there is formation of 2 molecules of Pyruvic acid, 2 $NADH_2$, and 4 ATP.
- Since 2 ATP are used in the 1^{st} and 3^{rd} reactions, the net gains of ATP is 2 ATP.
- In the presence of oxygen, the 2 $NADH_2$ are directly involved in ETS to form ATP.

Fate of Pyruvic acid

- In the presence of oxygen, pyruvic acid is oxidised in mitochondrial matrix to CO_2 and H_2O.
- In the absence of oxygen, it is converted to either Ethyl alcohol or other organic substance.

Oxidative decarboxylation of Pyruvic acid

- It is the second stage of aerobic respiration.
- 2 molecules of Pyruvic acid is transported into mitochondrial matrix through inner membrane by a protein called **Pyruvate translocator.**
- In the matrix, Pyruvic acid is first decarboxylated and then dehydrogenated. Finally it condenses with co-enzyme A to form Acetyl Co. A
- Acetyl Co.A is the connecting link of glycolysis and Krebs cycle.
- The reaction is catalysed by a cluster of **three enzymes** known as **Pyruvate dehydrogenase enzyme complex.**
- The three enzymes of the complex are **Pyruvate decarboxylase, Dihydrolipoyl transacetylase and Dihydrolipoyl dehydrogenase.**
- The **six** cofactors required for this reaction are **Mg^{+2}, FAD, NAD^+**, Thiamine pyrophosphate (**TPP**), **Lipoic acid** (oxidised) and **Co-enzyme A.**
- As a result of oxidative decarboxylation of pyruvate, there is formation of 2 $NADH_2$. These enter into ETS for the synthesis of ATP.

Krebs cycle

- Krebs discovered it. He was awarded Nobel prize in 1953 for this achievement. In his honour it is named as Krebs cycle.
- Since the first formed substance in this cycle is Citric acid, it is also known as Citric acid cycle.
- The first formed organic acids of this cycle have three carboxylic groups. Hence it is also known as Tricarboxylic acid cycle.
- It has the following 10-biochemical reactions.

1. **Condensation**

- 2 molecules of 2C compound Acetyl Co. A condenses with 2 molecules of 4C compound Oxaloacetic acid of mitochodrial matrix to form 2 molecules of the first stable 6C compound Citric acid in the presence of enzyme **Citrate synthetase.**
- It utilises 2 molecules of water and liberates Co.A.

2. Dehydration

- The enzyme **aconitase** removes 2 molecules of water from 2 molecules of Citric acid to form 2 molecules of Cis-aconitic acid.

3. Hydration

- 2 molcules of Cis – aconitic acid is hydrated to 2 molecules of Isocitric acid by the action of aconitase by using the 2 molecules of water released in the earlier reaction.

4. Oxidation I

- The enzyme **isocitrate dehydrogenase** converts Isocitric acid to Oxalo Succinic acid by removing hydrogen atoms.
- There is formation of 2 $NADH_2$ in this reaction. It is the **3rd** oxidation reaction of aerobic respiration.

5. Decarboxylation

- 2 molecules of OSA is decarboxylated to 2 molecules of α - keto glutaric acid by the action of **Oxalo succinate decarboxylase.**
- 2 molecules of CO_2 is released in this reaction.
- α - keto glutaric acid is the only 5C compound of this cycle.

6. Oxidative decarboxylation (Oxidation II and decarboxylation)

- The enzyme **α - keto glutaric dehydrogenase** decarboxylates, dehydrogenates (oxidation) and adds Co. A to α - keto glutaric acid to form 2 molecules of Succinyl Co.A.
- In this reaction there is formation of 2 $NADH_2$ and removal of 2 CO_2.
- 5C compound becomes a 4C compound.

7. Cleavage

- The enzyme **succinyl thiokinase** cleaves Succinyl Co. A into Succinic acid and Co. A.
- The energy liberated in this reaction is used for the synthesis of **GTP** from GDP and Pi.
- This is the only substrate level phosphorylation reaction of Krebs cycle and **3rd** substrate level phosphorylation reaction of aerobic respiration.
- In plants ATP is formed.

8. Oxidation III

- **Succinate dehydrogenase** enzyme converts Succinic acid to Fumaric acid.
- 2 $FADH_2$ are formed in this reaction.
- This is the only Krebs cycle enzyme present in the inner membrane but not in the matrix.

9. Hydration

- **Fumarase** hydrates Fumaric acid to Malic acid by using 2 molecules of water.

10. Oxidation IV

- **Malate dehydrogenase** removes hydrogen atoms from malic acid to form Oxalo acetic acid. The hydrogen atoms are accepted by NAD to form $NADH_2$.
- In this way upon oxidation of 2 molecules of Acetyl Co. A through Krebs cycle, there is fomation of 2 ATP, 6 $NADH_2$ and 2 $FADH_2$.
- $NADH_2$ and $FADH_2$ enter into ETS for oxidation.

Significance of Krebs cycle

- It is a central metabolic pathway and plays a key role in both catabolism and anabolism.
- It serves as a pathway for the oxidation of carbohydrates.
- The intermediate of this pathway known as α - keto glutaric acid is involved in the synthesis of amino acids.
- Since this pathway is involved in both catabolism and anabolism it is called as **amphibolic pathway.**

Electron Transport System

- It is the **4th** and final stage of aerobic respiration.
- It takes place in the **inner membrane** of mitochondrion.
- 12 high energy electron pairs generated in Glycolysis, Oxidative decarboxylation of Pyruvate and Krebs cycle as 10 $NADH_2$ and 2 $FADH_2$.
- These $NADH_2$ and $FADH_2$ are oxidised to form ATP.
- It is **oxygen dependent** process.

- There is involvement of **5** multi protein complexes and **2** mobile electron carriers known as **Cytochrome C** and **Ubiquinone.**

Components of Complexes

Complex I or NADH dehydrogenase

- This is a complex enzyme having **FMN** as prosthetic group.
- It consists of **6** Fe-S centres.
- It transfers electrons from NADH to Ubiquinone.

Complex II (Succinate dehydrogenase)

- It has FAD as prosthetic group and transfers electrons from Succinate to Ubiquinone.
- It has **2** Fe-S centres.

Ubiquinone

- It is a type of quinone **diffuses** freely in the inner membrane of mitochondrion.
- Structurally it is similar to **Plastoquinone** of chloroplast.
- It is mobile electron carrier and transfers electrons from complex I to complex III or complex II to complex III.

Complex III (Cytochrome 'C' reductase)

- It has 2 'b' type cytochromes (Cytochrome b_{560} and Cytochrome b_{565}) and Cytochrome C_1.
- It also has **1** Fe-S protein.
- This complex reduces Cytochrome C_1 by transferring electrons from Ubiquinol to Cytochrome C_1.

Cytochrome C

- It is mobile electron carrier between Complex III and complex IV

Complex IV (Cytochrome C oxidase)

- It has 4 units. They are Cytochrome 'a', Cytochrome 'a_3' and **2** Cu containing proteins.
- It transfers electrons from reduced Cytochrome C to O_2.

Complex V (ATP synthase or F_0 – F_1 ATP ase)

- It has 2 major components. They are F_0 or base piece and F_1 or head piece.
- F_0 is fixed in the inner membrane and serves as Proton channel.
- F_1 protrudes into the mitochondrial matrix. It is the actual catalytic site for forming ATP from ADP and Pi.

The method of electron transport and Proton translocation

- It has 7 steps.

Step I

- Complex I is the **gate way for electron transport.**
- 2 electrons of $NADH_2$ are transferred to FMN. FMN is the prosthetic group of Complex I.
- $NADH_2$ is oxidised to NAD

Step II

- Electrons present in Complex I are transferred to Ubiquinone. During this movement, 2 H^+ of mitochondrial matrix are transported to perimitochondrial space.
- It is generally agreed that for every 2 electrons transported through complex I, there is pumping of 4 H^+ into perimitochondrial space.
- UQ diffuses freely in the inner membrane and forms a pool of mobile electron acceptors.

Step III

- In this a Quinone cycle is operated like in thylakoid membrane.
- During this cycle, partially reduced ubiquinone known as Ubi-semiquinone accepts an electron from complex I and 2 H^+ from matrix and reduced to Ubiquinol.

Step IV

- Ubiquinol moves to complex III gives the electron to Cytochrome b and releases the 2 H^+ in to the perimitochondrial space.
- Due to this ubiquinol is changed to Ubi-semiquinone. It returns back to UQ pool to accept one more electron.
- In this way for every **two electrons** passing through ubiquinone cycle and complex III, there is pumping of $4H^+$ from matrix to perimitochondrial space.

Step V

- From complex III the electrons are transferred to mobile electron carrier II known as Cytochrome C. Due to this Cytochrome C is reduced.
- Reduced cytochrome C moves towards complex IV and gives the electrons to complex IV. In this way cytochrome C is oxidised.

Step VI

- Electrons are transferred to oxygen from complex IV.
- For each pair of electrons transferred from complex IV to oxygen, there is pumping of 2 H^+ from matrix to perimitochondrial space in lieu of charge transfer.
- Actually 4 H^+ are pumped for each pair of electron transfer.
- Out of these 4 protons, 2 are used for the formation of a molecule of H_2O along with 2 electrons and one atom of oxygen. The other 2 H^+ are transported to perimitochondrial space.
- For each pair of electrons transport from mitochondrial NADH through ETS, there is formation of one water molecule and pumping of 10 (4+4+2) H^+ into perimitochondrial space.

Step VII

- The 2 electrons of $FADH_2$ are transferred to Ubiqunol pool through complex II during which 4 protons are pumped into perimitochondrial space.
- During the transfer of electrons to oxygen atom through complex IV, there is transport of 2 H^+ from matrix to perimitochondrial space.
- Now a total of 6 H^+ are pumped into inter membrane space from matrix during the oxidation of one $FADH_2$.
- Similarly during the oxidation of glycolytic $NADH_2$ the electrons are transported directly to ubiquinone pool through an external NADH dehydrogenase but not to complex I.
- Due to this there is pumping of 6 protons from matrix to perimitochondrial space during the oxidation of glycolytic $NADH_2$.

Oxidative Phosphorylation

- It is synthesis of ATP from ADP and Pi during the oxidation of $NADH_2$ and $FADH_2$ by utilising oxygen.
- It is based on Peter Mitchel's chemiosmotic theory.
- During the oxidation of $NADH_2$ and $FADH_2$ protons of matrix are pumped into perimitochondrial space.
- Inner membrane of mitochondrion is not permeable to Protons.
- A H^+ gradient is established across the inner membrane.
- F_0 of elementary particle acts as proton channel and permits the transport of protons.
- When the energy rich H^+ are transferred through this particles, the energy rotates F_1 (smallest rotatory machine in the universe) part. This rotating F_1 particle helps in combining ADP and Pi to form ATP.
- The energy present in 3 H^+ moving down the gradient is sufficient to form one ATP.
- In this way 10 H^+ formed in the perimitochondrial space during the oxidation of One molecule of $NADH_2$ is sufficient for the formation of 3 ATP.
- 6 protons formed during the oxidation of glycolytic $NADH_2$ and $FADH_2$ are sufficient for the formation of 2 ATP.
- During the oxidation of each $NADH_2$ of Krebs cycle and Pyruvate oxidative decarboxylation, there is synthesis of 3 ATP and formation of one H_2O by consuming one atom of oxygen.
- During the oxidation of Glycolytic $NADH_2$ and $FADH_2$ of Krebs cycle, there is synthesis of 2 ATP.

Balance sheet of Aerobic respiration

- Glycolysis yields a total formation of 4 ATP and 2 $NADH_2$ and net gain of 2 ATP and 2 $NADH_2$.
- 2 ATP are formed during the oxidation of 2 glycolytic $NADH_2$.
- Total net gain of ATP is 2 + 4 = 6.
- Oxidation of 2 $NADH_2$ formed during the conversion of 2 Pyruvic acid molecules to Acetyl Co.A yields 6 ATP.
- Oxidation of 6 $NADH_2$ and 2 $FADH_2$ of Krebs cycle yield 18 + 4 = 22 ATP. 2 ATP are formed in substrate level phosphorylation. Total ATP formed is 22 + 2 = 24.
- Upon complete aerobic break down of one molecule of Glucose yields a total of 8 + 6 + 24 = 38 ATP and a net gain of 6 + 6 + 24 = 36 ATP.

Energetics of Aerobic respiration

- 36 ATP molecules are formed upon oxidation of one molecule of glucose.
- Hydrolysis of each ATP to ADP and Pi yields 7.6 K.cal.
- Hydrolysis of 36 ATP yields 36 X 7.6 = 273.6 k.cal.
- Total amount of energy released during the oxidation of glucose molecule is 686 K. cal.
- The amount of energy released in the form of heat is 686 – 273.6 = 412.4 K. cal.

Mechanism of Anaerobic respiration

- Partial oxidation of glucose in the absence of oxygen is called as anaerobic respiration.
- The organisms that carry out this respiration are called as **Anaerobes.**
- Anaerobes that can not survive in the presence of oxygen are called as **Obligate anaerobes.** e.g. Clostridium botulinum

- Anaerobes that can tolerate aerobic conditions are called as facultative anaerobes. e.g. Yeasts.
- There are two stages in anaerobic respiration known as Glycolysis and Fermentation.
- Glycolysis results in the formation of 2 molecules of Pyruvic acid, 2 $NADH_2$ and net gain of 2 ATP.
- Ethyl alcohol is formed during fermentation by using the 2 $NADH_2$ of glycolysis.
- Fermentation is formation of ethyl alcohol and CO_2 from sugars.
- Fermentation is cytosolic process.
- It was first reported by **Gaylussac. Louis Pasteur** coined the term fermentation.
- The process of fermentation starts with Pyruvic acid.
- 2 molecules of Pyruvic acid is decarboxylated to Acetaldehyde by **Pyruvate decarboxylase.**
- 2 molecules of Acetaldehyde is hydrogenated to ethyl alcohol by **alcohol dehydrogenase** by using the 2 glycolytic $NADH_2$.
- The net gain of ATP in anaerobic respiration is 2.
- Alcohol fermentation is utilised for the preparation of beverages by using yeasts.
- It is also employed in bread making where the elimination of CO_2 helps to raise the dough.

Differences between Aerobic and Anaerobic respirations.

Aerobic respiration	*Anaerobic respiration*
$C_6H_{12}O_6 + 6\ H_2O + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 686$ K.Cal	$C_6H_{12}O_6 \rightarrow C_2H_5OH + 2CO_2 + 56$ K.Cal
1. It is common in higher plants. 2. Occurs in the presence of Oxygen. 3. Glucose is completely oxidised. 4. More energy (686 k.cal.) is released. 5. Net gain of ATP is 36. 6. End products are CO_2 and H_2O 7. It is efficient process. 8. It occurs in 4 steps known as Glycolysis, Oxidative decarboxylation of Pyruvate, Krebs cycle and Electron transport system. 9. Both cytosol and mitochondria are involved	1. Commonly found in lower organisms such as bacteria and yeasts. 2. Occurs in the absence of oxygen. 3. Glucose is partially oxidised. 4. Less energy (56 K.cal) is released. 5. ATP output is only 2 molecules. 6. The end products are CO_2 and organic substances like ethyl alcohol, acetic acid and lactic acid etc. 7. It is not efficient process. 8. It has two stages known as Glycolysis and Fermentation. 9. Mitochondria are not involved. Only cytosol is involved.

Respiratory Quotient

- It is the ratio between the volume of CO_2 liberated and the volume of O_2 utilised in respiration of a given weight of tissue in a given time at standard temperature and pressure during respiration.
- It is represented as:

$$RQ = \frac{\text{Number of } CO_2 \text{ evolved}}{\text{Number of } O_2 \text{ absorbed}}$$

- It is an index of the substrate oxidised in respiration.
- It is measured by using **Ganongs respirometer.**

RQ values of various substrates are as follows.

1. **Carbohydrates:** It is 1 or Unity. The amount of oxygen used is equivalent to the amount of carbondioxide