13. **KREBS’ CYCLE / CITRIC ACID CYCLE / TCA CYCLE**

The pyruvic acid produced in glycolysis enters into Krebs’ cycle for further oxidation. Krebs’ cycle is also known as citric acid cycle or Tri carboxylic acid (TCA) cycle. This aerobic process takes place in mitochondria where necessary enzymes are present in matrix.

11. Pyruvic acid reacts with CoA and NAD and is oxidatively decarboxylated. One molecule of CO$_2$ is released and NAD is reduced. Pyruvic acid is converted into acetyl CoA.

   \[
   \text{Pyruvate dehydrogenase} \\
   \text{Pyruvic acid + CoA + NAD} \rightarrow \text{Acetyl – COA + CO$_2$ + NADH$_2$}
   \]

12. Acetyl-CoA condenses with oxaloacetic acid in the presence of condensing enzyme and water molecule to form citric acid. CoA becomes free.

   \[
   \text{Condensing enzyme} \\
   \text{Acetyl CoA + Oxaloacetic acid} \rightarrow \text{Citric acid + CoA + H$_2$O}
   \]

13. Citric acid is dehydrated in the presence of aconitase to form cis – aconitic acid

   \[
   \text{Aconitase} \\
   \text{Citric acid} \rightarrow \text{Cis – Aconitic acid - H$_2$O}
   \]

14. Cis-aconitic acid reacts with one molecule of water to form Isocitric acid

   \[
   \text{Cis-aconitic acid + H$_2$O} \rightarrow \text{Isocitric acid}
   \]

15. Iso-citric acid is oxidized to oxalo succinic acid in the presence of Isocitric dehydrogenase. NADP is reduced to NADPH$_2$ in the reaction.

   \[
   \text{IC dehydrogenase} \rightarrow \text{Oxalo succinic acid + NADPH$_2$}
   \]
Isocitric acid + NADP

16. Oxalo succinic acid is decarboxylated in the presence of oxalo succinic decarboxylase to form α-ketoglutaric acid and a second molecule of CO₂ is released.

\[
\text{Oxalosuccinic acid} \to \alpha\text{-ketoglutaric acid} + \text{CO}_2
\]

Decarboxylase

17. α-ketoglutaric acid reacts with CoA and NAD in the presence of α-ketoglutaric acid dehydrogenase complex and is oxidatively decarboxylated to form succinyl CoA and a third mole of CO₂ is released. NAD is reduced in the reaction.

\[
\alpha\text{-keto glutaric acid} + \text{CoA} \to \text{Succinyl-CoA} + \text{CO}_2 + \text{NADH}_2
\]

NAD  NADH₂

18. Succinyl CoA reacts with water molecule to form succinic acid. CoA becomes free and one molecule of GDP (Guanosine diphosphate) is phosphorylated in presence of inorganic phosphate to form one molecule of GTP.

\[
\text{H}_2\text{O}
\]

\[
\text{Succinyl-CoA} + \text{GDP} + \text{ip} \to \text{Succinic acid} + \text{GTP}
\]

GTP may react with ADP to form one molecule of ATP

\[
\text{GTP} + \text{ADP} \to \text{ATP} + \text{GDP}
\]

19. Succinic acid is oxidized to fumaric acid in the presence of succinic dehydrogenase and co enzyme FAD is reduced in this reaction.

\[
\text{Succinic acid dehydrogenase}
\]

\[
\text{Succinic acid} + \text{FAD} \to \text{Fumaric acid} + \text{FADH}_2
\]

20. One mole of H₂O is added to Fumaric acid in the presence of fumarase to form malic acid.
21. In the last step, malic acid is oxidized to oxaloacetic acid in the presence of malic dehydrogenase and one molecule of coenzyme i.e. NAD is reduced.

\[
\text{Malic dehydrogenase} \\
\text{Malic acid + NAD} \rightarrow \text{Oxaloacetic acid + NADH}_2
\]
Pentose phosphate pathway (ppp) / Hexose mono phosphate (hmp) shunt/
Phosphogluconate pathway / Warburg and Dicken’s pathway

The pentose phosphate pathway occurs in the cytoplasm outside the mitochondria and it is an alternative pathway to glycolysis and Kreb’s cycle. The presence of some compounds like iodoacetate, fluorides, arsenates etc. inhibit some steps in glycolysis and that leads to the alternate pathway. This pathway was discovered by Warburg and Dicken (1938). This pathway does not produce ATP but it produces another form of energy called reducing power in the form of NADPH. It is not oxidized in the electron transport system but, it serves as hydrogen and electron donor in the biosynthesis of fatty acids and steroids. The pentose phosphate pathway consists of two distinct phases. In the first phase, hexose is converted into pentose and in the second phase, pentose is reconverted into hexose.

In the process, oxidation of glucose 6 phosphate leads to the formation of 6 phosphogluconic acid (pentose phosphate). Since glucose is directly oxidized without entering glycolysis, it is called as direct oxidation.

\[
\text{6 Glucose 6 phosphate } + 12 \text{ NADP} \rightarrow \text{5 Glucose 6 Phosphate } + 12 \text{ NADPH} + 6 \text{ CO}_2
\]

It provides ribose sugars for the synthesis of nucleic acids and is also required for shikimic acid pathway. Although ATP is not produced, NADPH is produced and serves as hydrogen and electron donor in the biosynthesis of fatty acids and steroids. The pathway is also called as phosphogluconate pathway as the first product in this pathway is phosphogluconate.

OXIDATIVE PHOSPHORYLATION

C. TERMINAL OXIDATION OF THE REDUCED COENZYMES / ELECTRON TRANSPORT SYSTEM AND OXIDATIVE PHOSPHORYLATION

The last step in aerobic respiration is the oxidation of reduced coenzymes produced in glycolysis and Krebs’ cycle by molecular oxygen through FAD, UQ (ubiquinone), cytochrome b, cytochrome c, cytochrome a and cytochrome a₃ (cytochrome oxidase).

Two hydrogen atoms or electrons from the reduced coenzyme (NADH₂ or NADPH₂) travel through FAD and the cytochromes and ultimately combines with 1/2O₂ molecule to produce one molecule of H₂O. This is called as terminal oxidation.

The terminal oxidation of each reduced coenzyme requires 1/2O₂ molecule and 2H atoms (i.e. 2 e⁻ + 2H⁺) to produce one H₂O molecule. Except for flavoproteins (like FAD)
and ubiquinone (UQ) which are hydrogen carriers, the other components of electron transport chain (cytochromes) are only electron carriers i.e. they cannot give or take protons (H⁺)

During the electron transport, FAD and the iron atom of different cytochromes get successively reduced (Fe²⁺) and oxidized (Fe³⁺) and enough energy is released in some places which is utilized in the photophosphorylation of ADP molecules in the presence of inorganic phosphate to generate energy rich ATP molecules. Since, this oxidation accompanies phosphorylation; it is called as oxidative phosphorylation.

One molecule of ATP with 7.6 Kcal.energy is synthesized at each place when electrons are transferred from

1. Reduced NADH₂ or NADPH₂ to FAD
2. Reduced cytochrome b to cytochrome c
3. Reduced cytochrome a to cytochrome a₃

Thus, oxidation of one molecule of reduced NADH₂ or NADPH₂ will result in the formation of 3 ATP molecules while the oxidation of FADH₂ lead to the synthesis of 2 ATP molecules.

According to the most recent findings, although in eukaryotes terminal oxidation of mitochondrial NADH / NADPH results in the production of 3 ATP molecules but that of extra mitochondrial NADH / NADPH yields only 2 ATP molecules. Therefore, the two reduced coenzyme molecules (NADH) produced per hexose sugar molecule during Glycolysis will yield only 2x2:4 ATP molecules instead of 6 ATP molecules. Complete oxidation of a glucose molecule (hexose sugar) in aerobic respiration results in the net gain of 36 ATP molecules in most eukaryotes.

One glucose molecule contains about 686 Kcal. Energy and 38 ATP molecules will have 273.6 Kcal energy. Therefore about 40% (273.6/686) energy of the glucose molecule is utilized during aerobic breakdown and the rest is lost as heat. Since huge amount of energy is generated in mitochondria in the form of ATP molecules, they are called as Power Houses of the cell.

ATP molecules contain energy in terminal pyrophosphate bonds. When these energy rich bonds break, energy is released and utilized in driving various other metabolic processes of the cell.

**Differences between oxidative phosphorylation and Photophosphorylation**

<table>
<thead>
<tr>
<th></th>
<th>Oxidative phosphorylation</th>
<th>Photophosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of ATP synthesis</td>
<td>Mitochondria</td>
<td>Chloroplasts</td>
</tr>
<tr>
<td>Energy source</td>
<td>Oxidation of electron</td>
<td>Light absorption</td>
</tr>
<tr>
<td>ATP molecules</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Glucose breakdown</td>
<td>6</td>
<td>2x2:4</td>
</tr>
<tr>
<td></td>
<td>It occurs during respiration</td>
<td>Occurs during photosynthesis</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>2</td>
<td>Occurs inside the mitochondria (inner membrane of cristae)</td>
<td>Occurs inside the chloroplast (in the thylakoid membrane)</td>
</tr>
<tr>
<td>3</td>
<td>Molecular O\textsubscript{2} is required for terminal oxidation</td>
<td>Molecular O\textsubscript{2} is not required</td>
</tr>
<tr>
<td>4</td>
<td>Pigment systems are not involved</td>
<td>Pigment systems, PSI and PSII are involved</td>
</tr>
<tr>
<td>5</td>
<td>It occurs in electron transport system</td>
<td>Occurs during cyclic and non cyclic electron transport</td>
</tr>
<tr>
<td>6</td>
<td>ATP molecules are released to cytoplasm and used in various metabolic reactions of the cell</td>
<td>ATP molecules produced are utilized for CO\textsubscript{2} assimilation in the dark reaction of photosynthesis</td>
</tr>
</tbody>
</table>

**Efficiency of respiration**

The total energy content of one molecule of glucose is 686 Kcal. Out of this energy, available free energy is 673.6 Kcal and the energy content of ATP molecule is calculated as 7.3 Kcal. The efficiency of respiration may be expressed as follows.

Kcal of energy conserved in ATP

Efficiency of respiration: \( \frac{38 \times 7.3}{673.6} \times 100 \): 41 %

Efficiency of aerobic respiration: \( \frac{2 \times 7.3}{47} \times 100 \): 31 %

Efficiency of fermentation: \( \frac{2 \times 7.3}{40} \times 100 \): 36.5 %

**Respiratory quotient**
The ratio of the volume of CO₂ released to the volume of O₂ taken during respiration is called as respiratory quotient and is denoted as RQ

\[
RQ = \frac{\text{Volume of CO}_2}{\text{Volume of O}_2}
\]

**Value of RQ**

The value of RQ depends upon the nature of the respiratory substrate and the amount of O₂ present in respiratory substrate.

1. When **carbohydrates** such as hexose sugars are oxidized in respiration, the value of RQ is 1 or unity because volume of CO₂ evolved equals to the volume of O₂ absorbed.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}
\]

Glucose

\[
RQ = \frac{\text{volume of CO}_2}{\text{volume of O}_2} = \frac{6}{6} = 1 \text{ or unity}
\]

2. When **fats** are the respiratory substrate, the value of RQ becomes less than one because fats are poorer in O₂ in comparison to carbon and they require more O₂ for their oxidation,

\[
2\text{C}_{51}\text{H}_{98}\text{O}_6 + 145\text{O}_2 \rightarrow 102\text{CO}_2 + 98\text{H}_2\text{O}
\]

Tripalmitin

\[
RQ = \frac{\text{volume of CO}_2}{\text{volume of O}_2} = \frac{102}{145} = 0.7
\]

(Fats are oxidized in respiration usually during the germination of fatty seeds).

3. When **organic acids** are oxidized in respiration, the value of RQ becomes more than one. It is because organic acids are rich in O₂ and require less O₂ for their oxidation.

\[
\text{C}_4\text{H}_6\text{O}_5 + 3\text{O}_2 \rightarrow 4\text{CO}_2 + 3\text{H}_2\text{O}
\]

Malic acid

\[
RQ = \frac{\text{volume of CO}_2}{\text{volume of O}_2} = \frac{4}{3} = 1.3
\]

**Energy budgeting**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Gain of</th>
<th>Consumption</th>
<th>Net gain of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FACTORS AFFECTING RESPIRATION

A. External factors

1. Temperature

Temperature has profound influence on the rate of respiration. Optimum temperature for respiration is about 30°C, minimum 0°C and maximum about 45°C. At low temperature, the respiratory enzymes becomes inactive, consequently the rate of respiration falls. It is due to this fact that the quality of fruits and vegetables stored at low temperature does not deteriorate. At very high temperature, respiration slows down and may even be stopped due to denaturation of the respiratory enzymes.

2. Oxygen

In complete absence of O₂, anaerobic respiration takes place while aerobic respiration stops. In higher plants, the anaerobiosis produces large amount of alcohol which is toxic to plants. If some amount of O₂ is available, anaerobic respiration slows down and aerobic respiration starts. The concentration of O₂ at which aerobic respiration is optimum and anaerobic respiration is stopped, is called as extinction point.
It is observed that under anaerobic conditions, much more sugar is taken up per quantity of yeast present than it is consumed in the presence of oxygen. The inhibition on the rate of carbohydrate breakdown by oxygen is called as Pasteur’s effect.

3. Carbon dioxide

Higher concentration of CO$_2$ in the atmosphere especially in the poorly aerated soil has retarding effect on the rate of respiration.

4. Inorganic salts

If a plant or tissue is transferred from water to salt solution, the rate of respiration increases (called as salt respiration).

5. Water

Proper hydration of cells is essential for respiration. Rate of respiration decreases with decreased amount of water, so much so, that in dry seeds, the respiration is at its minimum. It is because in the absence of a medium, the respiratory enzymes become inactive.

6. Light

The effect of light is indirect on the rate of respiration through the synthesis of organic food matter in photosynthesis.

7. Wound or injury

Injury or wounds result in increased respiration as the plants in such a state require more energy which comes from respiration. The wounded cells become more meristematic to form new cells for healing the wound.

Internal factors

1. Protoplasmic factors

The amount of protoplasm in the cell and its state of activity influence the rate of respiration.

- The rate of respiration is higher in young meristematic cells which divide actively and requires more energy. Such cells have greater amount of protoplasm and no vacuoles.
• In old mature tissues, the rate of respiration is lower because of lesser amount of active protoplasm

2. Concentration of respiratory substrate

Increased concentration of respirable food material brings about an increase in the rate of respiration.

Under starvation conditions, such as in etiolated leaves, the rate of respiration slows down considerably. If such etiolated leaves are supplied with sucrose solution for few days even in dark conditions, the rate of respiration increases.

Differences between Photorespiration and Dark respiration

<table>
<thead>
<tr>
<th>Photorespiration</th>
<th>Dark /Mitochondrial respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>It occurs in the presence of light</td>
</tr>
<tr>
<td>2</td>
<td>The substrate is glycolate</td>
</tr>
<tr>
<td>3</td>
<td>It occurs in chloroplast, peroxisome and mitochondria</td>
</tr>
<tr>
<td>4</td>
<td>It occurs in temperate plants like, wheat and cotton ( mainly in C3 plants)</td>
</tr>
<tr>
<td>5</td>
<td>It occurs in the green tissues of plants</td>
</tr>
<tr>
<td>6</td>
<td>The optimum temperature is 25- 35°C</td>
</tr>
<tr>
<td>7</td>
<td>This process increases with increased CO₂ concentration.</td>
</tr>
<tr>
<td>8</td>
<td>Hydrogen peroxide is formed during the reaction</td>
</tr>
<tr>
<td>9</td>
<td>ATP molecules are not produced,</td>
</tr>
<tr>
<td>10</td>
<td>Reduced coenzymes such as NADPH₂, NADH₂ and FADH₂ are not produced.</td>
</tr>
<tr>
<td>11</td>
<td>One molecule of ammonia is released</td>
</tr>
</tbody>
</table>
Differences between respiration and photosynthesis

<table>
<thead>
<tr>
<th>Respiration</th>
<th>Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 It is catabolic process resulting in the destruction of stored food</td>
<td>It is an anabolic process resulting in the manufacture of food.</td>
</tr>
<tr>
<td>2 Light is not essential for the process</td>
<td>Light is very much essential</td>
</tr>
<tr>
<td>3 Oxygen is absorbed in the process</td>
<td>Oxygen is liberated</td>
</tr>
<tr>
<td>4 Carbon dioxide and water are produced</td>
<td>Carbon dioxide is fixed to form carbon containing compound</td>
</tr>
<tr>
<td>5 Potential energy is converted into Kinetic energy</td>
<td>Light energy is converted into chemical energy (potential energy)</td>
</tr>
<tr>
<td>6 Glucose and oxygen are the raw materials</td>
<td>Carbon dioxide and water are the raw materials</td>
</tr>
<tr>
<td>7 Energy is released during respiration and hence it is an exothermic process.</td>
<td>Energy is stored during photosynthesis and hence it is an endothermic process</td>
</tr>
<tr>
<td>8 Reduction in the dry weight</td>
<td>Gain in the dry weight</td>
</tr>
<tr>
<td>9 Chlorophyllous tissues are not necessary</td>
<td>Chlorophyllous tissues are essential for the process</td>
</tr>
</tbody>
</table>

Differences between aerobic respiration and fermentation

<table>
<thead>
<tr>
<th>Aerobic respiration</th>
<th>Fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 It occurs in all living cells of the plants throughout the day and night</td>
<td>Occurs outside the plant cells and in certain microorganisms</td>
</tr>
<tr>
<td>2 It takes place in the presence of oxygen</td>
<td>Absence of oxygen</td>
</tr>
<tr>
<td>3 The end products are CO₂ and H₂O</td>
<td>End products are CO₂ and alcohol or other organic acids</td>
</tr>
<tr>
<td>4 It is not toxic to plants</td>
<td>It is toxic to plants</td>
</tr>
<tr>
<td>5 Complete oxidation is food material is</td>
<td>Incomplete oxidation is observed</td>
</tr>
<tr>
<td></td>
<td>observed</td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>Large amount of energy (673 kCal) is released per glucose molecule</td>
</tr>
<tr>
<td>7</td>
<td>The complete oxidation yields 38 ATP molecules</td>
</tr>
<tr>
<td>8</td>
<td>The enzyme, zymase is not required but many other enzymes and coenzymes are required</td>
</tr>
</tbody>
</table>