RESPIRATION IN PLANTS

Introduction

Mechanism of breakdown of food materials within the cell to release energy, and the trapping of this energy for synthesis of ATP is called cellular respiration.

The complete combustion of glucose, which produces CO_2 and H_2O as end products, yields energy most of which is given out as heat.

The compounds that are oxidised during this process are known as respiratory substrates.

Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substrates, under certain conditions.

Calorific Value of protein, carbohydrate and fat : The amount of heat liberated from complete combustion of 1 g food in a bomb calorimeter (a closed metal chamber filled with O_2) is its gross calorific values.

Respiratory substrate	Gross Calorific Value	Physiological Value
Carbohydrate	4.1 kcal/g	4.0 kcal/g
Protein	5.65 kcal/g	4.0 kcal/g
Fat	9.45 kcal/g	9.0 kcal/g

The actual amount of energy released by combustion of lg of food is the physiological value of food.

Features of Cellular respiration :

(i) All the energy contained in respiratory substrates is not released free into the cells or in a single step.

(ii) Energy is released in a series of slow step wise reactions controlled by enzymes, and it is trapped as chemical energy in the form of ATP. (ATP acts as the energy currency of the cell)

(iii) Cellular respiration is an amphibolic process.
Reason : The carbon skeleton (Intermediates of respiration) produced during respiration is used as precursors for biosynthesis of other molecules in the cell.

(iv) Cellular respiration is an exergonic process.
Reason : The breaking of C–C bonds of complex compounds through oxidation within the cells, leading to release of considerable amount of energy.

(v) Cellular respiration is a downhill process.
Reason : Oxygen is a strong electron acceptor.

Do plants breathe?

Yes, plants require O_2 for respiration to occur and they also give out CO_2. Hence, plants have systems in place that ensure the availability of O_2. Plants, unlike animals, have no specialised organs for gaseous exchange but they have stomata and lenticels for this purpose.

There are several reasons why plants can get along without respiratory organs.

(1) Each plant part takes care of its own gas-exchange needs. There is very little transport of gases from one plant part to another.

(2) Plants do not present great demands for gas exchange. Roots, stems, and leaves respire at rates far lower than animals do. Only during photosynthesis are large volumes of gases exchanged and, each leaf is well adapted to take care of its own needs during these periods. When cells photosynthesis, availability of O_2 is not a problem in these cells since O_2 is released within the cell.

(3) The distance that gases must diffuse even in large, bulky plants is not great. Each living cell in a plant is located quite close to the surface of the plant.
Most cells of a plant have at least a part of their surface in contact with air. This is also facilitated by the loose packing of parenchyma cells in leaves, stems and roots, which provide an interconnected network of air spaces.

Type of respiration :

(A) On the basis of type of respiratory substrates :

(1) Floating respiration :
When carbohydrate or fats are oxidized inside the cell. Carbohydrates and fats are floating inclusions of cell thus, this is called floating respirations.

(2) Protoplasmic respiration :
When protein is oxidized inside the cell. This accurs in starved cell. Protein is constituent of protoplasm thus this is called protoplasmic respiration.

(B) On the basis of presence or absence of O_2 :
(1) Aerobic (2) Anaerobic / Fermentation

Aerobic		Anaerobic / Fermentation	
(1)	This accounts for complete oxidation (end products are inorganic) of food (glucose) to CO_2 and H_2O	(1)	This accounts for only a partial breakdown of glucose to either lactic acid or ethanol and CO_2
(2)	Its an intermolecular respiration.	(2)	Its an intramolecular respiration
(3)	36 or 38 molecules of ATP gain for each molecule of glucose	(3)	There is gain of only two molecules of ATP for each molecule of glucose
(4)	NADH is oxidised to NAD^+ vigorously	(4)	NADH is oxidised to NDA^+ rather slowly
(5)	O_2 remove hydrogen form the system and acts as the final hydrogen acceptor.	(5)	O_2 is absent. Hydrogen acceptor in the system is either acetaldehyde (during alcoholic fermentation) or Pyruvate (during lactic acid fermentation)
(6)	Reaction $C_6H_{12}O_6 + 6O_2 + 6H_2O \longrightarrow 6CO_2 + 12H_2O + 686$ kcal	(6)	Reaction $C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2$ + less than 7% of energy of glucose 'or' $C_6H_{12}O_6 \longrightarrow 2C_3H_6O_3$ + less than 7% of energy of glucose

Aerobic respiration : It is divided into following stages :
(1) Glycolysis (2) Link reaction
(3) Krebs cycle (4) Electron transport system and oxidative phosphorylation

(1) Glycolysis
(i) The term glycolysis has originated from the greek words. glycos for sugar and lysis for splitting.

(ii) The scheme of glycolysis was given by Gustav Embden. Otto Meyerhof and J.Pamas. Thus it is often referred to as the EMP pathway.
(iii) This process takes place inside cytoplasm of all living cells.
(iv) Glycolysis is called common pathway because it is a common step between aerobic and anaerobic respiration.
(v) Glycolysis is a chain process of ten chemical reactions, where 1, 3 and 10 reactions are irreversible.
(vi) In this process, glucose undergoes partial breakdown I oxidation to form two molecule of pyruvic acid. In plants this glucose is derived from sucrose lend product of photosynthesis) or from storage carbohydrates (starch).
(vii) Sucrose is converted into glucose and fructose by the enzyme, invertase and these two monosaccharides readily enter the glycolytic pathway. Glucose is the favoured substrate for respiration.

Gross products of gylcolysis	Net products of glycolysis
2 molecules of pyruvic acid ($CH_3CO.COOH$)	2 molecules or pyruvic acid ($CH_3CO.COOH$)
2 molecules of NADH	2 molecules of NADH
4 moleucles of ATP	2 molecules of ATP

Q. Calculate how many ATP molecules are directly synthesised in glycolysis from one glucose molecule?
Ans. 4 ATP

- Pyruvic acid is the key product of glycolysis. Metabolic fate of pyruvate depends on the cellular needs and availability of O_2.
- There are three major ways in which different cells handle pyruvic acid produced by glycolysis :-
  (i) Lactic acid fermentation (ii) Alcoholic fermentation (iii) Aerobic respiration

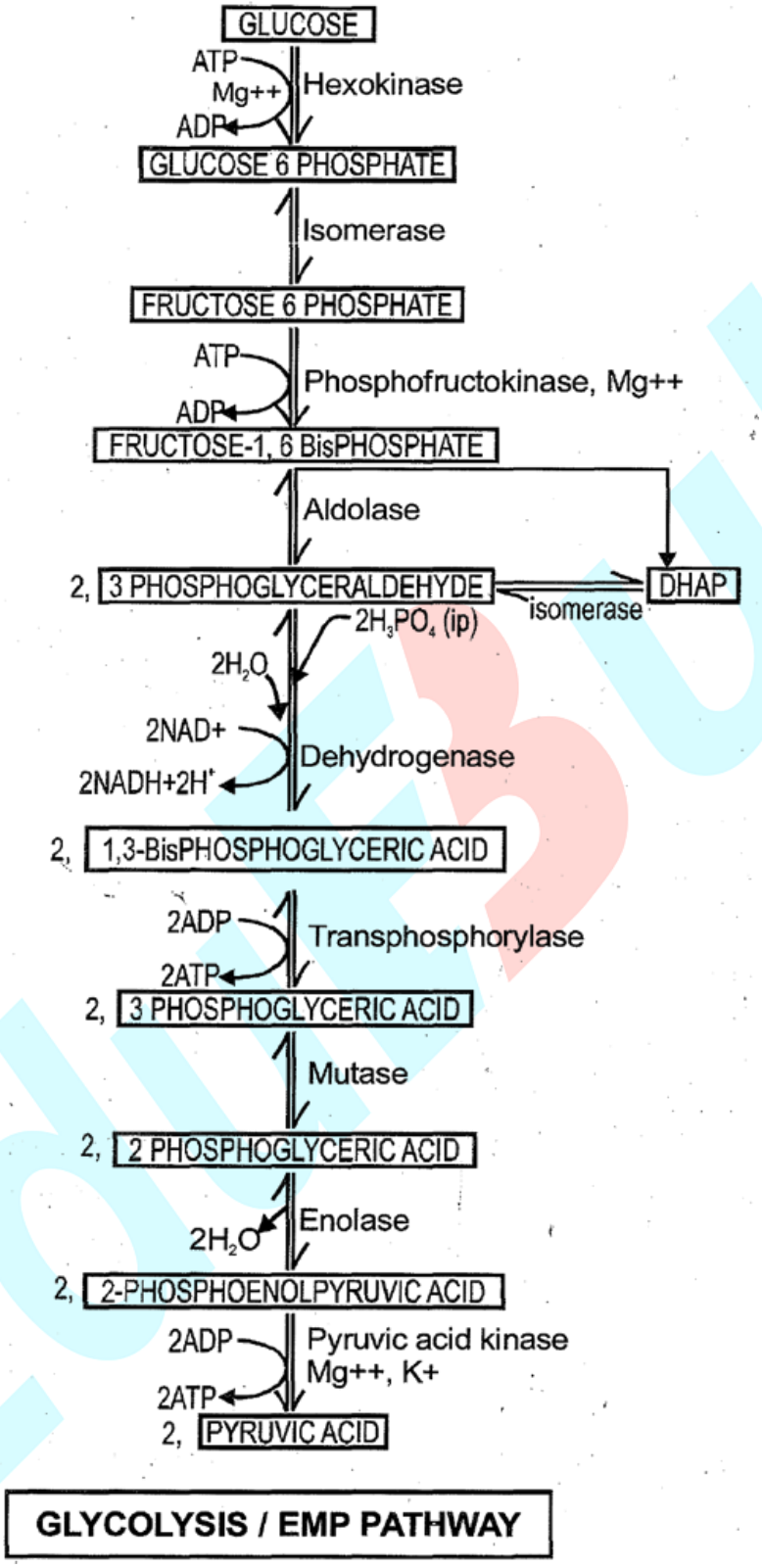
Q. What is the metabolic fate of pyruvate ?
Ans. This depends on the cellular need and availability of oxygen. There are three major ways in which different cells handle pyruvic acid produced by glycolysis. These are lactic acid fermentation, alcoholic fermentation and aerobic respiration.
Here we are going through aerobic respiration therefore next step is link reaction.

(2) Link reaction /Gateway step/ Transition reaction

Pyruvate, which is formed by the glycolytic catabolism of carbohydrates in cytosol, after it enters mitochondrial matrix undergoes oxidative decarboxylation by a complex set of reactions catalysed by pyruvate dehydrogenase.
This reaction require participation of several co-enzymes, including $NAD^{\pm}$ and CoA.

$$\text{Pyruvic acid} + \text{CoA} + NAD^+ \xrightarrow[\text{Pyruvate dehydrogenase}]{Mg^{2+}} \text{Acetyl CoA} + CO_2 + NADH(H^+)$$

During this process, two molecules of NADH are produced from the metabolism of two molecules of pyruvic acid (produced from one glucose molecule during glycolysis).
The acetyl CoA is called connecting link between glycolysis and Krebs cycle.

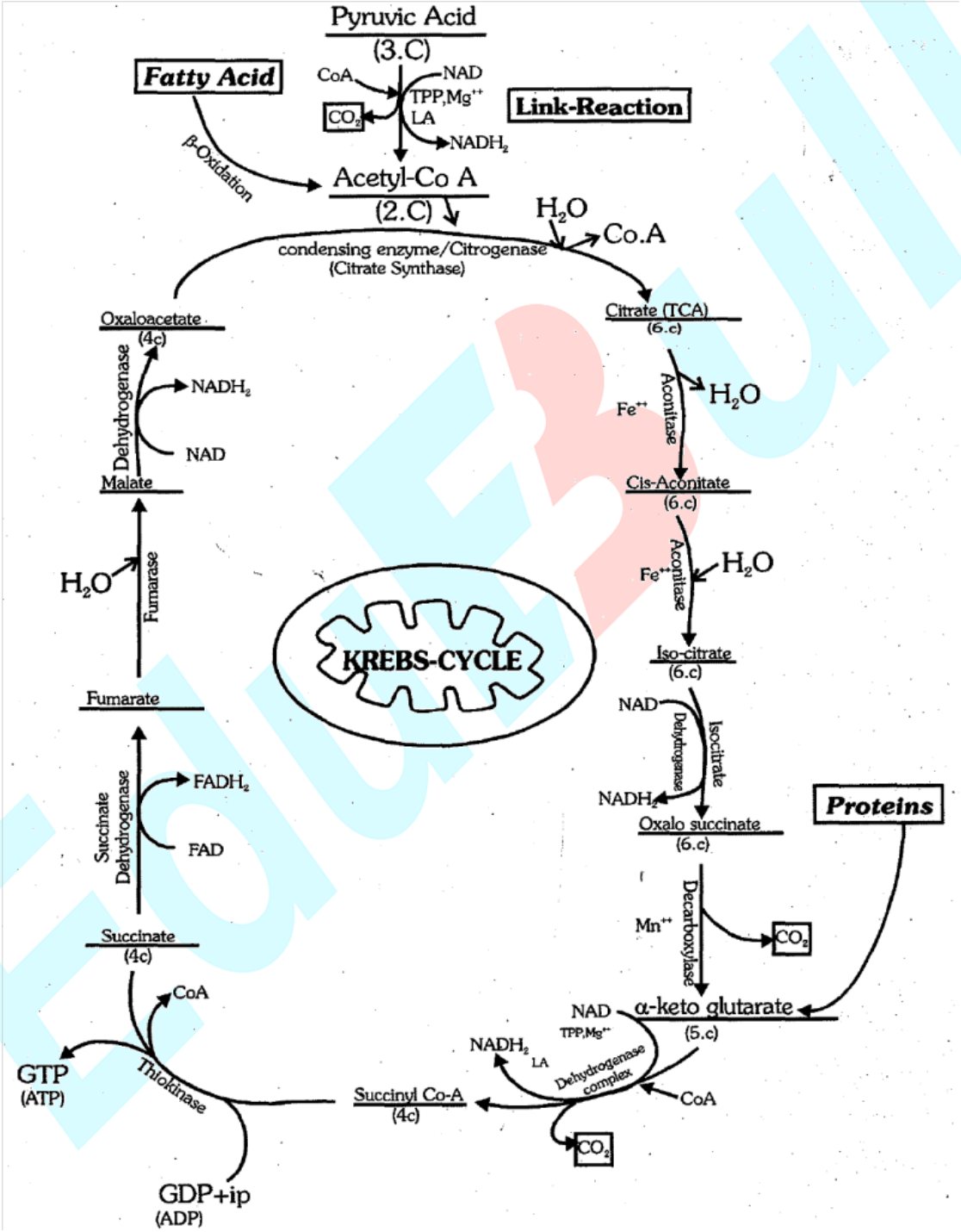


GLYCOLYSIS / EMP PATHWAY

(3) Krebs cycle

(i) Named after the scientist Hans Krebs who first elucidated it. It is also called TCA (tri carboxylic acid) cycle or CA (citric acid) cycle.

(ii) Krebs cycle occurs inside mitochondrial matrix of eukaryotic cells and cytoplasm of prokaryotic cells.
(iii) One turn of Krebs cycle involve four dehydrogenation , two decarboxylation and one substrate level phosphorylation.
(iv) OM is considered as the first member of the cycle.
(v) All enzymes of Krebs cycle are located inside mitochondrial matrix except succinate dehydrogenase (Marker enzyme), which is located in inner membrane of mitochondria.



BEGINNER'S BOX-1

1. In Embden, Meyerhof and Pamas pathway respectively at how many steps ATP is synthesised, NAD^+ is reduced and ATP is utilised ?
(1) Two, One and Two (2) One, Two and One
(3) Five, Two and Two (4) Three, One and Two

2. In mitochondrial matrix when pyruvic acid undergoes oxidative decarboxylation catalysed by pyruvate dehydrogenase, which of the following are the products of this process ?
(1) Ethanol, Lactic acid and CO_2 (2) $NADH + H^+$, CO_2 and Acetyl CoA
(3) Citric acid, $FADH_2$ and H_2O (4) Acetyl CoA, $NADH + H^+$ and $FADH_2$

3. The Tricarboxylic acid cycle starts with the condensation of acetyl group with :-
(1) Coenzyme A and water to yield citric acid
(2) Water and oxaloacetic acid to yield citric acid
(3) Pyruvic acid and NAD^+ to yield 9-cetyl CoA
(4) Coenzyme A and NAD^+ to yield acetyl CoA

4. The energy content in Kcal/g of carbohydrate : protein : Fat respectively is approximately in the ratio of-
(1) 1 : 2 : 2 (2) 1 : 1 : 2 (3) 2 : 2 : 1 (4) 2 : 1 : 1

5. The correct sequence of use of respiratory substrates in cellular respiration is :-
(1) Carbohydrate, Protein, Fat (2) Fat, Protein, Carbohydrate
(3) Carbohydrate, Fat, Protein (4) Protein, Fat, Carbohydrate

(4) ETS and oxidative phosphorylation (Terminal oxidation of NADH and $FADH_2$)

(i) It is associated with release and utilisation of the energy stored in $NADH + H^+$ and $FADH_2$.
(ii) $NADH + H^+$ and $FADH_2$ are oxidised through the electron transport system (ETS) and the electrons are passed on to O_2 resulting in the formation of H_2O.
(iii) ETS is present in the inner mitochondrial membrane of eukaryotes and plasma membrane of prokaryotes.

Electron carriers of ETS :

(i) Havins (FMN)
(ii) FeS
(iii) Quinone (Ubiquinone or CoQ)
(iv) Cytochromes (Cyt b $\longrightarrow$ Cyt c_1 $\longrightarrow$ Cyt c $\longrightarrow$ Cyt a $\longrightarrow$ Cyt a_3)

ETS is consists of four complexes and fifth complex is ATP synthase which is associated with ATP synthesis.

Name of complexes	Components of ETS	Inhibitors
Complex-I	FMN-NADH dehydrogenase	Rotenone & animal
Complex-II	$FADH_2$ dehydrogenase / Succinate dehydrogenase	
Complex-III	Cytochrome b-Cyto c_1	Antimycin
Complex-IV (Cytochrome C oxidase) Above four complexes are	Cyto. A, Cyto.a_3 and 2 Cu centres	Cyanide, CO

coupled with complex-V		
Complex-V	ATP synthase/ATPase	Oligomycin

Special features of ETS :

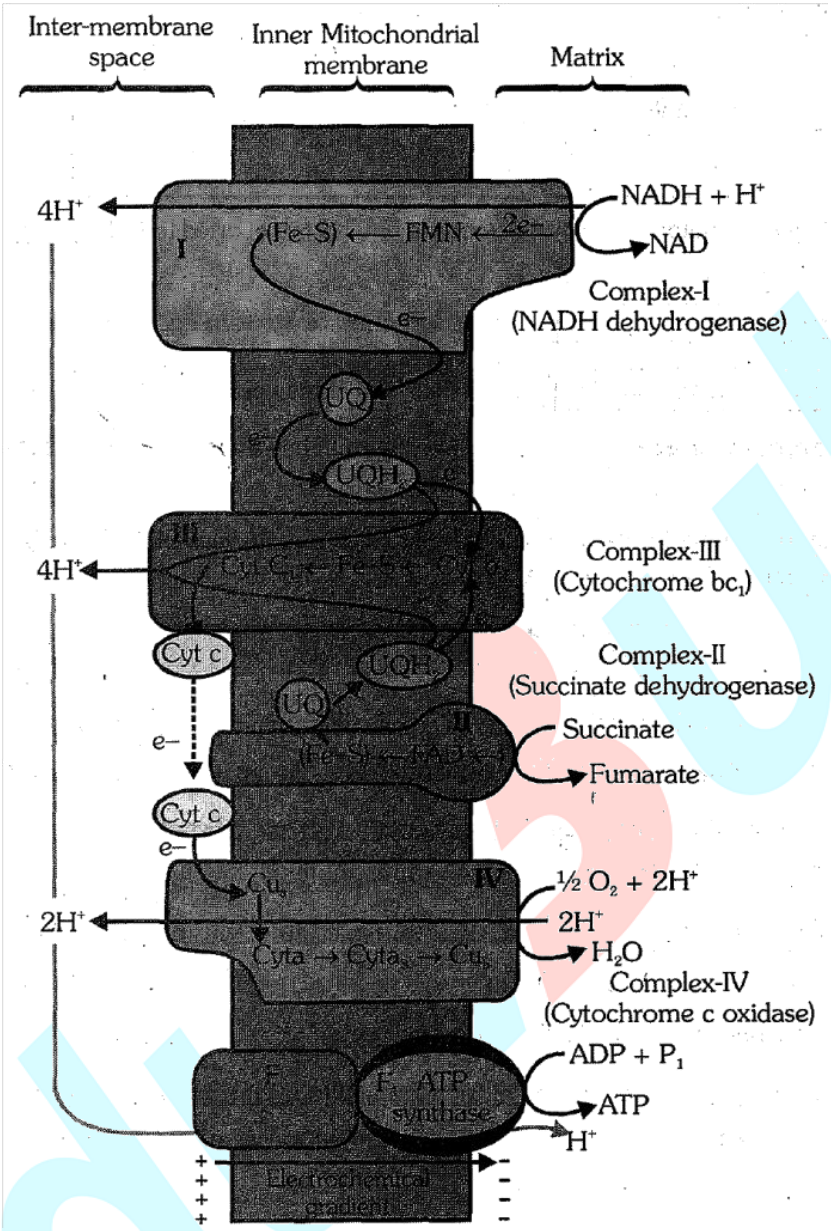
(i) UQ (Co.Q) and Cyt care mobile carrier of ETS.

(ii) Cytochrome c is a small protein attached to outer surface of the inner membrane and acts as a rriobile carrier for transfer of electrons between complex-m (cytochrome bc_1) and complex-IV (cytochrome c oxidase)

(iii) Every cytochrome has iron with variable valency $\left(Fe^{+++} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} Fe^{++}\right)$, Thus, helpful in transfer of electrons.

(iv) The role of O_2 is limited to the terminal stage of the process. The presence of oxygen is vital. Since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor.

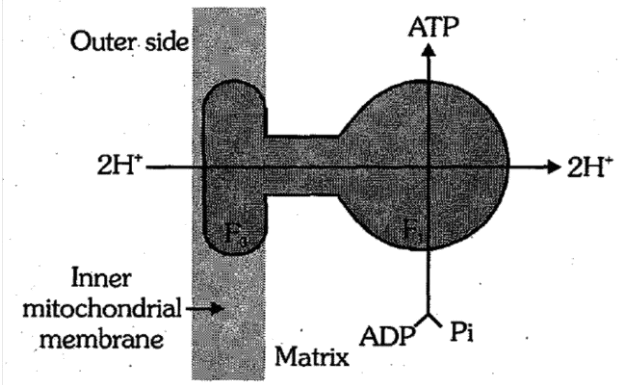
Cyanide inhibits the activity of cytochrome c oxidase which catalyse the oxidation of cytochrome a_3 and reduction of oxygen. In mitochondria of some plants alternative oxidase system is present in which ETS continues even in presence of cyanides. This type of respiration is known as cyanide resistance respiration or alternate electron pathway. eg. Spinach, Pisum



Oxidative phosphorylation (Chemiosmotic theory/Coupling theory)

(i) During ETS of respiration CoQ (UQ) & FMN can releases H+ ions in perimitochondrial space and leads to differenctial H^+ ion concetration across inner mitochondrial membrane. This differential H^+ ion concentration across inner mitochondrial membrane leads to creation of proton gradiant (pH gradient) and Electrical potential (diffrence of charge). Both are collectively known as Proton motive force (PMF).

(ii) PMF do not allow stay of H^+ ions in Perirnitochondrial space (PMS) so they return towards the matrix through F_0 part of ATPase selectively. Passage of $2H^+$ ions through F_0 part or proton channel leads to synthesis of 1 ATP.



(iii) Cytosolic or extra mitochondrial or glycolytic NADH transported to ETS by two type of shuttles (Only in eukaryotes) :
(a) Glycerol phosphate shuttle - Common shuttle system eg.- all plants, nerves and muscles.
(b) Malate aspartate shuttle - Heart, liver and kidney etc.

(iv) In prokaryotes, shuttle mechanism is absent. They always get 38 ATP from aerobic respiration of 1 glucose.

(v) Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of $FADH_2$ produces 2 molecules of ATP.

The respiratory balance sheet

It is possible to make calculations of the net gain of ATP for every glucose molecule oxidize & but in reality this can remain only a theoretical exercise. These calculations can be made only on certain assumptions that:

- There is a sequential, orderly pathway functioning, with one substrate forming the next and with glycolysis, TCA cycle and ETS pathway following one after another.
- The NAPH synthesised in glycolysis is transferred into the mitochondria and undergoes oxidative phosphorylation.
- None of the intermediates in the pathway are utilised to synthesise any other compound. Only glucose is being respired- no other alternative substrates are entering in the pathway at any of the intermediary stages.

But this kind of assumptions are not really valid in a living system: all pathways work simultaneously and do not take place one after another: substrates enter the pathways and are withdrawn from it as and when necessary : ATP is utilised as and when needed; enzymatic rates are controlled by multiple means. Yet, it is useful to do this exercise to appreciate the beauty and efficiency of the living system in extraction and storing energy.
Hence, there can be a net gain of 36 ATP molecules during aerobic respiration of one molecule of glucose.
Theoretical energy calculation for complete oxidation of one glucose molecule :

Step	Number of turn	ATP synthesis through substrate level phosphorylation	ATP gain through oxidative phosphorylation	ATP consumed	Net gain
EMP pathway	1	4	6 or 4	2	8 or 5
Link	2	0	6	0	6

reaction					
Krebs cycle	2	2	22	0	24

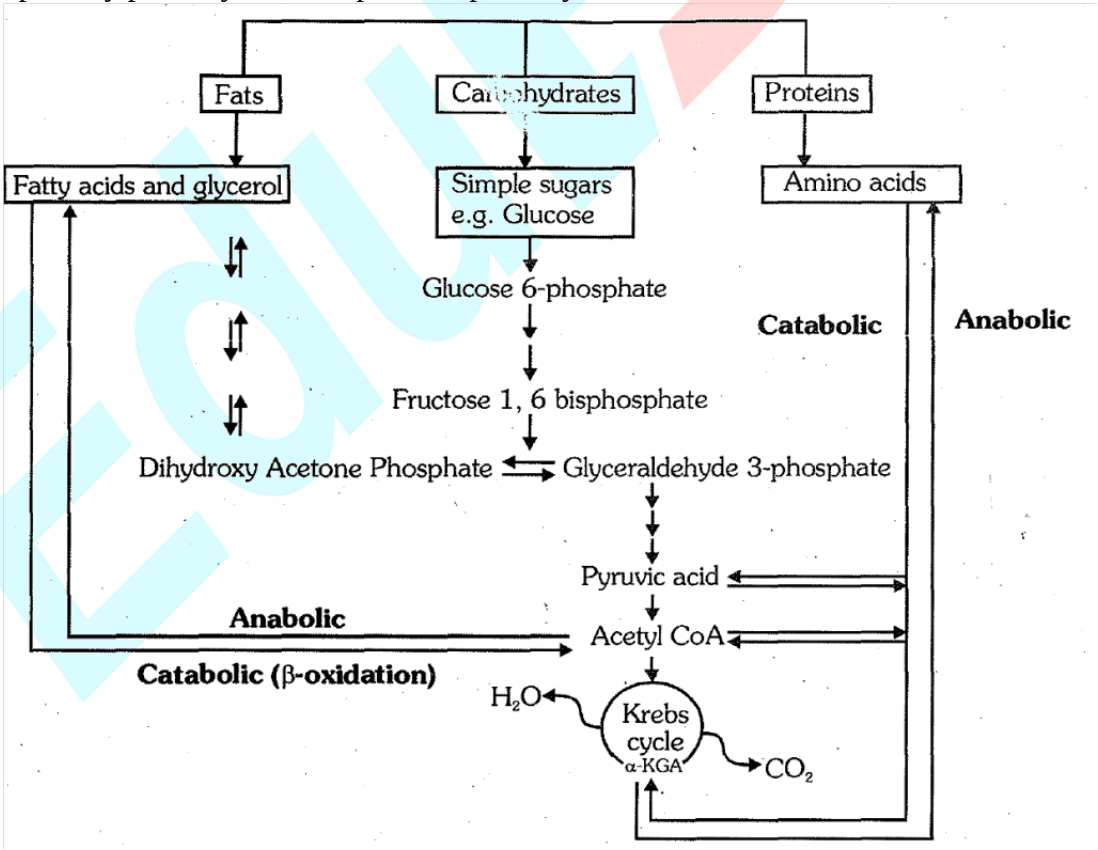
Amphibolic Pathway

(i) Glucose is the favoured substrate for respiration. All carbohydrates are usually first converted into glucose before they are used for respiration. Other substrates can also be respired, but then they do not enter the respiratoty pathway at the first step.

(ii) Fats would need to be broken down into glycerol and fatty acids first. If fatty acids were to be respired they would first be degraded to acetyl CoA and enter the pathway. Glycerol would enter the pathway after being converted to PGAL.

(iii) The proteins would be degraded by proteases and the individual·amino acids (after deamination) depending on their structure would enter the pathway at some stage within the Krebs cycle or even as pyruvate or acetyl CoA.

(iv) Since respiration involves breakdown of substrates, the respiratory process has traditionally been considered a catabolic process and the respiratory pathway as a catabolic pathway. Fatty acids would be broken down to acetyl CoA before entering the respiratory pathway when it is used as a substrate. But when the organism needs to synthesise fatty acids, acetyl CoA would be withdrawn from the respiratory pathway for it, Hence, the respiratory pathway comes into the picture both during breakdown and synthesis of fatty acids. Similarly, during breakdown and synthesis of protein too, respiratory intermediates form the link. Breaking down processes within the living organism is catabolism, and synthesis is anabolism. Because the respiratory pathway is involved in both anabolism and catabolism. it would hence be better to consider the respiratory pathway as an amphibolic pathway rather than as a catabolic one.



(v) Glycolysis is also known as oxidative anabolism or catabolic resynthesis, because it links with anabolism of fats and amino acids. An intermediate PGAL is used for the synthesis of glycerol later forms fats or lipid. PGA is used for synthesis of Serine, Glycine, Cysteine, Alanine forms from pyruvate.

(vi) Acetyl CoA is common meeting point (connecting link) between fat, carbohydrate and protein metabolism.

Amphibolism of Krebs cycle-

(1) Acetyl CoA- Synthesis of fatty acids & GA (Gibberellic acid)

(2) Succinyl CoA- Synthesis of chlorophyll, Cytochromes, Phytochromes

(3) OAA & α-Ketoglutaric acid- Synthesis of Amino acids.

(4) OAA-Synthesis of Alkaloids.

Anaerobic respiration / Fermentation

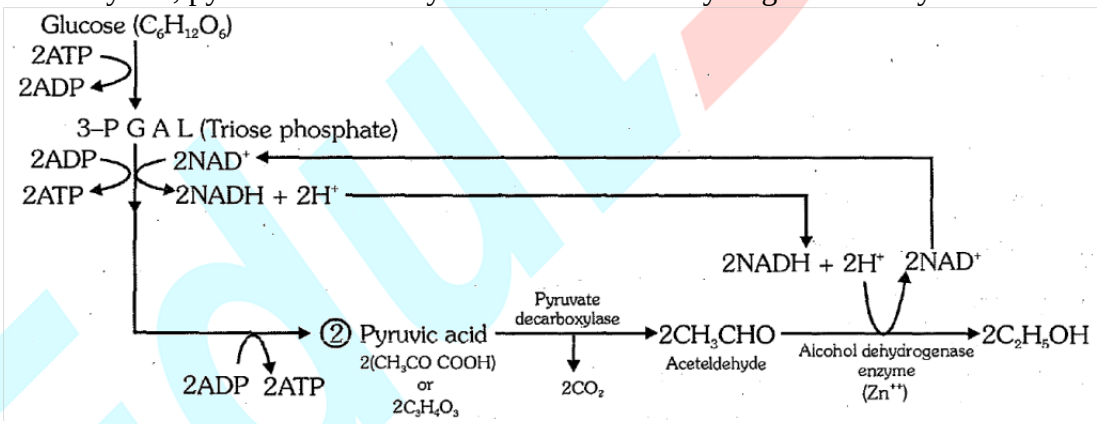
In fermentation not much energy is released; less than seven percent of the energy m glucose is released and not all of it is trapped as high energy bonds of ATP.

The processes are hazardous. either acid or alcohol is· produced. Yeasts. poison themselves to death when the concentration of alcohol reaches about 13% percent.

Fermentation is of two types :-

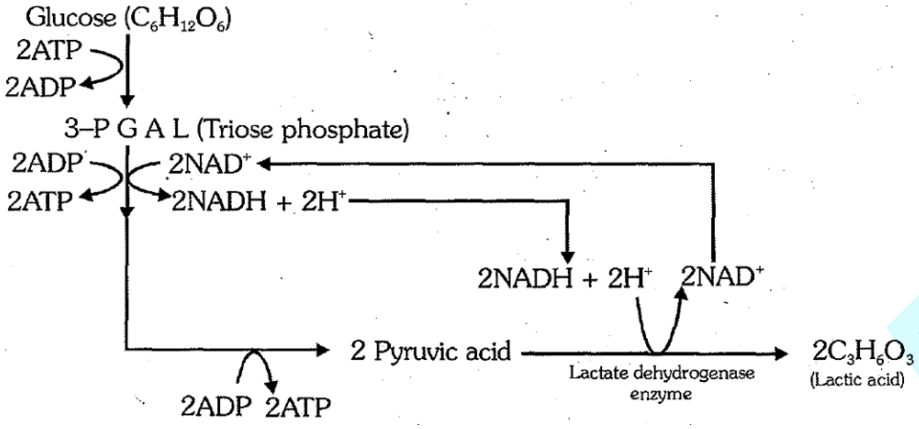
(A) Alcoholic Fermentation :

In this fermentation, say by yeast, the incomplete oxidation of glucose is achieved under anaerobic conditions by sets of reaction where pyruvic acid is converted to CO_2 and ethanol. The enzymes, pyruvate decarboxylase and alcohol dehydrogenase catalyse these reactions.



(B) Lactic acid Fermentation :

Some bacteria produce lactic acid from pyruvic acid. In animal cells also like in muscles during exercise, when oxygen is inadequate for cellular respiration pyruvic acid is reduced to lactic acid by lactate dehydrogenase.



- The reducing agent is NADH + H^+ which is reoxidised to NW in both the processes.
- During alcoholic fermentation triose phosphate (3PGAL) is the electron donor and acetaldehyde is acceptor, while during lactic acid fermentation although electron donor is triose phosphate but acceptor is pyruvic acid.

Q. What is the net ATPs that is synthesised when one molecules of glucose is fermented to alcohol or lactic acid?

Ans. 2 ATP

Q. What would be the maximum concentration of alcohol in beverages that are naturally fermented ?

Ans. ≤ 13%

Q. What is the way to obtain alcoholic beverages of alcohol content greater than 13 percent concentration ?

Ans. Distillation

Q. What is the process by which organisms can carry out complete oxidation of glucose and extract the energy stored to synthesise a larger number of ATP molecules needed for cellular metabolism ?

Ans. Aerobic respiration is the process that leads to a complete oxidation of organic substances in the presence of oxygen and release CO_2, water and a large amount of energy present in the substrate.

Pasteur effect :

It is an inhibitory effect of oxygen on the fermentation process.

Explanation :

The effect can be easily explained; as the yeast being facultative anaerobes can produce energy using two different metabolic pathways.

(i) While the oxygen concentration is low, the product of glycolysis, pyruvate is turned into ethanol and CO_2 and the energy production efficiency is low (2 moles of ATP per mole of glucose).

(ii) If the oxygen concentration grows, pyruvate is converted into acetyl CoA that can be used in the citric acid cycle, which increases the efficiency to 36 moles of ATP per mole of glucose.

Respiratory Quotient

The ratio of the volume of CO_2 evolved to the volume of O_2 consumed in respiration is called the respiratory quotient (RQ) or respiratory ratio.

$$RQ = \frac{\text{Volume of } CO_2 \text{ evolved}}{\text{Volume of } O_2 \text{ consumed}}$$

The respiratory quotient depends upon the type of respiratory substrate used during respiration.

[RQ = 1]

$C_6H_{12}O_6 + 6O_2 + 6H_2O \longrightarrow 6CO_2 + 12H_2O + 686$ kcal

$$RQ = \frac{6CO_2}{6O_2} = 1.0$$

[RQ = ∞]

$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH + 2CO_2 + 59$ kcal

$$RQ = \frac{6CO_2}{\text{Zero } O_2} = \infty$$

[RQ = Zero]

In succulent plants due to availability of insufficient O_2 glucose oxidise partially and RQ will be zero.

$2C_6H_{12}O_6 + 3O_2 \longrightarrow 3C_4H_6O_5 + 3H_2O +$ Energy

Malic acid

[RQ = Less than one]

- During complete oxidation of protein and fat
- During protoplasmic respiration (In case of a starved cell)
- In case of mixed diet.
- In case of germinating fatty seeds.

$2(C_{51}H_{98}O_6) + 145\ O_2 \longrightarrow 102\ CO_2 + 98\ H_2O +$ Energy

Triplamitin

$$RQ = \frac{102CO_2}{145O_2} = 0.7$$

When proteins are respiratory substrates the ratio would be about 0.8 or 0.9.

Pure proteins or fats are never used as respiratory substrates because before entering the respiratory pathway they must be converted into such compounds which can enter into the glycolysis or link reaction or Krebs cycle at their respective stages.

[RQ = More than one]

- During complete oxidation of organic acids.
- In case of maturing fatty seeds.

$2(COOH)_2 + O_2 \longrightarrow 4CO_2 + 2H_2O +$ Energy

Oxalic acid

RQ = 4.0

$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O +$ Energy

Malic acid

RQ = 1.33

$2C_4H_6O_4 + 7O_2 \longrightarrow 8CO_2 + 6H_2O +$ Energy

Succinic acid

RQ = 1.14

Energy efficiency of cellular respiration :-

$ADP + iP \xrightarrow{8.1Kcal} ATP$

Thus, 8.1 Kcal energy trapped for each molecule of ATP formation. Therefore, during arabic respiration if total net gain is 36 ATP molecules then total trapped energy is 36×8.1 = 291.6 Kcal.

$$\text{Efficiency} = \frac{291.6}{686} \times 100 = 42.50\%$$

During arobic respiration if total net gain is 38 ATP molecules then total trapped energy is 38×8.1 = 307.8 Kcal.

$$\text{Efficiency} = \frac{307.8}{686} \times 100 = 44.86\ \%$$

The total energy content of 1 molecule of glucose is 686 Kcal.

DYNAMIC STATE OF BODY CONSTITUENTS-CONCEPT OF METABOLISM

- All biomolecules have a turn over. This means that they are constantly being changed into some other biomolecules and also made from some other biomolecules. This breaking and making is through chemical reactions constantly occurring in living organisms. Together all these chemical reactions are called meta.
- Each of the metabolic reactions results in the transformation of biomolecules. A few examples for such metabolic transformations are; removal of CO_2 from amino acids making an amino add into an amine removal of amino group in a nucleotide base; hydrolysis of a glycosidic bond in a disaccharide, etc.
- Majority of these metabolic reactions do not occur in isolation but are always linked to some other reactions. In other words metabolites are converted into each other in a series of liked reactions called metabolic pathways.
- These metabolic pathways are similar to the automobile traffic in a city. These pathways are either linear or circular. These pathways criss-cross each other. i.e., there are traffic junctions. Flow of metabolites through metabolic pathway has a definite rate and direction like automobile traffic. This metabolites flow is called the dynamic state of body constituents.
- There is no uncatalysed metabolic conversion in liying systems. Even CO_2 dissolving in water a physical process is a catalysed reaction in living systems.

METABOUC BASIS FOR LIVING

- Metabolic pathways can lead to a more complex structure from a simpler structure - (for example, acetic acid becomes cholesterol) or lead to a simpler structure from a complex, structure (for example. glucose becomes lactic acid in our skeletal muscle). The former cases are called biosynthetic pathways or anabolic pathways. The latter constitute degradation and hence are called catabolic pathways.
- Anabolic pathways as expected consume energy. Assembly of a protein from amino acids requires energy input. On the other hand. catabolic pathways lead to the release of energy. For example. when glucose is degraded to lactic acid in our skeletal muscle. energy is liberated.
- How do living organisms derive their energy? What strategies have they evolved? How do they store this energy and in what form? How do they convert this energy into work? All this study comes under a sub-discipline called 'Bioenergetics'.

THE LIVING STATE

- Chemical compounds in a living organism, otherwise called metabolites, or biomolecules are present at concentrations characteristic of each of them. For example, the blood concentration of glucose in a normal healthy individual is 4.5-5.0 mM, while that of hormones would be nanograms/mL.
- The most important fact of biological systems is that all living organisms exist in, a steady-state characterized by concentrations of each of these biomolecules. These biomolecules are in a metabolic flux. Any chemical or physical process moves spontaneously to equilibrium.
- One should remember from physics that systems at equilibrium cannot perform work. As living organisms work continuously, they cannot afford to reach equilibrium. Hence the living state is a non-equilibrium steady-state to be able to perform work.
- Living process is a constant effort to prevent falling into equilibrium. This is achieved by energy input. Metabolism provides a mechanism for the production of energy. Hence the living state and metabolism are synonymous. Without metabolism there cannot be a living state.

ENZYMES

History

- Buchner discovered and isolated the enzyme zymase from yeast cells, while Kuhne coined the term enzyme.
- J.B. Sumner purified and crystalized urease enzyme from canavalia/Jack bean/Lobia plant.

Characteristics of enzymes

(1) Almost all enzymes are proteins. Though, there are some nucleic acids that behave like enzymes. These are called ribozymes.

- L_{19} RNAase (a ribozyme) was discovered by T.Cech from rRNA of a protozoan, Tetrahymena thermophila.
- RNAase P or Ribonuclease P (a ribbzyme) was & covered by Altman from a prokaryotic cell.

(2) Enzymes are colloidal substances, which are macromolecules of amino acids and are synthesised by ribosomes under genetic control.

(3) Encyme can be depicted by a line diagram. An enzyme like any protein has -

(a) Primary structure : Amino acid sequence of the protein. Its lack active sites.

(b) Secondary structure : It is a helical structure which also lack active sites.

(c) Tertiary structure : In this structure backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence many crevices or pockets are made such pockets represent active sites.

The catalytic structured most ofthe enzymes are tertiary and globular.

(d) Quarternary structure : Represented by, isoenzymes and active sites are present.

(4) Active site : An active site of an enzyme is a crevice or pocket into which the substrate fits. Thus enzymes through their active site, catalyse reactions at a high rate.

(5) Enzymes are very specific to their substrate or reactions. They are required in very small amount to catalyse a reaction. Catalytic power of an enzyme depends upon -

(a) Turn over number (b) Km constant

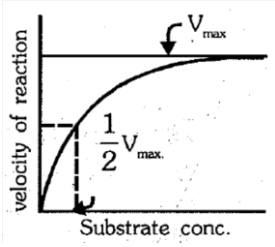
(a) Turn over number : It is the number of substrate molecules converted into products per unit time by a molecule of enzyme. Thus, catalytic power is directly proportional to turn over number. Carbonic anhydrase enzyme is considered as the fastest enzyme.

Enzyme	Turnover number
Carbonic anhydrase	306 lakh/minute
Catalase	50 lakh/minute

Flavoprotein	50/minute
Lysozyme	30/minute

(b) Km constant: This was coined by Michaelis and Menten. It is the concentration of substrate at which rate of reaction attains half of its maximum velocity.

$$K_m = \frac{1}{2} V_{max.}$$



Catalytic power of an enzyme id inversely proportional to its Km value.

(6) Enzyme increase the rate of reaction several times by lowering down activation energy.

(7) Catalytic power of an enzyme remains same even outside the living system.

(8) Enzymes when not in use, represent inactive form, called zymogen or pro-enzyme. Pepsinogen is an inactive form of pepsin, similarly trypsinogen is an inactive form of trypsin.

Enzyme (Biocatalyst)	Inorganic catalyst
Enzymes are thermo-sensitive and get damaged at high temperatures (say above 40°C)	They work efficiency at high temperatures and high pressures.

However enzymes isolated from organisms who normally live under extremely high temperatures (eg. hot vents and sulphur springs). are stable and retain their catalytic power even at high temperature. Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms. e.g. tag polymerase.

Uncatalysed reaction versus catalysed reaction

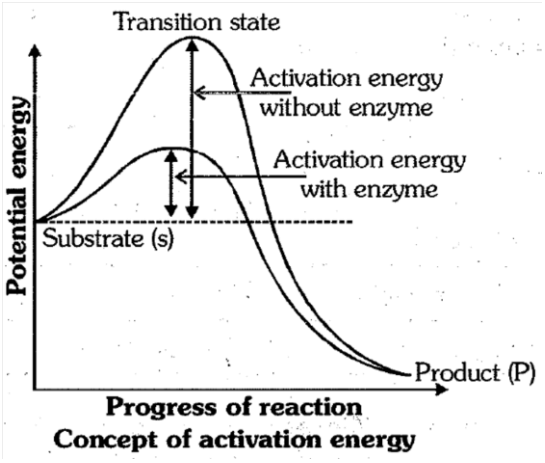
$$\underset{\text{Carbon di oxide}}{CO_2} + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} \underset{\text{Carbonic acid}}{H_2CO_3}$$

In the absence of an enzyme this reaction is very slow with about 200 molecules of H_2CO_3 being formed in an hour. However in the presence of enzyme carbonic anhydrase inside cytoplasm the reaction speeds dramatically with about 6,00,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times.

How do enzymes bring about such-high rates of chemical conversions ?

The chemical or metabolic conversion refers to a reaction. The chemical which is converted into a product is called a substrate. Hence enzymes i.e. proteins with three dimensional structures including an active site to convert a substrate (S) into a product (P) Symbolically, this can be depicted as:

$$S \rightarrow P$$



Concept of activation energy

They-axis represents the potential energy content. The x-axis represents the progression of the structural transformation or states through the 'transition state'. In above graph 'P' is at a lower level than 'S', thus this reaction is 1m exothermic reaction. (No need to supply energy in order to form the product.) However, whether it is an exothermic or spontaneous reaction or an endothermic or energy requiring reaction, the 'S' has to go through a much higher energy state or transition state. ·

"The difference in average energy content of 'S' from that of this transition state is called activation energy".

Enzymes eventually bring down this energy barrier making the transition of 'S' to 'P' more easy. All changes (ES complex, EP complex) occured during transition state are transient and unstable.

Nature of enzyme action

Each enzyme (E) has a substrate (S) binding site in its molecular structure so that a highly reactive enzyme- substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP). The formation 'of the ES complex is essential for catalysis.

Enzyme + Substrate ⇌ EPC ⟶ Enzyme + Product

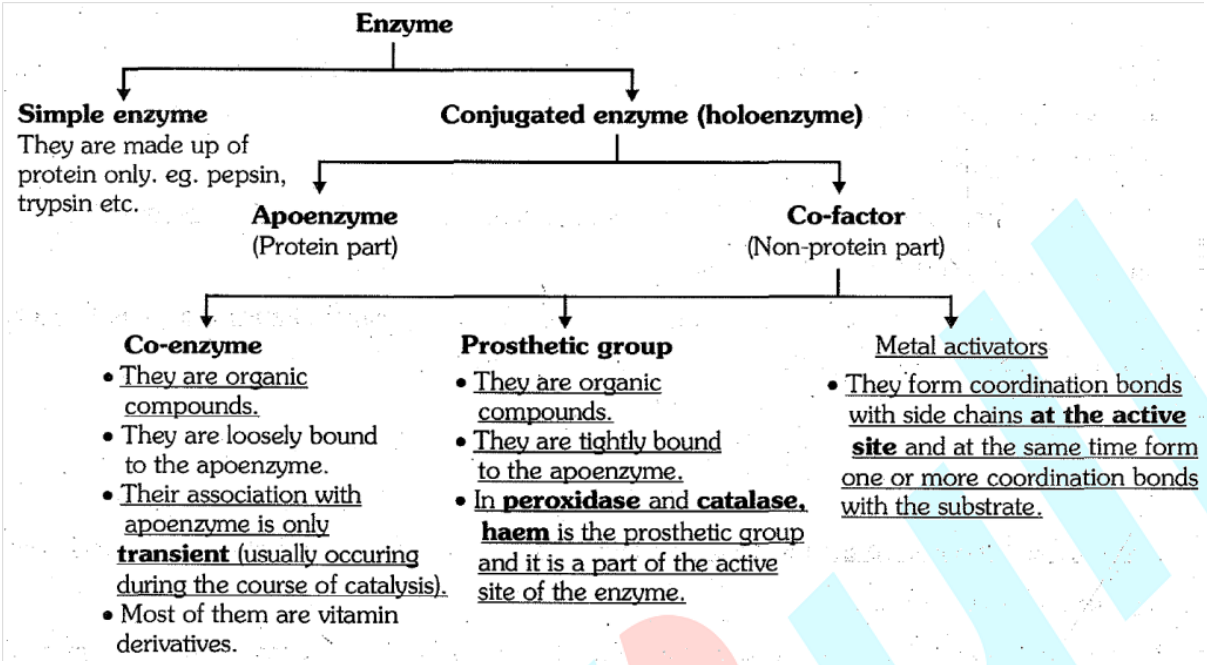
The catalytic cycle of an enzyme action can be described in the followirig steps:

1. First the substrate binds to the active site of the enzyme. fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity of the substrate breaks or form the chemical bonds of the substrate and the new enzyme-product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

Cofactors

Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called cofactors are bound to the enzyme to make the enzyme catalytically active. In these instances the protein portion of the enzymes is called the apoenzyme and non protein portion is called the cofactor.

Three kinds of cofactors may be identified : co-enzymes, prosthetic groups and metal ions.



Important examples of coenzymes and metal activators :

Examples of co-enzymes	Examples of metal activator
Co. I (NAD/DPN) : Derivative of niacin	Fe^{++} : Cytochrome c oxidase, peroxidase, aconitase
Co. II (NADP/TPN) : Derivative of niacin	Cu^{++} : Cytochrome c oxidasde, tyrosinase
FAD : Derivative of riboflavin	Zn^{++} : Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase
FMN : Derivative of riboflavin	Mg^{++} : Hexokinase, glucokinase, pyruvate kinase, PEPcase, RuBisCO
TPP : Derivative of thiamine	K^{+} : Pyruvate kinase
Co. R : Derivative of ubiquinone	Mn^{++} : arginase, ribonucleotide reductase, decarboxylase
Co. R : Derivative of biotin	Mo : Nitrogenase complex, nitrate reductase
Co. A : Derivative of panthothenic acid	Se : Glutathione peroxidase

Classification and Nomenclature

Thousands of enzymes have been discovered. isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

(I) Oxidoreductases/dehydrogenases : Enzymes which catalyse oxidoreduction (oxidation-reduction) between two substrates i.e. S and S' e.g. cytochrome c oxidase, dehydrogenase etc.

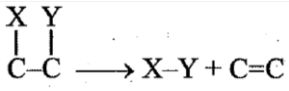
S reduced + S' oxidised $\rightarrow$ S oxidised + S' reduced

(II) Transferases : Enzymes catalysing a transfer of a group. G (other than hydrogen) between a pair of substrate S and S'. e.g. transaminase, hexokinase etc.

S–G + S' $\rightarrow$ S + S'–G

(III) Hydrolases : Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C–C, C–halide or P–N bonds. e.g. proteases, lipases, carbohydrases etc.

(IV) Lyases : Enzymes that catalyse removal of a group from substrate by mechanisms other than hydrolysis and leaving double bonds. e.g. aldolase.



(V) Isomerases : Includes all enzymes catalysing inter-conversion of optical geometric or positional isomers. e.g. hexoisomerase, mutase etc.

(VI) Ligases/synthase : Enzymes catalysing the linking together of two compounds. Such enzymes catalyse joining of C–O, C–S, C–N, P–O etc. bonds. e.g. citrate synthase, DNA ligase etc.

Factors affecting enzyme activity

The activity of an enzyme can be affected by a change in the conditions which can alter the tertiary structure of the protein. These include :

(1) Temperature
(2) pH
(3) Change in substrate concentration
(4) Inhibitor

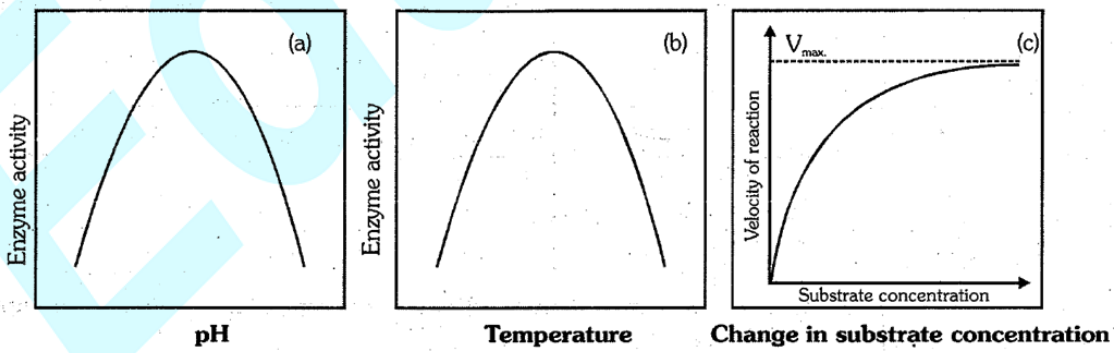
(1) Temperature:

Enzymes generally function in a narrow range of temperature. Each enzyme shows its highest activity at a particular temperature called the optimum temperature. Activity declines both below and above the optimum value. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature enzymatic activity because proteins are denature by heat.

A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Thus value of Q_{10} for enzymatic activities is 2.

(2) pH :

Enzymes generally function in a very narrow range of pH. Each enzyme shows its highest activity at a particular pH called the optimum pH. Activity declines both below and above the optimum value.



(3) Change in substrate concentration :

With the increase in substrate concentration. the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches· a maximum velocity (V_{max}).

This velocity is not exceeded by any further rise in concentration of the substrate.

Reason : The enzyme molecules are fewer than the substrate molecules and after saturation of these enzyme molecules, there are no free enzyme molecules to bind with the additional substrate molecules.

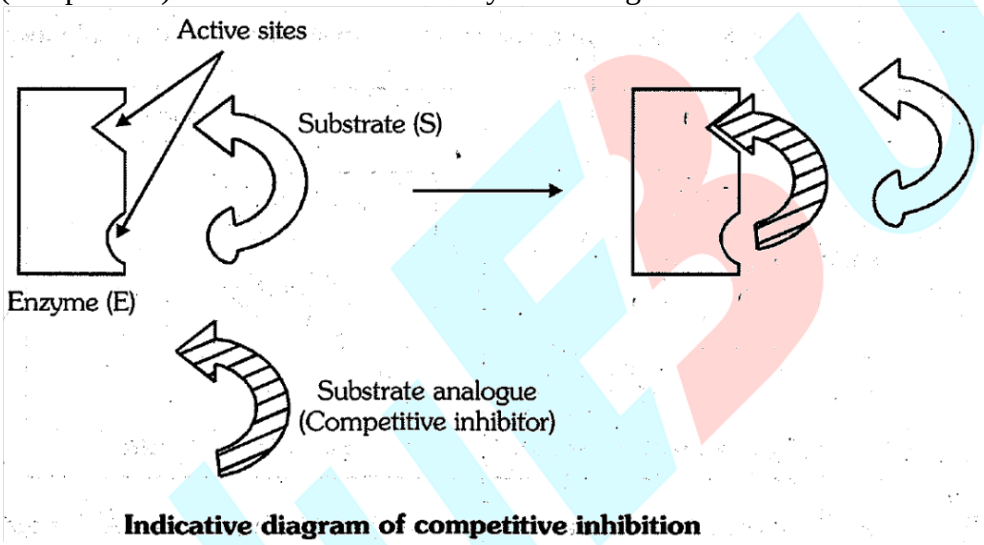
(4) Inhibitors/Enzyme inhibition :

The activity of an enz.yme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off (inhibits) enzyme activity, the process is called inhibition and the chemical is called an inhibitor.

It is of two types :

(1) Competitive inhibition:

- When the inhibitor closely resembles the substrate (substrate analogues) in its molecular structure and binds with active site of enzyme leads to inhibition of enzyme activity.
- It is competitive as well as reversible because inhibitor binds with active site (competitive) and can be deattached by increasing concentration of substrate. (reversible)



Indicative diagram of competitive inhibition

eg. (a) Inhibition of succinate dehydrogenase by malonate. Malonate and succinate are structural analogues.

(b) Inhibition of folic acid synthesis by sulpha drugs.

p-amino benzoic acid (PABA) is a precursor of folic acid. Sulpha drugs are structural analogues of p-amino benzoic acid, thus inhibits synthesis of folic acid. This competitive inhibition is often used in the controls of bacterial pathogens.

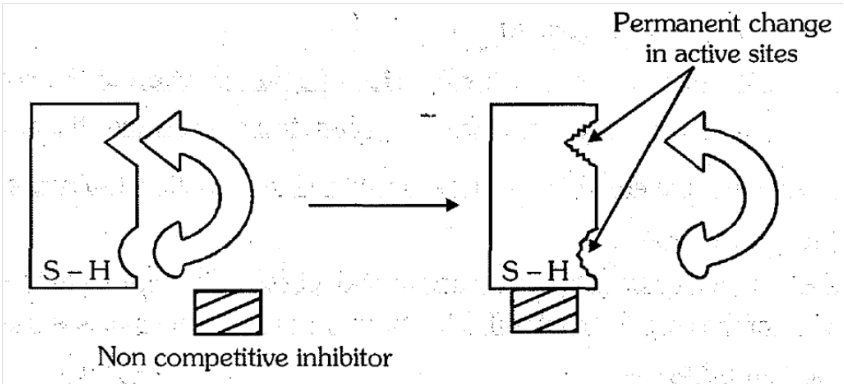
Reason : Bacteria requires folic acid for growth and multiplication.

[In the presence of competitive inhibitor Km increases while $V_{max.}$ remains unchanged.]

(2) Non competitive inhibition : It is again of two types :

(i) Non competitive irreversible :

- When the inhibitor binds with the site other than its active site (non competitive) and destroy the sulpha hydryl (S–H) group of enzyme.
- Here binding is irreversible because inhibitor can not be deattached by increasing substrate concentration.

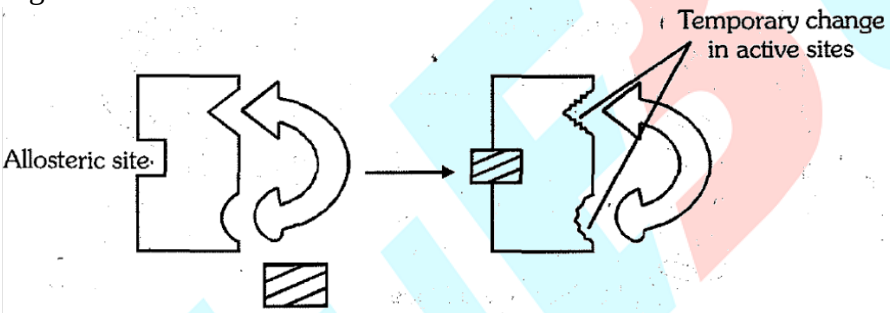


Indicative diagram of non competitive irreversible inhibition

eg. Cyanide binds with Cu center of Cyt a_3 of cytochrome c oxidpse and inhibits enzyme permanently.

(ii) Non competitive reversible : (Mostly)

- Some enzymes have allosteric site to control active site. This control is called modulation.
- Most of non competitive inhibitors bind reversibly with allosteric site and negatively change the configuration of active site. Such inhibition called non competitive reversible or negative allosteric modulation.



Indicative diagram of non competitive reversible inhibition

eg. Phosphofructokinase/PFK (pacemaker enzyme) inhibited by excess of ATP (negative modulation).

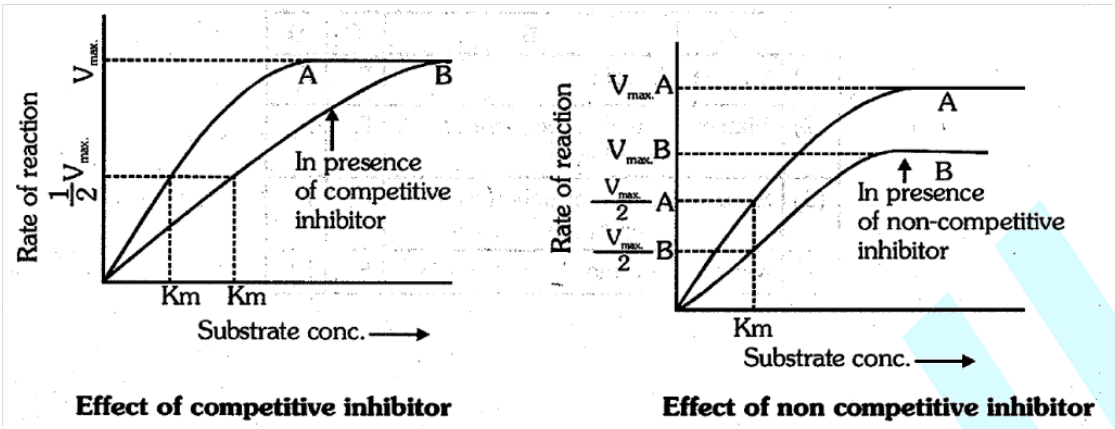
- Some allosteric enzyme inhibited by the product of that biochemical reaction which is catalysed by them, such inhibition called feedback inhibition or retro inhibition or product inhibition.

eg. Glucose + ATP $\xrightarrow[Mg^{++}]{\text{Hexokinae}}$ Glucose-6-phosphate + ADP

In above reaction the enzyme hexokinase inhibited reversibly by excess of product (glucose-6-phosphate)

"Therefore, all feedback inhibition are allosteric inhibition but all allosteric inhibiton are not feed back"

[In the presence of non competitive inhibitor Km remains unchanged while V_{max}. decreases. Km is not applicable for allosteric modulation.]



Effect of competitive inhibitor **Effect of non competitive inhibitor**

Note : Allosteric site may also be use to increase enzymatic activity such phenomenon is called positive allosteric modulation. eg. Phosphofructokinase/PFK activated by excess of AMP (positive modulation).

BEGINNER'S BOX-2

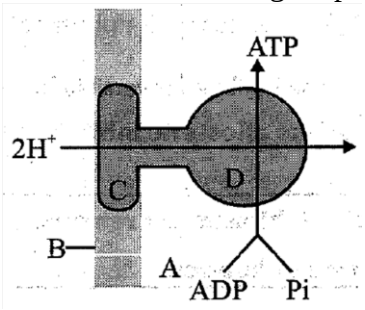
1. Non protein organic compound, which shows transient association with apoenzyme usually during the course of catalysis, is known as :-
(1) Coenzyme (2) Prosthetic group
(3) Metal ion (4) Pathogen

2. The full name of NAD is :-
(1) Nicotinamide adenosine dinucleotide (2) Nicotinamide adenine dinucleotide
(3) Nicotinamide adenine diamide (4) Nicotinamide adenosine diamide

3. Which of the following complexes of the respiratory electron transport system contain two copper centres?
(1) Complex-I (2) Complex-IV
(3) Complex-II (4) Complex-III

4. The correct relationship of value of Respiratory quotient is :-
(1) Glucose > Fats > Organic acid (2) Glucose < Fats < Organic acid
(3) Fats > Glucose > Organic acid (4) Fats < Organic acid > Glucose

5. Given below is the diagrammatic sketch of ATP synthesis in mitochondria. Identify the components labeled A, B, C and D and select the right option about them :-



Options :-

	A	B	C	D
(1)	Stroma	Matrix	F_1	F_0

(2)	Matrix	Inner mitochondrial membrane	F_0	F_1
(3)	Stroma	Outer mitochondrial membrane	F_0	F_1
(4)	Matrix	Stroma	F_1	F_0

ANSWER KEY

BEGINNER'S BOX-1

1. (1) 2. (2) 3. (2) 4. (2) 5. (3)

BEGINNER'S BOX-2

1. (1) 2. (2) 3. (2) 4. (4) 5. (2)