RESPIRATION

- Respiration is a **Amphibolic** & **Exergonic** cellular process.
- Respiration is an enzymatic process, which is also known as **internal respiration/tissue respiration/dark respiration/cellular respiration / mitochondrial Respiration.**
- An important feature of respiration is liberation of metabolic energy as **ATP.**

Reparatory substrate :

(Carbohydrates → Fats → Protein → others)

- When respiratory substrate are **carbohydrates** likes glycogen, starch, sucrose, hexose or fats, then respiration is know as **floating respiration.**
- When protein is oxidised in respiration, then respiration is Known as **protoplasmic respiration** protoplasmic components or cellular proteins may oxidised at the time of starvation & disease.
- Exceptionally oxidation of proteins in legume seeds in called **floating respiration.**

Types of respiration :

(A) **Aerobic respiration :** The complete oxidation of food with the use of oxygen and when entire carbon released, as CO_2 is called as aerobic respiration.

$$C_6H_{12}O_6 + 6O_2 + 6H_2O \xrightarrow[\text{cytp. \& mito}]{\text{Enzyme}} 6CO_2\uparrow + 12H_2O + 6\ 86\ \text{Kcal E (38/36 ATP)}$$

(B) **Aerobic respiration :** This is an incomplete oxidation.

- When food is oxidizded into alcohol or organic acids without use of oxygen.
  During it most of the energy is lost if form of heat. It occurs in cytoplasm and only 2ATP are produced.

$$C_6H_{12}O_6 \xrightarrow[\text{cytoplasm}]{\text{Enzymes}} 2C_2H_5OH + 2CO_2 + 21\ \text{Kcal (ATP)}$$

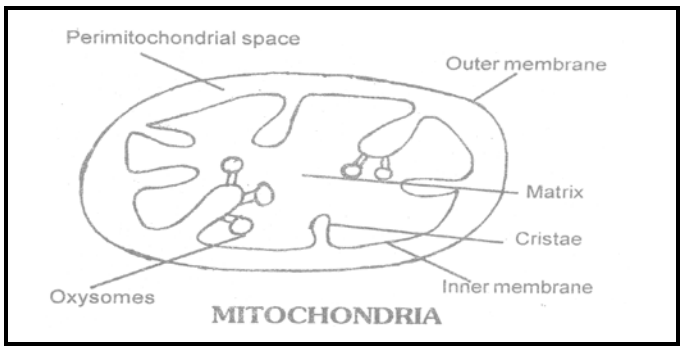
- Anaerobic respiration was first reported by **Kostychev.**
- Anaerobic respiration may takes place in bacteria, some lower parasitic animals (**Ascaris, Taenia**) plans, R.BCs. & muscles o human body. When oxygen is not available, then food in completely oxides in to some organic compounds like ethanol, acetic acid, lactic acid.
- In muscle cells & some bacteria, the energy is produced by breaking of glucose into lactic acid inside the cells.
- The amount of energy released in anaerobic respiration is much less than aerobic respiration.
- **Fermentation** is performed by some fungi & some bacteria (only by microbes) and is an extra cellular process. No ATP is produced.

$$C_6H_{12}O_6 \xrightarrow[\text{Bact.}]{\text{Yeast}} 2C_2H_5OH + 2CO_2 + \text{Heat}$$

- Both anaerobic respiration and fermentation are **incomplete oxidations.**
- Inhibitory effect on respiration (anaerobic respiration) of oxygen is called **Pasteur effect.**
  (Anaerobic → Aerobic)

STEPS OF RESPIRATION

(1) **Glycolysis -** Occurs in cytosol/cytoplasm
(2) **Formation of Acetyl COA -** (**Link Reaction**) Perimitochondiral space (outer chamber)
(3) **TCA cycle or Kreb's cycle -** Matrix of mitrochondria & cytosol in bacteria
(4) **ETS** - Occurs in cristae or inner memberance of mitochondria and **Oxidative phosphorylation -** Occurs in Oxysome head (F_1 particle)



(1) Glycolysis - EMP (Embden, Meyerhof, Parnas) Pathwa.

- The glycolysis is common phse for eaerobic & anaerobic respirations both.
- Glycolysis involves a series of ten biochemical reactions in cytoplasm.
- In glycolysis, neither consumption of oxygen nor liberation of CO_2 take place.
- In glycolysis, 1 glucose, produces 2mol, of pyruvic acids (3C)
- $2NADH_2$ & 2ATP are generated is glycolysis, which are equal to 8 ATP
- **Substrate level phosphorylation** from 4 ATP : [When the substrate release energy for phosphrylation of ADP Or formation of ATP, without ETS then calels a **substrate level phosphorylation**]
- 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 glycerola laterl forms **fats** or **lipid. PGA** is used for synthesis of **Serine, Glycine, Cystine. Alanine** forms from **pyruvate.**

Biochemical reactions of Glycolysis

1. Glucose $\xrightarrow[ATP \rightarrow ADP]{Hexo\ Kinase,\ Mg^{++}}$ Glucose - 6P

2. Glucose - 6P $\xrightarrow{Isomerase}$ Fructose - 6P

3. Fructose - 6P $\xrightarrow[ATP \rightarrow ADP]{Phosphoftuctokinase,\ Mg^{++}}$ Fructose - 11,6-Bip

4. Fructos-1, 6-BiP $\xrightarrow{Aldolas}$ PGAL $\rightleftharpoons$ DHAP

5. 2 PGAL $\xrightarrow[+\ 2ip]{Nonenzymati}$ 1,3 Bi PGAL (2 mol.)

[Phosphoglyceraldehyde]
3c

Starting of energy conserving phase :

6. 2,1,3 BiPGAL $\xrightarrow[2NA \rightarrow 2NADH]{dehudrogenasse,\ Mg^{+}}$ 1,3 BiPGA (2mol.)
(lodoacetate inhibits this reaction)

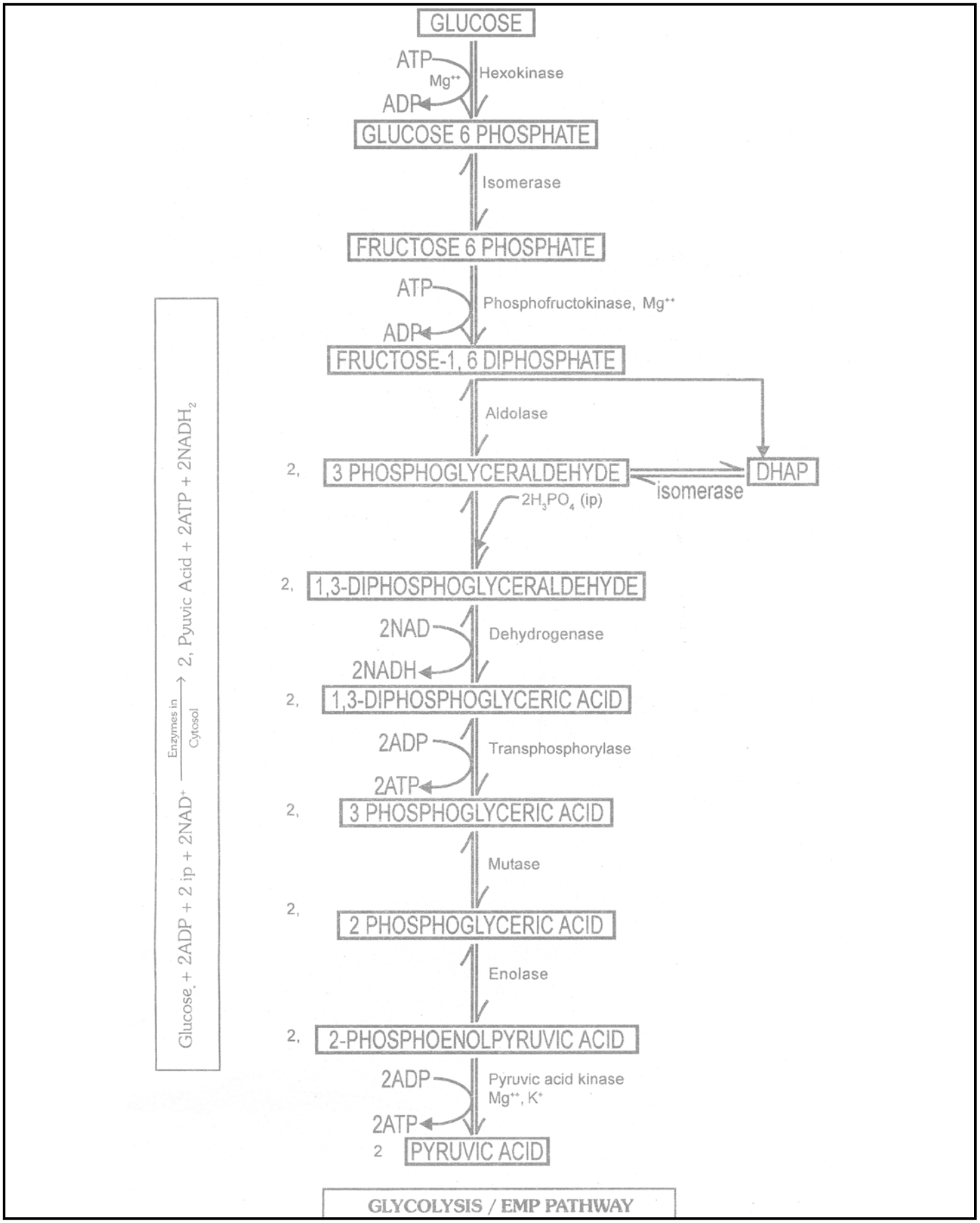
7. 2,1,3 BiPGA $\xrightarrow[2ADP+ip \rightarrow 2AT]{Transphosphorylase\ kinase}$ 3- PGA (2mol.

8. 2,3 -PGA $\xrightarrow{Phosphoglyceromutas}$ 2,2-PEP

9. 2,2-PGA $\xrightarrow[Mg^{++}]{Enolase}$ 2,2-PEP

10. 2-PEP $\xrightarrow[2ADP+ip \rightarrow 2AT]{Pyruvate\ kinase,\ K^{+},Mg^{++}}$ 2, Pyruvic Acid (3C)

- Phosphofructokinase is an **allosteric enzyme.** The phosphorylation of fructose 6 phosphate is the msot important control point of glycolysis.
- Phosphofructokinase has multiple allosteric modulator. It's activity is inhibited by ATP (-ve modulator) and stimulated by ADP & AMP (+ve modulator). Most of the biochemical reaction catalsed by allosteric enzymes are irreversible type and these are control point of glycolysis.
- Futher oxidation of pyruvic acid and $NADH_2$ after glycolysis in mitochondria required oxygen. So the fate of puruvic acid is decided by presence of absence of Oxygen.



GLYCOLYSIS / EMP PATHWAY

(2) Formation of Acetyl -Co-A : (Link/Gateway reaction)

- When respiration is aerobic, then pyruvic acid is oxidised to form 2C compound - **Acetyl Co-A.** It occurs in presence of O_2 and CO_2 is released first time druin it.
- Aetyl Co-A is a **connecting linnk between glycolysis & Krebs-cycle. Decarboxylation an dehydrogenatiin** (Oxidative decarboxylation) take palce during formation of acetyl Co-A.
- Acetyl Co-A is formed in **perimitochondrial space** by enxyme **pyruvate dehydrogensase complex (Mg^{++}, LA (Lipoic Acid). TPP (Thiamine pyrophosphate), NAD, CoA)**

$$2\text{Pyruvic Acid} + 2\text{CO - A} \xrightarrow[2\text{NAND} \quad \text{Mg++,LA, TPP} \quad 2\text{NADH}_2]{\text{Pyruvated dehy. complex}} 2\text{ Acetyl Co-A} + 2\text{CO}_2$$

- Acetly Co-A is also common intermetiate between fat & carbohydreate metabolism.

(3) Kreb cycle/TCA (Tricarboxylic acid) Cycle/Citric acid cycle :

- This cycle was discovered by **H.A. Kreb.** (Nobel prize)
- TCA cycle occurs in **mitochondiarl matrix** or power house of cell.
- Kreb cycle beings by **formation of citric acid** [TCA (Tri caboxylic cid)] & **O.A.A. is the acceptor** molecule of Acetyl CoA is Kreb's cycle.
- A number of Krebs cycle intermediates are used in synthetic (anabolic) pathways, thus TCA cycle is also called **amphibolic pathywa** or **anaplerotic pathway.**
- **Succinyl CO-A** is important for synthesis of porphyirin ring compounds like **Chlorophylls, Phytochromes, Ctochromes, Haemoglobin** etc.
- α -**ketoglutaric acid** (5c) involves in Amino Acid formation (Nitrogen-metabolism)
- Oxidation occurs at 4 sites in Kreb cycle.
- $3NADH_2$, $1FADH_2$ & 1GTP (ATP) produced by each tur of TCA cycle. (-12 ATP)
- All the enzymes of TCA cycle, except marker enzyme **Succinic dehydrogenase** (or inner mitochondrial membrane) present in matrix.

Bio Chemical reactions in Krebs Cycle ⇒

1. Acetyl Co-A + OAA $\xrightleftharpoons[\text{Synthase}]{\text{Citrate}}$ Citric Acid + CO -A

(2C) (4C) (TCA)

2. Citric Acid $\xrightleftharpoons[Fe^{++}]{\text{Aconitate}}$ Cis Aconitic Acid $\longrightarrow$ Isocitrate

(6C)

3. Isocitrate + NAD^+ $\xrightleftharpoons[\text{Dehydrogenase}]{\text{Isocitric}}$ Oxalosuccinic Acid + **$NADH_2$**

(6C)

4. Oxalouccinic Acid $\xrightleftharpoons[\text{Decartoxylase}]{Mg^{++}}$ α-Ketoglutarate + **Co_2**

(6C) (5C)

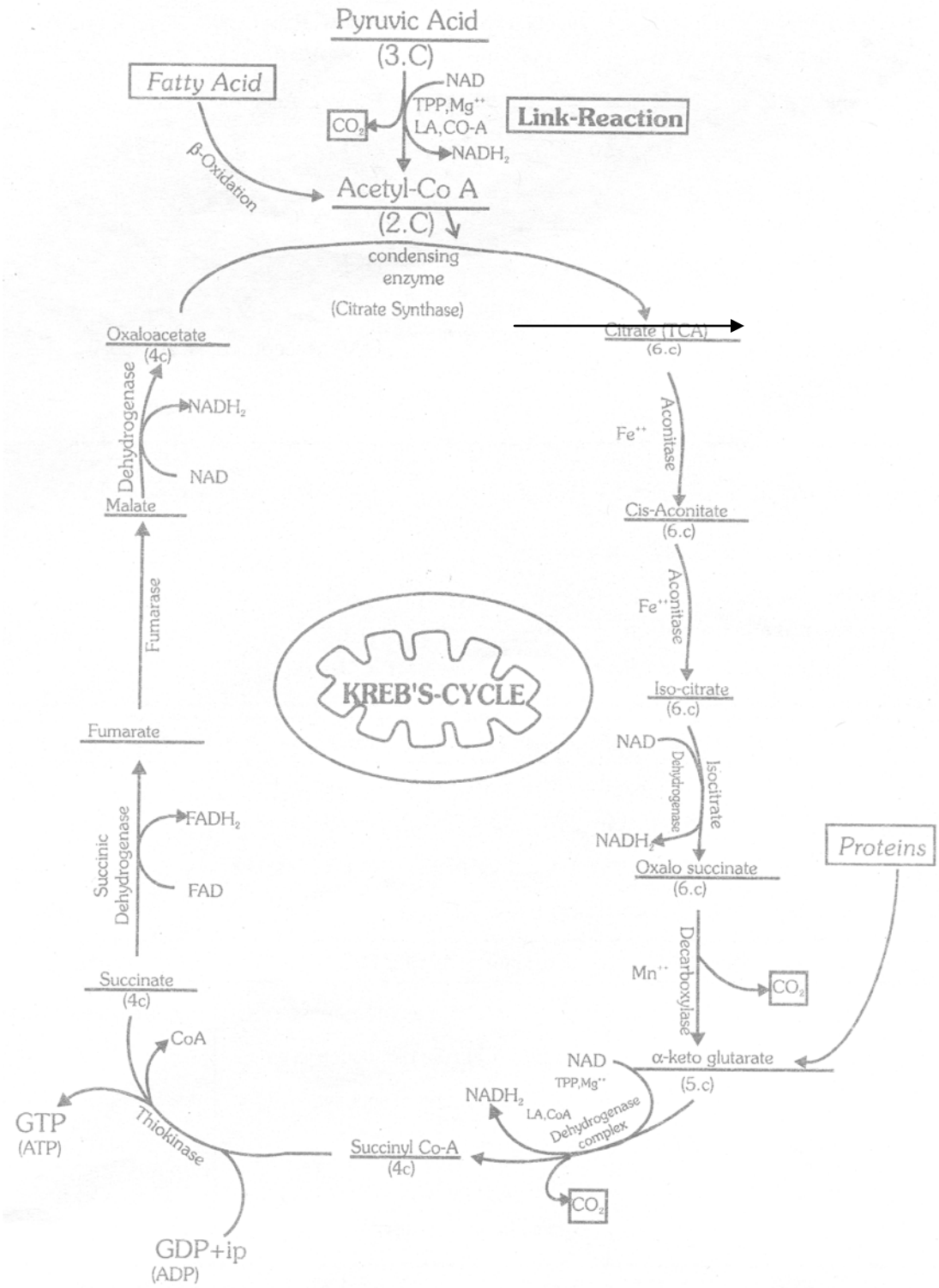
5. α Ketoglutaric Acid $\xrightarrow[NAD \quad TPP,LA,\ Mg^{++}CoA \quad NADH_2]{\text{Dehydrogenase comp.}}$ Succinyl Co A + Co_2

(5C) (4C)

(This reaction similar to link reaction)

Kreb's Cycle/Citric Acie Cycle

$2Acety1\ Co\text{-}A + 6NAD^{+} + 2\ FAD^{+} + 2\ GDP \text{ or } ADP + 2ip \xrightarrow{ENZYME} 4CO_2 + 6NADH_2 + 2FADH_2 + 2GTP \text{ OR } ATP + CO\text{-}A\ (2C + 2)$



6. Succinyl Co A (4C) $\xleftrightarrow[GDP + ip \quad GTP]{Tholdnase}$ Succinic Acid + C oA (4C)

[Energy of thioester bond is released, which used in formation of GTP]

(GTP + ADP $\xleftrightarrow[\text{diphosphokinase}]{\text{Nucleoside}}$ DGP + ATP)

The GTP formed in reaction 6, reacts with ADP to form ATP and GDP, as GTP and ATP have approximatly same energy.

7. Succinic Acid (4C) $\xleftrightarrow[FAD \quad FADH_2]{\text{Succinic dehudrogenase}}$ Fumaric Acid (4C)

8. Fumaric Acid (4C) $\xleftrightarrow{Fumarase}$ Malic Acid (4C)

9. Malate (4C) $\xleftrightarrow[NAD \quad FADH_2]{\text{dehydrogenase}}$ OAA (Acceptor of Acetyl CoA) (4C)

- α - Ketoglutaric acdi + NH_4^+ + NAD (P)H $\xleftrightarrow[\text{dehydrogenase}]{\text{Glutamate}}$ Glutamate + H_2O + $NADP^+$
- This is known as **reductive animation.**
- The first formed amino acid (in plants)is **glutamic acid,** which forms other types of amino acids by the process of **trasamination.**
  Ex. Glutamic acid + Pyruvic acid α - Ketoglutarate + alanine

(4) **ETS & Oxidative phosphorylation : (Terminal oxidation of $NADH_2$ $FADH_2$)**

- ETS (Respiratory chain) consists of four components
  (i) **FP (FMN),** (ii) **Fe-S Protein,** (iii) **Co-Q** (UQ) & (iv) **Cytochromes.**
- Cytochromes are **cyto.-b, cyto. - C_1 & cyto.-C, cyto-a** & **cyto a_3.**

- Now components of ETS are categories as follows :

Name of complexes	Components of ETS	Inhibitors
Complex -I	FMN-$NADH_2$ dehydrogenase	Rotenone & amytal
Complex – II	CaQ/UQ-$FADH_2$ dehydrogenase/Sccinate dehydrogenase	
Complex - III	Cytochrome - Ctyto c_1	Antimycin
Complex - IV	Cyto. a and Cyto. a_3	cyanide, CO
Complex-V	ATP synthetase/ATPase	

- **UQ** and **Cyto. c** are **mobile e^- carriers** in mitochondrial **ETS** (PQ and PC is mobile in z-scheme)

- **Cytochrome a_3** is last cytochrome in repiratory chain or electron transport chain **(ETC)**

- **O_2 is last e^- acceptor** in oxidative phosphorylation & due to this metabolic water is formed.

- During the ETS, $NADH_2$ gives it's $2e^-/2H^+$ to FMN in respiratory chain, thus 3 ATP are generated, while $FADH_2$ give it's $2e^-/2H^+$ to CoQ hence only 2 ATP are formed during the process of oxidative phosphoryltion.
- Enzyme Cytochrome oxidase is responsible for oxidation of cyto. a_3 & reduction of O_2.
  Enzyme **Cytochrome oxidase** has **cyto. a** & $\mathbf{a_3}$ as its components. (Cu present in cyto a and cyto. a_3)
- Cytosolic or extra mitochondrial or **glycolytic $\mathbf{2NADH_2}$** comes at ETS by two type of shuttles (Only in eukaryotes) :

(i) **Glycerol phosphate shuttle :** In brain/muscle cells.

Glycolytic/Gytosolic $NADH_2$ + DHAP $\xrightarrow{\text{In cytosol}}$ Glycerol 3 phosphate + NAD^+

Glycerol 3 phosphate + FAD^+ $\xrightarrow{\text{In mitochodria}}$ $FADH_2$ + DHAP

$\therefore$ $2NADH_2 = 4ATP$ | 1 Glucose = 36 ATP, | (It had overs it's e^- to mitochondrial ETS.)

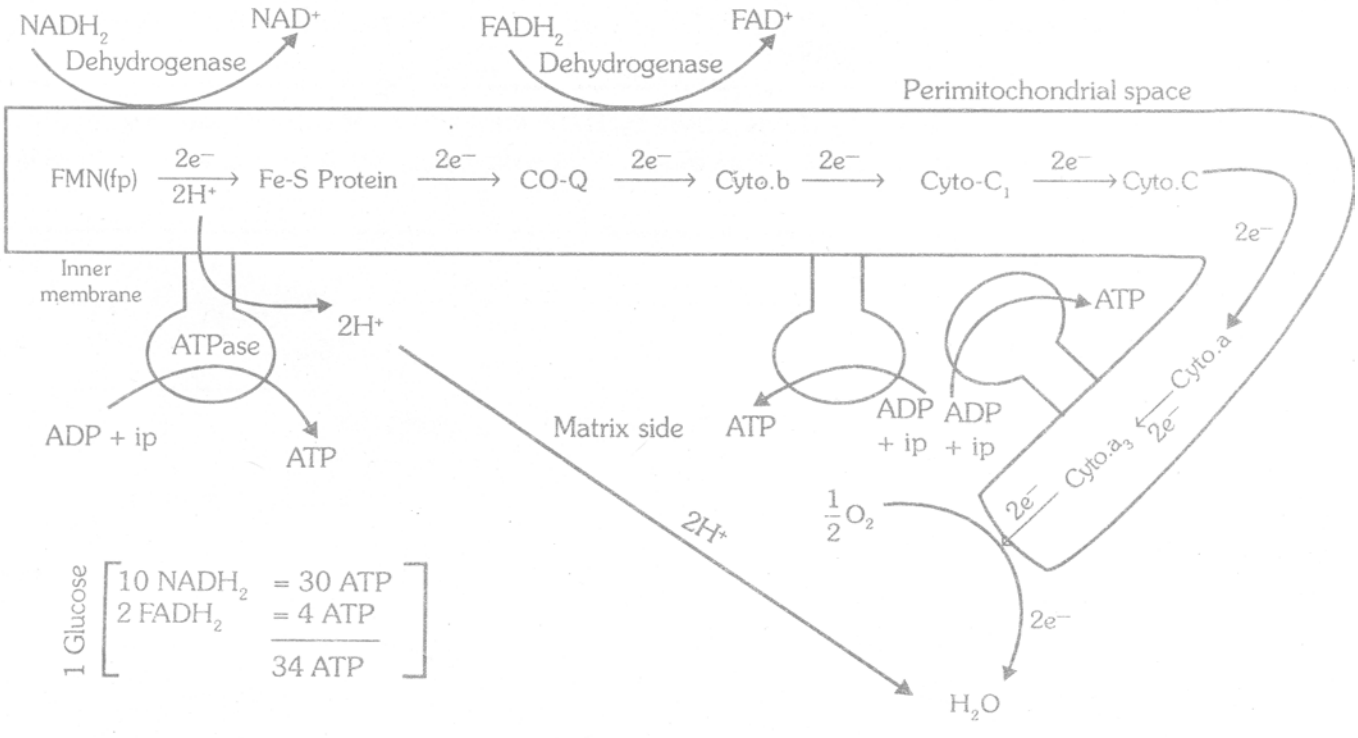
(ii) **Malate aspartate shuttle :**

Glycolytic/Cytosolic $NADH_2$ + O.A.A. $\longrightarrow$ Malate + NAD

Malate $\xrightarrow{\text{In mitochondria matrix}}$ O.A.A. + $NAHD_2$

$\therefore$ $2NADH_2 = 6ATP$ | 1 Glucose = 38 ATP |

- In **prokaryotes, shuttle mechanism is absent.** They always get 38 ATP from aerobic respiration of 1 glucose mol.
- Cynide inhibits the activity of cytochrome oxidase & inhibits the oxidation of cyto-a_3.
- In mitochondria of some plans **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.** Ex. **Spinacea, Pisum**



Schemetic diagram of mitochondrial ETS (respiratory chain) & oxidative phosphorylation

Bioenergetic of reparation - (1 mol. of glucose)

(1) EMP - Pathway

(i)	ATP formed at substrate level phosphorylation	⇒	4 ATP
(ii)	ATP produced via ETS ($2NADH_2$)	⇒	6 ATP
(iii)	ATP consumed in glycolysis	⇒	2 ATP
	10 ATP - 2 ATP	=	8 ATP
	Gross - Expenditure	=	Net or Total gain
	Direct Gain	=	2 ATP

(2) Link reactio or Gateway reaction -

$2NADH_2$ = 6ATP (via ETS)

(3) Kreb's Cycle -

(i) ATP produced at substrate level phosphorylation = 2 GTP/2ATP

(ii) ATP produced via ETS

$6NADH_2 \longrightarrow$ 18 ATP

$2\ FADH_2 \longrightarrow$ 4 ATP

24 ATP

Total $\longrightarrow$ 38ATP

- 1 Sucrose = 80 ATP
- 1 Fructose 1, 6 - Biphosphate = 40 ATP
- 1 Pyurvic acid = 15 ATP
- 1 Acetyl Co-A or 1 TCA cycle = 12 ATP

Pentose phosphate pathway (PPP)/HMP (Hexose mono phospahte) Shunt / Warburg-Dickens pathways

- PPP is also called a **Warburg - Dickens pathway/HMP shunt / Phosphogluconolactone pathway / Carbohydrate degadation withut mitochondria / Cytosolic oxidative decarboxylation / Horecker - Racker Pathway**

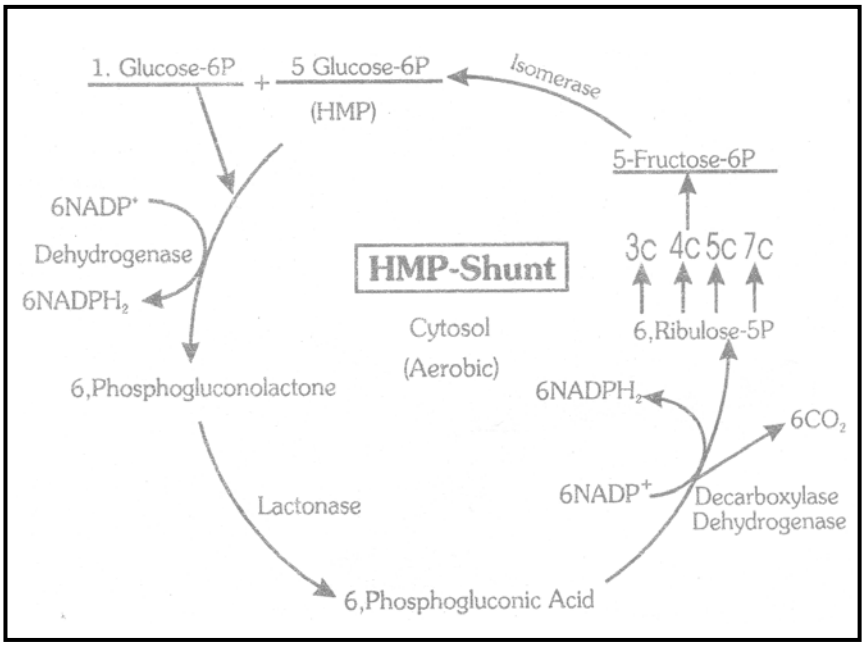
- Glycolysis & TCA cycle is the main route of carbohydrate oxidation, but Warburg & Dickens (1935) discovered an alternative route of carbohydrate break down, existing in plants, some animal tissues (Mammary glands, adipose, liver) & microbes

- **HMP/PPP occurs when**

(i) $NADPH_2$ requirement of cell increases during biosynthetic process.

(ii) When EMP pathway blocked by iodoacetate, fluorides, arsenates.

(iii) When mitochondria is busy in other pathways.



- Most of the intermeditaets are similar to Calvin cycle, but PPP is **catabolic** and **oxidative** process.
- One ATP is utilised in phosphorylation of glucose, so net gain equals to **35 ATP.** (12 $NADPH_2$)

Significance of HMP shunt :

(1) An intermediate **erythrose-P (4C)** of this pathway is precursor of **shikimic acid,** which goes to **synthesis of aromatic compounds and amino acids.**
(2) This cycle provides pentose sugars **Ribose-p** for synthesis of nucleotides, nucleosides, ATP and GTP.
(3) A five carbon intermediate **Ribulose-5-5phosphate** may used as CO_2 aceptor in green cells.
(4) This pathway **produces reducing power NADPH$_2$** for the various biosynthetic pathways, other than photosynthesis like **fats synthesis, starch synthesis, hormone synthesis** and **chlorophyll synthesis.**
(5) Intermediate like **PGAL** and **fructose - 6-6hosphate** of this pathway may link with glycolytic reactions.

β-Oxodation of Fatty acids

- β-Oxidation takes place mainly in **permitochondrial space** but also in **glyoxisome, peroxisome, cytosol.**
- Libertation of 2C segments from the fatty acid mol. in the form of acetly Co-A is known as β-oxidation. These acetyl-Co-A provides ATP after oxidation in kreb cycle.

- Acetyl CoA is oxidised in TCA cycle to CO_2 & H_2O with the release of 12 ATP molecules.

16C palmitic acid —(Enzymes)→ $NADH_2$ (=5 ATP), $FADH_2$, Acetyl Co-A (2C) oxidides in Krebs cycle

5 ATP × 7 tiems = 35 ATP
12 ATP × 8 Acetyl CoA = 96 ATP

131 ATP
-2 ATP consumed

1 palmitic acid = 129 ATP

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.
- Glyxylate cycle occurs in **glyoxisome, cytosol,** & **mitochondria.**

Fermentation

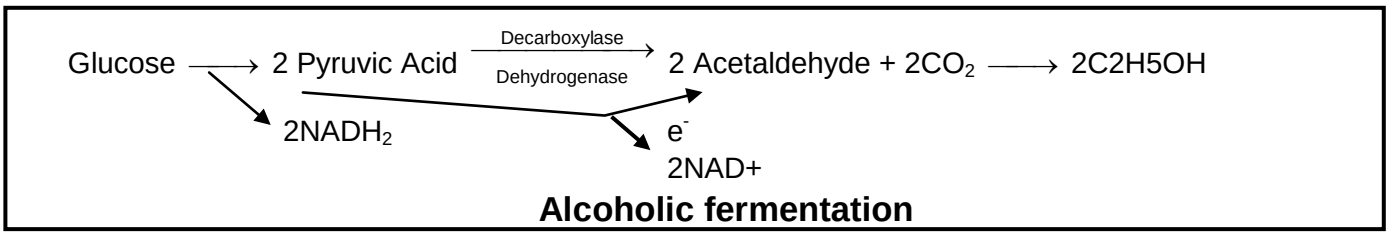
- **Cruick Shank** & Pasteur (1898).
- Fermentation in much similar to anaerobic respiration, but this is an extra cellular process & substrate is present outside the cell. Energy released as heat, no ATP generated.
- Buchner discovered the enzyme **zymase comple,** which is responsible for alcoholic fermentation.

- **Types of Fermentation : -**
  (i) When the one type of product formed in fermentation then it is called **homofermentation.**
  (ii) When the products of fermentation process are more than one type, then process is **heterofermentation.**

(1) **Alcoholic fermentation :**

This is the oldest & the best known type of fermentation performed by **yeast** & some bacteria.

$$C_6H_{12}O_6 \xrightarrow[\text{yeast}]{\text{Zymase (Bact).}} 2C_2H_5OH + 2CO_2 + \text{Energy}$$



Alcoholic fermentation

(2) **Lactic acid fermentation :**

It occurs during curd formation :

$$\text{Lactose} \rightarrow \text{Hexose} / C_6H_{12}O_6 \xrightarrow[\text{Lectate dehydrogenase}]{\text{Lactobacilus}} 2C_3H_6O_3$$

(3) **Acetic acid fermentation**

This is aerobic fermentation

$$C_2H_5OH + O_2 \xrightarrow{\text{Aceobacter sp.}} CH_3COOH + H_2O$$

Acetic acid

(4) **Butyric acid fermentation :**

In rancid butter, butyric acid fermentation takes place. Also in jute fiber.

$$C_6H_{12}O_6 \xrightarrow{\text{C butyricum}} C_4H_8O_2 + 2CO_2 + 2H_2$$

- Other eg. are **curring of tea, tanning of leather, retting of fibers, processing of Tobacco**

Respiratory quotient (R.Q.)

- The ratio of the volume of CO_2 released to the volume of O_2 taken in respiration is called R.Q.

$$\text{R.Q.} = \frac{\text{vol. oc } CO_2 \text{ liberated}}{\text{vol. of } O_2 \text{ consumed}}$$

Value of R.Q. depends upon the type of **respiratory substrate used** & measured by **Ganong's respirometer.**

(1) **Carbohydrates : - R.Q. = 1**

$C_6H_{12}O_6 + 6O_2 + 6H_2O \rightarrow 6CO_2 + 12\ H_2O + E$

(2) **Fat / Oil** $\rightarrow$ **R.Q. = 0.70** or **R.Q. < 1**

$2C_{51}H_{98}O_6 + 145O_2 \rightarrow 102\ CO_2 + 98\ H_2O + E$

- Fatty seeds germination castor, R.Q. < 1
- At the time of formation of fatty seeds, **(maturing fatty seeds)** R.W. is **more than 1** because more CO_2 is released than O_2 consumed

(3) **Organic acids :** (In succulent xerophytes at day time)

Malic acid : R.Q. = 1.33

$C_4H_6O_5 + 3O_2 \rightarrow 4CO_2 + 3H_2O + \text{Energy}$

Oxalic acid R.Q. = 4

Citric acid R.Q. = 1.3

(4) **Incomplete oxidation of carbohydrates :**
In CAM plants during night : R.Q. = 0
$\mathbf{2}C_6H_{12}O_6 + 3O_2 \rightarrow 3CO_4H_6O_5 + 3H_2O + Energy$

(5) **Protein : R.Q. = 0.8 or 0.9** or **< 1** Anaerobic respiration $\frac{2CO_2}{0O_2} = \infty$ (Infinite)

(6) **Anaerobic respiration :** RQ = ∞

FACTORS EFFECTING CELL RESPIRATION :

(1) Temperature :

- Optimum temp. for respiration is between 20-35^{0}C. Maximum temp. is around 45^{0}C.
- At low temp respiration is low due to inactivation of enzymes (Freeze preserve the food) while at very high temp. decrease, as enzyme denatured. Q_{10} = 2 to 3 for respiration.
- Production of potato crop is high on the hill areas due to low temp throughout year.
  CPRI (Central potato Research Institute is situated at Kufri-Shimla (H.P.)

(2) Oxygen :

- The inhibition of anaerobic respiration by O_2 concentration is called as **Pasteur's effect.**
- The minimum amount of oxygen, at which aerobic respiration takes place & anaerobic respiration become extinct is called as **extinction point.**
- Oxygen Conc^n at which both aerobic & anaerobic respiration take place simultaneously is called as **transition point**

(3) CO_2 :

- If CO_2 concentration increases, then rate of respiration decreases in plans, (because stomata get closed).

(4) Salts :
If a plant is transferred from water to salt solution, its respiration increase, this is known as **salt respiration.** Because absorption of ions requires metabolic energy.

(5) Hormnes :

- IAA, GA & cytokinin increase the respiration rate.
- The rapid increase in rate of respiration during ripening of fruits and senescence of leaves and plant organs is called s **"Climactric respiration".** This rate is decrease after sometime.
  It is due to production of **ethylene** hormone.

(6) Light :
Rate of respiration increases with increase in light intensity.
Light controls the stomatal opening & influence on temp and also produce respiratory substrates.

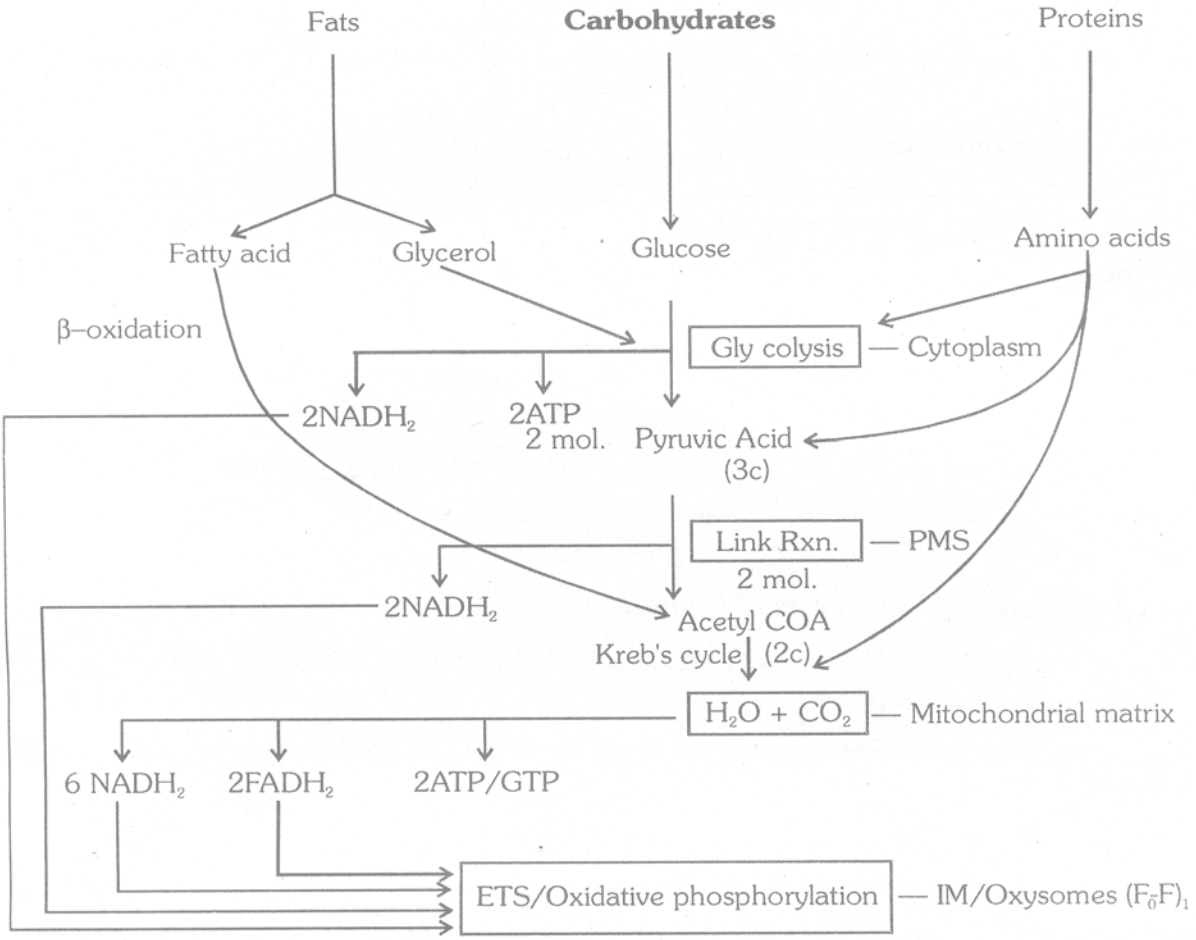
(7) Injury, disease & wounds :
the respiration increases due to injury wounding & infection.

(8) Inhibitors :
C.N. azides, DNP (Dinitrophenol), CO, rotenone, antimycin, amytal, etc inhibit the respiration.

(9) Age :
Rate of respiration is more in young cells. Rate of respiration at meristem apex in high.

(10) Water : Seeds are slow respiring part of plants, because dry seeds are deficient of H_2O.

Cell Respiration



ENZYMES

- Enzymes are proteinaceous, biocatalysts.
- Term **enzyme** was given by **Kuhne.**
- First of all isolated & discovered by **Buchner**
- **Zymase** (from yeast) was the **first discovered enzyme. (Buchner)**
- The first purifired and cystalized enzyme was **urease** (by **J.B. Summer)** From Canavalia/Jack Bean (LobiaM plant).
- **Proteinaceous nature** or enzyme was suggested by **Northrop** and **Summer.**

Definition : -

Enzymes are biocatalysts made up of proteins (exect ribozyme), which increase the rate of biochemical reactions by lowering down the activation energy.

- First discovered **ribozyme** was L_{19} **RNAase** by **T.Cech** from **RNA** of protozoan **Tetrahymena thermophila** and **RNAase P** or **Ribonuclease P** by **Altman** in prokaryotic cell (Nobel prize).

Characteristics of enzymes

1. All enzymes are proteins, but all proteins are not enzymes.
2. Enzymes accelerate the rate of reaction, without undergoin and change in themselves.
3. M. wt. of enzymes ranges from 6000 (bacterial fd) to 46 lack (Pyruvate dehydrogenase comp.)
4. Enzymes are colloidal substances, which are very sensitive to **pH** & **temperature.** Optimum temp. for enzymes is 20-35^{0}C.
5. Most of enzymes are active at neutral pH, **hydrolytic enzyes** of lysosomes are active on acidic pH (5).
6. Enzymes are **macromolecules of amino acids,** which are **synthesized on ribosomes** under the control of genes.
7. All enzymes are **tertiary** & **globulr proteins** (Isoenzymes quaternary protein)
8. Enzymes are required in very minute amount for bio-chemical reactions. Their catalytic power is represented by **Michaelis Menten** constant or **Km constant** and **turn over number.**
   "The number of substrate molecules converted into products per unit time by one molecule of the enzyme in favourable conditions is called **turn over number.**" The maximum turn over number is of **Carbonic anhydrase.** is 360 lakh, for **Catalase** is 50 lack, for **flavoprotein** is 50 & for **lysozyme** is 30 per minute.
9. Enzymes are very specific to their substrate or reactions.
10. Enzymes **lower down the activation energy** of substrate or reactions.

Structure of enzyme

(1) **Simple enzymes** $\rightarrow$ They are made up of only proteins. eg. **pepsin, trypsin papain.**

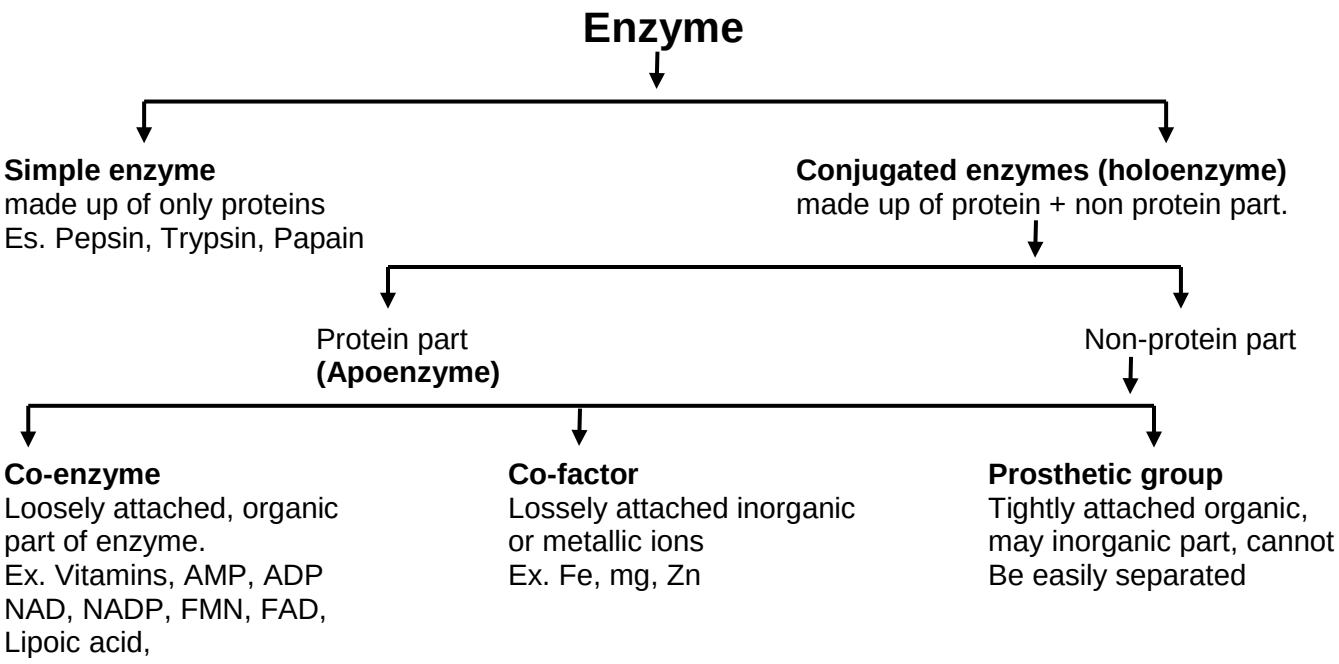
(2) **Conjugated enzymes** $\rightarrow$ They are made up of a protein part & non protein part.

   (i) **Co-enzymes :** Co-enzymes are non protein organic groups, which are loosely attached to apoenzymes. They are generally made up of vitamins.

   (ii) **Prosthetic group** - When non-protein part is lightly or firmly attached to apoenzyme.

   (iii) Metal activator/**Co-factors/** metallic factor : Loosely attached inorganic co - factor. eg. Mn, Fe, Co, Zn, Ca, Mg, Cu

- **Active site :** Specific part of amino acid chain in enzyme structure at which specific substrate is to be binded and catalysied, known as **active site.** Active site of enzyme is made up of **very specific sequence of amino acids,** determined by genetic codes.
- **Allosteric site :** Besides the active site's some enzymes posess additional sites, at which chemical **other than substate (allosteric modulators)** are bind. These sites are known as allosteric sites and enzyme with allosteric sites are called s **allosteric enzymes.** e.g. **hezokinase, phosphofructokinase.**



Terminology

- **Endoenzymes -** Enzymes which are functional only inside the cells.
- **Exoenzymes** - Enzymes catalysed the reaction outside the cell Eg : **enzymes of digestion some enzymes of insectivorous** plants, **Zymase** complex of fermentaion.
- **Proenzyme/Zymogen -** These are precursors of enzymes or inactive forms of enzymes. e.g. Pepsiongen, Trypsiogen etc.
- **Iso-enzymes-** Enzymes having similar action, but little difference in their molecular configuration are called isoenzymes. 16 forms of $\alpha-$**amylase** of wheat & 5 forms of **LDH (Lactate dehydrogenase)** are known. These all forms are synthesised by different genes.
- **Inducible enzymes :** When formation of enzyme is induced by substrate availability. eg. Lactase, Nitrogenase, β-galactosidase.
- **Extre mozymes -** Enzyems, which may also function at extremely adverse conditions e.g. Taq polymerase
- **Abzymes -** When the monoclonal atibodies are used as enzymes or reagents.
- **Biodetergents -** Enzymes used in was which powders are known as bio-detergents eg. - amylase, lipase, proteolytic enzymes.
- **House keeping / constitutive enzymes -** Which are always present in constant amount & are also esential to cell.

Nomenclature and classification

- Enzyme commision of IUB 1961 divides all enzymes into 6 manor classes and also propsoed an international code of 4 digits for each enzyme.

(I) **Oxido-reductases : These** enzymes involve in oxidation-reduction reactions.
In involves 3 sub classes (i) Oxidase (ii) dehydrogenase (iii) reductase
e.g. - Cytochrome oxidase.

(II) **Transfereases :** These Ensymes trnsfer specific group from one substrate to another.
e.g. - Transaminase, Hexokinse.

(III) **Hydrolases :** These Enzymes involve in hydrolysis reactioins with help of H_2O.
.e.g Proteases, Lipases, Carbohydrases.

(IV) **Lyases :** Split the substrate molecule without water. These Enzymes splits the specific covalent bonds without hydrolysis or H_2O additiion.

e.g. Aldolse.

(V) **isomerses :** Rearrangement of molecular structure to form isomers.

(VI) **Ligases (Synthases) :** Covalent bonding of two substates to form a large molecule e.g. Citrate synthetase, Ligase

Mode of action of Enzyme

(1) Lock & Key theory or template theory :

- Given by **Emil Fischer**
- According to this theory **active stides of enzymes** serve as a **lok** into, which the **reactant/substate fits like a key. Supported by completive inhibition.**

(2) **Enzyme - substrate complex theory :**

-Given by Henry explained by **Michaels** & **Menten**

E+S $\longrightarrow$ ESC $\longrightarrow$ EPC $\longrightarrow$ E + P

(3) **Induced fir theory**

-Given by **D.e. Koshland** (1973-74)

-Acc^n to this theory active site is not static, but is undergoes a conformational change which is induced by specific substrate. The active site has two groups, (1) **Buttressing** (supporint) group & (2) **Catalytic group**

-Buttresswing group is meant for supporting the substrate, while the catalytic group break the subtrate into product.

Factors

(1) **pH** - Enxymes very sensitive to pH.

(2) **Temperature -** High temp inactivates enzyme causing their denaturation. They also get inactive at lower temp. Generally all enzymes better perform at body temp. of organsm.

(3) **Enxyme concentration -** Increase in conc. of enzymes will increase the rate of enzymatic reaction till enough the subsrate.

(4) **Substrate conc^n -** Increase in substrate conc^n increases the activity of enzymes until all the ative sites ot enzyme are saturated.

(5) **Inhibitors / Enzyme inhibitioin :**

(i) Competitive inhibitors or competetrive inhivition and reversible type :

- These are **substrate analogues,** which bind to the active site of enzymes & enzymes get inhibited, such inhibition is called as **competitive inhibition.**
  Eg. **Succinic dehydrogenase** is inhibited by its competitor **malonate.**
- This reversible inhibition. **Malonate** is known as **substrate analogue of succinate.**

- Similary **sulpha drugs** are substrate analogue of p-amino benzoic acid **(PABA)** sued in folic acid synthesis in bacterial cells. Hence these drugs are used to kill bacterial cells.

(ii) Non-competitive inhibitors or non competitve inhibition and irreversible type :

- In this type of inhibitin, inhibitor substance can bind simultaneoulsy to an enzyme, other than it's active site and destroy he **sulfhyddrayl (S - H) group** of enzyme.
  Example : Toxic metals, CO, CN poisoning of cytochrome oxidase.
- Such inhibition are **irreversible inhibition.**

(iii) Non competitve & reversible type : When inhibitor binds at allosteric site reversibly.

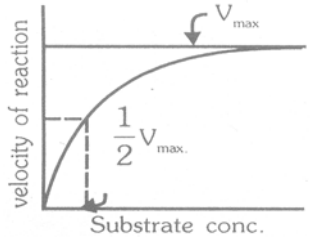
- When product of biochemical reaction inhibits the enzyme action, it is known as **product inhibition** or **retro inhibition** or **feed bak inhibition.**
- The p roduct may bind at **allosteric site** of alloster5ic enzyme then it is **non-competitive, reversible, allosteric inhibitiion.** Example : **inhibition of hexokinase by glucose 6P.**

Allosteric Modulation

- **Jacob & Monad.** First discovered L-threonine dehydratase, inhibited by its product isoleucine.
- In the allosteric modulation, chemical or products fits in allosteric sties & bring a change in **shape** of active site of enzyme.
- Chemicals which bind at allosteric site of allostreic enzymes are known as **allosteric modulatros.**
- These modulators may be **activator** (positive modulator) or **inhibitors** (negative modulators) of allosteric enzymes.
- If allosteric modulator postively change the configuraiton of active site, then called **positive allosteric modulation** and if negatively chagne then called **nagetice allsoteric modulatio** respectively by + ve modulator (activator) and -ve modulatro (inhibitors).
  Ex. **Phosphofructokinase** inhibited by ATP activiated by AMP and ADP.
- Some times product also binds at allosteric site and inhibits the enzyme.
  Ex. **Hexokinase** inhibited by glucose - 6P and exhibits feed back inhibition.
  This is atype of reversible and non-competi tive inhibition found in allosteric enzymes.
- All allosteric modulation are **not feed-back inhibition**

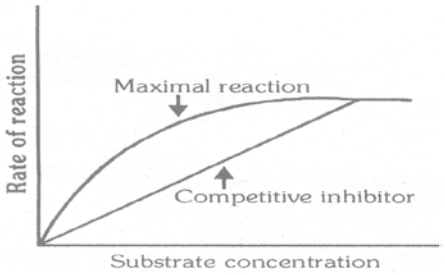
Km Constant (Michaelis & Menten Constant)

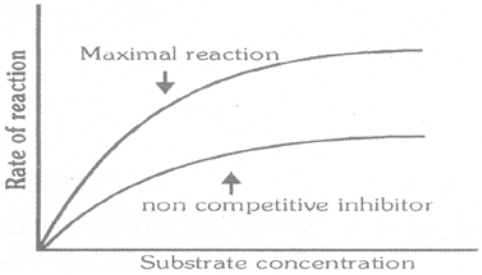
"Km constnat of an enzyme, is the concentration of substrate at which rate of reaction of that enzyme atains half of its maximum velocity". It is given by **Michaelis & Menten.** The value of **Km should be lower for an enzyme.**
Km exhibits catalytic activity of an enzyme.



- **Ki constant** (Enzyme inhibitor complex dissociation constant) indicates the dissociation of enzyme inhibitor complex (EIC). of reversible inhibitors.
- **Km and Ki constant** of an enzyme should be low.

- If **competitive inhibitor** is present, then, **km - ↑ and $V_{max.}$- No change.**
- If **Non competitive** (reversible) inhibitor is present, then **Km - No change, $V_{max.}$ - Decrease**
- **Competitive inhibition** is overcome by increase in concentration of substrate.





SPECIAL POINTS OF RESPIRTION OF ENZYMES

NAD = Nicotinamide Adenine Dinucleotide
DPN = Diphospho Pyridine Nucleotide
NADP = Nicotinamide Adenine Dinucleotide Phosphate
TPN = Triphospho Pyridine Nucleotide
FAD = Flavin Adenin Dinucleotide
FMN = Flavin Mono Nucleotide
Co-A = Adenosine Triphospho Pnato Thenylthio Ethanol Acetyl Amine

- ATP discovered by **Lohman,** while importance of ATP in metabolism by **Lipman.**
- 1 gram of fat equals of 9.8 K.Cal.
  Proteins = 4.8 K. Cal
  Carbohydrate = 4.4 K. Cal (Old 3.8 K.Cal)
  (Fat is energy rich respiratory substrate)
- Almost all enzymatic reactions are **rversible** type.
- Cytochromes are Iron - porphyrin protein discovered by macMunn (Terned by Keilin)
- **Entener - Doudoroff pathway,** occurs only in bacteria (**Pseudonas Anotobacter** for Carbohydrate oxidation. (Bact. respiration)

1 ATP = 7.6 K. Cal. (now 1 ATP = 8.9 K. Cal.)

Imp. Coenzymes are

Co - I (NAD)/DPN.....Niacin
Co - II (NADP)/TPN .....Niacin
FAD .....Riboflavin
FMN .....Riboflavin
TPP .....Thiamine
CO - Q .....Ubiquinone
CO - R .....Biotine
CO - A .....Pantothenic Acid

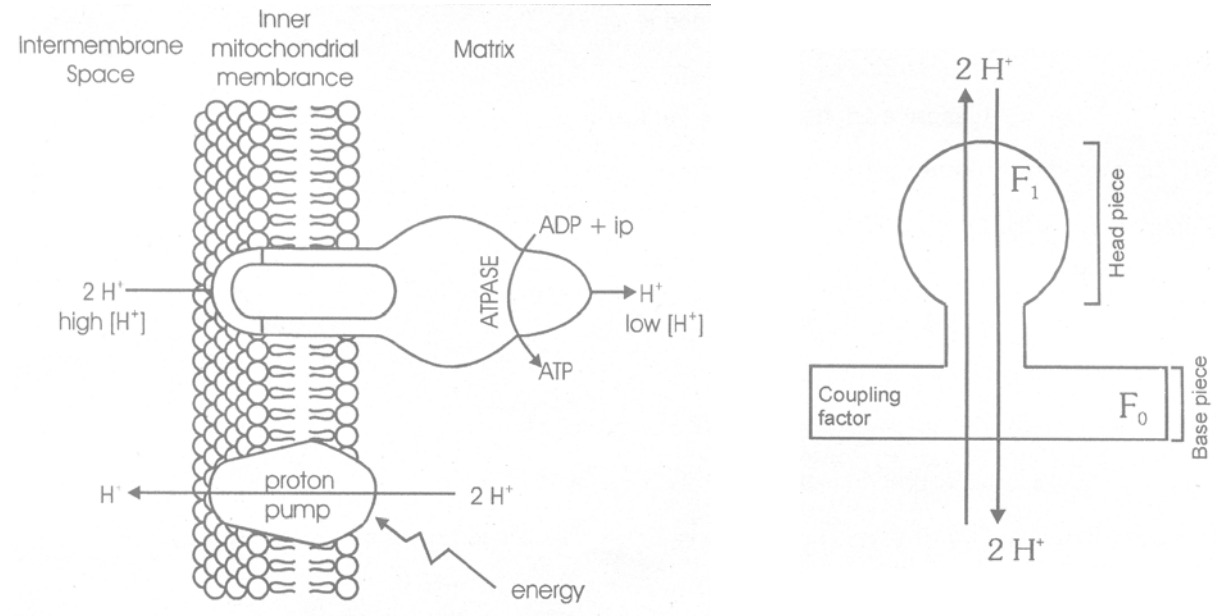
Important Co-factor :

Fe^{++} = Cytochrome oxidase, catalase, peroxidase, aconitase
Cu^{++} = Cytochrome oxidatse, tyrosinase
Zn^{++} = Carbonic anhydrase, alcohol dehydrogenase
Mg^{++} = Hexokinase, blucokinase, pyruvate kinase, pepcase, Rubisco.
K^{++} = Pyruvate kinase
Mn^{++} = arginase, ribonucleotide reductase, decarboxylase.
Mo = Nitrogenase complex, Nitrate reductase.
Se = Glutathione perozidase

- When respiratory substrate is fats or proteins, then level of Hg rises in Gango's respirometer, because more O_2 absorbed than CO_2 released.

- ATP synthesis during **oxidative phosphorylation & photophosphorylation** is explained by **Chemi-osmotic theory of P. Mitchell 1978.** According to this theory energy liberated during ETS, is used in creation of a **proton gradient** (pH gradient) & membrane potential which constitutes **protean motive force (pmf)** due to this formation of ATP takes place in F_1 particle of oxysome.

Chemiosmotic mechanism



- **Coupling factor :** ATP formation requires H^+ transport. These H^+ only passes through the **proton tunnel** or **Coupling factor** or **F_0 particle** in **mitochondrial** or **thylakoid membrane**. It is estiamted (By P. Mitchel 1978) that one mole of ATP is formed for every **3 H^+ pair passing through the coupling factor.**
- The process of electron transport and oxidative phosphorylation in mitochondria is tighly couped. Some chemical like 2,4 Dinitrophenol (2,4 DNP) and oligomycin acts as **uncouplers** for this process.

CHEMIOSMOSIS DURING PHOTOPHOSPHYRYLATION

- The Synthesis of ATP is couped with electron transport system and creation of **proton gradient** ctoss the membrane during **photophosphorylation** and **oxidative phosphorylation.** Both are same but the difference is the during oxidative phosporylation high H^+ ion concentration at intermembrane space / perimitochondria space and low H^+ concentration in mitochondiral matix. While during ptophosphorylation High H^+ conc. Inside the thylakoid lumen (due to photolysis of water at thylakoid lumen) and low H^+ ion conc. in stroma.
- Thylakoid membrane is impermeable for protons so platoquinone (which acts as both e^- and H^+ carrier) and Cf_0 particle (similar to F_0 particle of **oxysome** in mitochondria) helps in proton transport across the thylakoid membrane.
- Due to free energy of electrons, protons or H^+ ions transported from storma side towrds lumen of thylakoid by Pq or CF_0 ($PQ + 2H^+ \rightarrow PQH_2$ then $PQH \rightarrow PQ + 2H^+$)

  So within the chloroplastprotons in the stroma decreses in number, while in the lumen there in accumulation of protons. This event creates a **proton gradient** (ΔPH) across the thylakoid membrane, which results in development of membrane protential. Bot **proton gradient** and membrane potential constitute proton motive force (Pmf) or electro-chemical gradient.

- Due to high protin motive force (Pmf) inside the lumen results in collapse's of proton gradient and this potential is used in phosphorylation of ADP, results in synthesis of **ATP** (ADD + iP $\rightarrow$ ATP) catalysed by enzyme **ATP synthase.**

- The retuned protons are accepted by NADP leads to formation of $NADPH_2$ in stroma side.

- In bacteria site of ETS si **mesosome.**

- **Respiration efficiency :**

- 1 glucose = 686 Kcal.

  38 ATP × 7.6 Kcal = $\frac{288 \text{ Kcal}}{686} \times 100 = 42\%$

  Thus efficiency of aerobic respiration is 42% $\pm$.
- For the complete oxidation of one glucose if, in option 38 or 36 ATP are not given then the answer goes to 32 or 30 ATP.

Significance of respiration

1. The energy released during respiration is used for the various metabolic processes.
2. Various chemical substances are formed in this process which are important for cellular components.
3. CO_2 released in this process maintains a balance in the atmosphere.
4. Complex insoluble food materials are converted into simple soluble molecules by this process.
5. It converts the stored (static) energy into more useful (kinetic) form.