Metabolism

- chapter- 1

Membrane Transport & Bioenergetics.

* Membrane Transport mechanism :—

1) Passive Transport :—

- passive transport is the fundamental movement of ions and other molecular substances within the cells along the concentration gradient without any external energy

- conc. gradient ⟹ diff. between high & low concentrations.

- Types :—

(i) Diffusion —

Diffusion is the movement of substances from a region of higher conc. to lower concentration.

- The difference in the concentration of two areas is term a concentration gradient & the process of diffusion continues until this gradient neutrilise.

(ii) facilitated diffusion / Transport

facilitated diffusion is the part of passive transportation of ions or molecules across the cell membrane through specific transmembrane integral proteins.

- The molecules which are large & insoluble require a carrier substances for their transportation through the plasma membrane.

→ Membrane allows small & polar molecules.

→ semipermeable membrane only allows solvent molecules to transport through it.

This process does not require any cellular or external energy.

- Example : Glucose transporter, ion channels aquaporins
- facilitated diffusion occurs through trans-membrane proteins.

(iii) Osmosis —

In the process of Osmosis water and other solvent molecules pass through a selectively permeable membrane in order to balance the concentration of other substances.

- low concentration to higher concentration movement.
- Temperature, conc. gradient affects the process of osmosis.

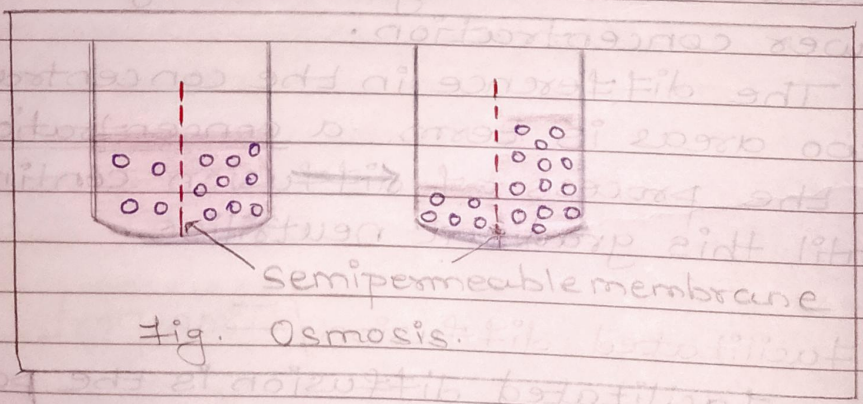

fig. Osmosis.

• Passive transport —

fig. Diffusion.

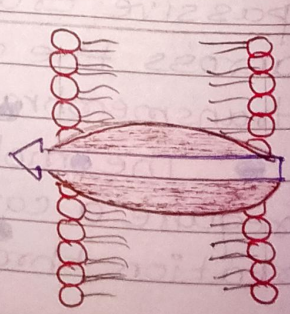

fig. facilitated diffusion

- Active & passive transport —

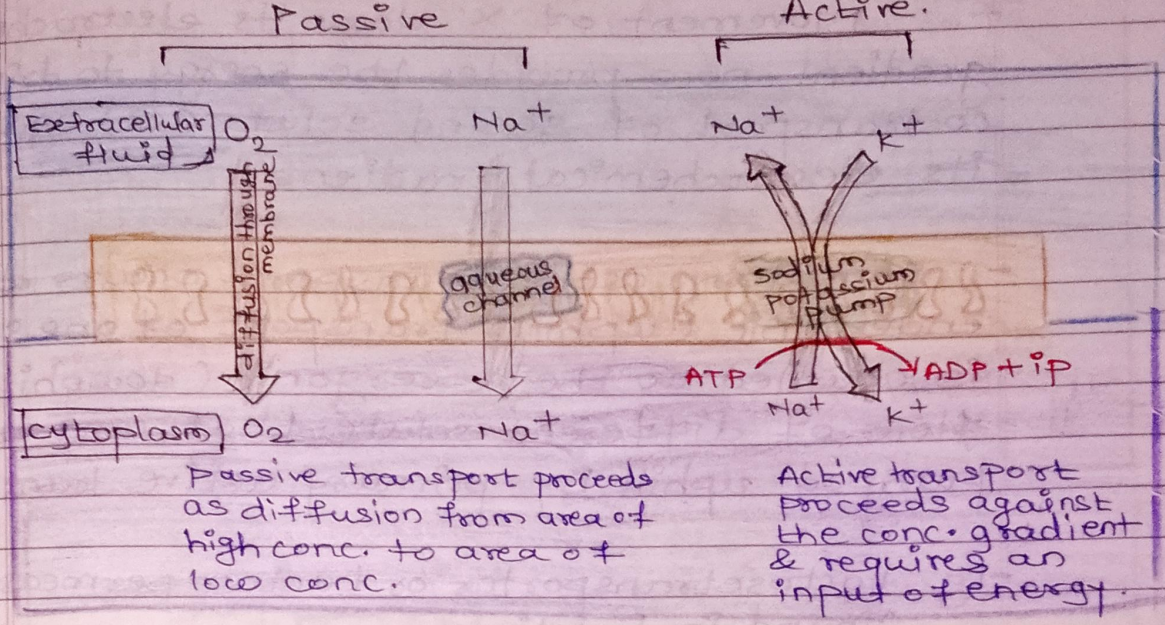

fig. Active & passive transport.

Monday
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2** Active Transport :— (lower to higher)

Active transport uses specific transport proteins called pumps, which use metabolic energy (ATP) to move ions or molecules against their concentration gradient.

- Endergonic process (utilise energy)

(i) primary Active transport :—

In primary active transport solute accumulation is coupled directly to an exergonic chemical reactions such as conversion of ATP to ADP and pi.

(ii) secondary Active transport :—

In Secondary Active transport a gradient of ion 'X' (often Na^+) has been established by primary active transport.

- uniport → single molecule movement.
- symport → two molecule movement in same direction.
- Antiport → two molecule movement in opposite direction.

- Movement of 'x' down its electrochemical gradient now provides the energy to drive co-transport of second solute ('s') against its electrochemical gradient.

- secondary active transport occurs when endergonic (uphill) transport of one solute is coupled to the exergonic (downhill) flow of different solute that was originally pumped uphill by primary active transport.

-Eg. Lactosetransporter or Lactose permease found in E. coli

- explanation →

Lactose uptake in E. coli —

(i) The primary transport of H^+ out of the cell driven by the oxidation of a variety of fuels establish both a proton gradient & an electrical potential across the membrane.

- (ii) Secondary active transport of lactose into the cell involves symport of H^+ and lactose by the lactose transporter.

The uptake of lactose against its conc. gradient is entirely dependant on this flow in flow of protons driven by the electrochemical gradient.

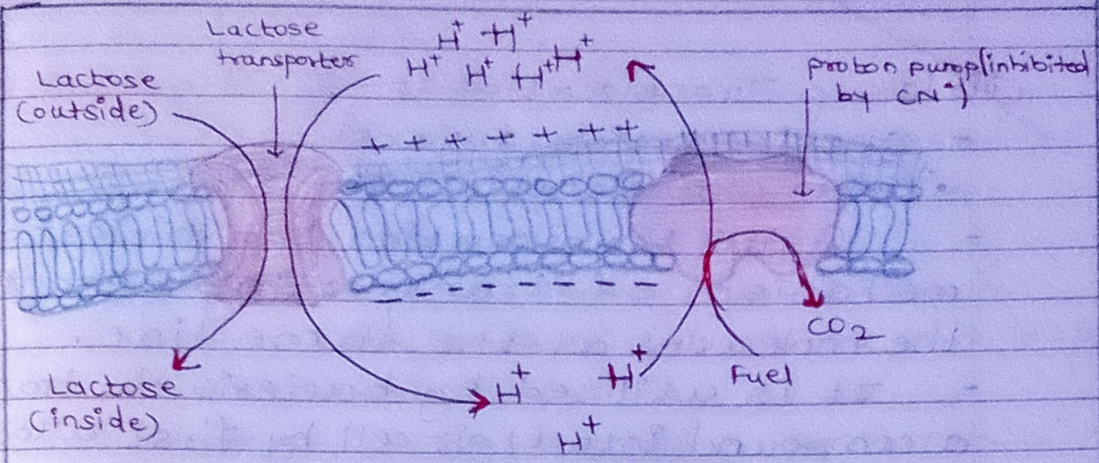


fig. Lactose uptake in E. coli.

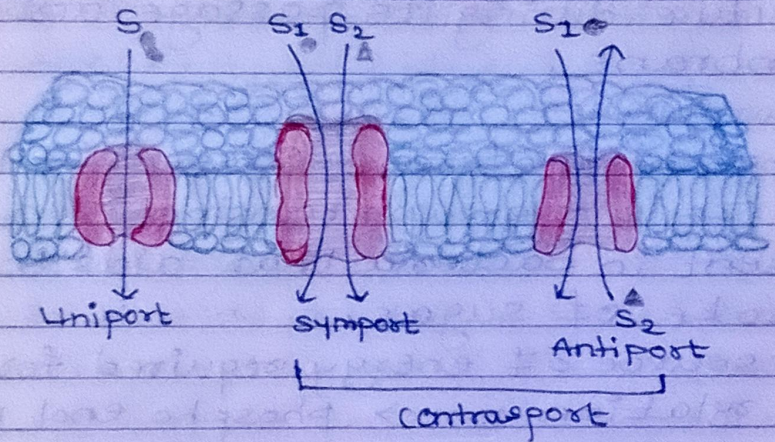
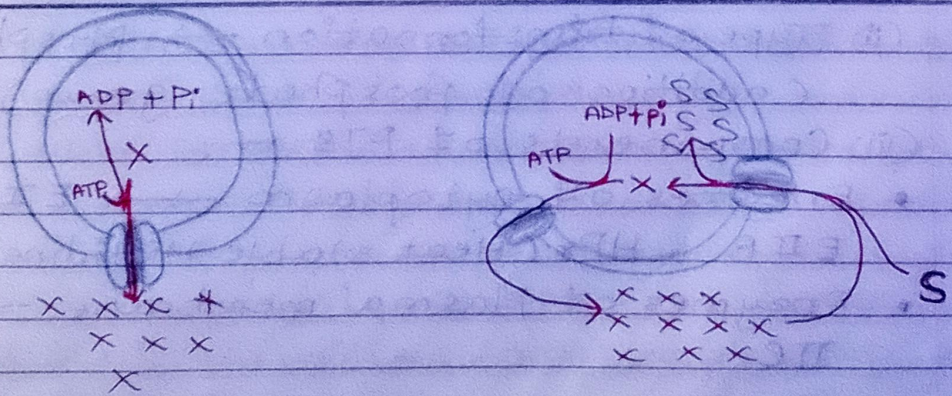


fig Three general classes of transport systems.



(a) primary active transport

(b) Secondary active transport

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(iii) Group Translocation :—
- specially for sugars.
• Group translocation :—
- Group translocation is a biological mechanism of transporting & transforming the molecules at the same time.
- It is utilised by bacteria to transport a compound into their cell by first allowing the compound to bind with protein on the cell surface followed by altering its chemical structure during its passage across the membrane.
• Example :—
• Phosphotransferase system (PTS)
- It is a group translocation system present in bacteria that aids in the uptake of sugar.
- (i) source of energy required for phosphorylation ⟶ phospho enol pyruvate (PEP)
∴ Hence it is also called the PEP group translocation.
(ii) Type of transformation ⟶ Phosphorylation (addition of phosphate group)
(iii) Components of PTS —
• Enzymes of cytoplasm ⟶ EI, EIIA, EIIB, HPr (Heat stable Histidine protein)
• Enzymes of Plasma membrane ⟶ IIC

- Role of PTS :—
  - (i) Transportation of various sugars like mannose, glucose, fructose, cellobios, kitobios, lactose
  - (ii) Regulation of carbon metabolism & Nitrogen metabolism in bacteria with respect to sugar availability.
  - (iii) Acts as a chemoreceptive system aiding in bacterial translocation and motility towards sugar concentrated enviroment
  - (iv) Helps in regulation of other non PTS enzymes.

(iv) General channel of phosphoryl group transport → PEP

- PEP → Enzyme I → HPr → EII A ↵
  Sugar ← EII B + II C
  (glucose → 6-phosphate)

- Advantage of PTS :—
  - Glucose-1 transforms to Glucose-6-Phosphate can't escape the cell and is the bacteriums way to concentrate & store glucose inside its body
  - only present in bacteria lessely present in Archaebacteria.

- Example → E. coli, Salmonella, Enterica, Bacillus subtilis, Streptomyces sp., Listeria monocytogenes

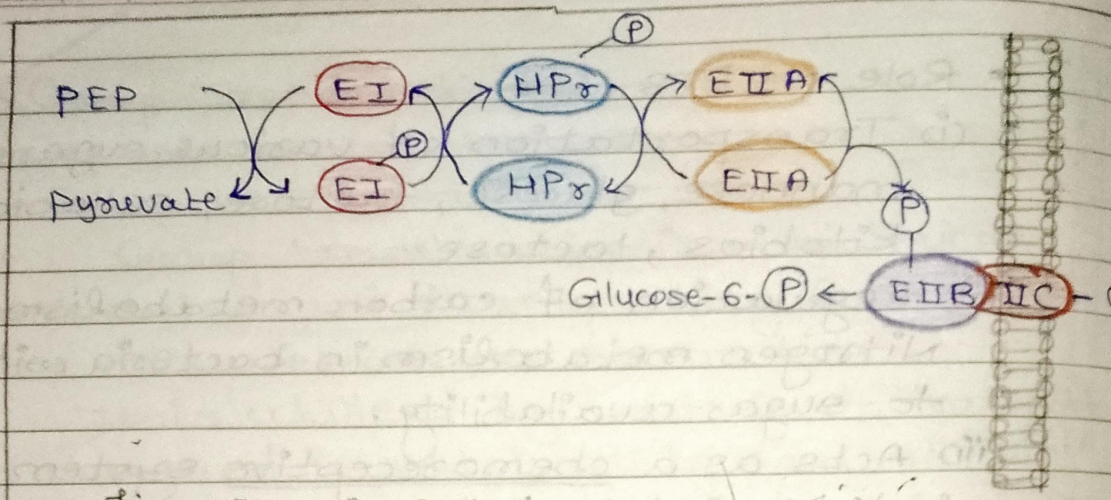


fig. PTS in E. Coli

22/2/23
Wednesday

* Ionophores Mechanism :—

- Inophores are specific molecules that complex or carry specific cations & facilitated there transport through biological membranes.
- They are molecules that acts as a membrane shuttles for particular ions across the lipid membranes without expenditure of energy.

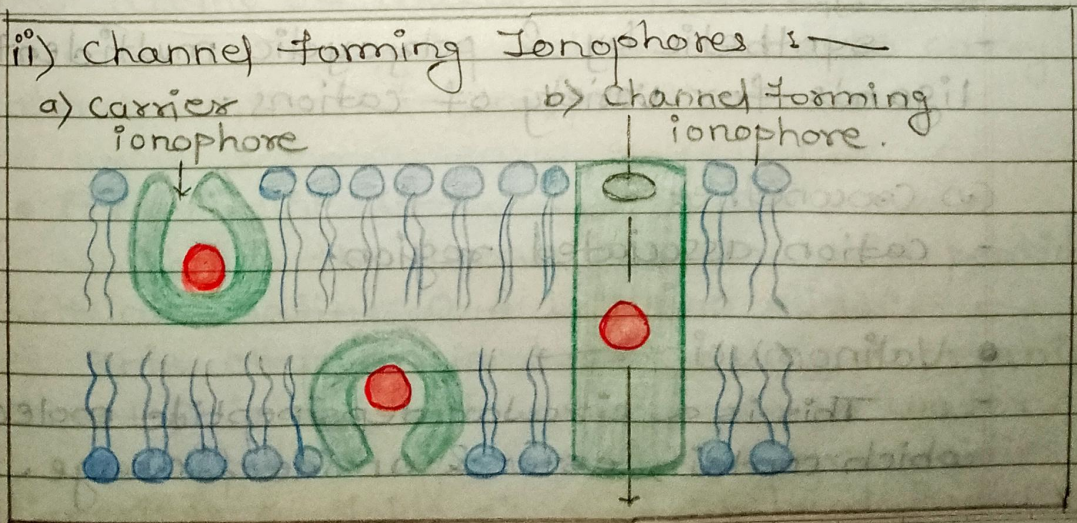
• Mechanism :—

- Ionophores contain Hydrophilic centers that bind specific ions via hydrophobic portion that interacts with a lipid interior of the membrane.
- Most ionophores adopt cyclic ring formation by concentrating oxygen or nitrogen functional groups at the centre of their structure to associated with the cation.
- With the hydrophobic group in contact with the acyl group of the molecule.
- The ionophores is able to dissolved & diffuse to the opposite side of the membrane.

- Classification of Ionophores :—
- Based on Mechanism of their action :
i) Mobile carrier Ionophores :—
- This bond to a particular ion & shield its charge from the hydrophobic and environment of the membrane.
- They form a lipid soluble complex with the cation which then diffuses across the membrane.
- E.g. Valinomycin
K^+ by reversible binding to the ion to form lipid soluble complexes which rapidly diffuses across the membrane they catelized passive transfer of cations across the otherwise impermeable hydrophobic membrane.

- Three steps involved :
1) Complexation of ionophore with the ion.
2) Diffusion of the complex via the membrane interphase to the other side of the membrane
3) Reverse complexation process.

ii) Channel forming Ionophores :—



- This introduced the hydrophilic pore into the membrane allowing specific cations to pass through without come in contact with the hydrophobic membrane.
- Eg. Gramicidin -
channel forming ionophores are usually large molecules.

- classification based on chemical structure :—

(i) polyether ionophore —
eg. Monensin & modulacin.

(ii) peptide ionophores —

(a) cyclodepsi peptide ionophore —
eg. Vallinomycine.

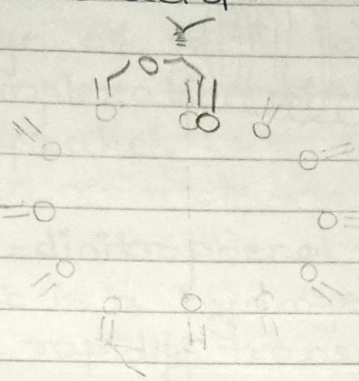
(b) Macrotetrolcads —
- macrocyclc compounds containing tetrahydro furanil, carboxylic acid residue link together.

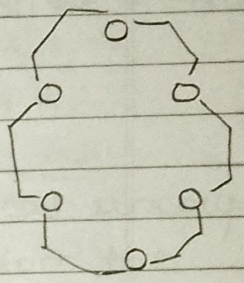
(iv) Cryptates —
- synthetic by & polycyclic multidentat ligands for variety of cations.

(v) Croconether —
- cation associated region.

- Valinomycin :—
- This is a circular depsipeptide molecule which contain ester & amide linkage.

- It contains D-valine, L-Lactic acid. hydroxy isovalleric acid.



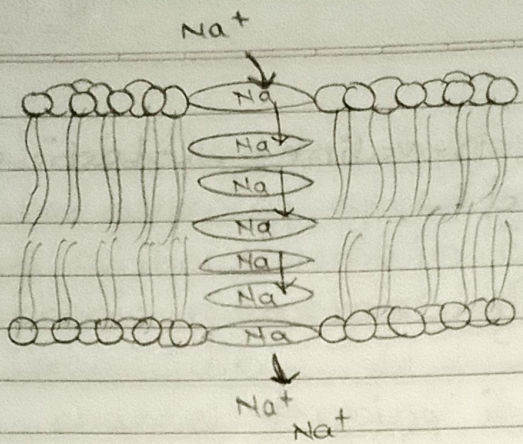


18-crown-6.

- valinomycin specific for K^+ ion, which is transfer in complex & uncomplex state.
- It has a plucked ring structure. stabilize by hydrogen bond. which therefore suits it to surround single unhydrated K^+ ion.
- The six oxygen atom of the ionophore interact with the bound K^+ ion. replacing the 'O' atoms of water of hydration.
- Each valinomycin molecule able to carry about 10,000 K^+ ions/~~per~~sec.

• Gramicidine :—

- This is the linear 15 amino acid peptide with alternating D & L amino acids.
- This structure is double helide inorganic. solvent & is a end to end dimer in water.
- In lipid bilayer membrane gramicidine dimerises & folds as a right hand β-helix.



- The dimer spans the bilayer the hydrophobic outersurface gramicidine interacts with the core of the lipid bilayer while the ions pass through the more polar lumen of the helix.
- gutting (opening & closing) of a gramicidine channel is through to involved reversible dimerasation. & open ion channel forms when gramicidine molecules join end to end to span the membrane.
- cations therefore move through the channel in a single file along with the single file of bottom molecule.

• Ionomycin (calcimycin) —

- Ionomycin carries Ca^{2+} ions into the cells & organelles.

• Monansin ⟶ Na^+, H^+ ions transportation.

- Sidophores —
  - This are bacterial ionophores specific for carriage of Fe^{3+} ions.
  - complexation of Fe^{3+} ions solublizes for its uptake.

- 3,5-dinitrophenol —
  - It is a hydrogen ion & chemical uncoupler.
  - It rapidly transport proton from the cytosolic side to the matrix side of the inner mitochondrial membrane.
  - High hydrogen ions on the cytosolic side causes dNP to ~~the~~ be protonated.
  - Low hydrogen ions concentration in the matrix causes dNP associates releasing protons.

Chapter-2

27/2/23

Bioenergetics

- The study of energy transfer within the living the living things.

- 1st Law of Bioenergetics :—

- Energy cannot be created or destroyed but can be change from one form to another form.

Eg.

(i) Muscle contraction (chemical → Mechanical)
(ii) Vit. D production (Light → chemical)
(iii) Sweating. (Water → water vapour)
(iv) Vision (Light - chemical → electrical)

- 2nd Law of Bioenergetics :—

Energy transfer will always proceede in the direction of increased entropy (Radomness) and the release of free energy.

- Entropy —

Entropy is a form of energy that cannot be reused in chemical reaction & is defined synonomously with increased randomness or disorder.

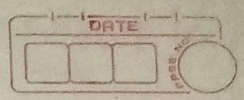
- Free Energy — (G)

Free energy is referred to a Gibb's free energy and is abbreviated as 'G'

- The maximum energy that can be derived from a particular molecule capable of doing work under conditions of constant temperature and pressure.

- Temp — 25°c , pressure — 1 atm.

$$K'eq = \frac{\text{conc. of P}}{\text{conc. of S}} = \frac{[P]}{[S]}$$



- Change in free energy (ΔG) :—

The change in free energy that takes place during a chemical reaction (equal to free energy of the product of reaction — (minus) free energy of reactant)

∴ ΔG = free energy (Kcal/mol) of product — free energy of reactant.

- Standard free energy (ΔG°)

- change in free energy that takes place under the following standard conditions —

(i) reactants & products are maintain at 1 molar concentration
(ii) Temperature 25°C
(iii) pressure 1 atm.
(iv) PH - 7.

- ΔG° can be calculated from equilibrium constant ($K'eq$) of a reaction using the relationship

$$\Delta G^\circ = -RT \ln K'eq$$
$$= -2.303 RT \log_{10} K'eq$$

Where,

R = Gas constant. (8.314 J/mole/degree)
T = absolute temperature (in degree Kelvin)
$K'eq$ = Equilibrium constant.

*1) The more Negative the ΔG the greater the release of free energy during a chemical reaction.

* 2) Chemical reactions that have a $-\Delta G$ are termed exergonic reactions.

* 3) By convention reaction that require free energy into to proceede are termed as energo endergonic reaction.

* 4) The free energy not used to do coork is expressed as heat.

5) Reactions that have no net change in substrate or product (conc.) are termed equilibrium reactions & have no change in free energy

6) If the equilibrium constant is 1 then, the $\Delta G^\circ = 0$

7) If the equilibrium constant is greater than 1 then, ΔG° value is negative & reaction is exergonic.

8) When equilibrium constant value is less than 1, then ΔG° value is positive. & reaction is endergonic.

- High Energy compound :—
  1) Pyrophosphate
  2) enolic phosphatase.
  3) Acyl phosphatase.
  4) Thioester compounds.
  5) Guanidium compounds.

*HEC stores large amount of energy in high energy bond & represented as symbol ~

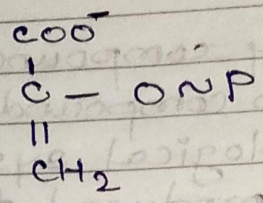
- High energy compounds are also called energy rich compounds. compounds present in the biological system that when hydrolysed produced free energy that is greater or equal to that of ATP (ΔG = -7.3 kilocal/mol) are term high energy compounds.
- Low energy compounds have an energy yeild of less than -7.3 kcal/mole.
- High energy bonds are found in the majority of high energy compounds that produced energy upon hydrolysis.
- Most of the high energy compounds contain phosphate groups & they are also term high energy phosphates.
- They are mainly classified into 5 compounds :—

1> Pyrophosphate - (Bond C-P-P)

- The energy bond in the pyrophosphate are acid unhydried bond.
- This bonds are form by the condensation of acid groups. mainly phosphoric acid or its derivatives
e.g. ATP.

2> Enolic Phosphate —

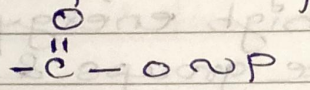
- The bond present here is enol phosphate bond it is form when phosphate group attaches to hydroxyl group which is bound to or bounded to a carbon atom having double bond.



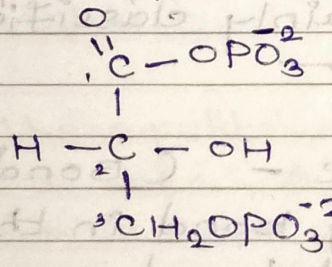
eg. phosphoenol pyruvate.
(ΔG = -14.8 kcal/mole)

3) Acyl phosphate —
(ΔG = -11.8 kcal/mole)

- The high energy bond in this compound form by the reaction between carboxylic acid group & phosphate group.

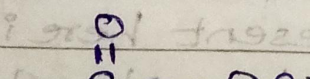


eg. 1,3- biphosphoglycerate

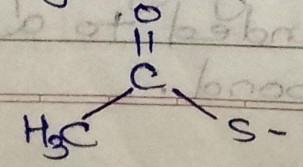


4) Thioester compound —

- High energy phosphate bond is absent instead high energy thioester bond is present.
- Thioester bond result from the reaction between thiol & carboxylic acid group.



eg. acetyl Co.A (ΔG = -7.7 kcal/mole)

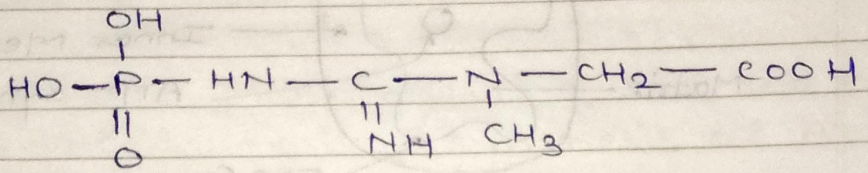


5) Guanidium compounds — (phophagens)

- This bond is known as guanidium phosphate bond.
- It is form by the attachment of phosphate group to guanidium group.

(-N∼P)

Eg. phosphocreatine ($\Delta G = -10.3$ kcal/mole)

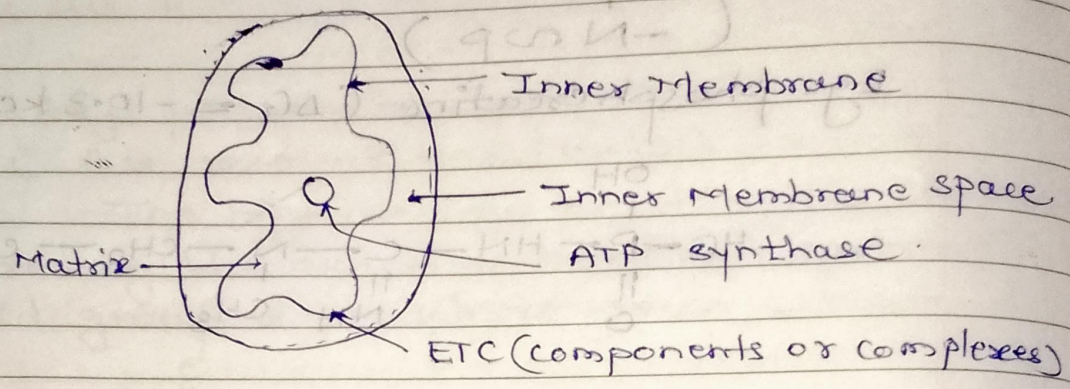


Class	Bond	Examples.
1) Pyrophosphatase	-C-P-P	ATP, pyrophosphate.
2) Acyl phosphatase	-C(=O)-O∼P	1,3-bisphospho-glycerate.
3) Enol phosphatase	-C(=CH)-O∼P	PEP (phospho enol pyruvate)
4) Thiol esters	-C(=C)-O∼S-	Acetyl Co-A, Acyl Co-A.
5) Guanido phosphatase	-HN∼P	phosphocreatine phosphoargenine.

- Electron Transport chain :—

- Matrix is negatively charged, hence it is denoted by 'N matrix'

- ETC complex present on inner membrane (N site) of mitochondria.



- The electron transport chain is a series of four protein complexes that couple redox reactions creating an electrochemical gradient that leads to the reaction of ATP in a complex system, named as oxidative phosphorylation.

- It occurs in mitochondria in both cellular respiration.

- Aerobic cellular respiration is made up of 3 parts —
  1) Glycolysis
  2) Citric Acid cycle.
  3) Oxidative phosphorylation.

- In glycolysis glucose is metabolised into 2 molecules of pyruvate with an output of ATP & NADH. (Nicotamine Dihydroxy phosphate)

each pyruvate is oxidizes into acetyl co-A & additional molecule of NADH & CO_2.

- The acetyl coA is then used in citric acid cycle which is a chain of chemical reaction that produce CO_2, NADH & $NADH_2$ & ATP.

- In the final step 3 NADH & 1 $FADH_2$ are used in oxidative phospho-rylation to make water & ATP.

- Oxidative phosphorylation has too parts ETC & chemiosmosis. (synthesis of ATP) by using ATP synthase.

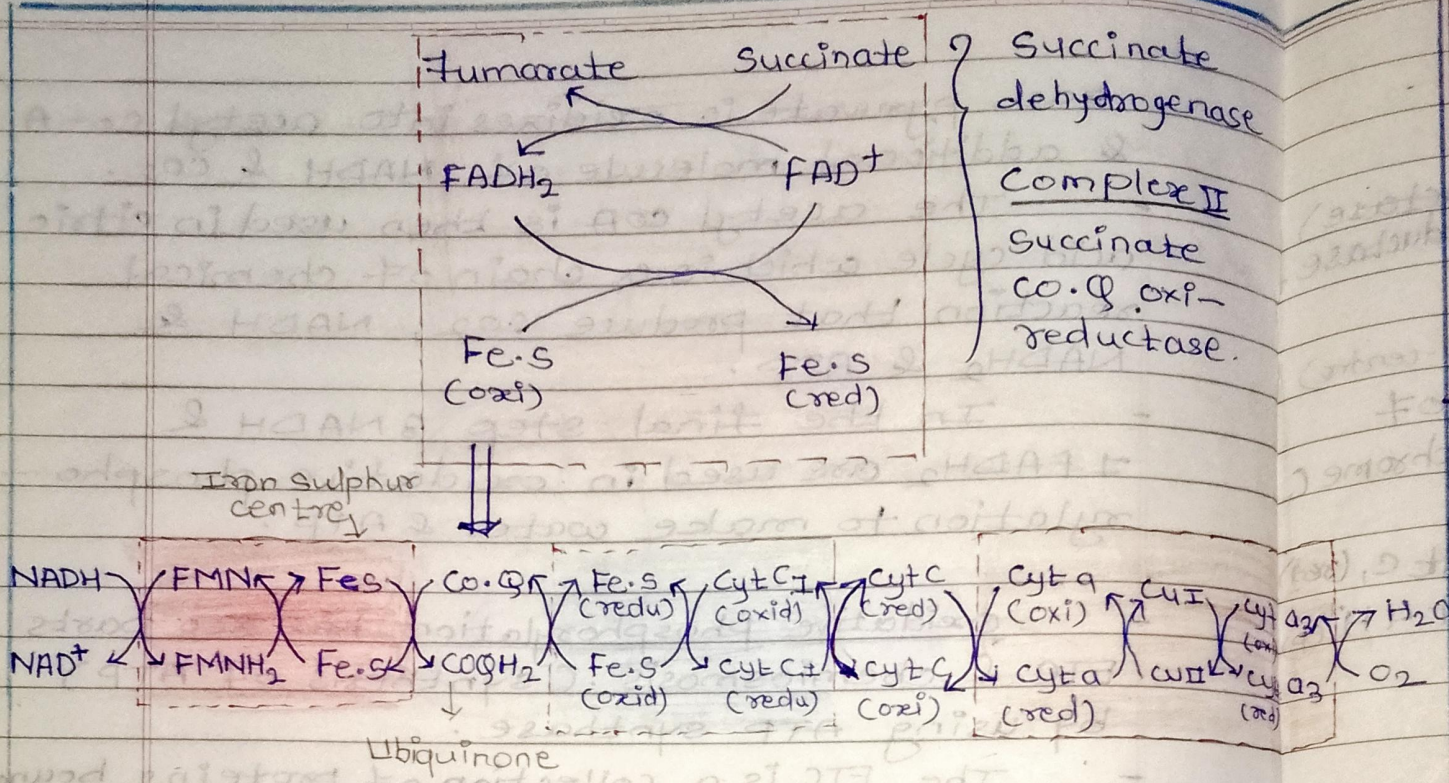
- The ETC is a collection of proteins bound to the inner mitochondrial membrane & organic molecules which electron pass through in a series of redox reactions & release energy.

- The energy release from proton forms a proton gradient which is used in chemo-osmosis to make a large amount of ATP by the protein ATP synthase.

• Electron Transport Chain —
(Diagram explanation)

1) oxidation of NADH & $FADH_2$ is carried out by electron transport chain, a set of protein complexes containing redox centre with progressively greater affinity for electron.
(In increasing standard reduction potential)

- ETC process also called oxidative phosphorylation.



Complex I
NADH : Ubiquinone oxidoreductase
(NADH dehydrogenase)

Complex III
Ubiquinone : cytochrome C oxireductase.

Complex IV
cytochrome oxidase.

Fig. Electron Transport chain.

2) complexes I & II catalyse electron transfer to ubiquinone from two different electron donors:
(NADH complex I & Succinate complex II)

3) Complex III carries electrons from reduced ubiquinone to cytochrome C & complex IV complete the sequence by transferring electrons from cytochrome C & O_2.

• Complex I :— (NADH = ubiquinone oxireductase or NADH dehydrogenase)

• Mass - 850 KDa
• subunit - 43
• prosthetic group - FMN, fe S

- Complex I catalyses two simultaneous & obligately coupled processes :-

1) The exergonic transfer to ubiquinone or hydride ion from NADH & proton from the matrix expressed by,

$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$

H_2O
O_2

2) The endergonic transfer of four protons from the matrix to the inter-membrane space, expressed by,

$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_{(P)}$

∴ N for the negative side of the matrix
P for the positive side of the inter-membrane space.

• Complex II :—

- Succinate to Ubiquinone (succinate dehydrogenase)
- It catalyses the oxidation of $FADH_2$ by Q. (ubiquinone)

$FADH_2 + Q \longrightarrow FAD + QH_2$

- This redox reaction does not release sufficient free energy to synthesized free ATP it function only to inject e^- from $FADH_2$ to ETC

• Mass — 140 KDa
• subunit — 4
• prosthetic group — FAD·FeS

• Complex III :—

- Ubiquinone to cytochrome C
(Ubiquinone :— cytochrome C ~~oxireductase~~ oxidoreductase.

. Mass — 250 KDa
. subunit — 11
. prosthetic group — Heme, fe-S (Iron-S-centre)

- complex III catalyses oxidation of QH_2 to Q and two molecule of cytochrome C are reduced.

$$QH_2 + 2\ cyt.C_1(oxi.) + 2H^+_N \longrightarrow Q + 2Cyt\ C_1(red) + 4H^+_{(P)}$$

• Complex IV :—

- cytochrome C to O_2
(cytochrome oxidase)

. Mass — 160 KDa
. subunit — 13
. prosthetic group — Hemes, CuA, CuB.

(Cu^I) (Cu^{II})
(Cu^+) (Cu^{2+})

- complex IV carrier electrons from cytochrome C to molecular oxygen (terminal e^- acceptor of ETC), reducing it to H_2O at the same time four protons are pumped from the matrix (N) to inter-membrane space (P)

$$4\ cyt.C\ (red.) + 8H^+_N + O_2 \longrightarrow 4\ cyt.C\ (oxi^n) + 4H^+_{(P)} + 2H_2O.$$

DATE 26/3/23

* Oxidative phosphorylation :—

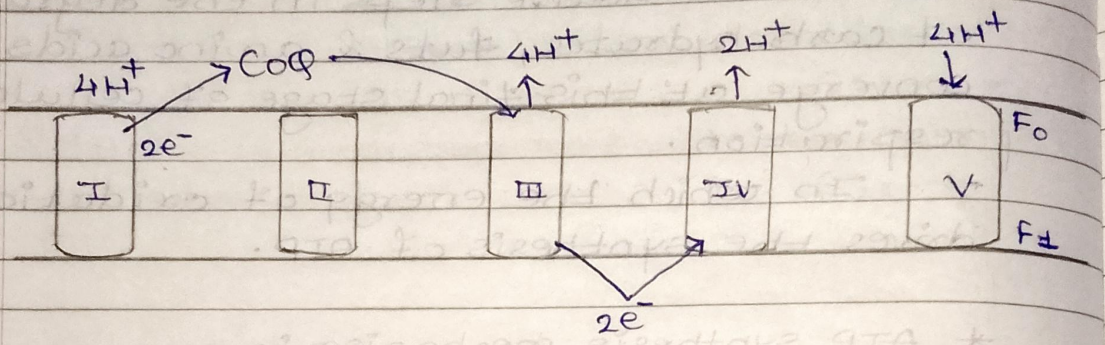
- Oxidative phosphorylation is the culmination of energy yeilding metabolism in aerobic organisms.
- All oxidative steps in the degradation of carbohydrates, fats & amino acids converge at this final stage of cellular respiration.
- In which the energy of oxidation drives the synthesis of ATP.

* ATP synthesis mechanism :—

- It is based on chemiosmotic model proposed by Pitter Mitchell. according to the model The electrochemical energy inharent in the difference in proton conc. and the separation of charge across the inner mitochondrial membrane.
- The proton motive force ⟶ drives the synthesis of ATP as protons flow passively back into the matrix through a proton core associated with ATP synthase.

- In ETC the flow of electrons through complexes I, III & IV results in pumping of protons across the inner mitochondrial membrane, making the matrix alkaline relative to the inter. membrane space.
- This proton gradient provides the energy for ATP synthesis from ADP & Pi by ATP synthase. (F_0F_1 complex) in the inner membrane. Total 10 H^+ are

pumped out by ETC chain when one NADH is oxidised & 6 H^+ are pumped out, when $FADH_2$ is oxidised.



1 ATP = $4H^+$ are pumped out
2.5 ATP = $10H^+$ are pumped (NADH oxidised)

- major function of F_0F_1 complex is ATP synthesis, for synthesis of 1 ATP $4H^+$ are pumped inside the matrix.

1 ATP = $4H^+$ are pumped out
2.5 ATP = $10H^+$ are pumped out (NADH oxidised)
1.5 ATP = $6H^+$ are pumped out (FADH oxidised)

- for Ideal condition,
  2 ATP = $6H^+$ (FADH oxidized)
  3 ATP = $10H^+$ (NADH oxidized)

* ATP synthase :—

- ATP synthase present on inner mitochondrial space.

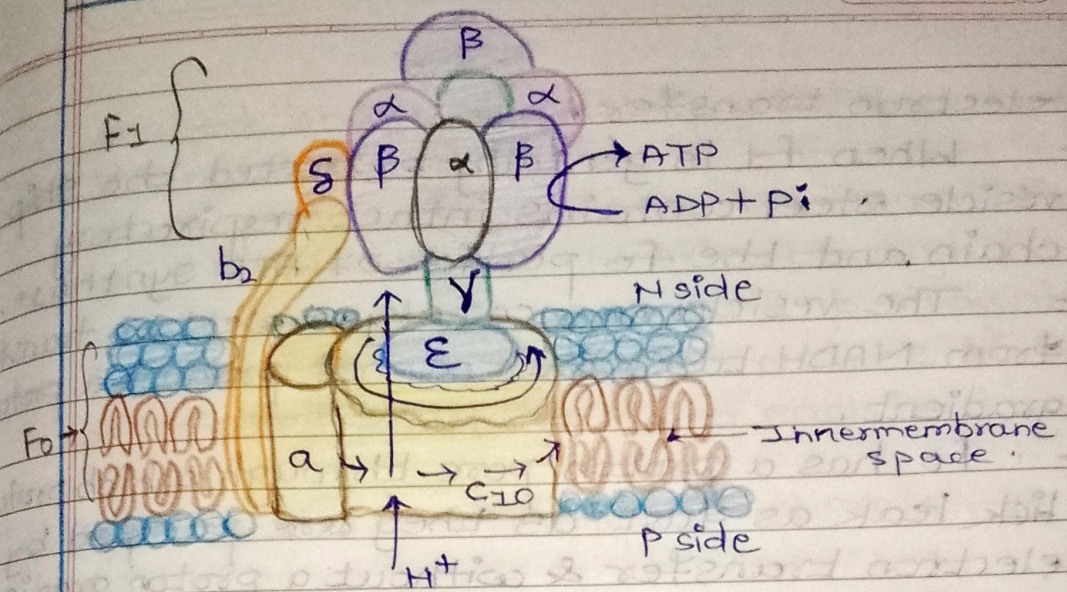


fig. ATP synthase

- ~~Structure~~ —
- ATP synthase has two functional domains: Fo and F1.
- This large enzyme complex of the inner mitochondrial membrane catalyses formation of ATP from ADP & pi accompanied by the flow of protons from P side to N side of inner membrane.
- ATP synthase also called complex V. has two distinct components : ~~F1~~ & Fo.
- F1 → A peripheral membrane protein & Fo → Zero denoting oligomycin sensitive, which is integral to the membrane.
- So F1 is the first factor recognize. as essential for oxidative phosphorylation (ETC + ATP synthesis)

- ~~function~~ —
- In laboratory small membrane vesicles ~~fro~~ form from the inner mitochondrial membrane carry out ATP synthesis couple to

electron transfer.

- When f1 is gentally extracted the strip vesicle steel contains intact respiratory chain and the fo portion of ATP synthase.
- The vesicle can catalyse electron transfer from NADH to O_2 but cannot produce a proton gradient.
- fo has a proton pore through which protons kick leak as fast as they are pumped by electron transfer & without a proton gradient the f1 depleted vesicles cannot make ATP.
- When purified f1 is added back to the depleted vesicles it reassociates with fo pluging its proton pore and restoring the membranes capacity to couple electron transfer & ATP synthesis.

• Structure :—

- The fof1 structure viewed end on in the direction p side to N side.
- The measure structure visible in the cross section are the two transmembrane helises of each of 10 C subunits arranged in a concentric circles..
- The above diagram of fof1 complex deduced by biochemical & crystallographic studies.
- The 2b subunits (b_2) of fo associated firmly with α & β subunits of f1 holding them fixed relative to the membrane.
- In fo the membrane embeded cylinder of c subunits (c_{10}) is attach to the

chated shaft made up of F1 subunit γ and ε

- A proton flow to the membrane from the P side to the N side through to the cylinder & shaft rotates & β subunit of F1 change confirmation as the γ subunit associated ~~which it~~ with each in turn.

Friday 4/8/23

* Inhibitors and Uncouplers of ETC :—

• Inhibitors :—

Inhibitors of ETC are the one which interupts the flow of electron through respiratory chain and thus block the respiratory chain at three sites (complex I, III and IV)

This results in the blockage of proton pumping, ATP synthesis and oxygen uptake.

• Some Important Inhibitors :—

1) Retonone —

- plant product → ~~extracted~~ from root of plant Derris elliptica (leguminous plant)
- Insecticide & fish poisonous in function.
- Inhibitor of → complex I ~~and~~ (NADH Ubiquinone reductase complex)

2) Amobarbital (Amytal) —

- Used as ~~An~~ Ansthethia agent.
- Inhibits complex I

3) Demerol —

- also inhibit complex I
- It works in brain
- It is also a Ansthecia.

- To reduce severity of pain.

4) Piericidin A —

- It also inhibit complex I and it is the antibiotic.
- It is a Neurotoxin
- Mode of action same as Retinone.

5) Antimycin —

- Antibiotic from Streptomyces sp.
- It blocks complex III i.e. inhibit the flow of electron from cytochrome b to cytochrome C1.

6) Cyanide —

- It blocks complex IV
- It blocks cytochrome oxidase (complex IV) which prevents both coupled & uncoupled respiration.
- cyanide binds with ions within this protein complex & prevents the regular activity of the complex system.
- It blocks the transport of electrons from NADH to Ubiquinone reductase complex ~~(complex I)~~ to oxygen
- As a result person deprive of energy & may lead to death of person.

7) Carbon Monooxide —

- It is respirator inhibitor which blocks complex IV. of ETC.