Electron Transport Chain.

vimpt → for exam. (must question).

Biological Oxidation.

High Energy Compounds: Compounds which release large amount of energy (at least -7Kcal) on hydrolysis.

ATP: most important high energy compound is constantly being utilized and regenerated.

High energy compound is represented by squiggle bond (~).

Vimpt • High energy compound. — standard free energy of hydrolysis in Kcal/mol.

Phosphate Compound.

• phosphoenol pyruvate.	-14.8	no need for values.
• Carbamoyl phosphate.	-12.3	
• Creatine phosphate.	-10.5	
• ATP, GTP, UTP.	-7.3	

- Creatine phosphate. - 10.5
- ATP, GTP, UTP. - 7.3

Substrate Level Phosphorylation.

- Energy from substrate is directly transferred to nucleoside diphosphate to form a triphosphate without the intervention of electron transport chain.

→ Bisphospho glycerate Kinase.
→ pyruvate Kinase.
→ Succinate thiokinase.

Oxidative Phosphorylation.

- Oxidative phosphorylation is the terminal process of cellular respiration.
- During oxidative phosphorylation electrons are transferred.

from NADH or $FADH_2$ - created in glycolysis, fatty acid metabolism and the Krebs cycle - to molecular oxygen.

- In eukaryotes, this is carried out by a series of protein complexes located in the inner mitochondrial membrane called E.T.C.

- Transfer of electrons from the reduced co-enzymes through respiratory chain to oxygen is known as biological oxidation.
- Energy released during this electron transfer process is trapped as ATP.
- Coupling of oxidation with phosphorylation is called oxidative phosphorylation.
- All enzymes involved in biological oxidation belong to major class of oxidoreductases.

They include:

– oxidases, aerobic dehydrogenases, Anaerobic dehydrogenases, hydroperoxidases, oxygenases.

Oxidases.

enzymes that catalyse the removal of hydrogen from substrate, but only oxygen can act as acceptor of hydrogen, so that water is formed.

$AH_2 + {}^1/_2O_2 \longrightarrow A + H_2O$.

eg: cytochrome oxidase,

(2) Aerobic dehydrogenases.

These enzymes are flavoproteins which catalyse removal of hydrogen from a substrate oxygen can act as the acceptor. The product is usually H_2O_2.

$AH_2 + O_2 \longrightarrow A + H_2O_2$. eg: L-amino acid oxidase. xanthine oxidase.

(3) Anaerobic dehydrogenases

→ catalyse removal of hydrogen from a substrate but oxygen cannot act as hydrogen acceptor.

eg: malate & Succinate dehydrogenase.

(4) hydroxyperoxidases. (i) peroxidases:

• remove free radicals like H_2O_2

$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$.

(ii) catalase $2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$

(5) Oxygenases → add O atoms to substrate

(i) monooxygenase: add 1 atom of O to substrate & other atom is reduced to H_2O

eg: phenyl alanine hydroxylase, Tyrosine hydroxylase.

(ii) Dioxygenases: add both atoms of O_2 into substrate $A + O_2 \longrightarrow AO_2$.

eg: tryptophan pyrrolase. homogentisic acid oxidase.

Components of the respiratory chain.

- The electrons flow through the respiratory chain in a stepwise manner.

for more electronegative components.

↓

to electropositive oxygen.

- All the components of etc are located in the inner mitochondrial membra
- Four multiprotein complexes: complex 1 complex 2 Complex 3 complex 4.
- they are connected by 2 mobile carriers cytochrome c & coenzyme Q.
- NADH. dehydrogenase (complex 1 → FMN → Fe-S
  Complex 2 → FAD → Fe-S → complex Q.

↓

Cytochromes
(a+b, c1, c; a-a3).

↓

molecular O2

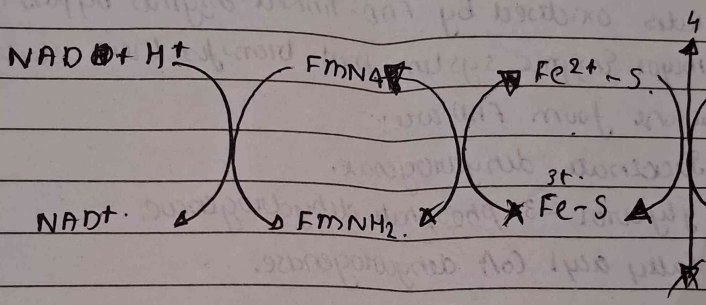
↓

H_2O.

↓
molecular O2
↓
H_2O.

Complex I

- NADH – Co-Q reductase or NADH dehydrogenase complex.



- Tightly bound to inner membrane of mitochondria.
- contains FMN, prosthetic group.
  Fe-S protein.
- NADH is the donor of electrons
- FMN accepts them and reduced to $FMNH_2$.

NAD+ 2H. quinone.

12 kcal/mol.

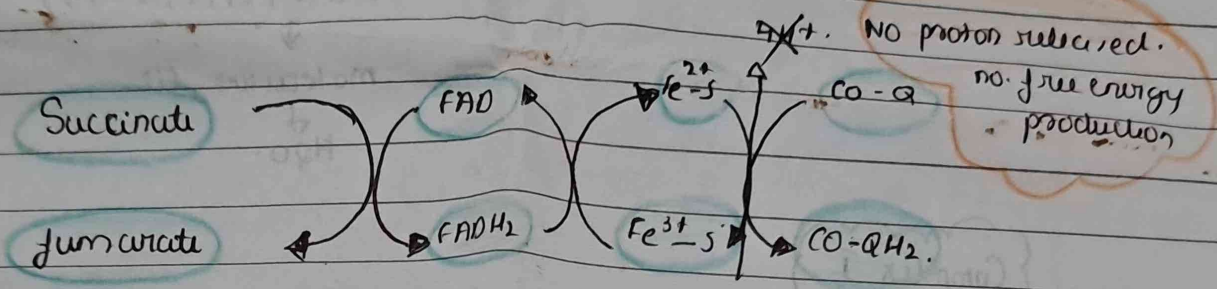
(47)

- When NAD+ accepts 2H atoms, one of them is removed from substrate as such.
- Other hydrogen atoms is split into one H^+ & one $\bar{e}$.
- $H_2 \longrightarrow H^+ + H^+ + \bar{e}$.
- $AH_2 + NAD^+ \longrightarrow A + NADH + H^+ + \bar{e}$. { reaction not needed } X
- $NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$.

- $\bar{e}$ from $FMNH_2$ are then transferred to FeS.
- Electrons are then transferred to co-enzyme Q) (co Q, ubiquinone)
- A large free energy change will release energy of 12 kcal/mol.
- This is used to drive 4 protons out of mitochondria.

Complex - 2

- Succinate - Q - reductase.



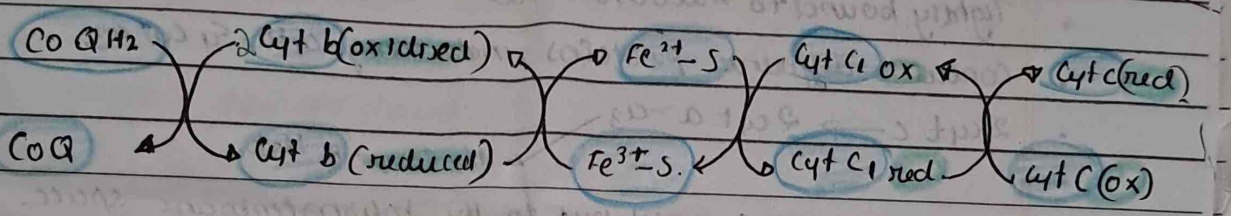
- Substrates oxidised by FAD-linked enzyme bypass complex-1.
- three major Enzyme systems that transfer their electrons directly to ubiquinone from FAD are!
  - Succinate dehydrogenase.
  - glycerol - 3 - phosphate dehydrogenase.
  - Fatty acyl CoA dehydrogenase.

Coenzyme Q

- also called ubiquinone.
- Lipid soluble quinone.
- Structurally similar to vitamin K.
- It is the only non protein electron carrier in respiratory chain
- It is a quinone derivative with long isoprenoid side chain.
- Coenzyme Q accept reducing equivalents from $FMNH_2$.

- In the oxidised form, coenzyme Q is designated as quinone form and reduced form as quinol form (QH_2).
- Q → QH (semiquinone) → QH_2.
- Coenzyme Q accepts 2H atom from $FMNH_2$ and gets reduced to quinol form (coenzyme QH_2).
- Coenzyme Q also function as acceptor of 2 H protons from several substrates include succinate, glycerol-3-PO4, fatty acyl CoA etc.

Complex -3. - Cytochrome Reductase.

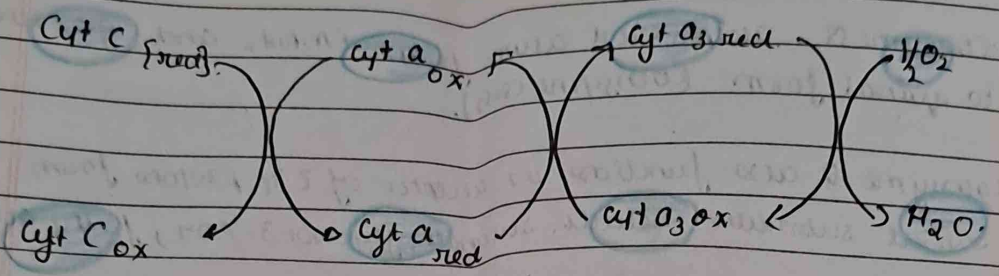


Cytochromes.

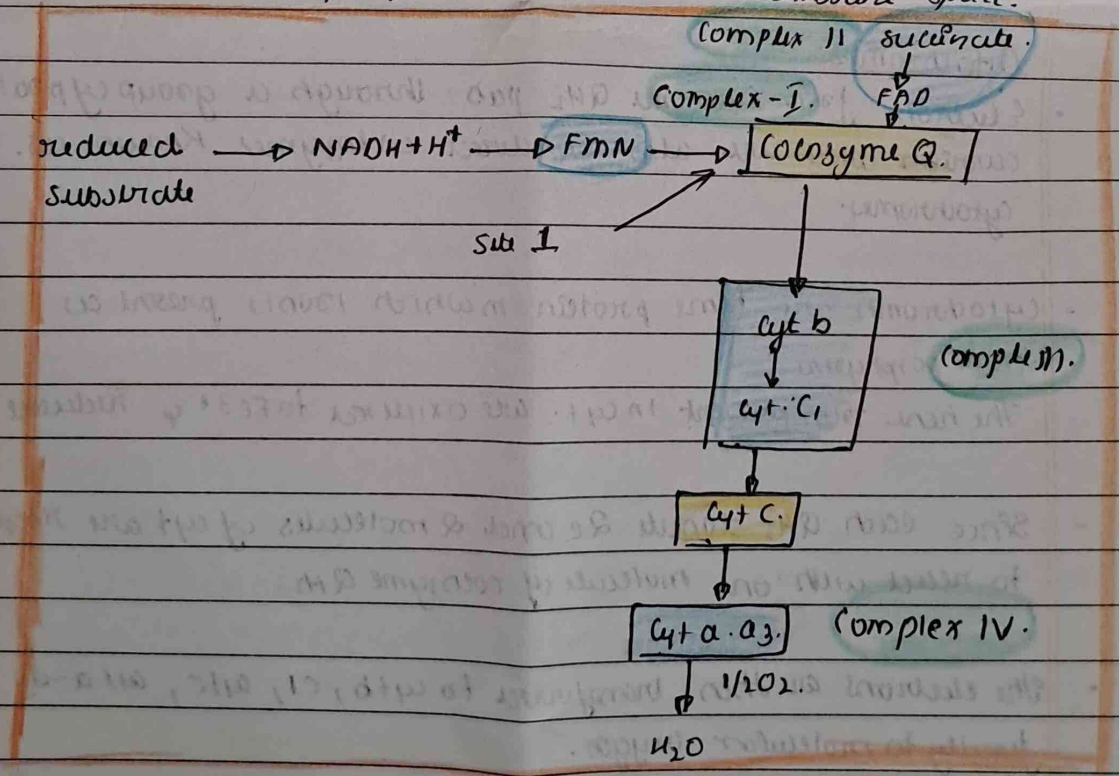
- Electrons from coenzyme QH_2 pass through a group of protein carriers which are also considered as enzymes known as cytochromes.
- Cytochromes are heme protein in which irons present as iron porphyrins.
- The heme iron present in cyt. are oxidised to Fe^{3+} & reduced to Fe^{2+} form.
- Since each QH_2 donate 2ē and 2 molecules of cyt are required to react with one molecule of coenzyme QH_2.
- The electrons are then transferred to cyt b, c1, cyt c, cyt a-a_3 & finally to molecular oxygen.
- Cyt C is a single electron carrier.
- Cyt a-a_3 is also called cytochrome oxidase complex.
- reduced cyt. aa_3 can donate e directly to oxygen to complete the process of ETC.
- $2H^+ + 2e + 1/2O_2 \rightarrow H_2O$

- free energy change in complex 3 is ~10 kcal/mol.
- ∴ 4 protons are pumped out.

COMPLEX-4 - CYTOCHROME OXIDASE.



- Tightly bound to mitochondrial membrane.
- Contains different proteins including cyt a. & cyt a3.
- 2 cyt c ⟶ 2 cyt a -a3 ⟶ 1/2 O2 ⟶ H2O.
- 2 protons are pumped out to the intermembrane space.

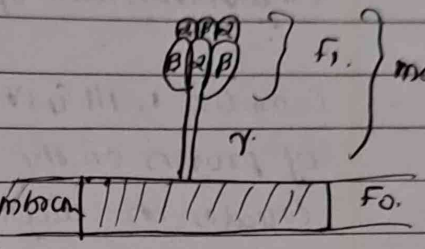


Site of ATP Synthesis in ETC.

- ETC provides 3 site at which oxidation is coupled with phosphorylation.
- at these sites, ATP is formed from ADP & Pi
- Site I : Electron transfer from $FMNH_2$ to coenzyme Q.
- site II : Electron transfer from cyt. c1 to cyt. c.
- site III : Electron transfer from cyt a-a3 to O_2.

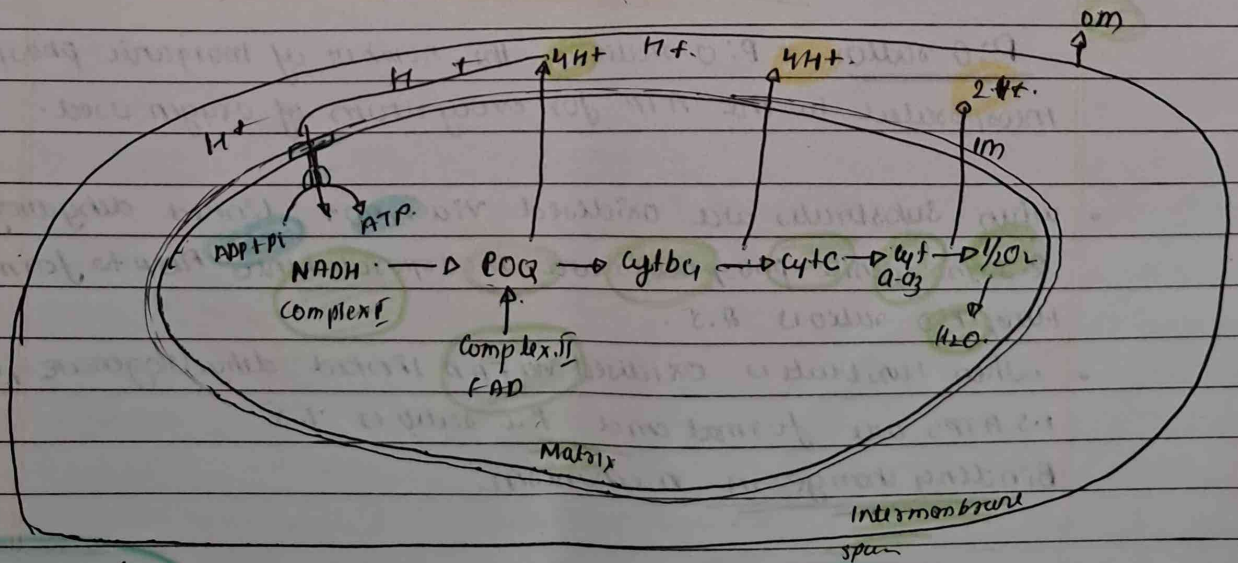
ATP Synthase Complex.

- Sometimes refered as complex 5.
- It has two fundional units - F_1 & F_o.



- F_o : Serves a proton channels through which protons enter mitochondria.
- Has 4 polypeptide chains connected to F_1.
- span the inner mitochondrial membrane.
- F_1 projects into the matrix.
- Has 9 polypeptide chains.
- 3 α, 3β, 1γ, 1 sigma, 1 epsilon.
- α has binding site for ADP and Pi
- β has catalytic activity. → ATP synthesis occurs here.
- ATP synthesis required Mg^{2+} ions.

Mechanism of Oxidative Phosphorylation.



If NADH + H+

1 → 2½ ATP.

1 → 1/2

4H+ → 1 ATP.

FAD. 1 → 1½ = 1.5

Mechanism of Oxidative phosphorylation.

- Chemi-Osmotic theory.
- widely accepted
- proposed by peter mitchell.
- According to this theory, electron transport in the respiratory chain leads to translocation or pumping of protons (H^+) from inner side. (matrix side) of inner mitochondrial membrane to the outer side (intermembrane space)

- Complex I, III & IV of ETC act as a proton pump. The accumulation of protons on the outer side of inner mitochondrial membrane. Creates an electrochemical potential difference across the membrane.

- The net effect is a change in pH and electrical charge across the inner mitochondrial membrane.
- The free energy stored in this gradient is called proton motive force or proton gradient.

- The link between electron transport and production of ATP is the movement of proton across inner mitochondrial membrane.

Regulation of ATP synthesis :- Availability of ADP regulates the process

* when ATP lvl is low & ADP is high, oxidative phosphorylation proceeds at a rapid state. This is called respiratory Control.

P:O ratio. : P:O ratio is the number of inorganic phosphates incorporated to the ATP for every atom of oxygen used.

- When substrates are oxidised via NAD+ linked dehydrogenase, 2.5 inorganic phosphates are incorporated into ADP to form 2.5 ATP. Here, P:O ratio is 2.5.
- When substrate is oxidised via FAD linked dehydrogenase, only 1.5 ATP's are formed and P:O ratio is 1.5.

Binding change in mechanism:

BINDING CHAIN MECHANISM.

According to this theory, 3 beta units are in 3 conformations.
O state - no substrate affinity
T state - binds substrates tightly
L state - binds loosely to substrate.

- ADP and Pi bind to L.
- T catalyses ATP
- T changes to O when ATP is released.

Inhibitors of respiratory chain.

- site specific inhibitors.
- inhibitors of oxidative phosphorylation.
- uncouplers of oxidative phosphorylation.

(1) site specific inhibitors.
these inhibitors are at 3 sites of ATP generation in ETC.

Imp. site I is inhibited by
Amobarbital - barbiturate derivative.
Rotenone - Fish poison
Piericidin - antibiotic
alkyl guanide - hypotensive drug.

Site II is inhibited:
- Antimycin A - antibiotic
- BAL - an antidote agent
- Napthoquinone.

Site III is inhibited by:
- CO
- CN
- H_2S
- Azide.

site between succinate dehydrogenase and coenzyme Q is inhibited by:
- Carboxin
- Malonate.

(2) INHIBITORS OF OXIDATIVE PHOSPHORYLATION.

atractyloside: it inhibit oxidation and phosphorylation by preventing the transport of ADP into mitochondria.

Oligomycin: An antibiotic which inhibit the enzyme ATP. synthase located in F_1-Fo complex of loner mitochondrial membrane.

Ionophores: lipophilic substances which promote the transport of ions across biological membrane.

- increase the permeability of lipid bilayer to certain ions, which dissipate the proton gradient.

2 types:

- Mobile ion carrier: valinomycin - pottasium ionophore.
- channel formers: gramicidin.

(3) Uncouplers of oxidative phosphorylation.

- action of uncouplers is to dissocuate oxidation in the respiratory chain. from phosphorylation.

- they allow oxidation to continue but prevent phosphorylation of ADP to ATP.

- Energy from oxidation is dissipated as heat.

Impt viva

eg: 2,4 - dinotrophenol
2,4 dihitro cresol.
CCCP - chloro carbonyl cyanide.
phenylhydrazine.

Impt viva

Physiological uncouplers:

- Thyroxine
- FFA
- Bilirubin.

PHYSIOLOGICAL SIGNIFICANCE OF UNCOUPLERS.

- maintenance of body temperature in hibernating animals and in new born infants.
- These animals posess a specialised tissue called brown adipose tissue.
- The MC of brown adipose tissue is rich in electron carriers and are specialized to carry out oxidation.
- This cause liberation of heat when fat is oxidised in adipose tissue.
- Thermogenesis is achieved by this process. Thermogenin (uncoupling protein) in MC is responsible for this.
- The presence of active adipose tissue in certain individual protect them from becoming obese.