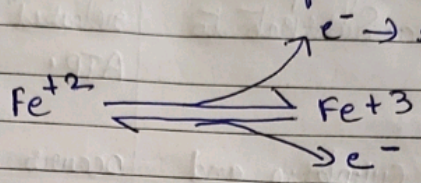
Chapter-5

Biological oxidation

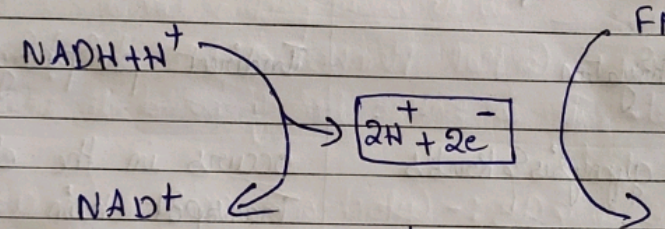
Oxidation is defined as the loss of electrons and reduction as the gain of electrons.

Eg:- Interconversion of ferrous ion & ferric ion



The electron lost in oxidation is accepted by an acceptor & it is reduced. Thus oxidation & reduction is a Coupled process. This is applicable to biological Systems also.

The oxidation of NADH to NAD^+ coupled with the reduction of FMN to $FMNH_2$



Two Redox pairs $NADH/NAD^+$ & $FMN/FMNH_2$ differ in their tendency to lose or gain electrons.

1. What is oxidative phosphorylation? How does it differ from Substrate level phosphorylation (5 Marks)

The process of Synthesizing ATP from ADP & Pi Coupled with the electron transport chain is known as oxidative phosphorylation.

Substrate level phosphorylation

ATP may be directly Synthesized during Substrate oxidations in the metabolism. The high energy Compounds such as phosphoenolpyruvate, & 1,3-biphosphoglycerate & Succinyl Co-A Can Transfer high Energy phosphate to ultimately produce ATP.

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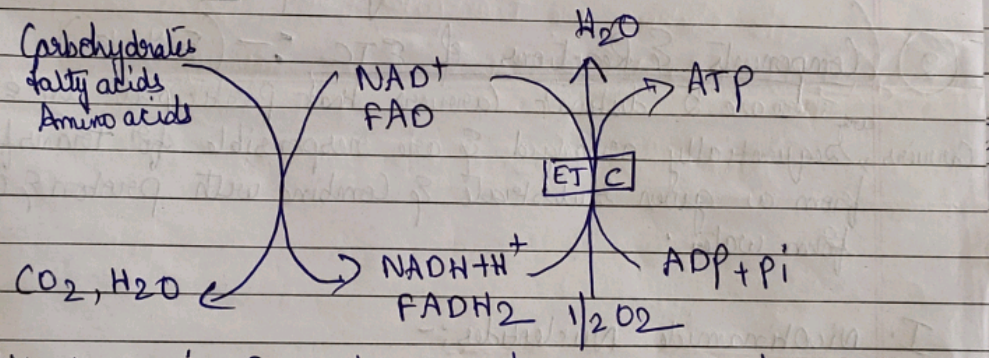
Substrate level phosphorylation	Oxidative phosphorylation
1. It refers to type of phosphorylation in which a phosphate group is transferred from a substrate to ADP.	It refers to a type of phosphorylation which uses the energy released from the ETC to generate ATP.
2. occurs in the cytoplasm and Mitochondrial Matrix	occurs on the inner membrane of the Mitochondria
3. A phosphate group is directly removed from a substrate by a coupled reaction & transferred into ADP.	phosphate groups are added from the energy released in the electron Transport chain.
4. Direct phosphorylation	Indirect phosphorylations
5. occurs in the glycolysis & Krebs cycle.	occurs in the electron Transport chain
6. NAD & FAD are reduced	NADH & FADH are oxidized
7. The change of the Redox potential of the Substrate is less.	The change of the redox potential of the Substrate is more
8. Partial oxidation of the Substrate occurs	Complete oxidation of the electron donors occurs.

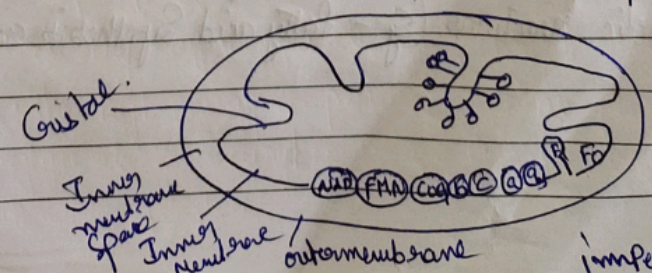
Overview of ETC (Electron Transport chain)

1. The energy rich carbohydrates, fatty acids & amino acids undergo a series of metabolic reactions & finally get oxidized to CO_2 & H_2O.
The reducing Equivalents from various metabolic intermediates are transferred to Coenzymes NAD^+ & FAD to produce $NADH_2$ & $FADH_2$. These Coenzymes pass through ETC or respiratory chain & finally reduce O_2 to water.
The passage of electrons through ETC is associated with loss of free energy.
A part of free energy is utilized to generate ATp from ADp & Pi.



Mitochondria Power houses of cells - Center for many metabolic oxidative reactions - generation of Reduced Coenzymes $NADH_2$ & $FADH_2$

Mitochondrial Organization :- 5 distinct parts.
Outermembrane, Innermembrane, Inner membrane Space, cristae & matrix.
Inner mitochondrial Membrane :- ETC, ATP Synthesizing System are located on inner membrane



Special proteins impermeable to H^+, K^+ & Na^+

D- A... L-amino acid oxidase ... FMNH2

→ folded - cristae - Surface area.
Specialized particles phosphorylating Sub unit (F1) → center for ATP production

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Mitochondrial Matrix :-
Interior ground Sub^n -
Rich in enzyme - TCA, β-oxidation of fatty acids & oxidation of amino acids.

Structural Organization of Respiratory chain

Inner mitochondrial membrane - disrupted - 5 distinct enzyme / Respiratory Complexes - Complex - I, II, III, IV, I
↓
Carriers of electrons

Mobile electron Carriers - NADH (CoQ), Cyt C & O_2

(2) Components & Reactions of ETC :- (5 Marks)
There are 5 distinct Carriers that Participate in ETC. Th... are sequentially arranged & are responsible for Transfer of e... from a given Substrate & Combine with proton & Oxygen form water.

I. Nicotinamide Nucleotides :-
The two Coenzymes derived from Niacin are NAD & $NADP^+$, out of these two NAD^+ actively involves in ETC. NAD^+ is reduced to $NADH + H^+$ by dehydrogenases with the removal of 2 hydrogen atoms from the Substrate (AH_2). The Substrates include glyceraldehyde-3-phosphate, pyruvate, isocitrate, α-Ketoglutara... & Malate.

$$AH_2 + NAD^+ \rightleftharpoons A + NADH + H^+$$

NADPH is not the Substrate for ETC it actively participates anabolic reactions (Eg:- Fatty acid Synthesis and cholesterol Synth...

II FLAVOP... The enz... is a Coenzym... NADH ... with ... Succinat... found ... FAD Succi...

IV I ron The ... The ... State. Coenzy... & Cytoch...

Coenzy... (Co... deriva... 10 iso...

CoQ FMN...

II FLAVOPROTEINS :-

The enzymes NADH dehydrogenase (NADH - Coenzyme Q reductase) is a flavoprotein with FMN as the prosthetic group. The Coenzyme FMN accepts two electrons & a proton to form $FMNH_2$. NADH dehydrogenase is a complex enzyme closely associated with iron Sulfur proteins (FeS)

$$NADH + H^+ + FMN \longrightarrow NAD^+ + FMNH_2$$

Succinate dehydrogenase (Succinate Coenzyme Q reductase) is found in inner mitochondrial membrane & is a flavoprotein with FAD as the coenzyme. This accepts 2 hydrogen atoms from Succinate.

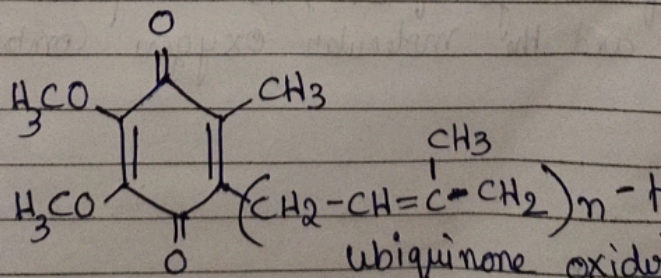
$$Succinate + FAD \longrightarrow Fumarate + FADH_2$$

III Iron Sulfur proteins :-

The mechanism of FeS proteins in ETC is not clearly known. The iron Sulfur proteins exist in oxidized (Fe^{+3}) or Reduced (Fe^{+2}) State. one FeS involves in transfer of electrons from FMN to Coenzyme Q. other FeS is associated with Cytochrome b & Cytochrome C_1 & participate in the transfer of electrons.

IV Coenzyme Q :-

Coenzyme Q is also known as ubiquinone. It is a quinone derivate with a isoprenoid Side chain. In mammals Quinone with 10 isoprenoid units is present which is known as Coenzyme Q_{10}.



ubiquinone oxidized form

CoQ is a lipophilic electron Carrier. It accepts electrons from $FMNH_2$ produced in the ETC by NADH dehydrogenase & $FADH_2$

produced outside ETC (Succinate dehydrogenase)

V. Cytochromes

The Cytochromes are Conjugated proteins Containing heme group. Heme consists of polphyrin ring with iron atom. The heme present in Cytochrome is different from Haemoglobin & Myoglobin.

The iron present in the heme of cytochrome is alternatively oxidized (Fe^{+3}) & reduced (Fe^{+2}), which is essential for the transport of electrons in ETC.

The electrons are transported from Coenzyme Q to Cytochromes b, c_1, c, a & a_3.

Cytochrome c is a small protein Containing 104 amino acids & a heme group. Central Member of ETC with an intermediate redox potential. It is rather loosely bound to inner mitochondrial membrane

cytochrome a & a_3 :- Cytochrome oxidase (a, a_3) is terminal Component of ETC. Cytochrome oxidase is the only electron Carrier, the heme iron of which Can directly react with Molecular O_2.

→ It also contains Copper ($Cu^{+2} \rightleftharpoons Cu^{+}$) that undergoes Oxidation - Reduction during the transport of electrons.

In the final Stage of ETC, the transported electrons, the free protons and the molecular oxygen Combine to produce water.

only L-amino acids
D-amino acids
(glycine, decarboxylic acid)
α-keto acid + NH_3
NH_2

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ETC.

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(1) Inhibitors of ETC :- (2 Marks) & name any two Sites

The inhibitors bind to one of the Component of ETC & block the transport of electrons. This leads to accumulation of ~~reducing~~ reduced Components before the block & oxidized Components after the block.

→ ~~It also inhibits~~
The Site Specific inhibitors of ETC also block ATP Synthesis. Three possible Sites of action for the inhibitors of ETC are identified.

1) NADH and Coenzyme Q : Fish poison rotenone, barbiturate drug amytal Antibiotic piercidin A inhibits this Site
2) Between Cytochrome b & c1 :- ~~Antimycin~~ - A (antibiotic) British Antilewisite (BAL)
3) Inhibitors of cytochrome oxidase :- Carbon Monoxide, Cyanide, Hydrogen Sulphide azide.

(2) What is Substrate level phosphorylation & oxidative phosphorylation (2 Marks)

Refer Question (1) under 5 Marks

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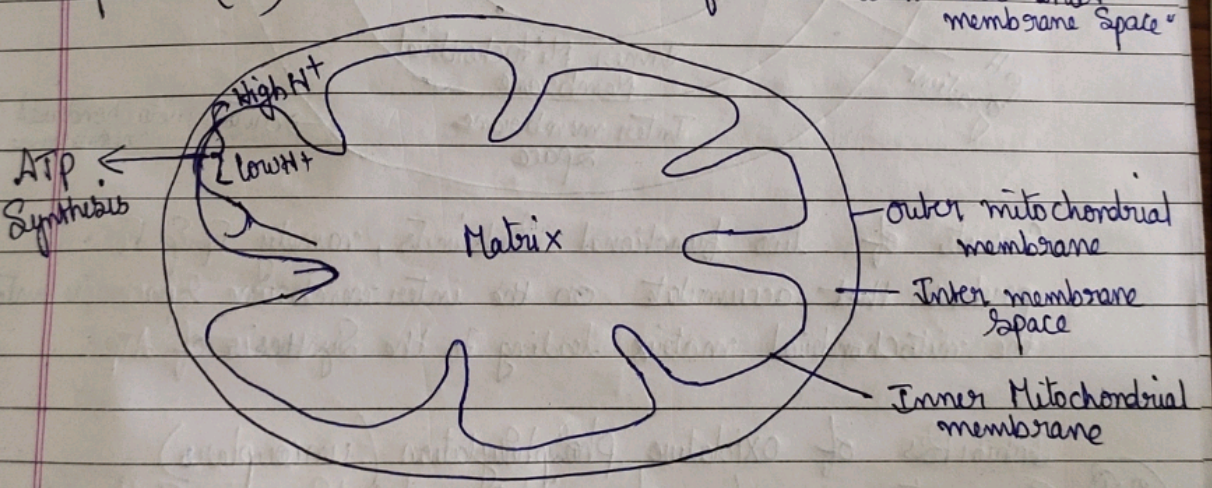
(3) oxidative phosphorylation (5 Marks)

Explain the chemiosmotic theory of oxidative phosphorylation

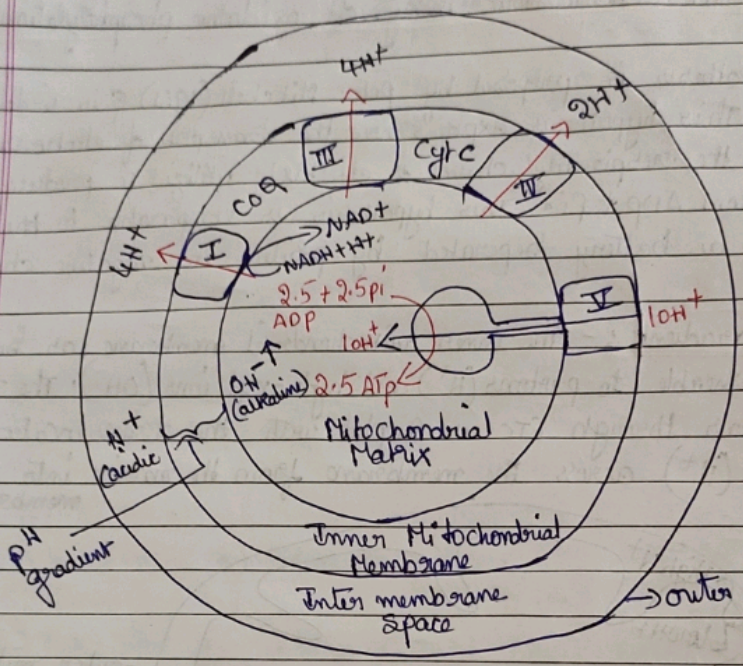
This hypothesis is proposed by peter Mitchell (1961) & is widely accepted. This hypothesis explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi. This hypothesis is comparable to the energy stored in a battery seperated by positive and negative charges.

proton gradient :- The inner mitochondrial membrane as such is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the translocation of protons (H^+) across the membrane from the matrix into inter membrane space.



This pumping of protons results in development of electrochemical proton gradient. This is due to accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side. The proton gradient developed due to electron flow is sufficient to result in the Synthesis of ATP from ADP & Pi.

Enzyme System for ATP Synthesis :- ATP Synthase present in the Complex V utilizes the proton gradient for the Synthesis of ATP. This enzyme is also known as ATPase. ATP Synthase



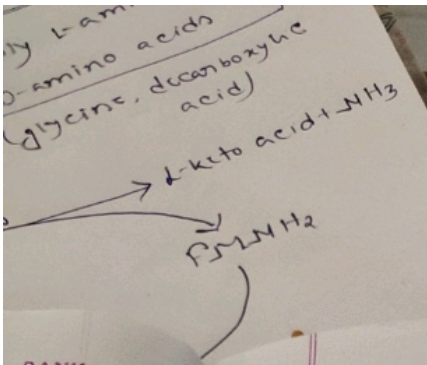
Consists of two functional subunits, namely F_1 & F_0. The protons that accumulate on the inter membrane space re-enter the mitochondrial matrix leading to the Synthesis of ATP.

(2M) Inhibitors of oxidative phosphorylation (uncouplers)

Enumerate uncouplers of oxidative phosphorylation & their Mechanism of action:-

The ETC is tightly coupled with oxidative phosphorylation. The oxidation & phosphorylation occurs simultaneously. There are certain compounds that can uncouple the ETC from oxidative phosphorylation. These compounds are known as uncouplers.

The uncouplers increase the permeability of inner mitochondrial membrane towards (H^+). This inhibits the Synthesis of ATP & allows oxidation of substrates, the energy linked with ETC is dissipated as heat.



Eg:- 2,4 dinitro phenol (DNP)
Dinitrocresol,
Penta chlorophenol
trifluoro carbonyl cyanide phenyl hydrazone (FCCP)
High doses of Aspirin.

physiological uncouplers:- Thermogenin,
Thyroxine,
long chain free fatty acids

Significance of uncoupling:-

1) Maintenance of body temperature is important in hairless animals, and the animals that are adapted to cold. They have specialized tissue called Brown adipose tissue in the upper back & neck portions.
The mitochondria of this tissue carries oxidation uncoupled from phosphorylation.
Thermogenin - is a physiological uncoupler located in the inner mitochondrial membrane of brown adipose tissue.

2) Antibiotics like Valinomycin, gramicidin A acts as ionophores for K^+ ions, dissipate proton gradient across the inner mitochondrial matrix & inhibit oxidative phosphorylation.