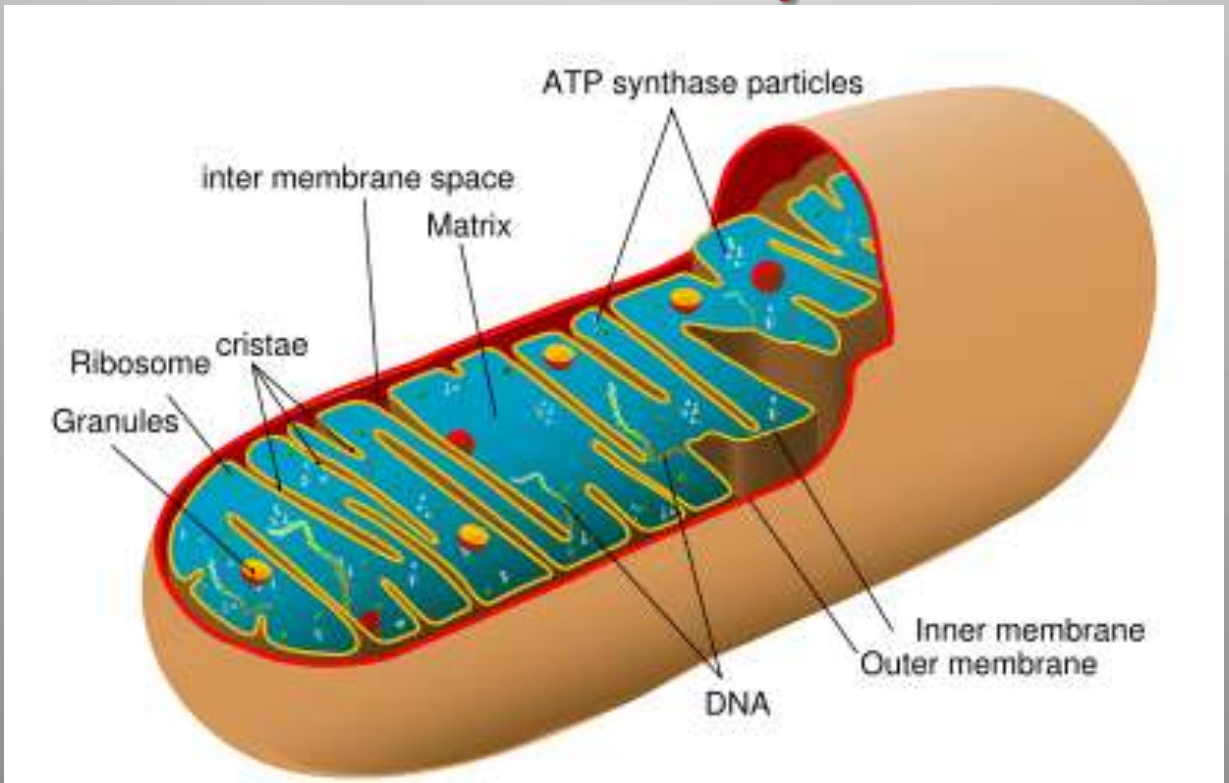
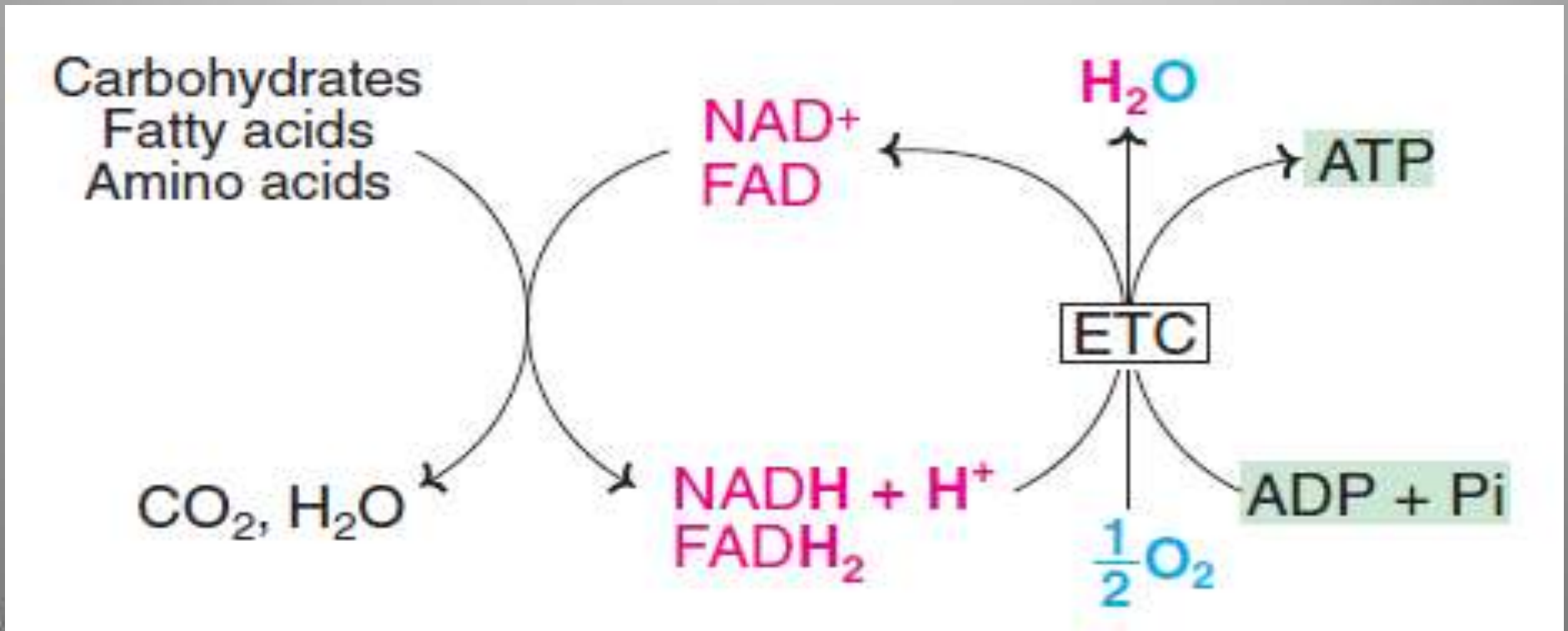
Oxidative Phosphorylation & Electron Transport Chain



Biological Oxidation & Oxidative Phosphorylation

- The transfer of electron from the reduced co-enzymes through the respiratory chain to oxygen is known as biological oxidation.
- Energy released during this process is trapped as ATP, this coupling of oxidation with phosphorylation is called oxidative phosphorylation.

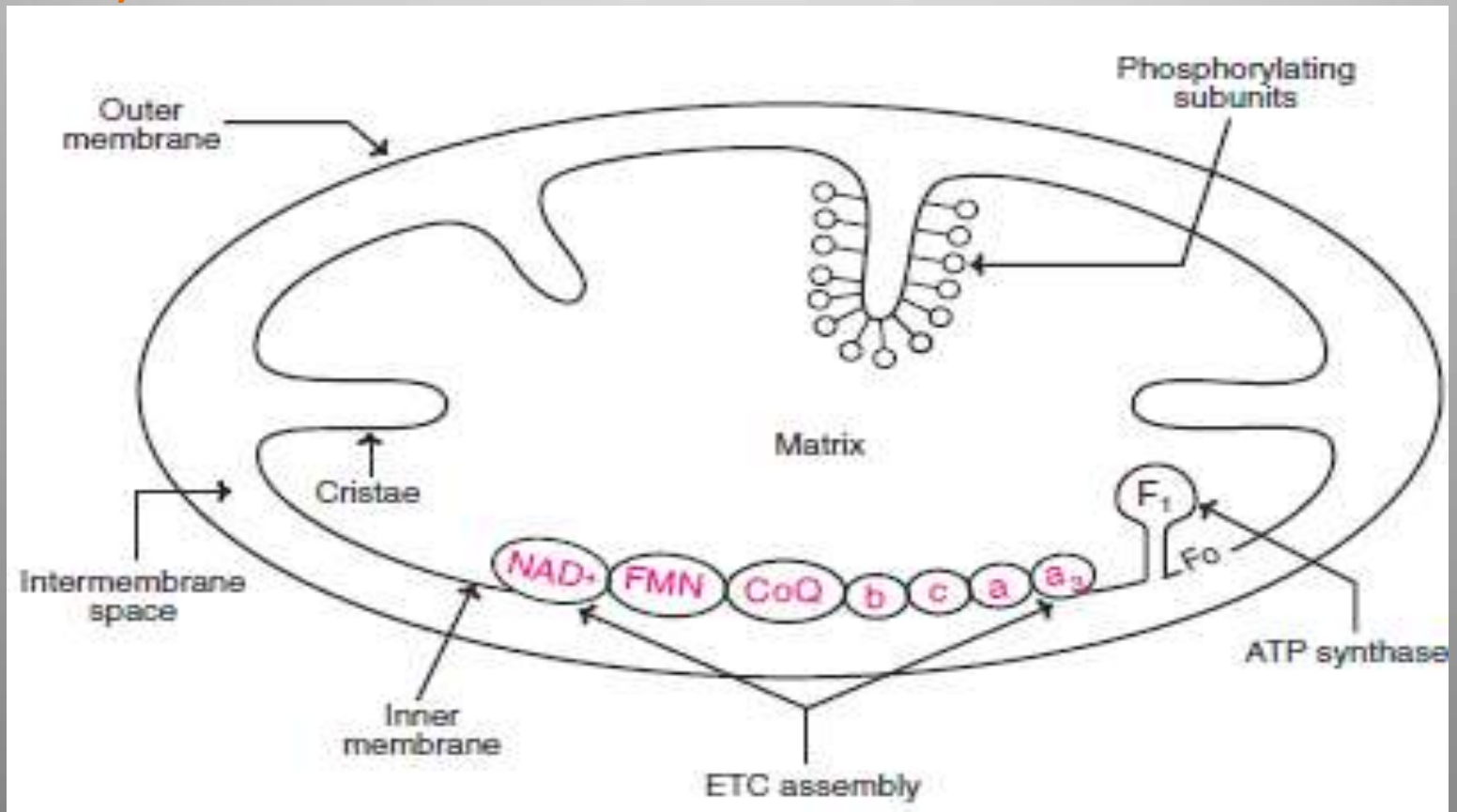


- The formation of ATP from ADP & Pi is termed phosphorylation.

- During biological oxidations, the reacting chemical systems move from a higher energy level to a lower energy and therefore liberation of energy.

SITES OF OXIDATIVE PHOSPHORYLATION IN ETC

- There are **three sites in the ETC that are** exergonic to result in the synthesis of 3 ATP molecules**.**
  - i. **Oxidation of FMNH2 by coenzyme Q.**
  - ii. **Oxidation of cytochrome b by cytochrome c1.**
  - iii. **Cytochrome oxidase reaction.**



Enzymes,Co-enzymes & Electron Carriers of Biological Oxidation

They can classified as:-

- **Oxidases:-** These enzymes catalysed the removal of hydrogen from substrates, but only oxygen can act as acceptor of hydrogen, so that water is formed. Ex. Cytochrome oxidase, tyrosinase, xanthine oxidase.
- **Dehydrogenases:-**

  Aerobic dehydrogenases:- these enzymes catalyse the removal of hydrogen from a substrate, but oxygen can act as the acceptor.

- **Anaerobic dehydrogenases:-** these enzyme catalyse the removal of hydrogen from a substrate but oxygen cannot act as the hydrogen acceptor. They therefore require co-enzymes as acceptors of the hydrogen atoms, when the substrate is oxidised, the co-enzyme is reduced.
- The coenzymes of dehydrogenases may be either NAD^+ or FMN or FAD

- **Cytochromes:-** cytochromes are electron transferring proteins, 3 classes- a, b, c
- All the cytochromes, except cytochrome oxidase, are anaerobic dehydrogenases. All cytochromes are hemoproteins having iron atom.
- Cytochrome b, C1,and C are in the mitochondria while cytP-450 & cyt b5 are in the endoplasmic reticulum.

- **Hydroperoxidases:-** have protective effect against peroxides. They use hydrogen peroxide or organic peroxide as substrate. Two types:-

  Peroxidases & catalases
- **Peroxidases:-** is a haemoprotein, having haem as a prosthetic group.
- Peroxidases catalyze the reduction of H_2O_2 to H_2O
- Ex. Glutathione peroxidase

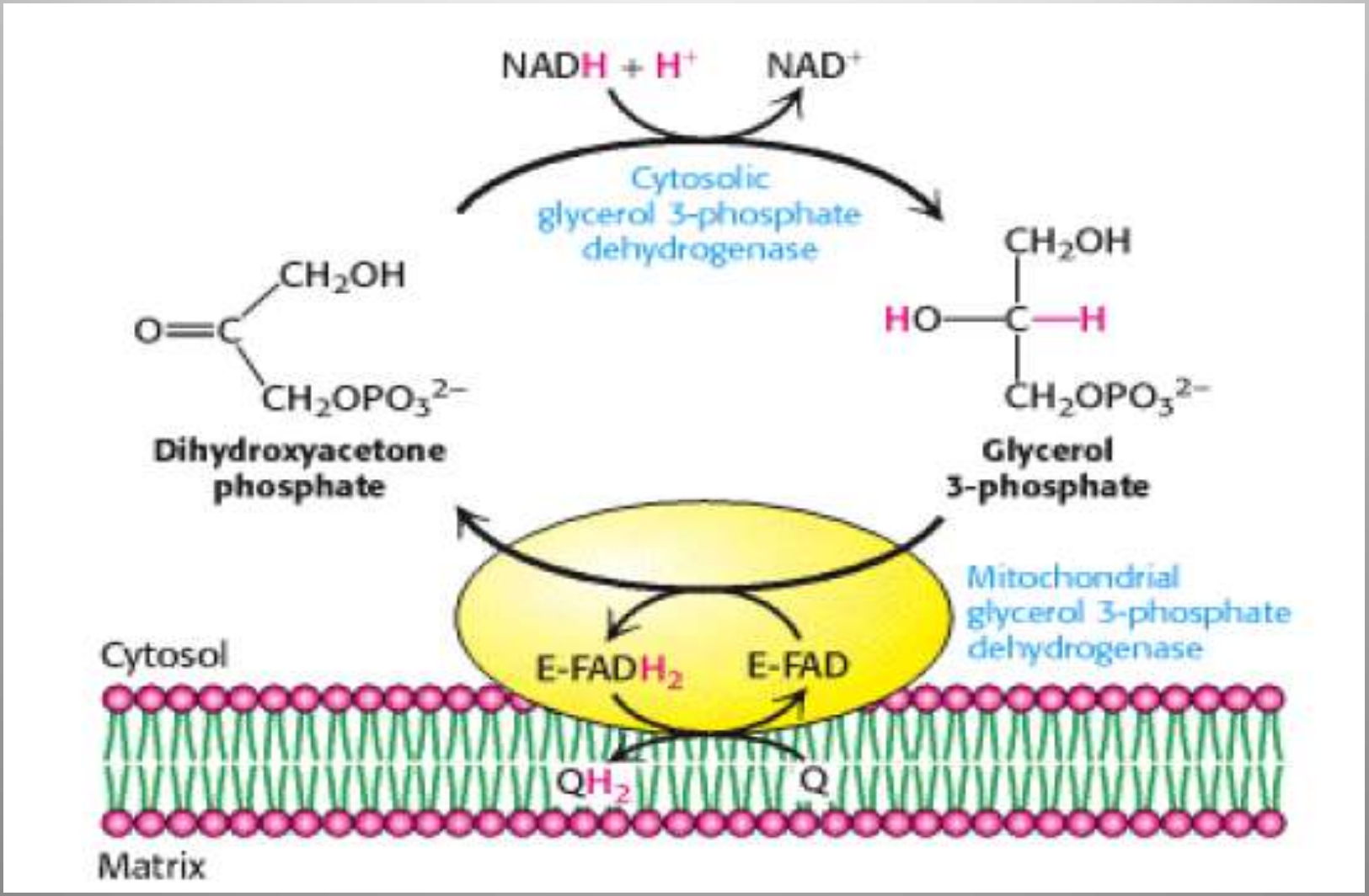
- **Catalases:-** also a haemoprotein containing 4 haem groups and uses 1 molecule of H_2O_2 as electron donor and another molecules of H_2O_2 as electron acceptor.
- Its function is to destroy H_2O_2 formed by the oxidases.

- **Oxygenases:-** oxygenases are a group of enzymes that catalyze the addition of one or both of the atoms of the O_2 molecule into the substrate.
- The former are called monooxygenases or hydroxylases or mixed function oxidases.
- The latter are called dioxygenases.

- **Monooxygenases:-** here one atom is incorporated into the substrate and the other oxygen atom is reduced to water. These enzymes are also called hydroxylases because OH group is incorporated into the substrate.
- The coenzymes may be NADPH, tetrahydrobiopterin, Cytb5, ascorbate or cytP450.
- Ex. Phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase.

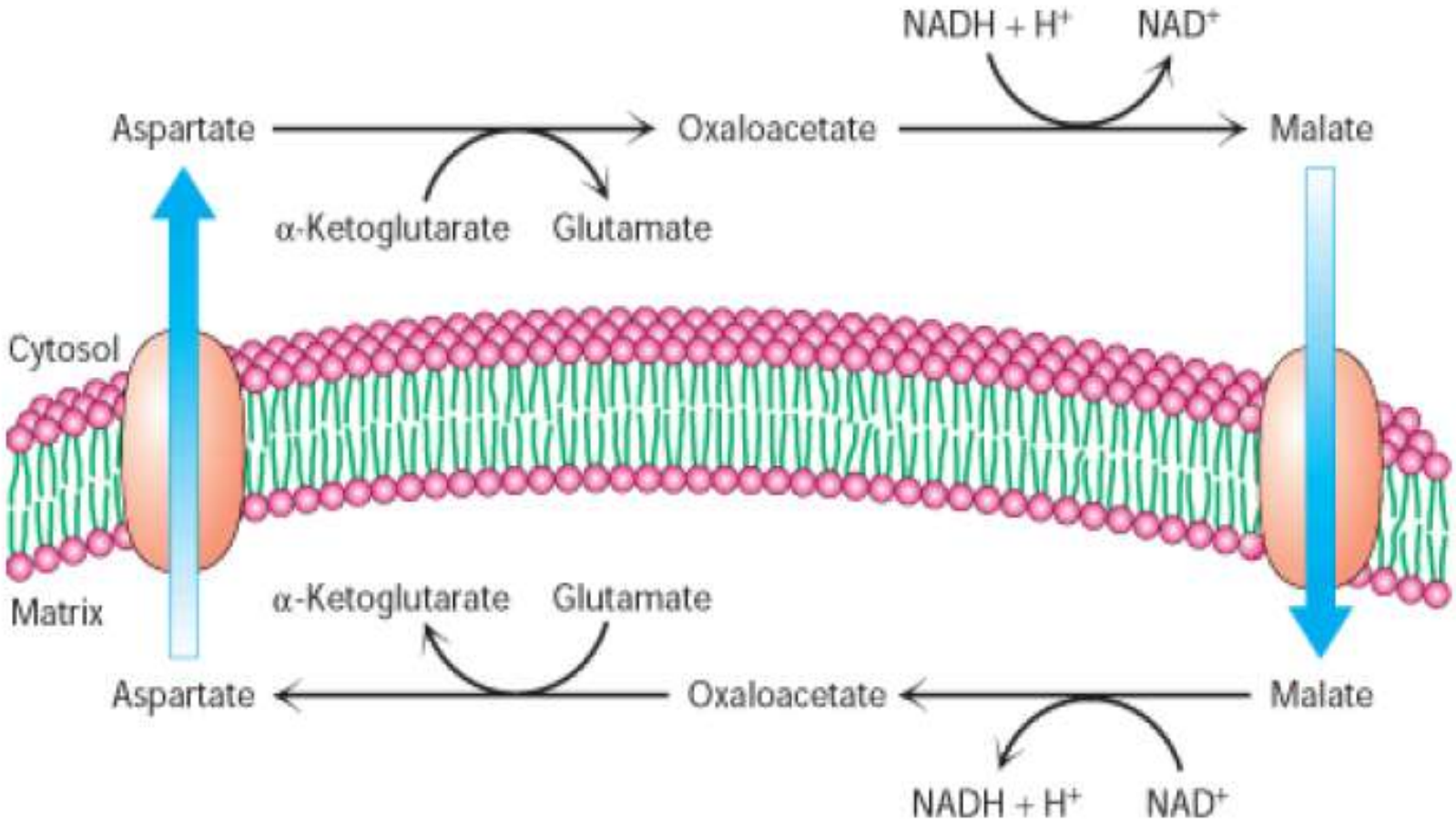
- **Dioxygenases:-** these catalyze the incorporation of both the atoms of O_2 into the substrate.
- Ex. Homogentisic acid oxidase, tryptophan dioxygenase.

Glycerol Phosphate shuttle



- In muscle and brain
- Each NADH converted to $FADH_2$ inside mitochondrion
  - $FADH_2$ enters later in the electron transport chain
  - Produces 1.5 ATP

Malate – Aspartate Shuttle



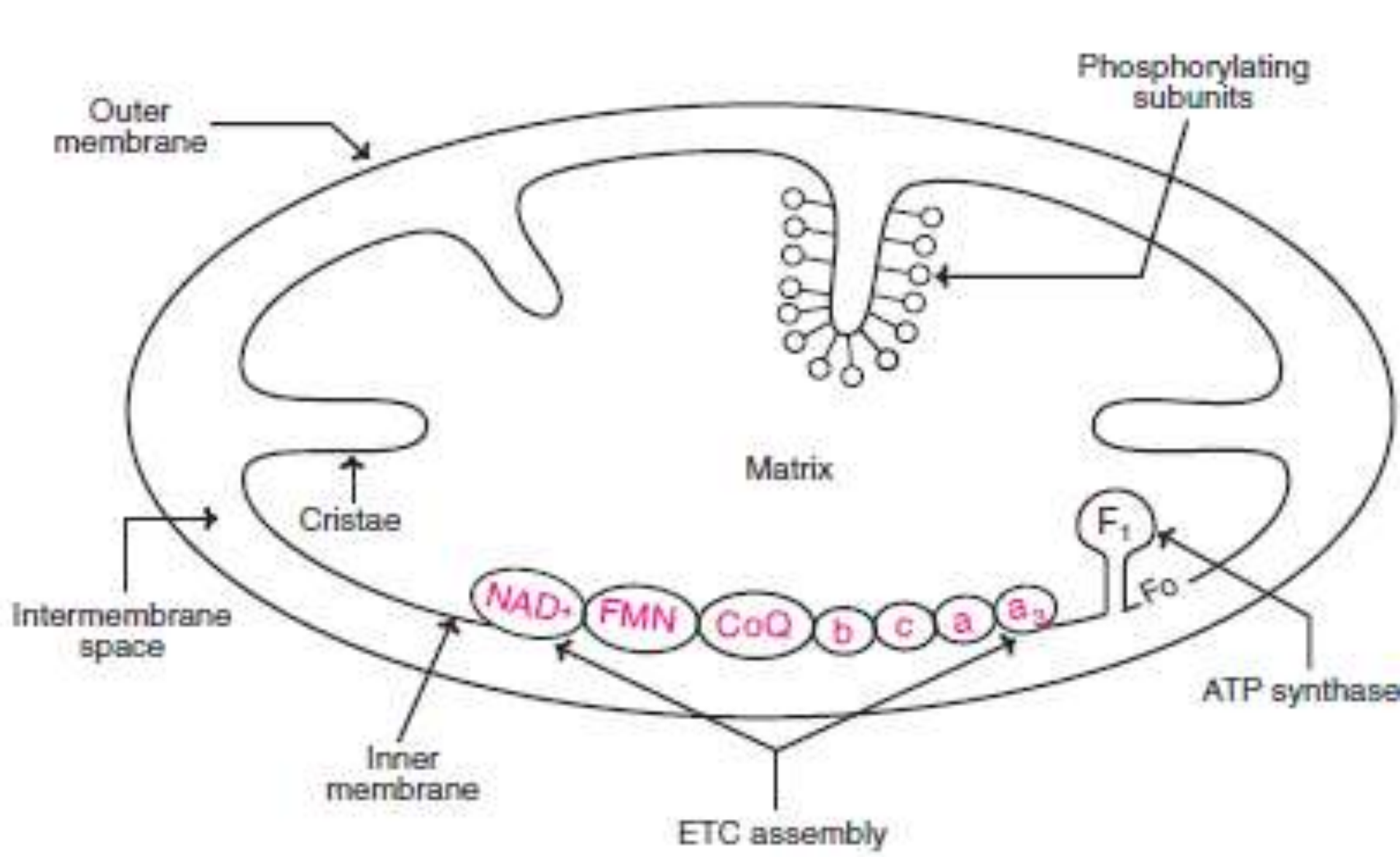
- In liver and heart
- NADH oxidized while reducing oxaloacetate to malate
  - Malate dehydrogenase
- Malate crosses membrane
- Malate reoxidized to oxaloacetate
  - Malate dehydrogenase
  - NAD^+ reduced to NADH
- NADH via electron transport yields 2.5 ATP

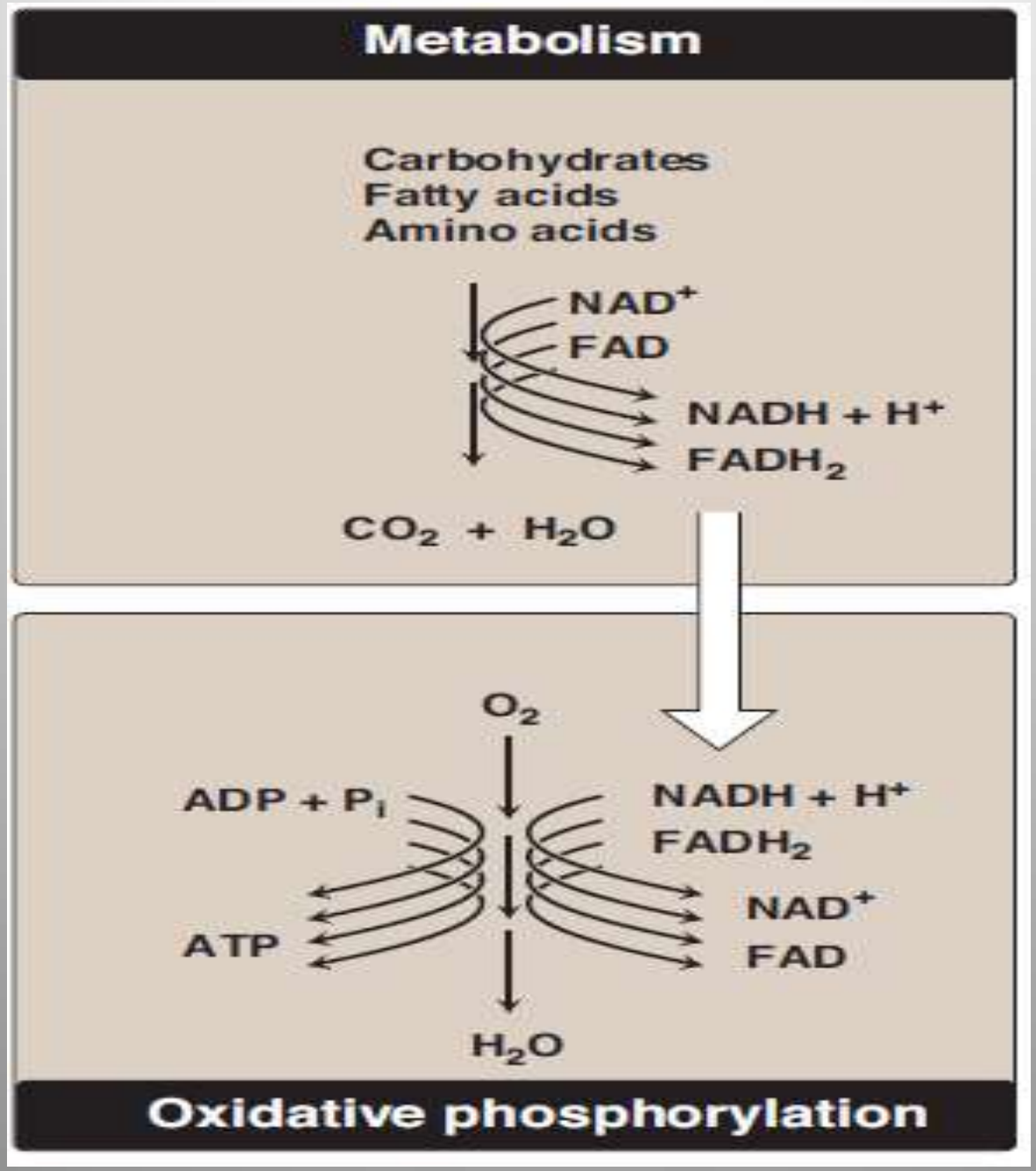
Organisation of ETC

- The electron flow occurs through successive dehydrogenase enzymes, together known as ETC.

Or

- Energy rich molecules, such as glucose, are metabolised by a series of oxidation reactions ultimately yielding CO_2 & water, the metabolic intermediates of these reactions donate electrons to specific coenzymes- NAD^+ or FAD to form the energy rich reduced coenzymes, NADH or $FADH_2$. these reduced co-enzymes can in turn, each donate a pair of electrons to a specialized set of electron carries, collectively called the ETC

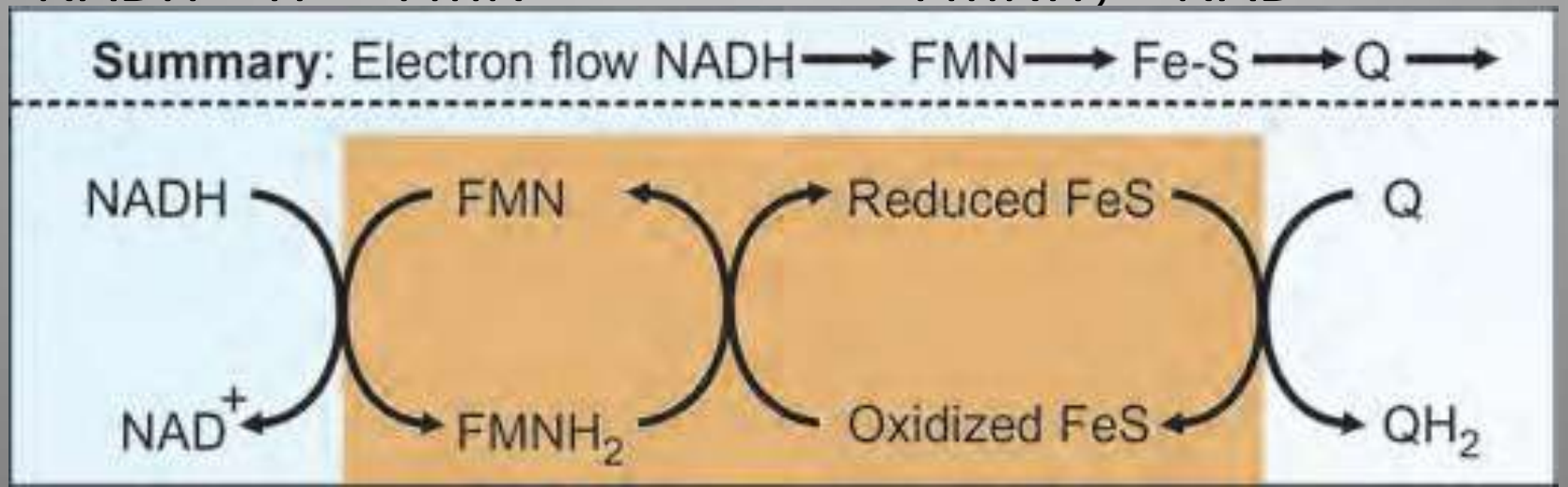

Outer membrane
Phosphorylating subunits
Matrix
Cristae
Intermembrane space
NAD+
FMN
CoQ
b
c
a
a3
F1
Fo
ATP synthase
Inner membrane
ETC assembly


Metabolism
Carbohydrates
Fatty acids
Amino acids
NAD+
FAD
NADH + H+
FADH2
CO2 + H2O
O2
ADP + Pi
ATP
NADH + H+
FADH2
NAD+
FAD
H2O
Oxidative phosphorylation

- In the ETC or respiratory chain, the electrons are transferred from NADH to a chain of electron carriers.
- All the components of ETC are located in the inner membrane of mitochondria.
- These are four distinct multi-protein complexes:
- these are complex I,II,III & IV. These are connected by two mobile carriers – coenzyme Q & cytochrome C

Complex I or NADH-CoQ oxidoreductase or NADH dehydrogenase complex

- It is tightly bound to inner membrane of mitochondria.
- It contains flavoprotein, consisting of FMN as prosthetic group and an iron sulphur protein(Fe-S). NADH donor of electrons, FMN accepts them & get reduced to $FMNH_2$.
- Two electrons & 1 H^+ are transfer from NADH to the flavin prosthetic group of the enzyme.
- $NADH + H^+ + FMN \longrightarrow FMNH_2 + NAD^+$

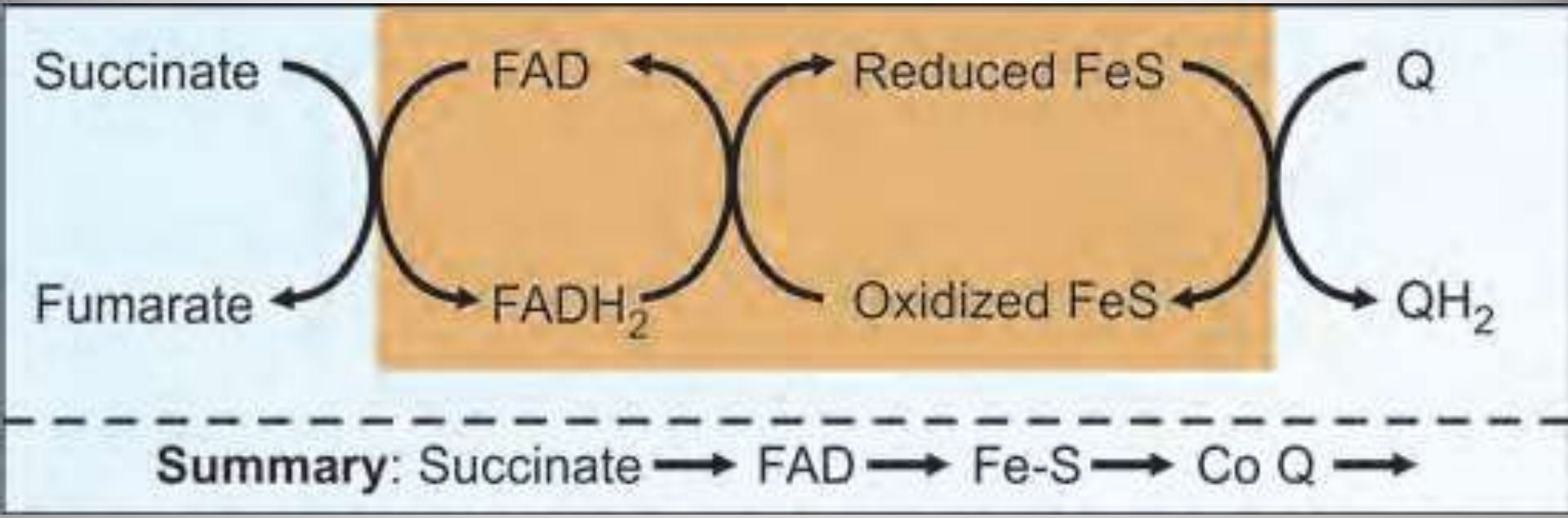


- The electron from $FMNH_2$ are then transferred to Fe-S. The electrons are then transferred to CoQ.

- Overall function of this complex is to collect the pair of electrons from NADH & pass them to CoQ.

Complex II or Succinate Q-reductase

- The electrons from $FADH_2$ enter ETC at level of CoQ.
- this step does not liberate enough energy to produce ATP.
- The 3 major enzyme system that transfer their electrons directly CoQ from the FAD prosthetic group are:-
- Succinate dehydrogenase
- Fatty acyl CoA dehydrogenase
- Mitochondrial glycerol-3- phosphate dehydrogenase



- oxidation of succinate from Krebs cycle to fumarate

  Succinate + FAD → Fumarate + $FADH_2$

  $FADH_2$ then tries to oxidize back into FAD by passing its electrons to $2Fe^{3+}$, which is reduced to $2Fe^{2+}$ (Like in Complex 1)

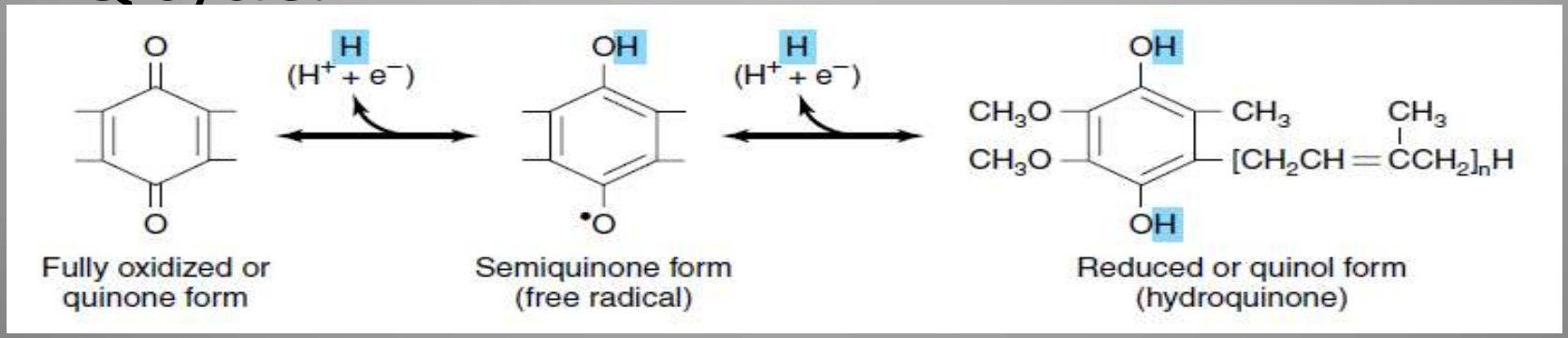
- $2Fe^{3+} + 2e^{-} \rightarrow 2Fe^{2+}$

Co-enzyme Q or Ubiquinone

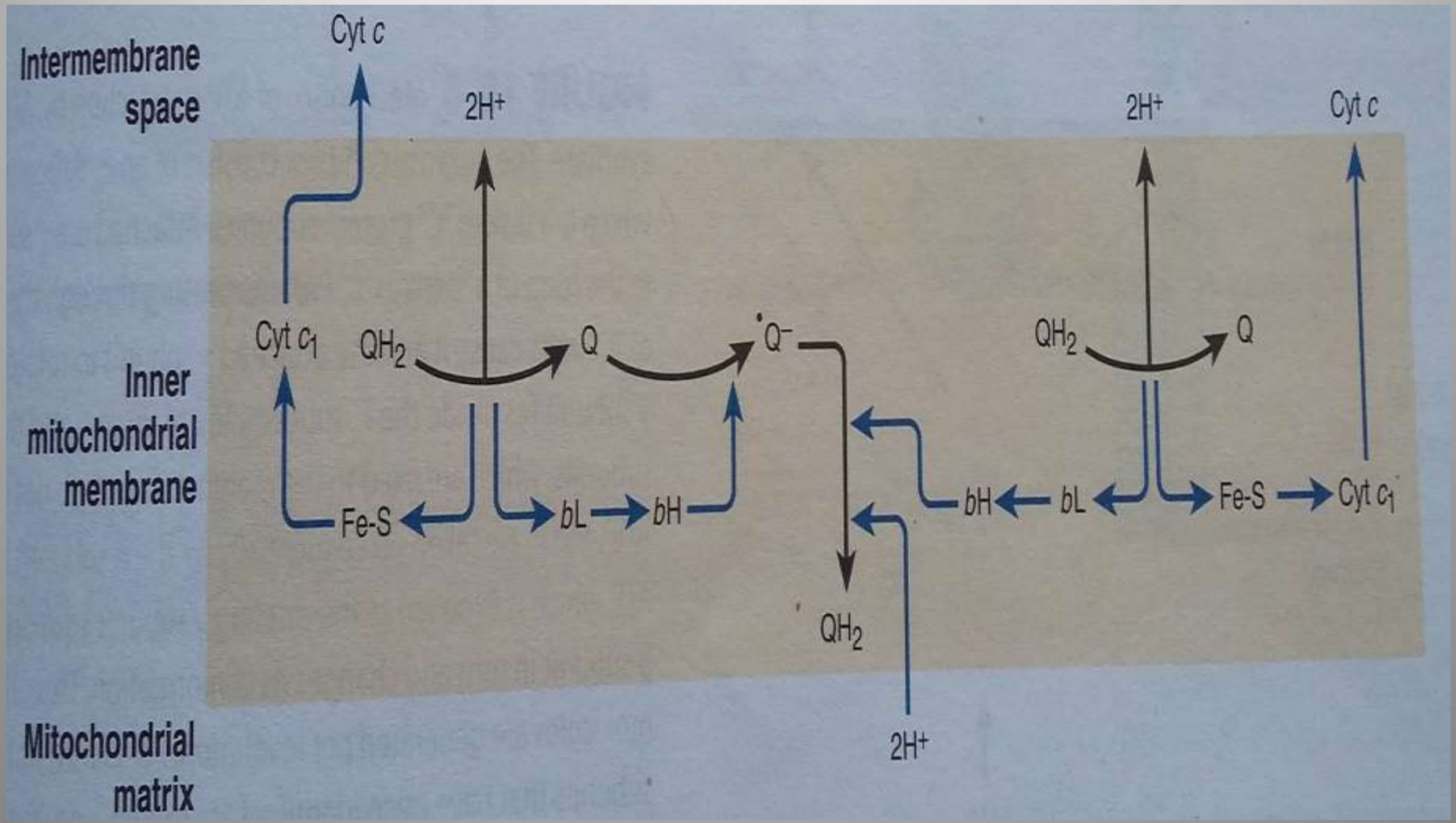
- The ubiquinone is reduced successively to semiquinone(QH) and finally to quinol(QH2).
- It accepts a pair of electrons from NADH or $FADH_2$ through complex I or II respectively.
- Coenzyme Q is a quinone derivative having a long isoprenoid tail.

Q cycle

- The electron are passed from QH2 to cytochrome C vi a complex III.
- The process is believed to involve cytochromes C1, bL & bH & a Rieske Fe-S (an unusual Fe-S in which one of the Fe atom is linked to two histidine –SH groups rather than two cystein –SH groups) and is known as the Q cycle.



Q cycle

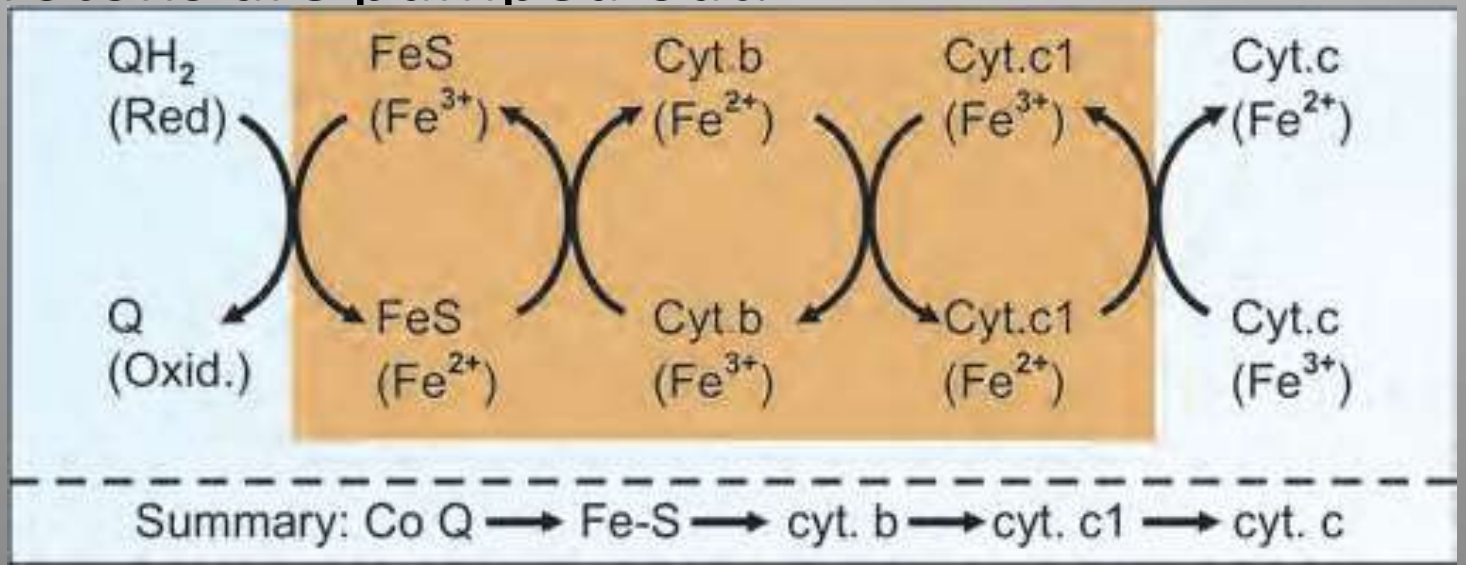

Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Cyt c
2H+
2H+
Cyt c
Cyt c1
QH2
Q
•Q−
QH2
Q
Fe-S
bL
bH
bH
bL
Fe-S
Cyt c1
QH2
2H+

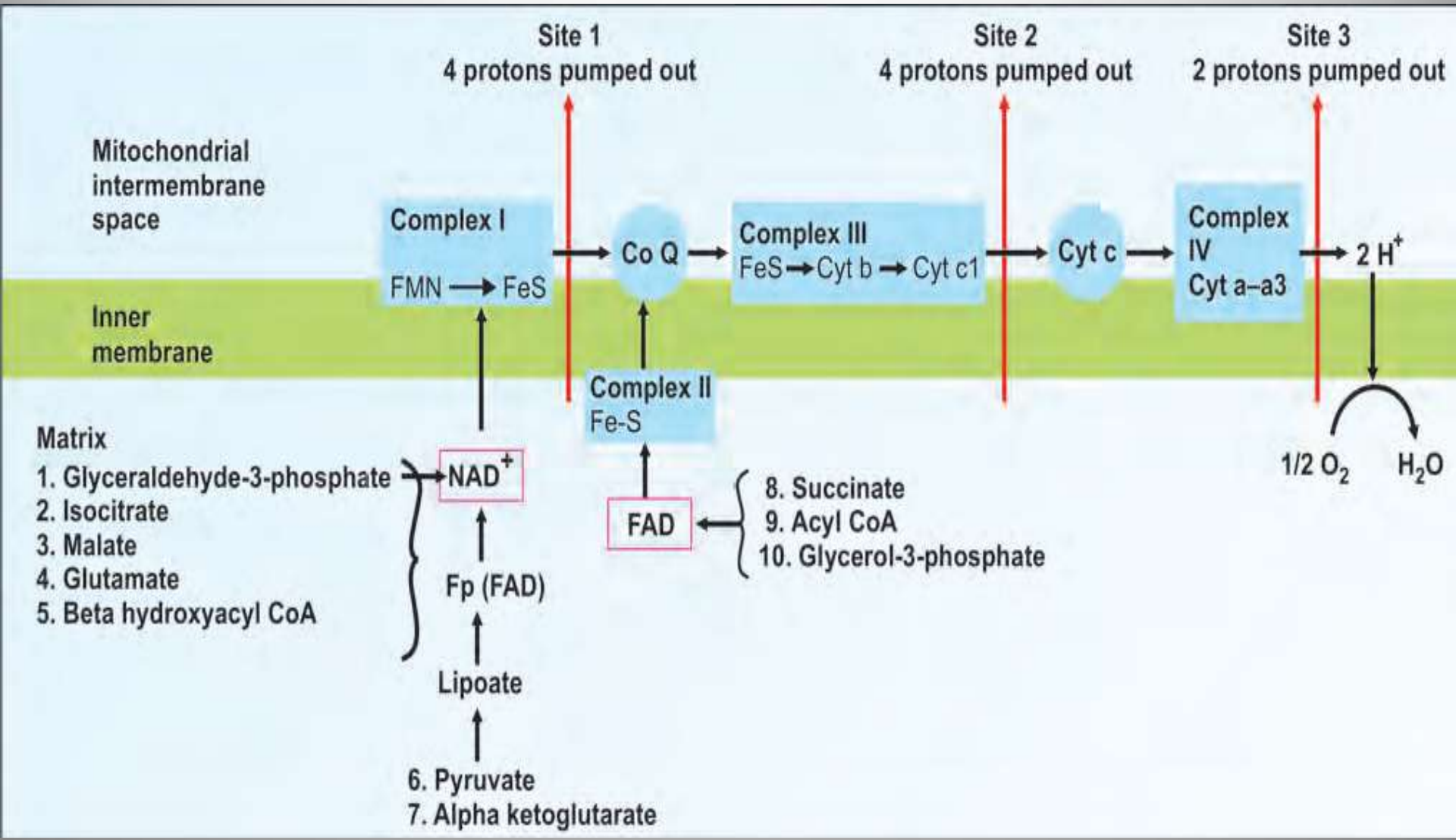
Complex III or Cytochrome Reductase

This is a cluster of iron-sulphur proteins, cytochrome b and cytochrome C1, both contain Heme prosthetic group.

During this process of transfer of electron the iron in heme group shuttles between fe^{3+} and fe^{2+} forms.

4 protons are pumped out.




Site 1
4 protons pumped out
Site 2
4 protons pumped out
Site 3
2 protons pumped out
Mitochondrial intermembrane space
Inner membrane
Complex I
FMN → FeS
Co Q
Complex III
FeS → Cyt b → Cyt c1
Cyt c
Complex IV
Cyt a–a3
2 H+
1/2 O2
H2O
Complex II
Fe-S
Matrix
1. Glyceraldehyde-3-phosphate
2. Isocitrate
3. Malate
4. Glutamate
5. Beta hydroxyacyl CoA
NAD+
Fp (FAD)
Lipoate
6. Pyruvate
7. Alpha ketoglutarate
FAD
8. Succinate
9. Acyl CoA
10. Glycerol-3-phosphate

Cytochrome C

- It contain one heme prosthetic group. The term cytochrome is derived from greek meaning cellular colors.
- It is one of the highly conserved proteins among different species.
- Cytochrome C collects electrons from complex III and delivers them to complex IV
- Cytochrome C is also the mediator of apoptosis.

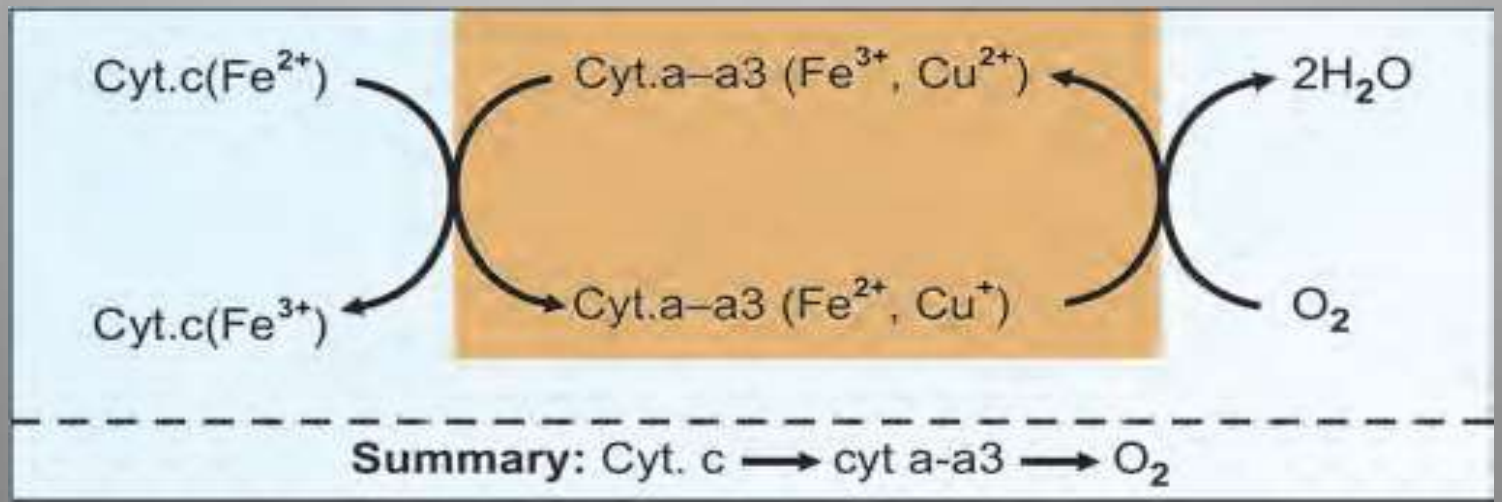
Complex IV or Cytochrome Oxidase

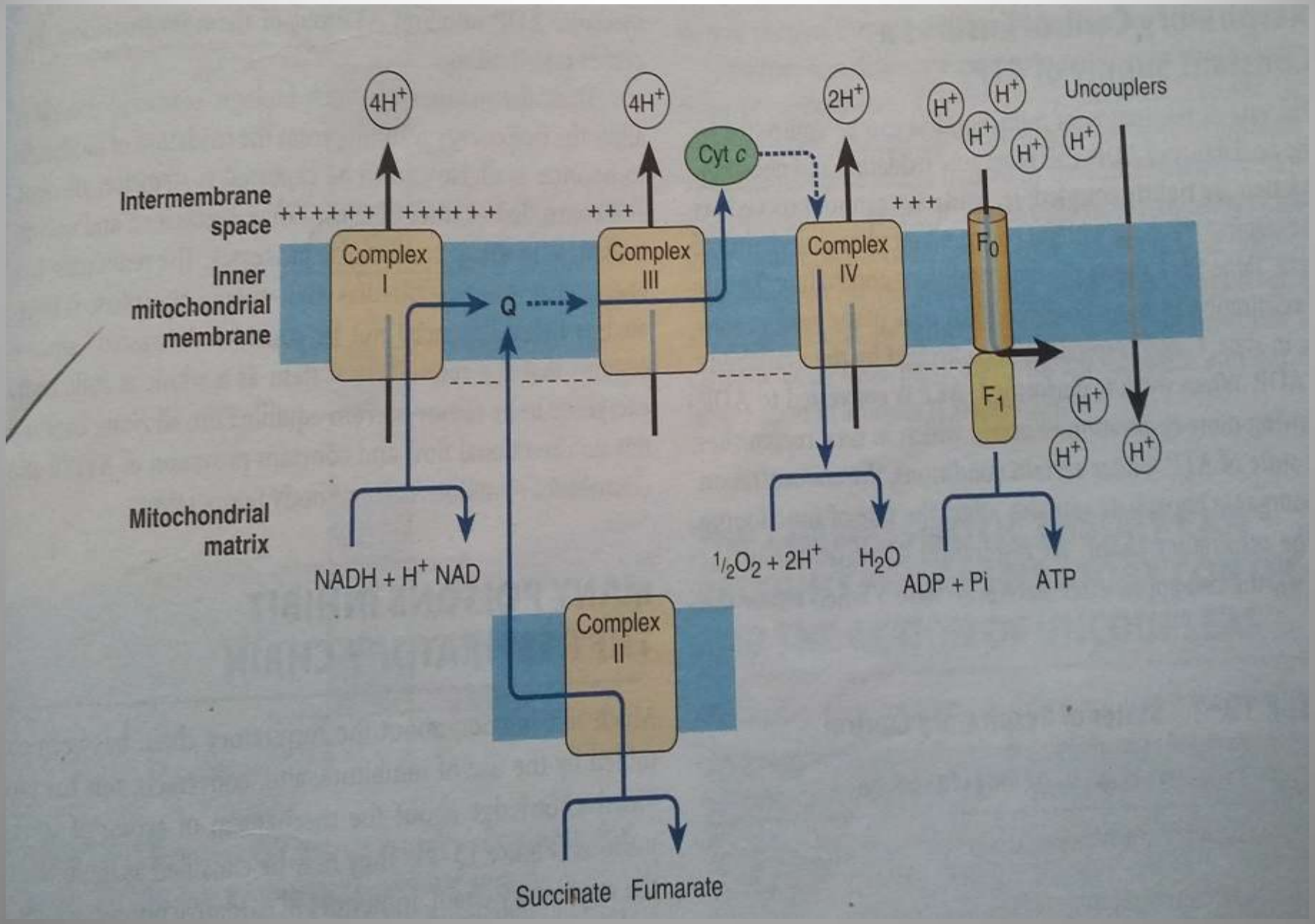
- It contains different proteins, including cytochrome a and cytochrome a3. the complex IV is tightly bound to the mitochondrial membrane.

- 4 electrons are accepted from cytochrome C, and passed on to molecular oxygen.

$$4H^{+} + O_2 + 4\ \text{Cyt C} - Fe^{2+} \longrightarrow 2H_2O + 4\text{CytC} - Fe^{3+}$$

2 protons are pumped out to the intermembrane space. Cytochrome oxidase contains 2 heme groups and 2Cu ions. These two heme groups are denoted as cytochrome-a and cytochrome-a3 the functional unit of the enzyme is a single protein and is referred to as cytochrome




4H+
4H+
2H+
Cyt c
Uncouplers
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Complex I
Complex III
Complex IV
Complex II
Q
F0
F1
NADH + H+ NAD
1/2O2 + 2H+
H2O
ADP + Pi
ATP
Succinate
Fumarate

Hypothesis for ATP Generation

Chemical coupling hypothesis:- this hypothesis, proposed by Edward Stater, postulates that the energy released from ETC causes formation of high energy covalent intermediates. These intermediates are subsequently cleaved to release their energy content, which is used for the synthesis of ATP.

Conformational coupling hypothesis:- the conformational coupling hypothesis put forth by Paulboyer proposes that the energy of electron transport is used for altering conformation of certain proteins that are located in innermitochondrial membrane the proteins with altered conformation have high energy content, which is subsequently used for ATP generation.

Chemi-osmotic hypothesis or theory:- proposed by the British biochemist Peter Mitchell. This is widely accepted. The coupling of oxidation with phosphorylation is termed oxidative phosphorylation explain by chemi-osmotic theory.

It postulates that the two processes are coupled by a proton gradient across the innermitochondrial membrane so the proton motive force caused by the electrochemical potential difference (-ve on the matrix side) drives the mechanism of ATP synthesis.

Proton pump & ATP Synthesis:- the proton pumps (complexes I,III & IV) Expel H^+ from inside to outside of the inner membrane, so there is high H^+ concentration. Outside the inner membrane. This causes H^+ to enter into mitochondria through the channels(fo), this proton influx causes ATP synthesis by ATP synthase.

ATP Synthase:- it is a protein assembly in the inner mitochondrial membrane. It is also referred to as the 5^{th} complex. It has two units.

1. **fo unit:-** the o stands for oligomycin, as fo is inhibited by oligomycin.

   It serves as a proton channel, through which proton enter into mitochondria.

   fo unit has 4 polypeptide chains and is connected to f1.

   the flow of protons through fo, causes it to rotate, driving the production of ATP in the f1 complex.

2. **f1 unit:-** it projects into matrix.

   it catalyses the ATP synthesis.

   f1 unit has 9 polypeptide chains (3α, 3β, 1γ, 1 delta, 1 epsilon).

   the α chains have binding sites for ATP and ADP and β chains have catalytic sites.

   ATP synthesis requires mg^{2+} ions.

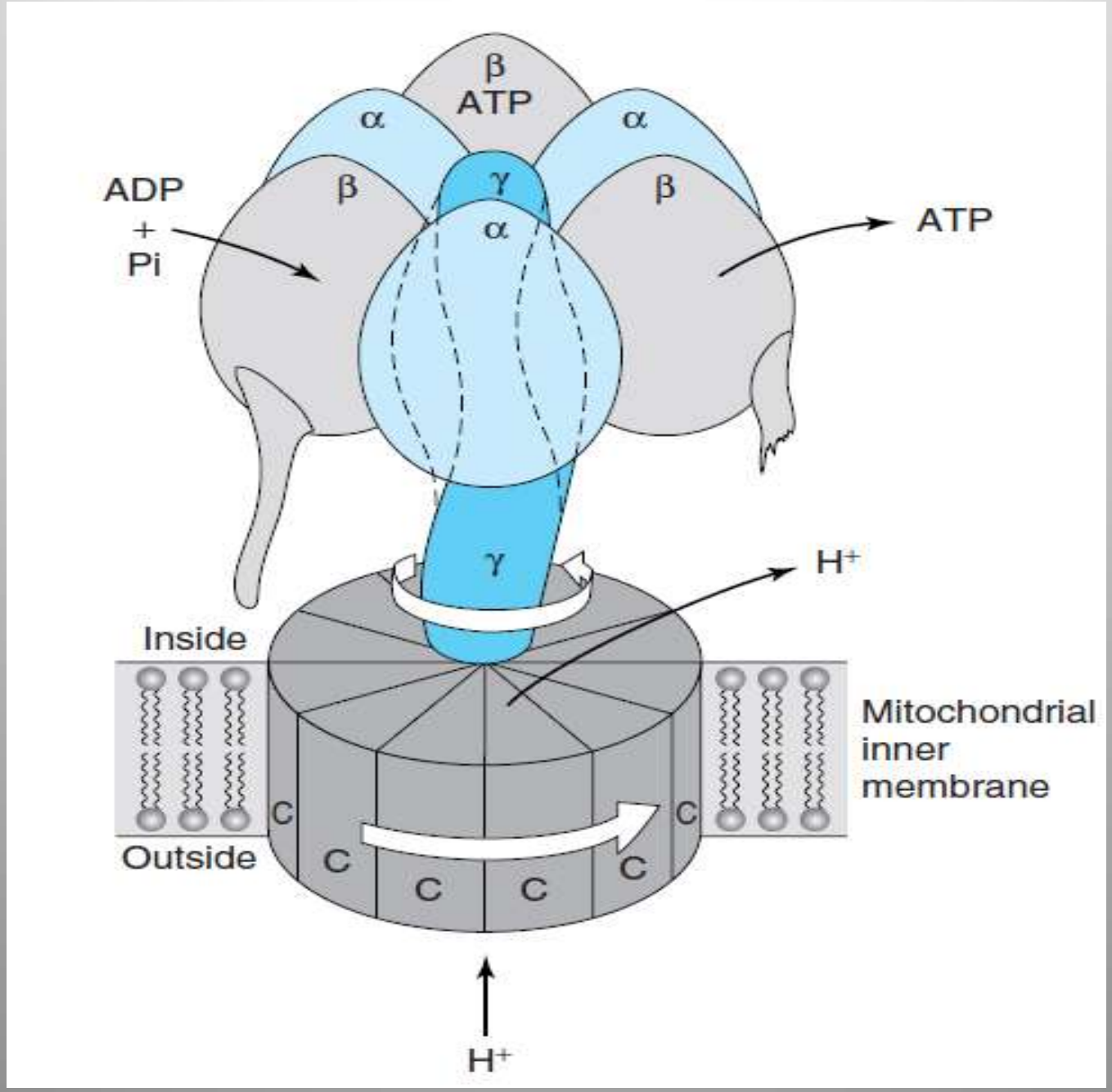
Binding change mechanism:- the ATP synthase is a molecular machine.

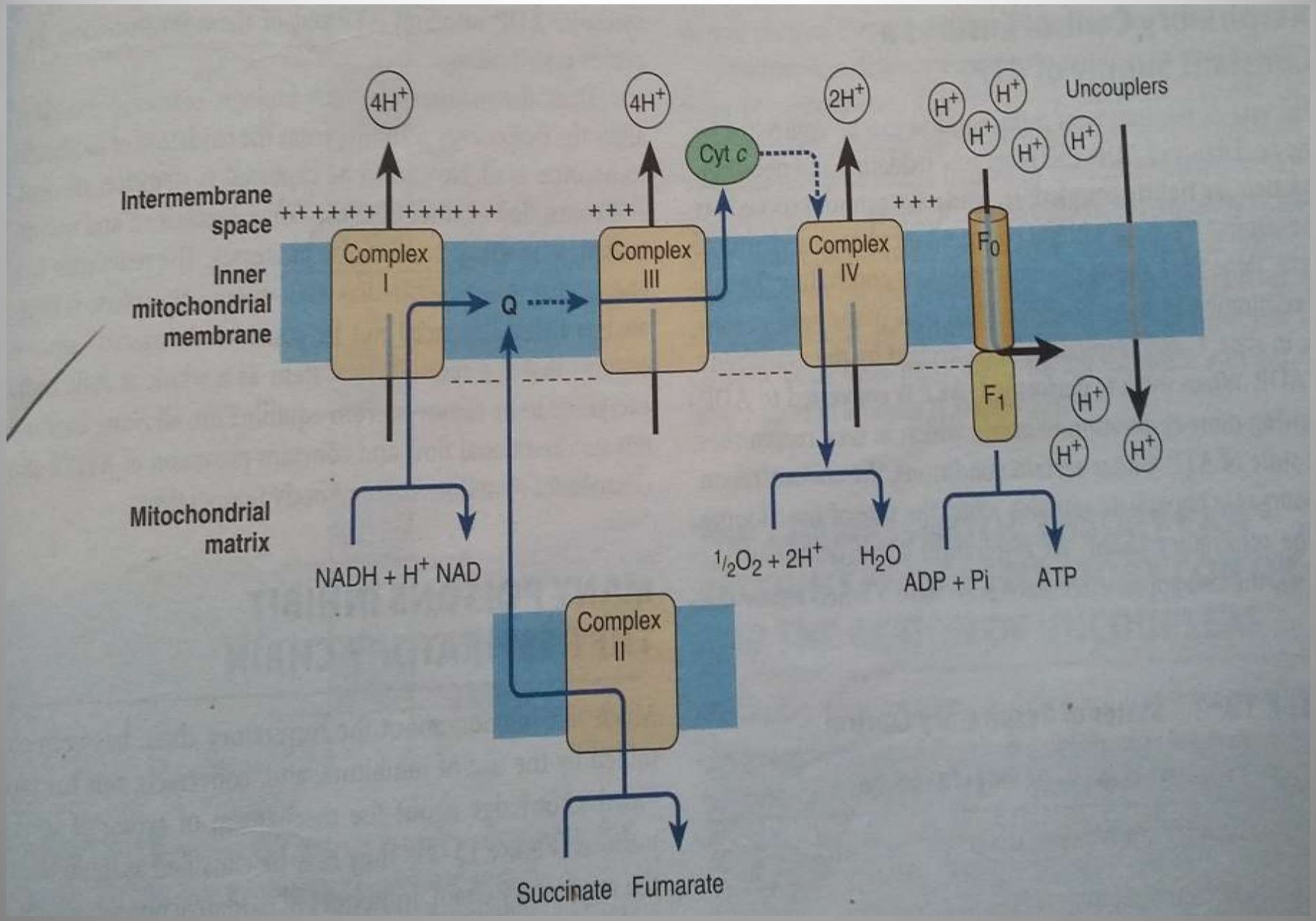
According to this theory, the 3β subunits catalytic sites, are in 3 funtional states, o form is open and has no affinity for substrates. L form binds substrate with sluggish affinity. T form binds substrate tightly and catalyses the ATP synthesis.

Protons entering the system, cause conformational changes in f1 particle. Initially the ADP and Pi are loosely bound to the catalytic site on f1.

As the f1 accepts proton the affinity for ADP is increased. Further conformational change induces catalytic activity and ATP is synthesised. This moves the proton to the matrix site as the ATPs are released. The original conformation of the enzyme is assumed.

Then ADP is again bound and the cycle repeats.


β
ATP
α
α
ADP
+
Pi
β
γ
α
β
ATP
γ
H+
Inside
Mitochondrial
inner
membrane
C
C
C
C
C
C
Outside
H+


4H+
4H+
2H+
Cyt c
Uncouplers
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Complex I
Complex III
Complex IV
Complex II
Q
F0
F1
NADH + H+ NAD
1/2O2 + 2H+
H2O
ADP + Pi
ATP
Succinate
Fumarate

Regulation of ATP synthesis or Respiratory control:- the rate of respiration of mitochondria can be controlled by the availability of ADP. This is because oxidation and phosphorylation are tightly coupled i.e. Oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP.

when ATP level is low and ADP level is high, oxidative phosphorylation proceeds at a rapid rate. This is called respiratory control or acceptor control.

the major source of NADH and $FADH_2$ is the TCA, the rate of which is regulated by the energy charge of the cell.

P : O ratio

Phosphate : oxygen ratio is defined as the number of inorganic phosphate molecules incorporated into ATP for every atom of oxygen consumed.

Inhibitors of ETC or Inhibitors of ATP Synthesis

Site specific inhibitors

Site 1 (complex I to CoQ) specific inhibitors:- Alkylguanides(guanethide) hypotensive drug, Rotenone- Insecticide and fish poison, barbiturates (amobarbital) sedative.

Site 2 (complex III to cyt C) inhibitors:- BAL(British anti-levisite), antidote of war gas, Napthoquinone.

Site 3 (complex IV) inhibitors:- carbon monooxide inhibits cellular respiration, Cyanide, Azide

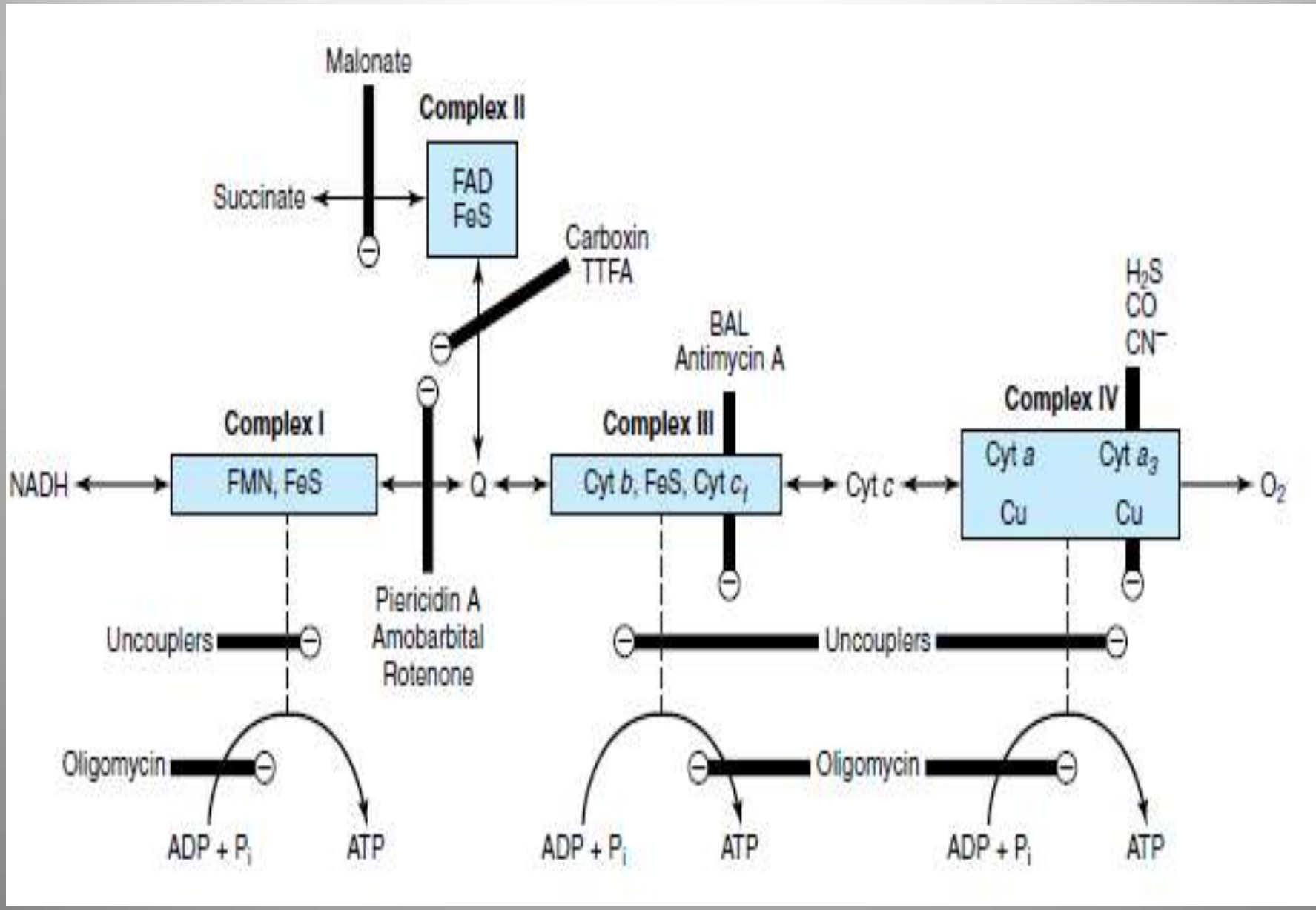
Inhibitors of oxidative phosphorylation:- Atractyloside inhibits the translocase, Oligomycin inhibits the fo subunit.

Ionophores are lipid soluble compounds that increase the permeability of lipid bilayers to certain ions. There are two types of ionophores. Mobile ion carries(eg. Valinomycin) and channel formers(eg. Gramicidin) valinomycin allows K to permeate mitochondria and dissipate the proton gradient.

Site between succinate dehydrogenase and Co-Q:-

Carboxin, inhibits transfer of ions from $FADH_2$

Malonate, competitive inhibitor of succinate dehydrogenase.


Malonate
Complex II
Succinate
FAD
FeS
Carboxin
TTFA
BAL
Antimycin A
H_2S
CO
CN^-
Complex IV
Complex I
Complex III
NADH
FMN, FeS
Q
Cyt *b*, FeS, Cyt c_1
Cyt *c*
Cyt *a*
Cyt a_3
Cu
Cu
O_2
Piericidin A
Amobarbital
Rotenone
Uncouplers
Uncouplers
Oligomycin
Oligomycin
ADP + P_i
ATP
ADP + P_i
ATP
ADP + P_i
ATP

Uncouplers of oxidative phosphorylation:- being linked through a proton gradient, the oxidation and the phosphorylation are said to be coupled processes. They can be uncoupled from each other by certain compounds called uncouplers.

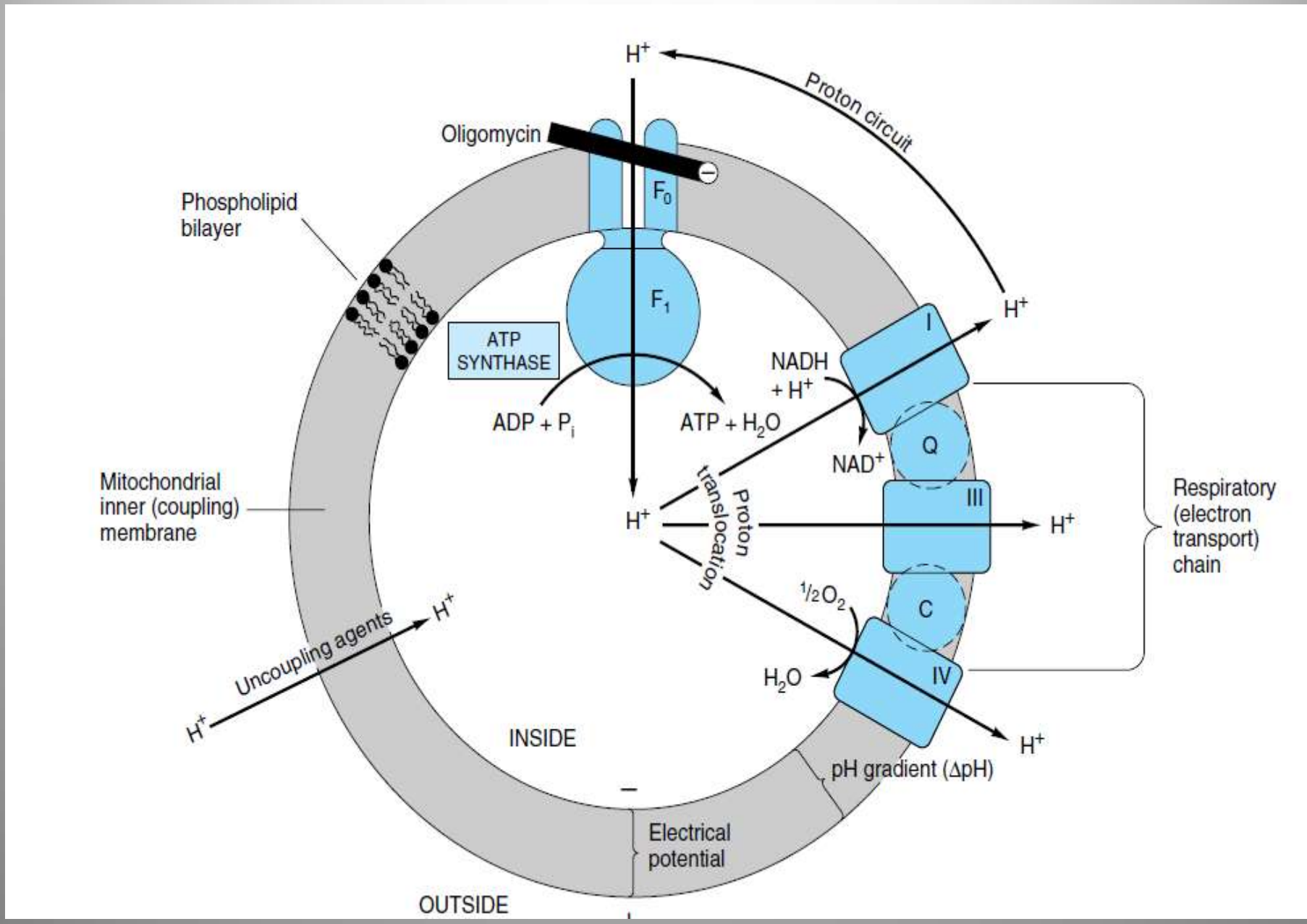
primary action of these compounds is to increase permeability of inner mitochondrial membrane to proton. As a result relatively free movement of proton across the IMM occurs, which prevents building of the electrochemical gradient. Since this gradient is essential for ATP generation, failure to build it stops ATP genretion. Ex. 2,4 dinitrophenol

other uncouplers are, pentachlorophenol, dinitrocresol and trifluorocarbonylcyanide.

Significance of uncoupling:- some time, the uncoupling of oxidative phosphorylation is useful biologically. In hibernating animals and in newborn human infants, the liberation of heat energy is required to maintain body temp. In brown adipose tissue, thermogenesis is achieved by this process.

Thermogenin, a protein present in the inner mitochondrial membrane of adipocytes provides an alternate pathway for protons. It is one of the uncoupling proteins.

Physiological uncouplers:- thyroxine , free fatty acids and bilirubin


H+
Proton circuit
Oligomycin
Phospholipid bilayer
F0
F1
ATP SYNTHASE
ADP + Pi
ATP + H2O
NADH + H+
NAD+
I
Q
III
C
IV
H+
Proton translocation
1/2 O2
H2O
Respiratory (electron transport) chain
Mitochondrial inner (coupling) membrane
Uncoupling agents
INSIDE
OUTSIDE
pH gradient (ΔpH)
Electrical potential
−

THANK YOU