Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Department

Biochemistry Lectures for 2nd Year Students

(2 Credit Hrs. Theory + 2 Credit Hrs. Practice / Week = 3 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

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Second Semester

Lecture No. 8

Date: April, 8th, 2025

Electron Transport Chain and Biological Oxidation

Objectives:

The reader will be able to answer questions on the following topics:

1. Redox potentials
2. Biological oxidation and its importance
3. Enzymes and co-enzymes
4. Organization of electron transport chain
5. Chemiosmotic theory
6. Proton pump
7. ATP synthase
8. Inhibitors of ATP synthesis

Stages of Oxidation of Foodstuffs

First Stage

Digestion in the gastrointestinal tract converts the macromolecules into small units. For example, proteins are digested to amino acids. This is called **primary metabolism** **(Fig. 1).**

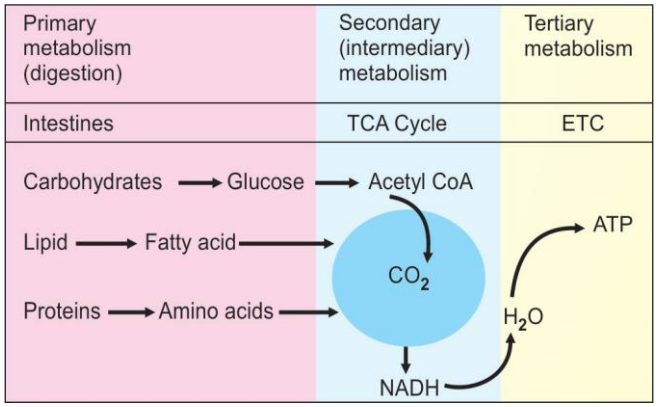

Fig. 1: Oxidation of foodstuffs in three stages.

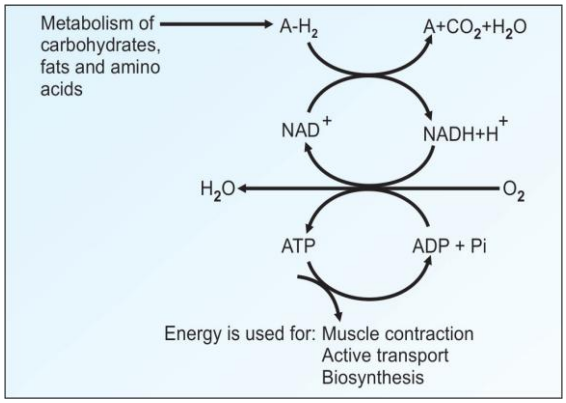

Fig. 2: ATP generation. Food is catabolized; energy from food is trapped as ATP; it is then used for anabolic reactions

Second Stage

The products of digestion are absorbed, catabolized to smaller components, and ultimately oxidized to CO_2. The reducing equivalents are mainly generated in the mitochondria by the final common oxidative pathway, citric acid cycle. In this process, NADH and FADH2 are generated. This is called **secondary or intermediary metabolism**.

Third Stage

These reduced equivalents (NADH and FADH2) enter into the **Electron transport** chain (ETC), or **Cytochrome** chain, or **Respiratory** chain, or **biological** oxidation chain where energy is released. This is the **tertiary metabolism** or **Internal respiration** or cellular respiration **(Fig. 1).**

The energy production by complete oxidation of one molecule of glucose is 2850 kJ/mol and that of palmitate is 9781 kJ/mol. This energy is then used for synthetic and metabolic purposes in the body **(Fig. 2).**

This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. **Note that many biologic oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations.**

Table-1. Redox potentials

Oxidant	*Reductant*	*Eo' (in V)*
NAD^+	$NADH + H^+$	–0.32
Cytochrome b^{+++}	Cytochrome b^{++}	+0.07
Co-enzyme Q	Coenzyme QH_2	+0.10
Cytochrome c^{+++}	Cytochrome c^{++}	+0.22
Cytochrome a^{+++}	Cytochrome a^{++}	+0.29
$O_2 + 2H$	H_2O	+0.82

Box 1. Summary of bioenergetics

1. **Free energy** is a measure of the energy available to perform useful work
2. ΔG can predict the direction of a chemical reaction
3. Chemical reactions can be coupled, which allows an energetically unfavorable reaction to conclusion
4. ΔG measured under physiological conditions may be different from that at a standard state.

Biological Importance

1. Biological oxidation deals with the uses of respiratory O_2 in the body.
2. Several important biological oxidation reactions are directly associated with respiratory O_2.
3. Biological oxidation provides means for the regeneration of coenzymes, which are used in metabolism.
4. It is the final aspect of all energy-producing compounds.
5. Transfer of electrons is impaired in certain disease like encephalopathy, lactic acidosis and mitochondrial myopathy.
6. In myocardial infarction O_2 supply to cardiac muscle is impaired. As a result, energy production in cardiac cells is blocked, which lead to necrosis.
7. In some instances, like high altitudes, surgeries to maintain normal functioning of body or cells O_2 supply are essential.
8. Though O_2 is essential for survival of cells at high concentration it is toxic to cells. Hence, it is used to treat tumors along with radiation.

The life of higher animals is absolutely dependent upon a supply of oxygen for **respiration,** the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as **oxygenases;** many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the **cytochrome P_{450} system.**

Energy is essential for living cells to perform vital cellular functions. Living cells obtain energy by burning foodstuffs. The foodstuffs are made up of carbohydrates, fats and proteins.

The degradation of foodstuffs is accompanied by production of reduced coenzymes like $FADH_2$, $FMNH_2$, NADH + H^+ and NADPH + H^+. Since the continuation of metabolic pathways depends on availability of FMN, FAD and NAD^+, the reduced coenzymes must be re-oxidized. The oxidation of $FMNH_2$, $FADH_2$ and NADH + H^+ by respiratory O_2 with simultaneous production of H_2O is the final stage of biological oxidation reactions. The oxidation of $FADH_2$ and NADH + H^+ by O_2 accompanies release of energy, which is used for the formation of ATP and a small amount of energy is released as heat **(Fig. -3-).**

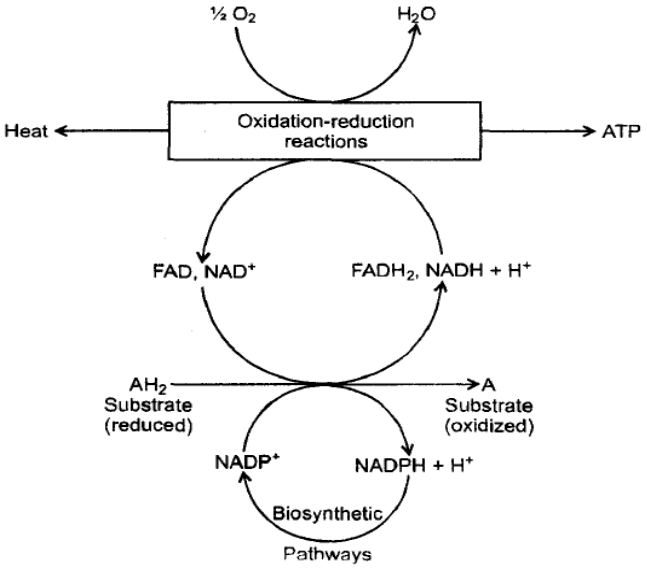

Fig. 3- Schematic diagram showing production of reduced coenzymes on oxidation of substrate with subsequent oxidation of them by O_2 and formation of ATP

The NADPH + H^+ is re-oxidized back by biosynthetic pathways that require reduced $NADP^+$. The transfer of hydrogen atoms or electrons or reducing equivalents of $FADH_2$ and NADH + H^+ to respiratory O_2 is a stepwise process. Specific carrier molecules are arranged in a sequence to carry hydrogen (electrons) atoms from $FADH_2$ and NADH + H^+ to O_2. During the transfer of electrons from reduced coenzymes to O_2, the carrier molecules undergo coupled oxidation-reduction reactions because whenever one carrier is oxidized the other carrier is simultaneously reduced. Therefore, the electron transfer in biological systems involves coupled oxidation-reduction reactions. The coupled oxidation and reduction reaction during transfer of electrons is shown briefly **(Fig. -4-).**

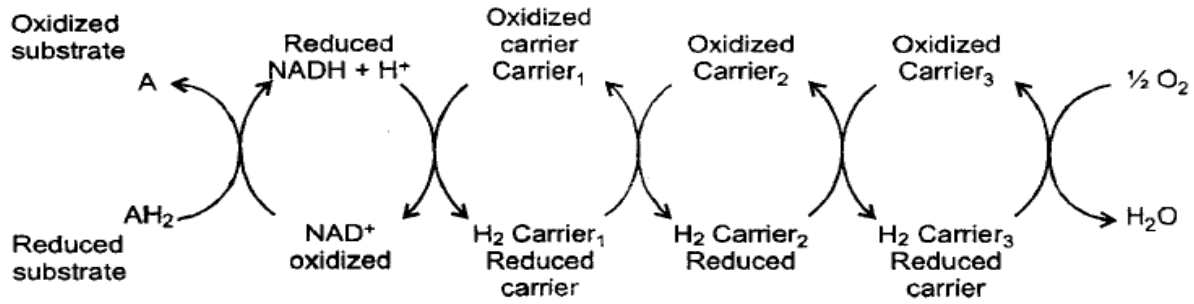

Fig. 4- Transfer of electrons from substrate to O_2 in coupled oxidation-reduction reactions

A. In the respiratory chain, O_2 is used as final electron acceptor and reduced to water.

B. Parts from respiratory chain, several enzymes use O_2 as final electron acceptor and produce H_2O_2.

C. Several new compounds are synthesized by directly incorporating O_2 into certain substances.

D. Respiratory O_2 is also required for the removal of toxins and drugs from the body.

E. Superoxide ion derived from 0_2 functions as microbicide.

In Electron Transport Chain (ETC) the oxygen-dependent oxidation-reduction reactions involve intermediate electron carriers intervening in the flow of electrons between reduced metabolite and the electron acceptor, the oxygen. Each intermediate electron carrier would first participate in its oxidized state as an acceptor of electrons and then be converted to its reduced state. In the reduced state the carrier, as a donor, would then transfer electrons to the next carrier, as a donor, would then transfer electrons to the next carrier in its oxidized state and in doing so, it would be reconverted back to original oxidized state. The final carrier would transfer electrons to molecular oxygen, the terminal electron acceptor in respiration which would be reduced to water.

In eukaryotes:

1. Oxidative phosphorylation occurs in mitochondria, photo-phosphorylation in chloroplasts.
2. Oxidative phosphorylation involves the *reduction* of O_2 to H_2O with electrons donated by NADH and $FADH_2$; it occurs equally well in light or darkness. Photo-phosphorylation involves the *oxidation* of H_2O to O_2, with $NADP^+$ as ultimate electron acceptor; it is absolutely dependent on the energy of light. Despite their differences, these two highly efficient energy-converting processes have fundamentally similar mechanisms.

All oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP as shown in (Fig. 5-).

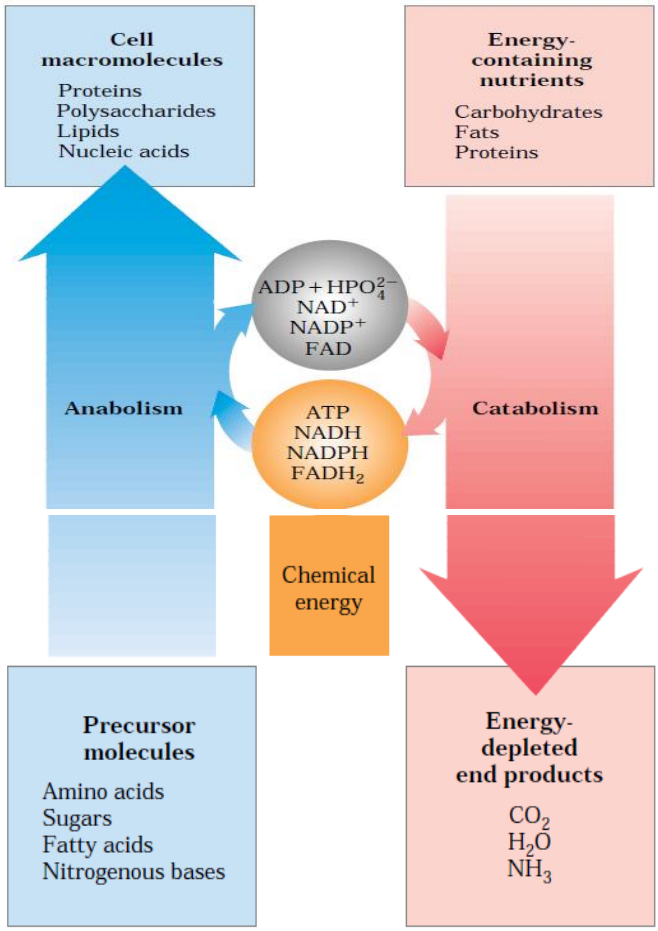

Fig. 5- Role of ATP in anabolic and catabolic pathways

Oxidative phosphorylation and photo-phosphorylation are mechanistically similar in three respects.

1. Both processes involve the flow of electrons through a chain of membrane-bound carriers.
2. The free energy made available by this “downhill” (exergonic) electron flow is coupled to the “uphill” transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential.
3. The transmembrane flow of protons down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.

While photophosphorylation is the means by which photosynthetic organisms capture the energy of sunlight—the ultimate source of energy in the biosphere— and harness it to make ATP. Together, oxidative phosphorylation and photo-phosphorylation account for most of the ATP synthesized by most organisms most of the time.

Enzyme and Carrier Molecules Involved in Electron Transport:

Many enzymes, coenzymes and several carrier molecules are involved in oxidation-reduction (electron transfer) reactions of biological system; they can be classified into the following 5 headings such as:

Oxidases:

Aerobic Dehydrogenases:

Anaerobic Dehydrogenases

These enzymes catalyze the removal of hydrogen from a substrate but **oxygen cannot** act as the hydrogen acceptor such as:

Examples of NAD^+ linked dehydrogenases are:

i. Glyceraldehyde-3-phosphate dehydrogenase
ii. Isocitrate dehydrogenase
iii. Malate dehydrogenase
iv. Glutamate dehydrogenase
v. Beta hydroxyacyl CoA dehydrogenase
vi. Pyruvate dehydrogenase
vii. Alpha ketoglutarate dehydrogenase.

Examples of FADH2 linked enzymes:

i. Succinate dehydrogenase.
ii. Fatty acyl CoA dehydrogenase.
iii. Glycerolphosphate dehydrogenase.

They catalyze the removal of hydrogen from substrates. They use FMN and FAD as hydrogen carriers. FMN and FAD are tightly bound (prosthetic group) to apo-enzymes.

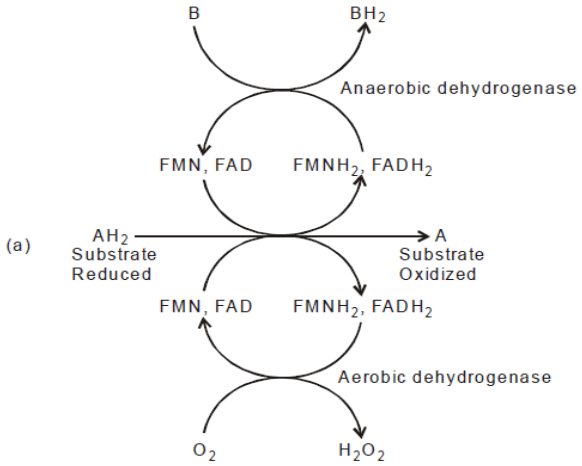

Fig. 6. (a) Hydrogen transfer from AH_2 to B and O_2 using FMN, FAD as carriers by anaerobic and aerobic dehydrogenases

They are of two types.

1. Some of them transfer hydrogen to another substrate in a coupled oxidation and reduction reaction. Since oxygen is not (electron) hydrogen acceptor these are referred as riboflavin dependent anaerobic dehydrogenases.

Few of the riboflavin-dependent dehydrogenase use oxygen as hydrogen acceptor and produce H_2O_2. Hence, these can be referred as riboflavin dependent aerobic dehydrogenases **(Fig. -6-).**

a. **Cytochromes:** All the cytochromes, except cytochrome oxidase, are anaerobic dehydrogenases. All cytochromes are hemoproteins having iron atom. Cytochrome b, cytochrome c1, and cytochrome c are in mitochondria while cytochrome P_{450} and cytochrome b5 are in endoplasmic reticulum.

The cytochromes are b, c_1 and c. They are components of electron transport chain present in mitochondria. They are heme proteins with characteristic strong absorption of visible light; due to their iron containing heme prosthetic groups **(Fig.7-).** Cytochrome c is a peripheral protein. Cytochrome b and c_1 are integral membrane proteins and they are constituents of cytochrome reductase complex.

They are involved in transfer of electrons from ubiquinone to cytochrome oxidase. The iron of the cytochromes participates in oxidation-reduction reactions. The iron oscillates between Fe^{2+} and Fe^{3+} states. There are three types of cytochrome b. They are cytochrome b_{560}, cytochrome b_{562} and cytochrome b_{566}.

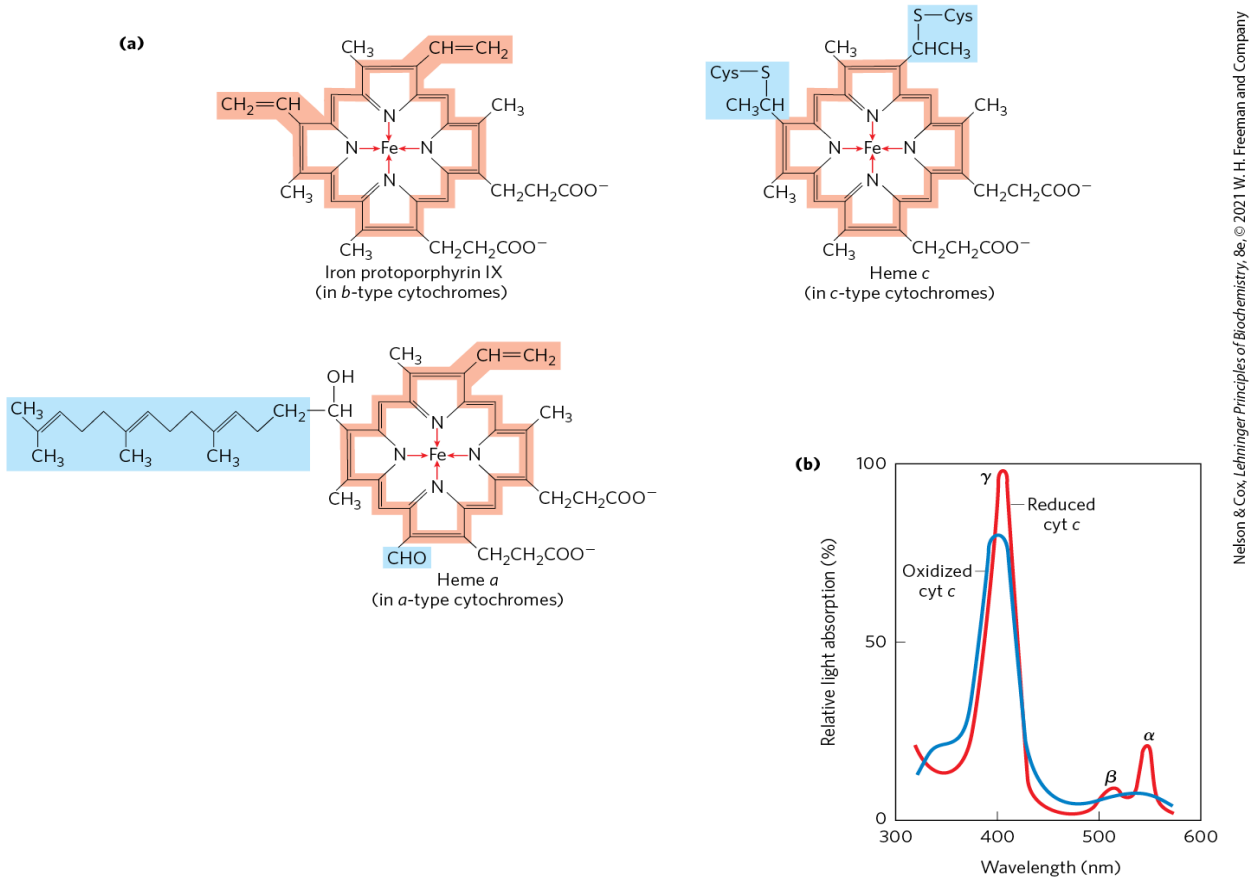

Fig. 7: Prosthetic groups of cytochromes. (a) Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a porphyrin. The four nitrogen atoms are coordinated with a central Fe ion, either Fe^{2+} or Fe^{3+}. Iron myoglobin. Heme *c* is covalently bound to the protein of cytochrome *c* through

thioether bonds to two Cys residues. Heme *a*, found in *a*-type cytochromes, has a long isoprenoid tail attached to one of the five-membered rings. The conjugated double-bond system (shaded light red) of the porphyrin ring has delocalized *π* electrons that are relatively easily excited by photons with the wavelengths of visible light, which accounts for the strong absorption by hemes (and related compounds) in the visible region of the spectrum. (b) Absorption spectra of cytochrome *c* (cyt *c*) in its oxidized (blue) and reduced (red) forms. The characteristic α, β, and γ bands of the reduced form are labeled.

Cytochromes other than the components of respiratory chain are, **Cytochrome P_{450} ; Cytochrome b_5.** Mitochondria contain three classes of cytochromes, designated *a, b,* and *c,* which are distinguished by differences in their light-absorption spectra. Each type of cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range **(Fig. -12-).** The longest wavelength band is near 600 nm in type *a* cytochromes, near 560 nm in type *b,* and near 550 nm in type *c.* To distinguish among closely related cytochromes of one type, the exact absorption maximum is sometimes used in the names, as in cytochrome b_{562}. The heme cofactors of *a* and *b* cytochromes are tightly, but not covalently, bound to their associated proteins; the hemes of *c*-type cytochromes are covalently attached through Cys residues.

Hydroperoxidases:

a. **Peroxidase:** Examples of peroxidases are glutathione peroxidase in RBCs (a selenium containing enzyme), leukocyte peroxidase and horse radish peroxidase. Peroxidases remove free radicals like hydrogen peroxide.

$$H_2O_2 + AH_2 \text{ ------- (peroxidase) -------►} 2H_2O + A$$

b. **Catalase:** Catalases are hemoproteins. Peroxisomes are subcellular organelles having both aerobic dehydrogenases and catalase.

$$2H_2O_2 \text{ --------- (catalase) ----------►} 2H_2O + O_2$$

c. **Glutathione peroxidase** It is present in RBC. It is involved in the removal of H_2O_2 present in RBC. It contains selenium. Glutathione serve as hydrogen donor.

$$H_2O_2 + 2G\text{-}SH \xrightarrow[\text{Peroxidase}]{\text{Glutathione}} 2H_2O + G\text{-}S\text{-}S\text{-}G$$

Oxygenases

a. **Mono-oxygenases:** These are otherwise called mixed function oxidases. Here, one oxygen 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.

$$A\text{-}H + O_2 + BH_2 \text{ -------(hydroxylase)-------►} A\text{-}OH + H_2O + B$$

i. Phenylalanine hydroxylase

ii. Tyrosine hydroxylase
iii. Tryptophan hydroxylase
iv. **Microsomal** cytochrome P_{450} mono-oxygenase is concerned with **drug metabolism**.

These enzymes are loosely referred as hydroxylases and (or) mixed function oxidases.

$$R-H \xrightarrow[\downarrow]{H_2,\ O_2} R\text{-}OH + H_2O$$

b. Di-oxygenases: They are enzymes which incorporate both atoms of a molecule of oxygen into the substrate, e.g. tryptophan pyrrolase and homogentisic acid oxidase.

$$A + O_2 \text{ ----------►} AO_2$$

Examples

Homogentisate dioxygenase ; Cyclooxygenase ; Hydroxy anthranilate dioxygenase ; Tryptophan dioxygenase.

Ubiquinone or Coenzyme Q (CoQ):

It is a constituent of mitochondrial lipids. It is a component of respiratory chain. It is the only non-protein component of electron transport chain, **Fig. 8.**

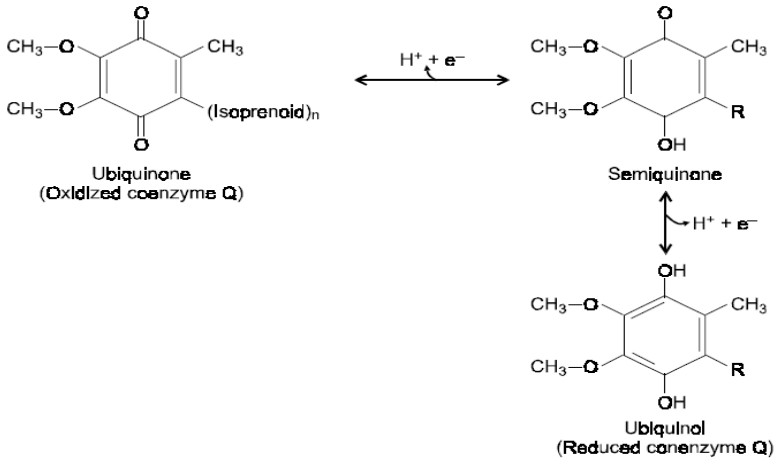

Fig. 8- Oxidation-reduction of ubiquinone via semiquinone

Because of its ubiquitous nature it is called as ubiquinone. It is a mobile electron carrier of respiratory chain. It collects electrons from NADH and $FADH_2$ and transfers to cytochromes. It participates in coupled oxidation reduction reactions of respiratory chain via semiquinone intermediate **(Fig. 8).**

Iron-sulfur proteins:

They are proteins containing iron-sulfur centers. Iron and sulfur are present as clusters in these proteins. Iron of these proteins is referred as

non-heme iron (NHI). Iron is complexed with organic sulfur and inorganic sulfur.

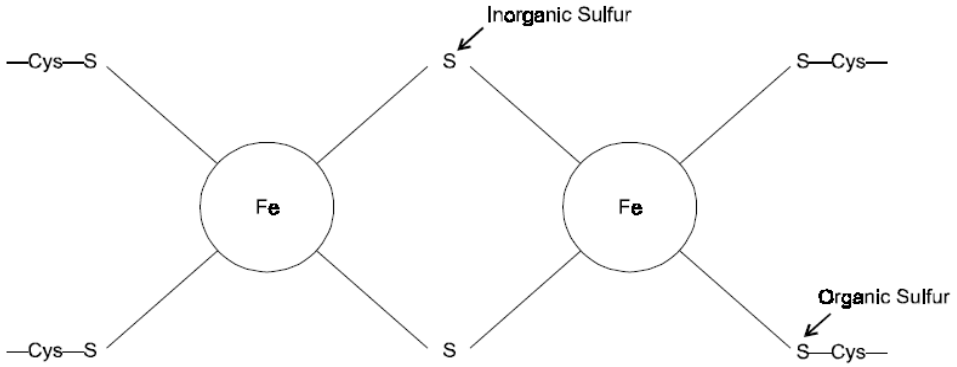

Fig. 9- An iron sulfur center of protein containing two iron atoms and two sulfur atoms (Fe_2Se_2)

Organic sulfur is contributed by the cysteine residue of protein **(Fig.9-).** Iron and sulfur are present in equimolar amounts. They participate in one electron transfer reactions. Iron oscillates between Fe^{2+} and Fe^{3+}. The oxidized center accept one electron **(Fe^{3+} + e^- --------->Fe^{2+}).**

In **iron-sulfur proteins,** the iron is present not in heme but in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both. These iron-sulfur (Fe-S) centers range from simple structures with a single Fe atom coordinated to four Cys -SH groups to more complex Fe-S centers with two or four Fe atoms **(Fig.10-).** All iron-sulfur proteins participate in one-electron transfers in whom one iron atom of the iron-sulfur cluster is oxidized or reduced. At least eight Fe-S proteins function in mitochondrial electron transfer.

In the overall reaction catalyzed by the mitochondrial respiratory chain, electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O_2.

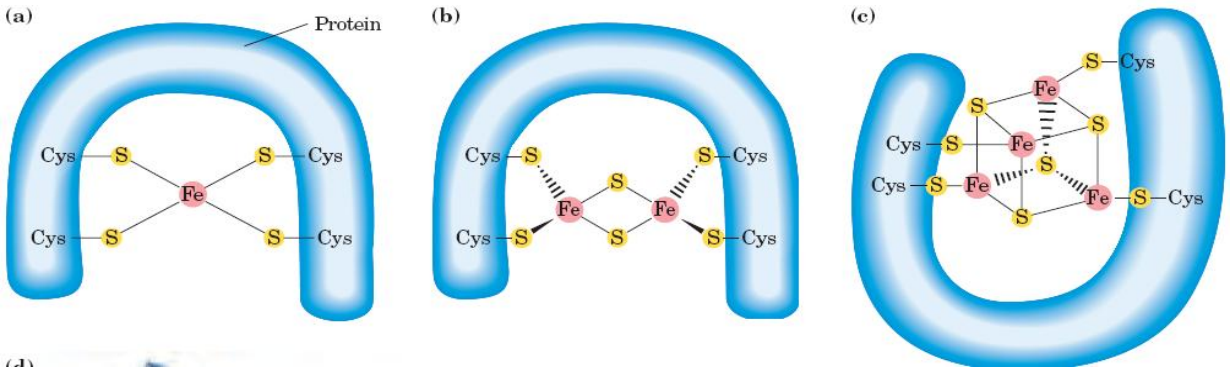

Fig. 10. Iron-sulfur centers. The Fe-S centers of iron-sulfur proteins may be as simple as (a), with a single Fe ion surrounded by the S atoms of four Cys residues. Other centers include both inorganic and Cys S atoms, as in (b) 2Fe-2S or (c) 4Fe-4S centers.

First, the standard reduction potentials of the individual electron carriers have been determined experimentally, **See table-3-:**

Table 3. Indicates the Standard Reduction Potentials of Respiratory Chain and Related Electron Carriers.

Redox reaction (half-reaction)	*E'° (V)*
$2H^+ + 2e^- \longrightarrow H_2$	−0.414
$NAD^+ + H^+ + 2e^- \longrightarrow NADH$	−0.320
$NADP^+ + H^+ + 2e^- \longrightarrow NADPH$	−0.324
NADH dehydrogenase (FMN) + $2H^+ + 2e^- \longrightarrow$ NADH dehydrogenase ($FMNH_2$)	−0.30
Ubiquinone + $2H^+ + 2e^- \longrightarrow$ ubiquinol	0.045
Cytochrome *b* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *b* (Fe^{2+})	0.077
Cytochrome c_1 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome *c* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *c* (Fe^{2+})	0.254
Cytochrome *a* (Fe^{3+}) + $e^- \longrightarrow$ cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + $e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.8166

The carriers' functions in order of increasing reduction potential, because electrons tend to flow spontaneously from carriers of lower *E*'° to carriers of higher *E*'°. The order of carriers deduced by this method is **NADH → Q → cytochrome *b* → cytochrome c_1 → cytochrome *c* → cytochrome *a* → cytochrome a_3 → O_2.**

Biochemical Anatomy of Mitochondria:

The discovery in 1948 by Eugene Kennedy and Albert Lehninger that mitochondria are the site of oxidative phosphorylation in eukaryotes marked the beginning of the modern phase of studies in biological energy transductions. Mitochondria, like gram negative bacteria, have two membranes.

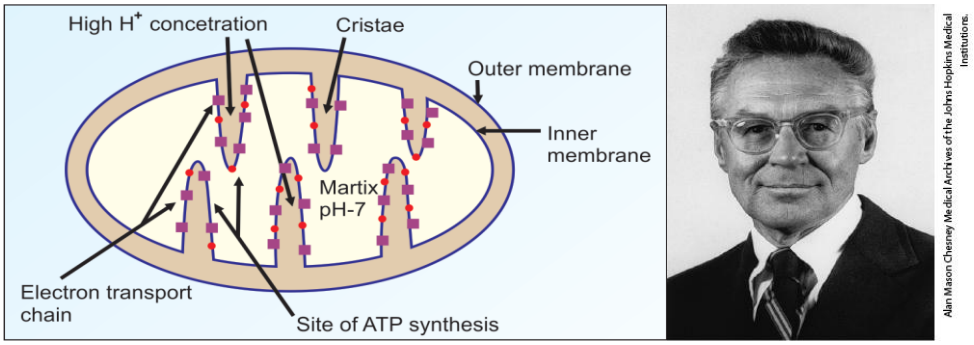

Fig. 11-a. The mitochondria and A. Lehninger

The outer mitochondrial membrane is readily permeable to small molecules (Mr < 5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins.

The inner membrane is impermeable to most small molecules and ions, including protons (H^+); the only species that cross this membrane do so through specific transporters. The inner membrane bears the components of the respiratory chain and ATP synthase.

The electron transport chain is functioning inside the mitochondria. The mitochondrion is a subcellular organelle having the outer and inner membranes enclosing the matrix **(Fig. 11-a and b).** The inner membrane is highly selective in its permeability, containing specific transport proteins. Certain enzymes are specifically localized in mitochondria **(Table 4).** The **inner membrane** contains the respiratory chain and translocating systems. The knob like protrusions represent the ATP synthase system **(Fig. 11).**

Inner and outer mitochondrial membrane differs greatly in their composition. Inner membrane is 22% cardiolipin and contains no cholesterol, whereas outer membrane is similar to cell membrane, with less than 3% cardiolipin and 45% cholesterol.

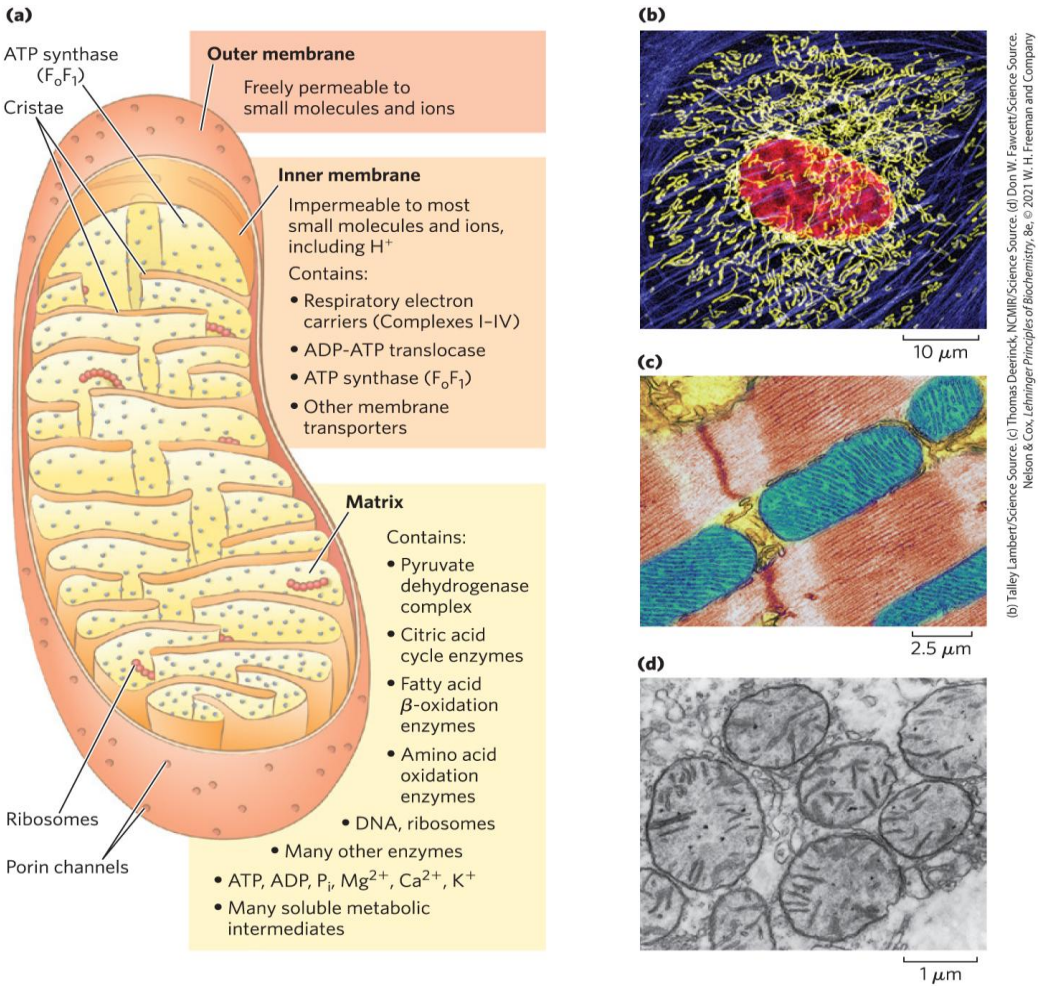

Fig. 11-b: Biochemical anatomy of a mitochondrion. (a) The outer membrane has pores that make it permeable to small molecules and ions, but not to proteins. The cristae provide a very large surface area. The inner membrane of a single liver mitochondrion may have more than 10,000 sets of electron-transfer systems (respiratory chains) and ATP synthase molecules, distributed over the membrane surface. (b) A typical animal cell has hundreds or thousands of mitochondria. This

endothelial cell from bovine pulmonary artery was stained with fluorescent probes for actin (blue), for DNA (red), and for mitochondria (yellow). Notice the variability in length of the mitochondria. (c) The mitochondria of heart muscle (blue in this colorized electron micrograph) have more profuse cristae and thus a much larger area of inner membrane, with more than three times as many sets of respiratory chains as (d) liver mitochondria. Muscle and liver mitochondria are about the size of a bacterium — 1 to 10 µm long. The mitochondria of invertebrates, plants, and microbial eukaryotes are similar to those shown here, but with much variation in size, shape, and degree of convolution of the inner membrane.

Table 4: Location of enzymes in mitochondria
Mitochondria, outer membrane: - Monoamino oxidase - Acyl CoA synthetase - Phospholipase A2 **In between outer and inner membrane:** - Adenylate kinase - Creatine kinase **Inner membrane, outer surface:** - Glycerol-3-phosphate dehydrogenase **Inner membrane, inner surface:** - Succinate dehydrogenase - Enzymes of respiratory chain **Soluble matrix:** - Enzymes of citric acid cycle - Enzymes of beta oxidation of fatty acid

Organization of Electron Transport Chain:

i. In the electron transport chain, or respiratory chain, the electrons are transferred from NADH to a chain of electron carriers. The electrons flow from the more electronegative components to the more electropositive components.

ii. All the components of electron transport chain (ETC) are located in the **inner membrane of mitochondria**.

iii. There are four distinct multi-protein complexes; these are named as **complex-I, II, III and IV.** These are connected by two mobile carriers, **co-enzyme Q** and **cytochrome c**.

iv. The arrangement is schematically represented in **Fig. 12.** The sequence of reaction is depicted in Box 2.

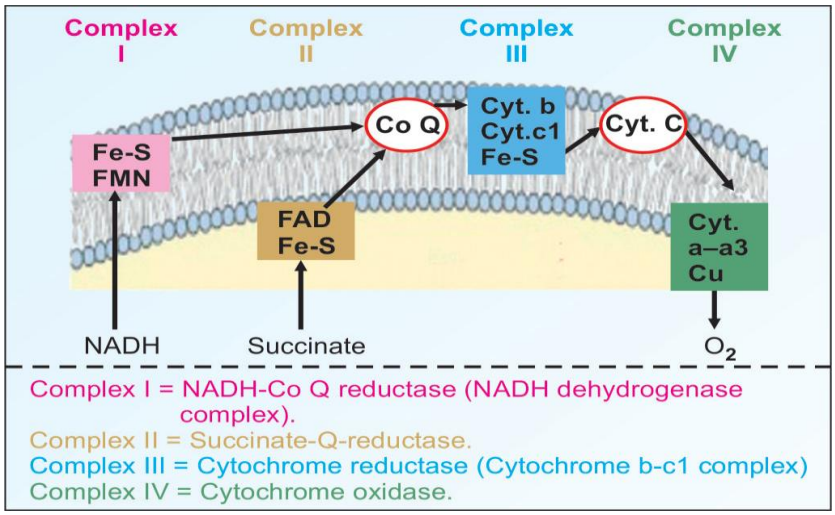

Fig. 12. Summary of electron flow

Box 2: Summary of electron flow in ETC
Complex I: NADH → FMN → Fe-S → Co Q →
Complex II: Succinate → FAD → Fe-S → Co Q →
Complex III: Co Q → Fe-S → cyt.b → cyt.c1 → cyt. c
Complex IV: Cyt. c → cyt a-a_3 → O_2

NADH Generation

The NADH is generated during intermediary metabolism **(Fig. 13).**

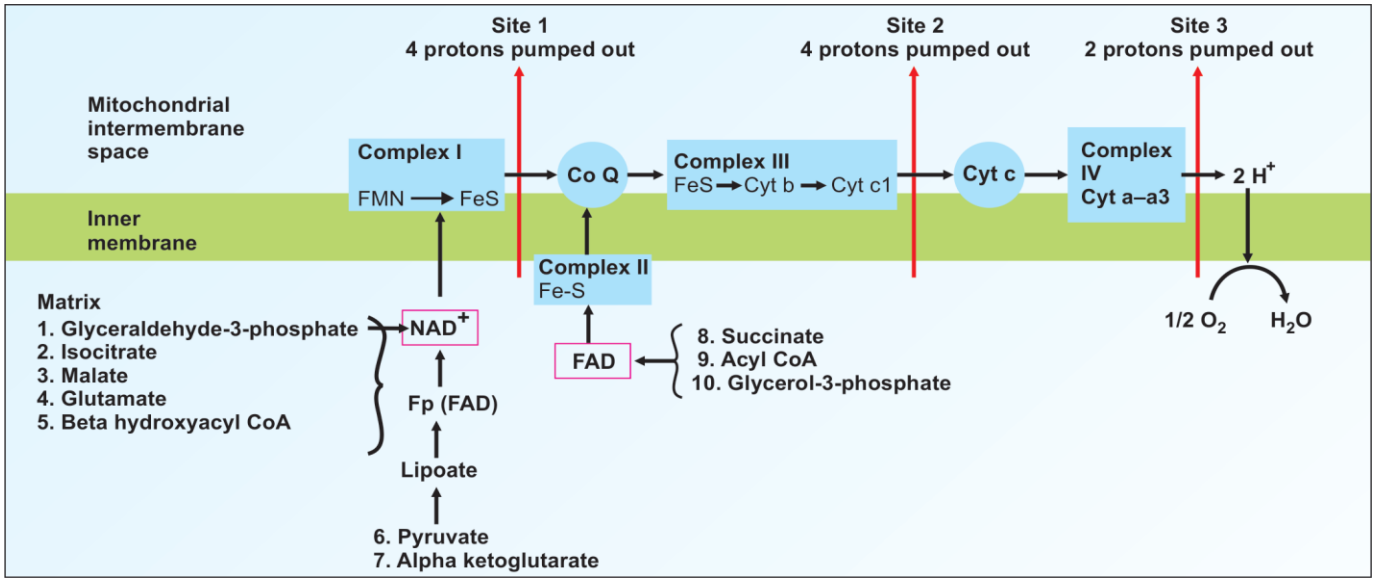

Fig. 13: Components and sequence of reactions of electron transport chain

ETC Complex-I or NADH to Ubiquinone:

Overall function of this complex is to collect the pair of electrons from NADH and pass them to CoQ. The reactions are shown in **Fig. 14.**

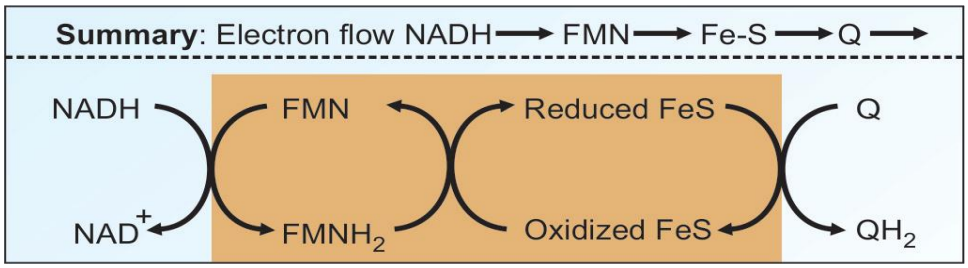

Fig. 14: Complex I or NADH-CoQ reductase (NADH dehydrogenase complex)

i. There is a large negative free energy change; the **energy released is 12 kcal/mol.** This is utilized to drive **4 protons** out of the mitochondria.

Complex II Succinate to Ubiquinone or Succinate-Q-Reductase

The reaction in Complex-II is represented in **Fig. 15.** We encountered **Complex II** in citric acid cycle as **succinate dehydrogenase**, the only membrane-bound enzyme. Complex II couples the oxidation of succinate at one site with the reduction of ubiquinone at another site about 40 Å away. Although smaller and simpler than Complex I, Complex II contains five prosthetic groups of two types and four different protein subunits, see **Fig. 25.** The electrons from FADH2 enter the ETC at the level of coenzyme Q. This step does not liberate enough energy to act as a proton pump.

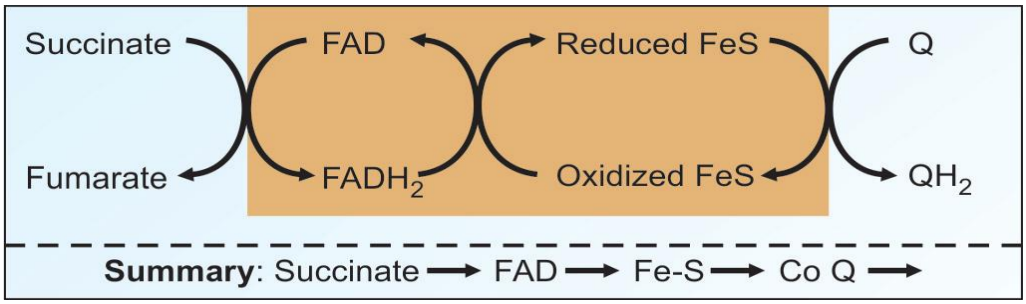

Fig. 15: Complex II; Succinate-Q-reductase

In other words, substrates oxidized by FAD-linked enzymes bypass complex-I.

Co-enzyme Q

i. The ubiquinone (Q) is reduced successively to semiquinone (QH) and finally to quinol (QH2).

ii. It accepts a pair of electrons from NADH or FADH2 through complex-I or complex-II respectively.

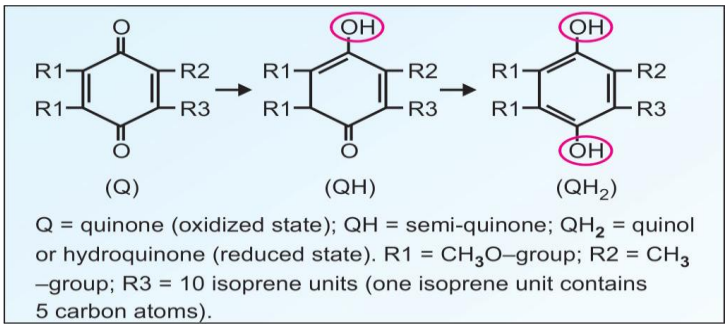

Fig. 16: Addition of H^+ to co-enzyme Q

iii. Co-enzyme Q is a quinone derivative having a long isoprenoid tail. The chain length of the tail is different in various species, mammals have 10 isoprene units **(Fig. 16).** Two molecules of cytochrome c are reduced.

iv. The **Q cycle** thus facilitates the switching from the two electron carrier ubiquinol to the single electron carrier cytochrome c.

Complex III or Ubiquinone to Cytochrome *c* or Cytochrome Reductase

This is a cluster of iron-sulfur proteins, **cytochrome b and cytochrome c1,** both contain **heme** prosthetic group. The sequence of reaction inside the Complex III is shown in **Fig. 17.**

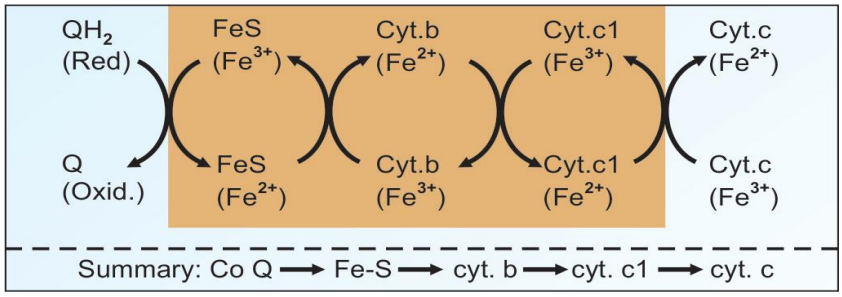

Fig. 17: Complex III or cytochrome reductase (cytochrome b-c1 complex) of respiratory chain

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

ii. The free energy change is—10 kcal/mol; and **4 protons are pumped out.**

Cytochrome c

It is a peripheral membrane protein containing 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.

Complex IV or Cytochrome *c* to O_2 or Cytochrome Oxidase

Generally ***Cytochrome c to O_2*** In the final step of the respiratory chain, **Complex IV,** also called **cytochrome oxidase,** contains the hemeproteins known as cytochrome *a* and cytochrome a_3, as well as copper-containing proteins in which the copper undergoes a transition from Cu^{+} to Cu^{2+} during the transfer of electrons through the complex to molecular oxygen. This carries electrons from cytochrome *c* to molecular oxygen, reducing it to H_2O. Oxygen is the final electron acceptor, with water being the final product of oxygen reduction. Complex IV is a large enzyme (13 subunits; *Mr* 204,000) of the inner mitochondrial membrane.

i. It contains different proteins, including **cytochrome a and cytochrome a3**. The Complex IV is tightly bound to the mitochondrial membrane.

ii. The reaction is depicted in **Fig. 18.** Four electrons are accepted from cytochrome c, and passed on to molecular oxygen.

$$4H^{+} + O_2 + 4\ Cyt.\ c\text{-}Fe^{++} \text{----------►}\ 2\ H_2O + 4\ Cyt.\ c^{+++}$$

iii. 2 protons are pumped out to the inter-membrane space.

iv. Cytochrome oxidase has 4 redox centers, namely, a, a3, CuA and CuB. The electron transfer in this complex is as shown

Cyt c -----► CuA -----► Cyt a -----► Cyt a3 ------► CuB

Cytochrome oxidase contains two heme groups and two copper ions. The two heme groups are denoted as **cytochrome-a** and **cytochrome a-3.** The functional unit of the enzyme is a single protein, and is referred to as cytochrome **a--a_3**.

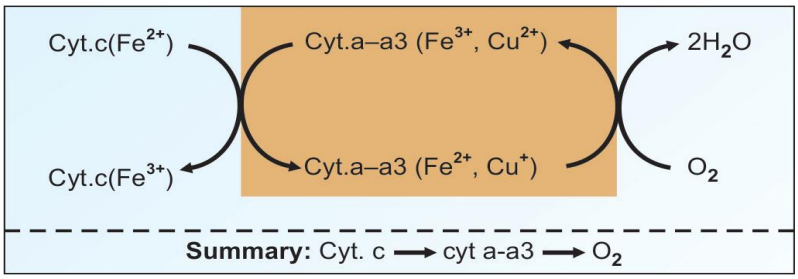

Fig. 18: Complex IV (cytochrome oxidase) of respiratory chain

In summary: Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from reduced ubiquinone to cytochrome *c,* and Complex IV completes the sequence by transferring electrons from cytochrome *c* to O_2, see **Fig. 19.**

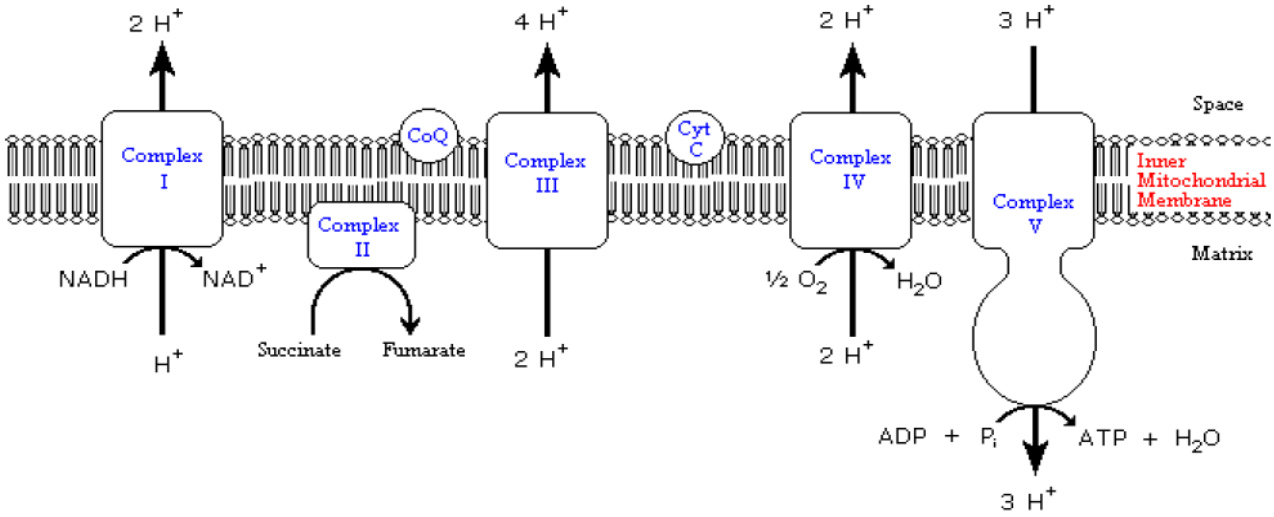

Fig. 19– Flow of pair of electrons from the reducing equivalents (FADH2 , NADH + H^{+}) through the four complexes to O_2.

The free energy available as a consequence of transferring 2 electrons from NADH or succinate to molecular oxygen is –57 and –36 kcal/mol, respectively. Oxidative phosphorylation traps this energy as the high-energy

phosphate of ATP. In order for oxidative phosphorylation to proceed, two principal conditions must be met.

1. The inner mitochondrial membrane must be physically intact so that protons can only re-enter the mitochondrion by a process coupled to ATP synthesis.
2. High concentration of protons must be developed on the outside of the inner membrane.

The energy of the proton gradient is known as the chemiosmotic potential, or proton motive force (PMF). This potential is the sum of the concentration difference of protons across the membrane and the difference in electrical charge across the membrane. The 2 electrons from NADH generate a 6-proton gradient. Thus, oxidation of 1 mole of NADH leads to the availability of a PMF with a free energy of about –31.2 kcal (6 x –5.2 kcal). The energy of the gradient is used to drive ATP synthesis as the protons are transported back down their thermodynamic gradient into the mitochondrion. Electrons return to the mitochondrion through the integral membrane protein known as ATP synthase (or Complex V), **Fig. 20.**

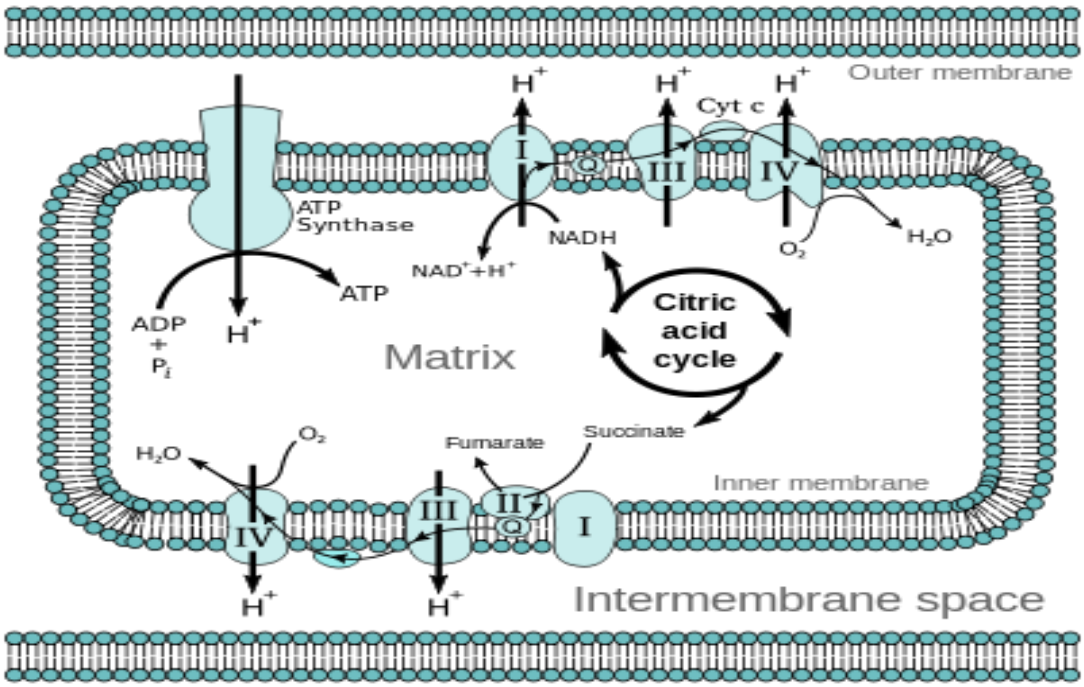

Fig. 20– The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, providing energy to power ATP synthase.

ATP Synthase : Mitochondrial **ATP synthase, also called complex V, Fig. 21** is an F-type ATPase similar in structure and mechanism to the ATP synthases of chloroplasts and eubacteria. This large enzyme complex of the

inner mitochondrial membrane catalyzes the formation of ATP from ADP and Pi, accompanied by the flow of protons from the P to the N side of the membrane.

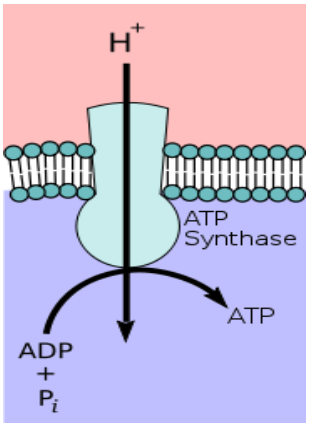

Fig. 21: Depiction of ATP synthase, the site of oxidative phosphorylation to generate ATP

ATP synthase, is a multiple subunit complex that binds ADP and inorganic phosphate at its catalytic site inside the mitochondrion, and requires a proton gradient for activity in the forward direction. ATP synthase is composed of 3 fragments: F_o, which is localized in the membrane; F_1, which protrudes from the inside of the inner membrane into the matrix; and oligomycin sensitivity-conferring protein (OSCP), which connects F_o to F_1. In damaged mitochondria, permeable to protons, the ATP synthase reaction is active in the reverse direction acting as a very efficient ATP hydrolase or ATPase.

The flow of electrons through Complexes I, III, and IV results in pumping of protons across the inner mitochondrial membrane, making the matrix alkaline relative to the intermembrane space. This proton gradient provides the energy (in the form of the proton-motive force) for ATP synthesis from ADP and Pi by ATP synthase (F_oF_1 complex) in the inner membrane.

- ATP formation on the enzyme requires little energy; the role of the proton-motive force is to push ATP from its binding site on the synthase.
- The ratio of ATP synthesized per 1/2 O_2 reduced to H_2O (the P/O ratio) is about 2.5 when electrons enter the respiratory chain at Complex I, and 1.5 when electrons enter at CoQ.
- Energy conserved in a proton gradient can drive solute transport uphill across a membrane.
- The inner mitochondrial membrane is impermeable to NADH and NAD^+, but NADH equivalents are moved from the cytosol to the matrix by either

of two shuttles. NADH equivalents moved in by the malate-aspartate shuttle enter the respiratory chain at Complex I and yield a P/O ratio of 2.5; those moved in by the glycerol 3-phosphate shuttle enter at CoQ and give a P/O ratio of 1.5.