

OXIDATIVE PHOSPHORYLATION

❖ Overview of cellular respiration

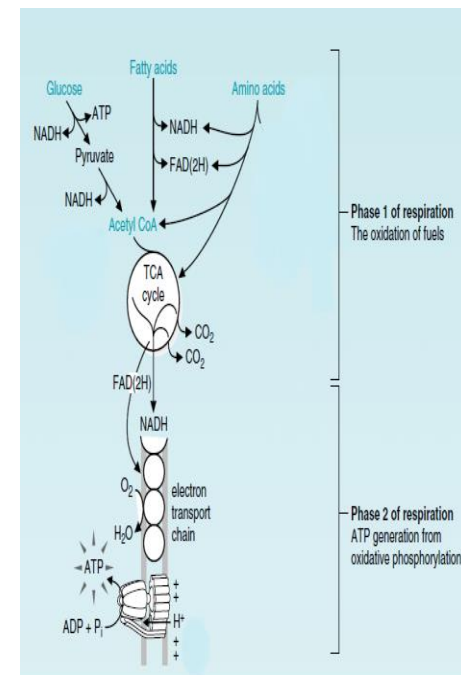
- ✓ Most of the **energy from oxidation of fuels** in the **TCA cycle** and other pathways is **conserved** in the form of NADH and FADH₂.
- ✓ Energy from fuel oxidation is converted to the high-energy phosphate bonds of adenosine triphosphate (ATP) by the process of **oxidative phosphorylation**.
- ✓ Through **ETC**, **NADH** and **FADH₂** are **oxidized**, and the electrons are donated to **O₂**, forming H₂O
- ✓ Part of the released energy during transfer of electrons through the ETC is captured for the phosphorylation of adenosine diphosphate (ADP) to ATP by **ATP synthase (F₀ F₁ ATPase)**.
- ✓ The net yield of oxidative phosphorylation is approximately **3 ATP moles of ATP** per mole of **NADH** oxidized, or **2 moles of ATP** per mole of **FADH₂** oxidized.

❖ Important definitions

- **Oxidation**: It is the addition of oxygen, or removal of hydrogen, or removal of electrons.
 - **Reduction** is the addition of hydrogen, or addition of electrons. or removal of oxygen.
- NB. Oxidation** of a compound is always **accompanied** by the **reduction** of another compound. This is why we usually refer to the reactions as **oxidation-reduction** reactions.
- **Oxidoreductases**: are enzymes concerned with the catalysis of oxidation-reduction reactions. They are subdivided into **4 subgroups**:

1- Oxidases:

- Enzymes concerned with **removal of hydrogen**, using **oxygen** as a **hydrogen acceptor**.
- They form **water** or hydrogen peroxide.
- An important example of oxidases is the **cytochrome oxidase**. It presents at terminal components of the respiratory chain that is responsible for the reaction with molecular oxygen to produce water.



2- Dehydrogenases:

- They **transfer hydrogen** from one substrate to another, in a **reversible**, coupled oxidation-reduction reaction.
- Oxygen is not used** as a hydrogen acceptor.
- Many dehydrogenases use **nicotinamides** as coenzymes. These may be either **NAD⁺ or NADP⁺**.
- NAD⁺** is usually linked to dehydrogenases responsible for **oxidative pathways** as glycolysis, TCA cycle and the respiratory chain. While, **NADP⁺** is used by dehydrogenases linked to **reductive synthetic pathways** as in the cytoplasmic synthesis of fatty acids.
- Other dehydrogenases depend on **riboflavin as coenzymes (FAD, FMN)** as succinate dehydrogenase and mitochondrial glycerol 3-phosphate dehydrogenase

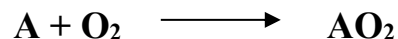
3- Hydroperoxidases:

- Two subgroups. They are responsible for **disposal of hydrogen peroxides** from the body (as **peroxidases and catalase**)

4- Oxygenases:

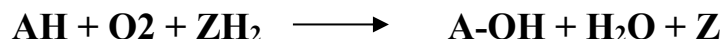
- They catalyse the **incorporation of oxygen** into substrate molecules, with the synthesis or degradation of different metabolites. Have no relation to energy in the body. They are subdivided into two subgroups;

a. **Di-oxygenases:** they incorporate **both atoms of oxygen molecule** into the substrate (in amino acid metabolism).



b. **Monooxygenase (hydroxylase):** These enzymes introduce **one atom of oxygen** to the **substrate** and the **other oxygen atom** is reduced to **water**.

e.g., **Microsomal cytochrome P450** is an important enzyme of this group that is responsible detoxification of many harmful substances and drugs....etc



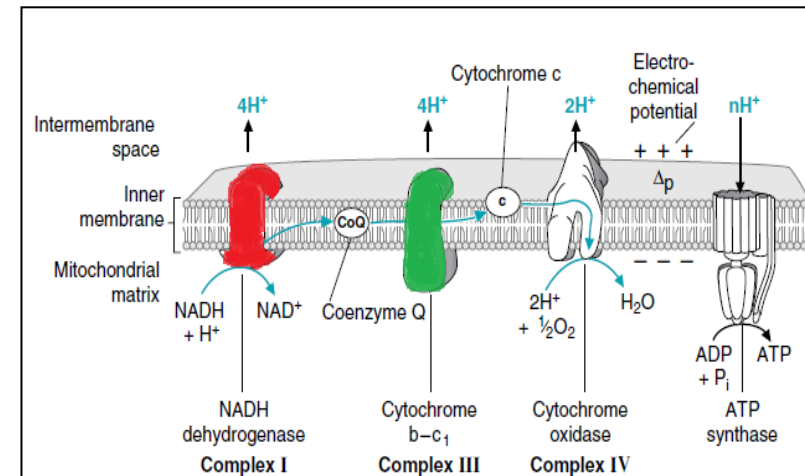
OXIDATIVE PHOSPHORYLATION IN ETC

Generation of ATP by oxidative phosphorylation requires:

- an electron donor (NADH or FADH₂)
- an electron acceptor (O₂)
- an intact inner mitochondrial membrane
- All the components of the electron transport chain
- ATP synthase
- Oxidative phosphorylation is **regulated** by the rate of ATP utilization.

❖ Overview of the electron transport chain (ETC)

- Electrons donated by NADH or FADH₂ are passed sequentially through a series of electron carriers embedded in the **inner** mitochondrial membrane.
- Each of the components of the ETC is **reduced** as it accepts an electron, and then **oxidized** as it passes the electrons to the next member of the chain, **The final acceptor is O₂, which is reduced to H₂O.**
- With the exception of coenzyme Q (CoQ), all members of the ETC are proteins.
- The ETC included 4 complexes: **NADH dehydrogenase (Complex I)**
Succinate dehydrogenase (Complex II)
Cytochrome b-c₁ complex (Complex III)
Cytochrome oxidase (Complex IV).
- In oxidation-coupled phosphorylation, the **energy for ATP synthesis** is provided by an **electrochemical gradient** across the **inner mitochondrial** membrane.
- This **electrochemical gradient** is **generated** by the **components of the electron transport chain**, which **pump protons** across the inner mitochondrial membrane as they sequentially **accept and donate electrons**.



REACTIONS OF THE ETC

- NAD^+ is reduced to NADH by a dehydrogenase (e.g., isocitrate dehydrogenase of TCA cycle) that removes two hydrogen atoms from their substrate.
- Both electrons but only one proton (a hydride ion $[:\text{H}-]$) are transferred to the NAD^+ , forming NADH plus a free proton H^+ .

Components and Carriers of ETC

1-COENZYME Q:

- Coenzyme Q (CoQ) is a quinone derivative with a long, hydrophobic isoprenoid tail.
- It is also called **ubiquinone** because it is **ubiquitous** in biologic systems.
- **CoQ** is a **mobile** electron carrier and can **accept hydrogen atoms** from NADH dehydrogenase (**Complex I**), from succinate dehydrogenase (**Complex II**) and from other **mitochondrial dehydrogenases**: as glycerol-phosphate dehydrogenase
- **CoQ** transfers electrons to **Complex III** (cytochrome bc1).

N.B: The free energy drop between **NADH** and **CoQ** is able to pump protons. However, the **FADH₂** in succinate dehydrogenase (as well as glycerophosphate dehydrogenase) is at roughly the same energy level as **CoQ**, and there is no energy released as they transfer electrons to CoQ.

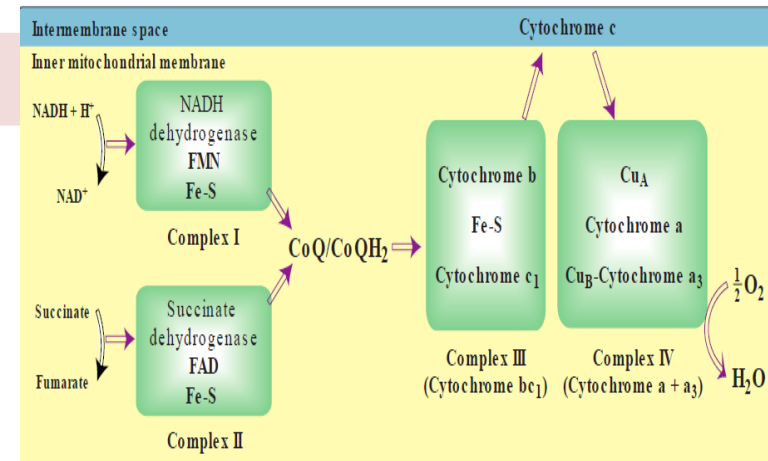
2- CYTOCHROMES :

- The remaining members of the ETC are cytochrome proteins, Each contains a **heme** group (a **porphyrin** ring& **iron**).
- Ex: Cytochrome C (the only free carrier), a-a3, b-c1
- **Unlike** the iron of the heme groups of haemoglobin, the cytochrome iron is **reversibly** converted from its **ferric** (Fe^{3+}) to its **ferrous** (Fe^{2+}) form as a normal part of its function as an **acceptor and donor of electrons**.

3- Other electron Carriers: Iron-sulfur protein, FMN

1. *NADH DEHYDROGENASE (COMPLEX I)*

- The **free proton plus the hydride ion** carried by NADH are transferred to NADH dehydrogenase, a protein complex (**Complex I**) that is embedded in the inner mitochondrial membrane.
- **Complex I** has a tightly bound molecule of **flavin mononucleotide (FMN)**, a coenzyme structurally related to FAD that accepts the two hydrogen atoms ($2e^- + 2H^+$), becoming **FMNH₂**. NADH dehydrogenase also contains peptide subunits with **iron-sulfur centers**



- **At Complex I**, electrons move from **NADH** to **FMN** to the **iron of the iron-sulfur centers** and then to **coenzyme Q**.
- As **electrons** flow, they **lose energy**. This energy is used to **pump protons** across the inner mitochondrial membrane, from the **matrix** to the **intermembrane space**.

2. *SUCCINATE DEHYDROGENASE (COMPLEX II)*

- Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate and the reduction of the coenzyme, **FAD to FADH₂**. Then, hydrogen atoms are moved from FADH₂ to an **iron-sulfur** protein, and then to **coenzyme Q**. [Note: *No energy is released in this process, and, therefore, no protons are pumped at Complex II*].

3. *CYTOCHROME b-c₁ (COMPLEX III)*

- Electrons move from **cytochrome b** to the **iron-sulfur centers** then to **cytochrome c₁** then to **cytochrome c**, and then to **cytochromes a + a₃** (Complex IV).
- As electrons flow, **protons** are pumped across the inner mitochondrial membrane at Complexes **III and IV**.
- The cytochromes of the **b-c₁** complex have a **higher energy level** than those of **cytochrome oxidase (a + a₃)**. Thus, energy is released by electron transfer between complexes III and IV.

Note: Cytochrome c is located in the intermembrane space, **loosely** associated with the outer face of the inner membrane. As seen with CoQ, cytochrome c is also a mobile carrier of electrons.

4- CYTOCHROME a + a3 (COMPLEX IV)

- This cytochrome complex (**Complex IV**) is the **only** electron carrier in which the heme iron has an available coordination site that can react directly with **O₂** and so also is called **cytochrome oxidase**.
- At Complex IV, the transported electrons, O₂, and free protons are brought together, and O₂ is reduced to water
- **Note: Four electrons are required to reduce one molecule of O₂ to two molecules of water.**
- Cytochrome oxidase contains copper (**Cu**) **atoms**.
- **Electrons** move from **CuA** to **cytochrome a** to **cytochrome a3** (in association with **CuB**) to **O₂** which is the **final acceptor**.

How is oxidation coupled to the production of the high-energy compound ATP?

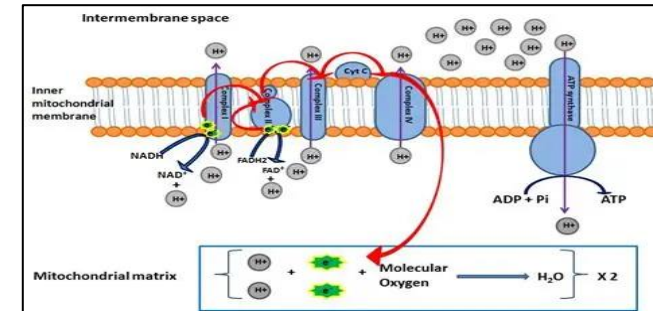
- ✓ The transfer of electrons down the ETC is energetically favored because **NADH** is a **strong electron donor** and **O₂** is a **strong electron acceptor**. However, the flow of **electrons** does **not** directly result in **ATP synthesis**.

CHEMIOSMOTIC HYPOTHESIS

The chemiosmotic hypothesis (also known as the **Mitchell hypothesis**) explains how the **free energy** generated by the **transport of electrons** by the ETC is used to **produce ATP** from $\text{ADP} + \text{P}_i$.

1. Proton pump:

- **Electron transport** is **coupled** to the **phosphorylation** of ADP by the pumping of protons across the inner mitochondrial membrane from the **matrix** to the **intermembrane space** at Complexes I, III, and IV.
- This process creates **an electrical gradient** (with more positive charges on the outside of the membrane than on the inside) and a **pH gradient** (the outside of the membrane is at a lower pH than the inside)
- **The energy generated by this proton gradient is sufficient to drive ATP synthesis (Complex V)**
- Thus, the **proton gradient** serves as the **common intermediate** that **couple**s oxidation to phosphorylation.



2. ATP synthase (Fo F1ATPase)

- The multi-subunit enzyme ATP synthase (**Complex V**) synthesizes ATP using the energy of the proton gradient.
- It contains a domain (**F_o**) that spans the inner mitochondrial membrane, and an extra-membranous **domain (F₁)** that appears as a sphere that protrudes into the mitochondrial matrix
- The chemiosmotic hypothesis proposes that after protons have been pumped to the cytosolic side of the inner mitochondrial membrane, they re-enter the matrix by passing through a proton channel in the F_o domain, driving rotation of F_o and, at the same time, dissipating the pH and electrical gradients.
- **F_o rotation** causes **conformational changes** in the β subunits of the F₁ domain that allow them to bind $\text{ADP} + \text{P}_i$, phosphorylate ADP to ATP, and release ATP.

❖ Several findings support the chemiosmotic theory:

- **Addition of protons** (acid) to the external medium of intact mitochondria stimulates the formation of **ATP**.
- Oxidative phosphorylation **does not occur** in case of **solubilizing** the mitochondrial membranes.

❖ Respiratory control ensures a constant supply of ATP

- ✓ It is to be noted that there is **no mechanism** for **storage** of ATP in the body.
- ✓ Actually all the **ATP present at any moment is only enough to meet the needs of the body for a few seconds**.
- ✓ So, there must be an efficient and controlled way for the production of ATP. The respiratory chain allows the free energy resulting from the oxidation of food stuffs to become available in a stepwise, efficient and controlled way.

CONTROL OF THE RESPIRATORY CHAIN (REGULATION OF ETC)

- Mainly by the **availability of ADP**, that constitutes the **main** controlling factor.
- When doing work, **ATP** is converted to **ADP**. This allows more **respiration** (oxidation reactions) to **occur**, which in turn **replenishes** the stores of **ATP**.
- Under certain conditions, the **concentration of inorganic phosphate** could affect the rate of ATP formation.



INHIBITORS OF RESPIRATORY CHAIN

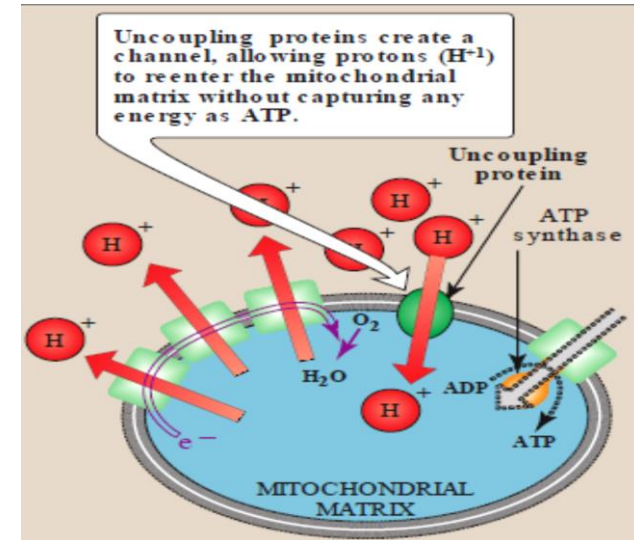
1- UNCOUPLERS:

When protons leak back into the mitochondrial matrix **without** going through the **ATP synthase (proton channel in the Fo domain)**, they dissipate the electrochemical gradient across the membrane **without** generating ATP. This phenomenon is called “**uncoupling**” of oxidation to phosphorylation

- It occurs with chemical compounds, known as **uncouplers**, and it may occur physiologically or not, with uncoupling proteins that form proton conductance channels through the membrane.
- Uncoupling of oxidative phosphorylation results in **increased oxygen consumption** and **heat production** as electron flow and proton pumping attempt to maintain the electrochemical gradient.

❖ Examples of uncouplers:

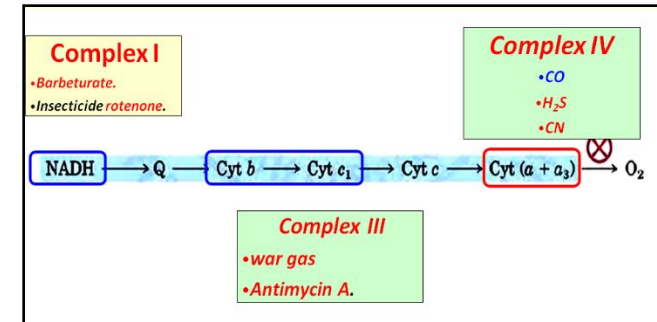
- **Thermogenin (UCP1):**
 - It is considered as a **physiological** uncoupling protein.
 - It is present in the **brown adipose tissues** of newly born humans.
 - It allows **protons** to pass to the **mitochondrial matrix** without passing by the **F0-F1 complex**.
 - **No ATP** is produced and **energy is dissipated** in the form of **heat**.
- **Synthetic uncouplers: 2,4-dinitrophenol**, a lipophilic proton carrier that readily diffuses through the mitochondrial membrane.



2-INHIBITORS OF THE RESPIRATORY CHAIN COMPONENTS

These inhibitors act on the three sites that produce the electrochemical gradient; i.e., complexes I, III and IV.

- **Complex I** is inhibited by: **barbiturates**, and the insecticide **rotenone**.
- **Complex II** is inhibited by: **malonate**
- **Complex III** is inhibited by: **the war gas and antimycin A**.
- **Complex IV** is inhibited by: **H₂S**, **carbon monoxide (CO)** and **cyanide (CN)**.



3- OLIGOMYCIN

- This drug binds to the **F₀ domain of ATP synthase**, closing the **proton channel** and preventing **re-entry of protons into the matrix**, thereby **preventing** phosphorylation of **ADP to ATP**.
- Because the pH and electrical gradients cannot be dissipated in the presence of this drug, electron transport stops because of the difficulty of pumping any more protons against the steep gradients.

INHERITED DEFECTS IN OXIDATIVE PHOSPHORYLATION

- 13 of the approximately 90 polypeptides required for oxidative phosphorylation are **coded for by mitochondrial DNA (mtDNA)** and synthesized in **mitochondria**.
- The **remaining** proteins are coded for by **nuclear DNA**, synthesized in the **cytosol**, and transported into the **mitochondria**.
- **Defects** in oxidative phosphorylation are more likely a result of **alterations in mtDNA**, which has a **mutation rate about 10 times greater** than that of **nuclear DNA**.
- **Tissues** with the greatest ATP requirement (central nervous system, skeletal and heart muscle, and liver) are most **affected by defects** in oxidative phosphorylation.

- **Mutations in mtDNA** are responsible for several diseases, including some cases of **mitochondrial myopathies**, and **Leber hereditary optic neuropathy** (a disease in which bilateral loss of central vision occurs as a result of neuroretinal degeneration, including damage to the optic nerve).

Note: mtDNA is **maternally-inherited** because **mitochondria** from the **sperm cell** **do not** enter the fertilized egg.