BIOCHEMISTRY **1st Year, 2nd Semester**

Prof. Catalin MARIAN, MD PhD Dr. Habil.
Email: cmarian@umft.ro

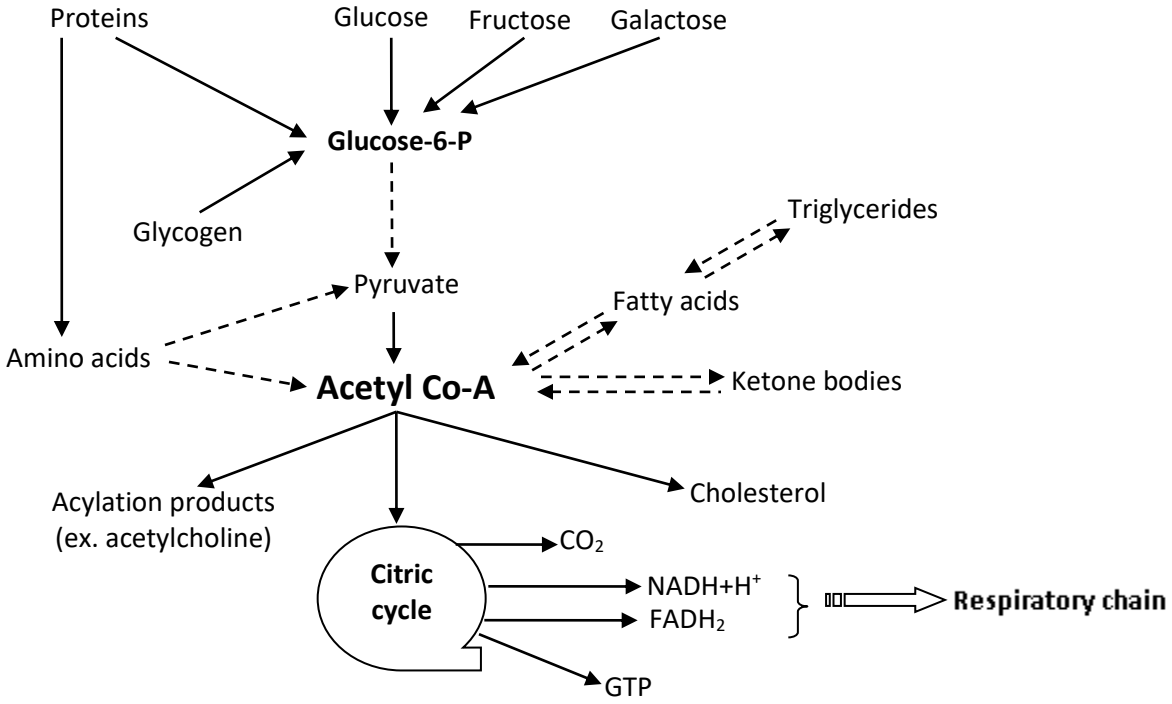
Lecture 2

I.3.3. Citric acid cycle (tricarboxylic acid cycle – TCA or Krebs cycle)

TCA is located into the mitochondrion, in the matrix and on the internal side of the internal mitochondrial membrane, in the vicinity of the respiratory chain compounds onto which it directly transfers the resulting reducing equivalents.

TCA oxidizes acetyl-CoA to CO_2, producing energy. The hydrogen from the NAD^+ or FAD coenzymes is immediately transferred to the respiratory chain, and part of the resulted energy is coupled with the GDP phosphorylation reaction, resulting GTP. Figure 2 presents the sources of acetyl-CoA.

The precursor molecule for acetyl-CoA is the final product of the carbohydrates, lipids and proteins metabolism, and also their convergence point. Therefore, the citric acid cycle represents the intersection of the main metabolic pathways, being also called the turntable of intermediary metabolism.



Sources of acetyl-CoA

Citric acid cycle steps

1. Citric acid formation

It takes place by a condensation reaction at the methylene carbon atom (acetyl-CoA) with carbonyl carbon atom of oxalic acid. This is an irreversible reaction of hydrolysis of a macroergic thioester bond and thus it can be considered as the **rhythm reaction** of the entire citric acid cycle. The reaction is catalyzed by citrate synthase, an enzyme regulated by the inhibitory action of ATP, acyl-CoA and succinil CoA.

2. Isomerization of citric acid to isocitric acid

It takes place in two steps: citric acid dehydration, resulting cis-aconitic acid, followed by the addition of water with the formation of isocitric acid. Both steps are catalyzed by aconitase, an enzyme specifically inhibited by fluoroacetate.

3. Oxidation of isocitric acid to α-ketoglutaric acid

This is a dehydrogenation reaction and secondarily a decarboxylation in which the NAD^+ dependent enzyme isocitrate dehydrogenase transfers the hydrogen to the coenzyme. This enzyme is positively allosteric regulated by AMP and ADP, and negatively allosteric regulated by ATP.

1. Oxidative decarboxylation of α-ketoglutaric acid

During this reaction, the elimination of a carboxyl group is accompanied by the oxidation of the adjacent carbon atom. It represents a substrate phosphorylation reaction in which the reaction energy is directly coupled by phosphorylation with the formation of a phosphorylated nucleotide compound, GTP. This step consists of two reactions:

- **the oxidative decarboxylation of α-ketoglutaric acid** resulting succinyl-CoA, $NADH+H^+$ and CO_2. the reaction is catalyzed by a multienzymatic complex called α-ketoglutarate dehydrogenase and contains several coenzymes (lipothiamine pyrophosphate, NAD^+, FAD) that successively transfer reducing equivalents among them.
- **the thiolysis of succinyl-CoA,** coupled with the phosphorylation of GDP and resulting succinic acid, CoA and GTP. In all tissue excepting liver, GTP is replaced with ATP. The reaction is catalyzed by succinyl-CoA synthetase or thiokinaze, enzyme stimulated by AMP, ADP.

2. Oxidation of succinic acid to fumaric acid

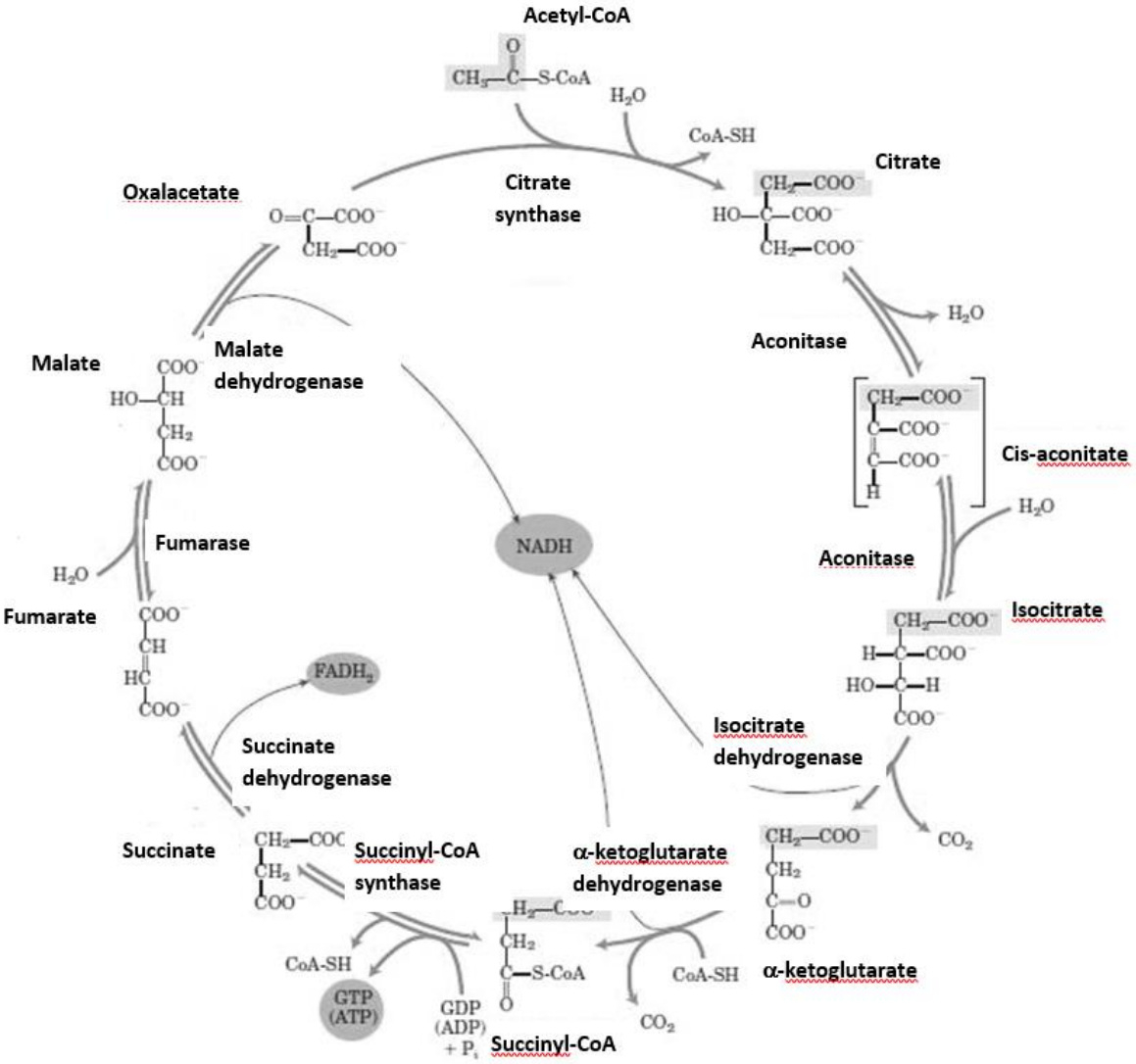
This is a dehydrogenation reaction catalyzed by succinate dehydrogenase, a FAD dependent enzyme. Fumaric acid and $FADH_2$ is being produced, and the enzyme can be competitively inhibited by malonic acid.

6. Hydration of fumaric acid

The reaction takes place by the addition of water to fumaric acid, resulting malic acid, and is catalyzed by fumarase.

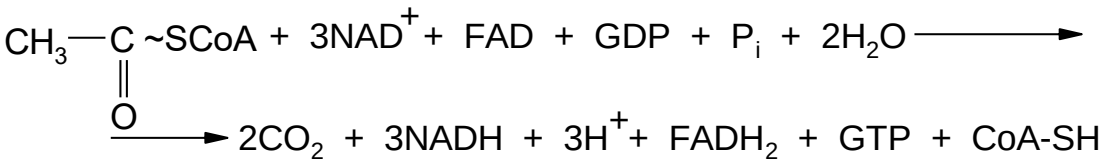
7. Oxidation of malic acid to oxaloacetic acid.

This is a dehydrogenation reaction catalyzed by the NAD_+ dependent malate dehydrogenase; malic acid being converted into oxaloaceitc acid and $NADH+H^+$. This reaction closes the citric acid cycle, the oxaloacetic acid being available for condensation with a new acetyl-CoA molecule, thus initiating a new cycle of reactions.



Krebs cycle (citric acid cycle).

The global reaction is the following (the first two CO_2 molecules coming from the first round of the cycle are from the oxaloacetate and not from acetyl-CoA):



TOTAL ATP = 1ATP + 3 X 2,5 ATP + 1X 1,5 ATP = 10 ATP

The functioning of the citric acid cycle is strictly dependent on the presence of oxygen. The citric acid cycle is an amphibolic pathway, being involved in both catabolism (energy producing) and anabolism (energy consumption). As part of the anabolism:

a) the intermediary compounds in the cycle can be precursors in different synthesis pathways:

- all intermediaries can be precursors in gluconeogenesis
- most intermediaries can be precursors in the synthesis of amino acids
- succinyl-CoA is a precursor for porphyrins synthesis
- α-ketoglutaric acid is a precursor in the synthesis of glutamic acid, purine and pyrimidine bases
- oxaloacetic acid is a precursor in the synthesis of aspartic acid, purine and pyrimidine bases

b) some of the reactions from the cycle are used in synthesis processes such as:

- gluconeogenesis
- fatty acids synthesis
- ureogenesis
- purine nucleotides synthesis

Regulation of the citric acid cycle

The cycle can be regulated in two ways: substrate regulation and enzymatic regulation.

1. **Substrate regulation** consists of:
   - the availability of acetyl-CoA, which is dependent on the catabolic contribution of glucose (pyruvic acid), amino acids or fatty acids.
   - the availability of B vitamins which is supplying the coenzymes TPP, lipoic acid, NAD^+, FAD.
   - the availability of oxidized coenzymes NAD^+ and FAD.
   - the concentration and lifespan of all the intermediaries in the cycle. It has been determined that excepting oxaloacetic acid, all other intermediaries have a relatively constant concentration of 10^{-4} mole/l and a lifespan of a few seconds. Oxaloacetic acid has a much smaller lifespan and concentrations, thus being the main regulator reactant of this pathway. Therefore, **the mechanisms regulating oxaloacetic acid concentration** will automatically also regulate the rate of the citric acid cycle. In the case of increased energy needs part of the excess pyruvic acid will be converted into oxaloacetic by the anaplerotic reaction:
   -

$$\text{pyruvic acid} + CO_2 + ATP \xrightarrow{\text{pyruvat carboxylase (biotin)}} \text{oxaloacetic acid} + ADP + P_i$$

The oxaloaceitc acid can also be obtained through a transamination reaction:

$$\text{aspartic acid} + \alpha\text{ - ketoglutaric acid} \rightleftharpoons \text{glutamic acid} + \text{oxaloacetic acid}$$

The excess oxaloacetic acid can be reconverted into pyruvic acid by decarboxylation.

2. Enzymatic regulation

The enzymes with regulatory roles in the irreversible steps of the citric acid cycle are citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. They are all allosteric inhibited by the increase in ATP concentration.

Due to the main energy producing role of the citric acid cycle, and because ATP is the final product of energy producing metabolic pathways, the regulation through ATP represents a negative feedback type of regulation of the enzymes through the final product. Therefore, the rate of the cycle is correlated with the cell's need for ATP and thus energy through this type of regulation.

I.3.4. Oxidative phosphorylation in the respiratory chain

Compared to the oxidative phosphorylation of substrates, this type of phosphorylation is simpler, universal and much more efficient. It consists of the following steps:

1. The mobilization of H from substrates takes place in the cytosol under the action of pyridine and flavin dehydrogenases, which transfer it to the specific enzymatic cofactors NAD^+ and FAD.
2. The protons mobilized in the cytosol are then transferred in the mitochondrial matrix through shuttling systems.
3. The oxidation reaction of hydrogen ($2H + 1/2O_2 \rightarrow H_2O$) takes place in the mitochondrion in several steps that make up the respiratory chain, each step being represented by a redox system.
4. The components of the respiratory chain support with energy the transfer of H^+ in the mitochondrial intermembrane space, generating an electrochemical potential that constitutes the electromotive force for ATP synthesis by ADP phosphorylation.

The energy created from the oxidation of the hydrogen transferred by $NADH+H^+$ and $FADH_2$ by reacting with oxygen and coupled with the ADP phosphorylation is 2.5 ATP molecules for $NADH+H^+$ and 1.5 ATP molecules for $FADH_2$.

I.3.4.1. Hydrogen shuttling

The mitochondrial internal membrane is impermeable to $NADH+H^+$, therefore there is a need for a shuttling system. The most frequent shuttling system is the malate-aspartate shuttle system consisting of the malate- α-ketoglutarate transporter and the glutamate-aspartate transporter. Through this system:

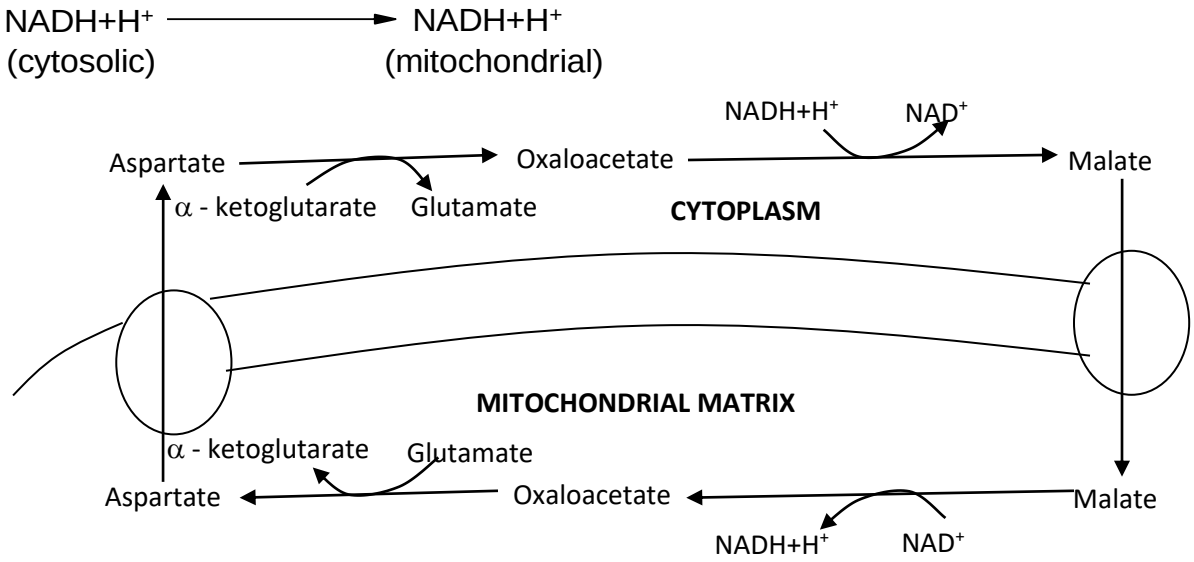


Figure 4. The malate-aspartate shuttle system

In muscle, brain and brown adipose tissue there is another shuttle system that uses the couple dihydroxyacetone phosphate-glycerol-3-phosphape (Figure 5).

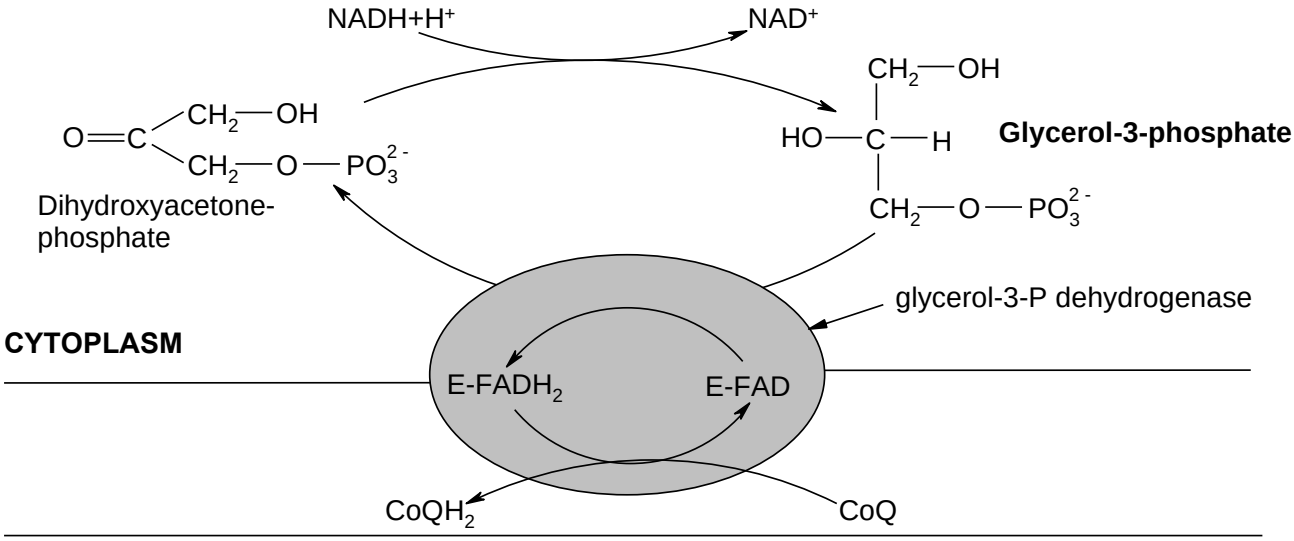


Figure 5. The glycerol-3-phosphate shuttle

Through this system:

$NADH+H^+$ (cytosolic) ⟶ $FADH_2$ (mitochondrial)

I.3.4.2. The respiratory chain

This is the final, aerobic phase of biological oxidation in which the hydrogen atoms mobilized from substrates and transported by coenzymes ($NADH+H^+$ or $FADH_2$) into the mitochondrion are finally combined with the oxygen:

$$\mathbf{CoH_2 + 1/2O_2 \rightarrow H_2O + Co_{ox}}$$

The reaction occurs in several successive steps in which the energy from the reaction $2H + 1/2O_2$ is gradually released. The following law makes **possible the segmentation of the reaction**:

$$\mathbf{\Delta G = -nF\Delta E}$$

This law specifies that useful energy (ΔG) is produced when reducing equivalents (electrons) pass from an electron donor (reducing agent) to an electron acceptor (oxidative agent), which is directly proportional to the potential difference between the donor and the acceptor. Substances that participate in such electron transfers are redox systems.

A **redox system** represents the couple of the oxidized and reduced form of the same substance, which can be converted among them in the presence of a donor or acceptor of electrons.

The availability of the redox system to donate or to accept electrons is characterized by the electronic potential of the system, called **redox potential**. When two redox systems are in contact, there is a transfer of electrons from the system with lower affinity for electrons (more negative) towards the one with higher affinity (more positive), this transfer constituting a redox reaction.

The comparison of the redox potential of several redox systems is done by measuring them against a reference redox system such as the standard hydrogen electrode. Conventionally, the redox potential of the hydrogen electrode is considered to be zero at 25°C, pH=0, [H]=1M, p_{H_2}=1atm. According the Nernst equation:

$$E = E^0 + \frac{RT}{nF}\ln\frac{[ox]}{[red.]} = E^0 + 0{,}059 \log\frac{[ox]}{[red]} \quad (1\ e^-)$$

$$= E^0 + 0{,}03 \log\frac{[ox]}{[red]} \quad (2\ e^-)$$

In condition when [ox]=[red], $E=E^0$, where E^0 is the standard redox potential of the system to be measured.

In biological systems pH=7, therefore the reference electrode needs to have $[H^+]=10^{-7}$ M; in this case we measure the **normal physiological redox potential** E'^0.

When measuring the potential of the H_2 half-cell at pH=7 against the standard hydrogen electrode, a value of -0.42 volts is obtained.

When several redox systems are couples, the reducing equivalents will pass successively from one redox system to the next according to increasing positivity of the system's redox potential (from – to +).

Redox potentials of some redox system in the respiratory chain

REDOX SYSTEM		E'^0 (volts)
$NAD(P)^+ + 2H + 2e^-$	$NAD(P)H + H^+$	- 0.32
Oxidized lipoic acid + 2H + $2e^-$	Reduced lipoic acid	- 0.29
$FMN+2H+2e^-$	$FMN\ H_2$	- 0.12
$FAD+ 2H+2e^-$	$FAD\ H_2$	0
Coenzyme Q+ 2H + $2e^-$	$CoQH_2$	+ 0.10
Cytochrome b (Fe^{3+}) + e^-	Cytochrome b (Fe_2^+)	+ 0.12
Cytochrome c (Fe^{3+}) + e^-	Cytochrome c ($Fe_{\ 2}^+$)	+ 0.22
Cytochrome a (Fe^{3+}) + e^-	Cytochrome a (Fe_2^+)	+ 0.29
½ O_2 + $2H^+$ + $2e^-$	H_2O	+ 0.82

In the mitochondrion, the redox potential of these couples also depends on the binding protein and the different concentrations [ox] ≠ [red], therefore the following statement is true:

The real intracellular redox potential is $E' = E'^0 + \frac{RT}{nF}\ln\frac{[Aox]}{[Ared,]}$.

Passing from one redox system to another is accompanied by energy release which can be calculated by the equation:

$$\Delta G^{0'} = -nF\Delta E^0$$

For the reaction:

$NADH_2 + ½\ O_2 \rightarrow NAD^+ + H_2O$ $\Delta G = -52,7$ kcal/mole

there is a transfer of reducing equivalents from the NAD^+/NADH, H^+ system towards the O_2 /O^{2-} system. Knowing that the standard physiological potential of the two systems is $E'^0_{NAD+/NADH,\ H+} = -0,32$ and $E'^0_{O2\ /O2-} = +0,82$, respectively, according to the equation $\Delta G^{0'} = -nF\Delta E^0$ we will obtain $\Delta G^{0'} = -52,7$ kcal/mole.

The **respiratory chain** consists of a chain of redox systems organized according to their affinity for electrons, thus ensuring the progress of the global reaction:

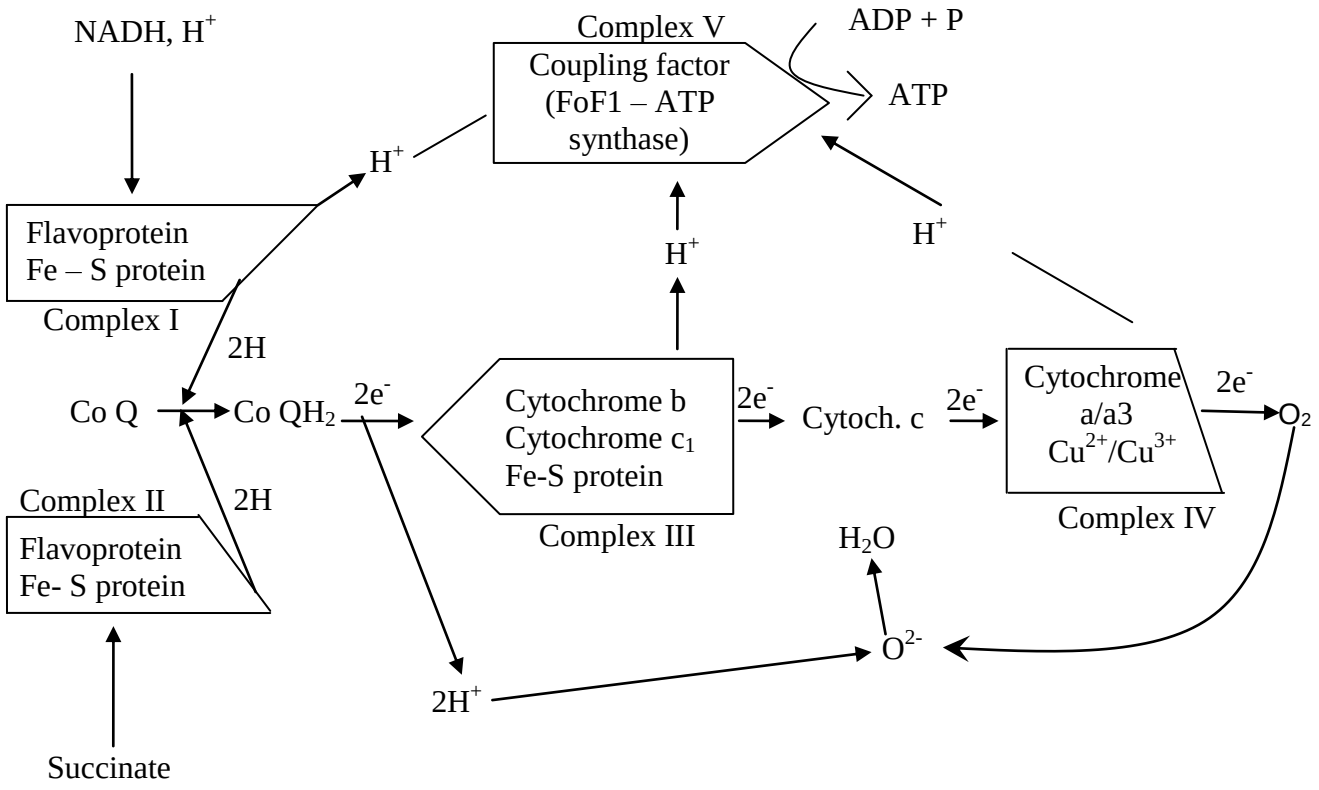
$2H + ½\ O_2 \rightarrow H_2O$.

The passing of electrons through the respiratory chain components is done according to the increase in electropositivity of the redox potential E'^0. The global variation of free energy in the respiratory chain is calculated based on the redox potential difference between $NADH/NAD^+ = -0,32$ V and $O_2 = 0,82$ V.

$$\Delta G^0 = -(2 \times 23,062 \times [(0,82 - (-0,32)] = -52,7 \text{ kcal/mol}$$

The respiratory chain is located on the internal mitochondrial membrane. When trying to isolate the components of the respiratory chain, one obtains two separate soluble components: **cytochrome c** and **Coenzyme Q (CoQ or ubiquinone)**, and 5 insoluble components called **complexes I-V**. The coupling of all these ensures the

complete oxidation of $NADH+H^+$ or succinate in the presence of O_2 to H_2O, accompanied with ATP production.



Complex I (NADH dehydrogenase) transfers electrons from NADH, H^+ to the CoQ, and contains as coenzyme FMN and four proteins with FeS in their structure.

$$\mathbf{NADH+H^+ + Enz\text{-}FMN \rightarrow Enz - FMNH_2 + NAD^+}$$

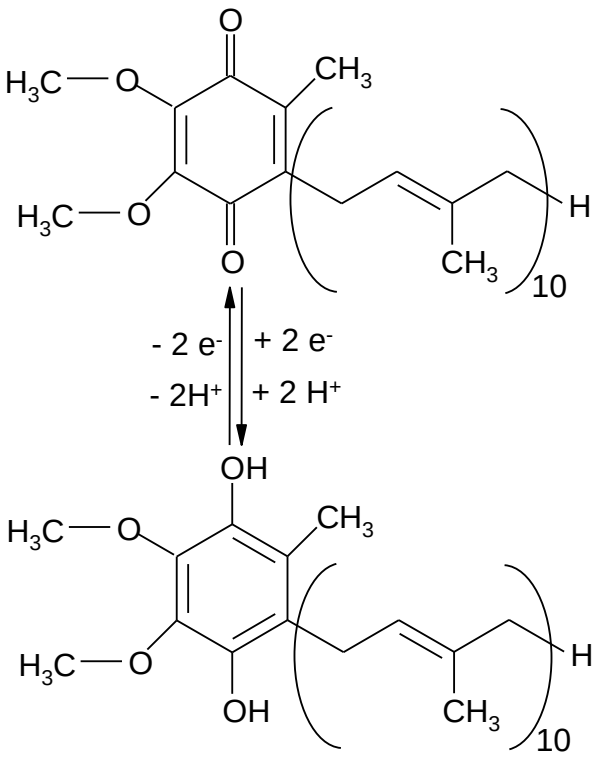
$$E\text{-}FMNH_2 \xrightarrow{-2e} (FeS)_1\ (FeS)_2\ (FeS)_3\ (FeS)_4 \text{ redus}$$

$$E\text{-}FMN + 2H^+$$

reduced $(FeS)_1(FeS)_2(FeS)_3(FeS)_4 + 2H^+ + CoQ \rightarrow CoQH_2 +$ oxidized $(FeS)_1(FeS)_2(FeS)_3(FeS)_4$

Complex II transfers electrons from $FADH_2$ to the CoQ. The FAD coenzyme is associated to enzymes such as succinate dehydrogenase (FAD dependent), glycorel-3-phosphate dehydrogenase and acyl-CoA dehydrogenase.

Coenzyme Q (CoQ), chemically is a derivative of 1,4 benzoquinone that contains a polyisoprene chain made of 10 isoprene molecules:



CoQ is located in the internal mitochondrial membrane and its role is to accept the reducing equivalents transported by $NADH+H^+$ (complex I) and $FADH_2$ (complex II), thus passing into the reduced form CoQH2. The hydrogen electrons are then transferred to the cytochromes system and H^+ are passing in the mitochondrial matrix.

$CoQH_2$ + 2cit b(Fe^{3+})ox → CoQ + 2cit b(Fe^{2+})red + $2H^+$ (released in the mitochondrial matrix)

The cytochromes system contains hemeproteins having heme as prosthetic group and a different structure from the heme in hemoglobin in order to allow the reversible conversion $Fe^{2+} \leftrightarrow Fe^{3+}$. This adaptation allows the existence of cytochromes as redox systems, able to transfer electrons according to the following equation:

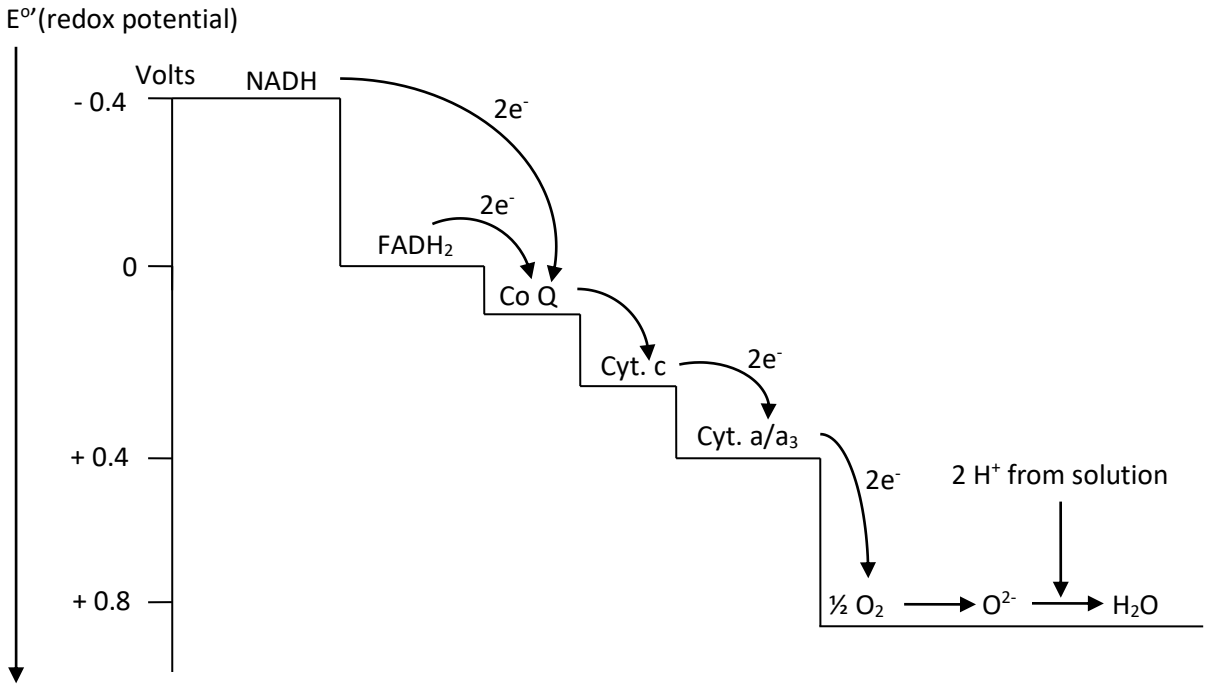
$$\textbf{citocrom } (Fe^{3+})\textbf{oxidat} \underset{-\,e^-}{\overset{+\,e^-}{\rightleftharpoons}} \textbf{citocrom } (Fe^{2+})\textbf{redus}$$

The passing of electrons from one cytochrome redox system to the next is done according to the increase in electropositivity of redox potentials. Therefore, the cytochromes systems is a chain of reactions in which electrons will pass from one redox system with more negative potential to the next one with a more positive potential.

reduced cyt b(Fe^{2+}) + oxidized 2 cyt c_1(Fe^{3+}) ↔ oxidized cyt b(Fe^{3+}) + reduced 2 cyt c_1(Fe^{2+})

The order of cytochromes succession is:

2cyt.b → 2cyt c_1 → 2cyt c → 2cyt a



The only cytochrome that could be experimentally separated and purified is cytochrome c, all other being deeply integrated in complexes III and IV.

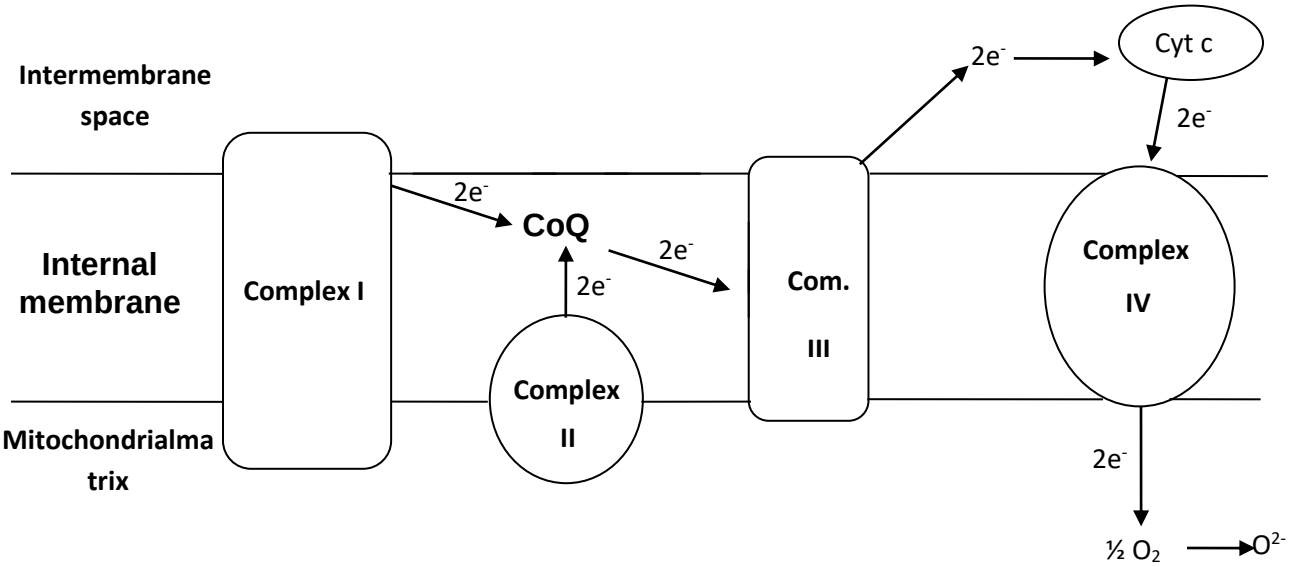
Cytochrome oxidase (cytochrome a/cytochrome a_3) is the cytochrome that is structurally the closest to hemoglobin, therefore being sensitive to the inhibitory action of CO, HCN, azide. This is also the cytochrome complex that transfers electrons directly to the oxygen atom. It contains two tightly linked components, cytochrome a and cytochrome a_3.

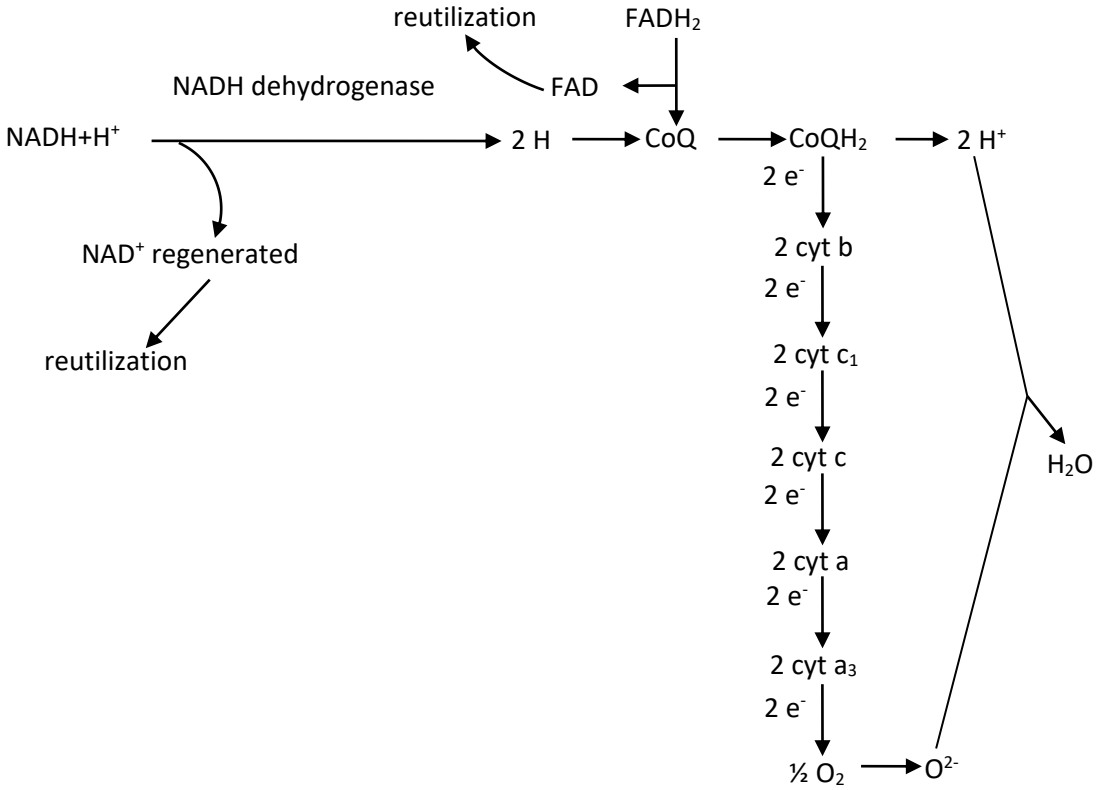
red. 2cyt. a(Fe^{2+}) + ox. 2cyt.a_3(Fe^{3+}) → ox. 2cyt.a(Fe^{3+}) + red. 2cyt.a_3(Fe^{2+})

red. 2cyt. a(Fe^{2+}) + 1/2O_2 → ox. 2cyt.a(Fe^{3+}) +O^{2-}

O^{2-} + 2H^+ → HOH

General schematic representation of the respiratory chain:





Coupling the respiratory chain with oxidative phosphorylation

This represents the process through which the energy released from oxidizing hydrogen in the respiratory chain is used as support for the ADP phosphorylation reaction in order to obtain ATP.

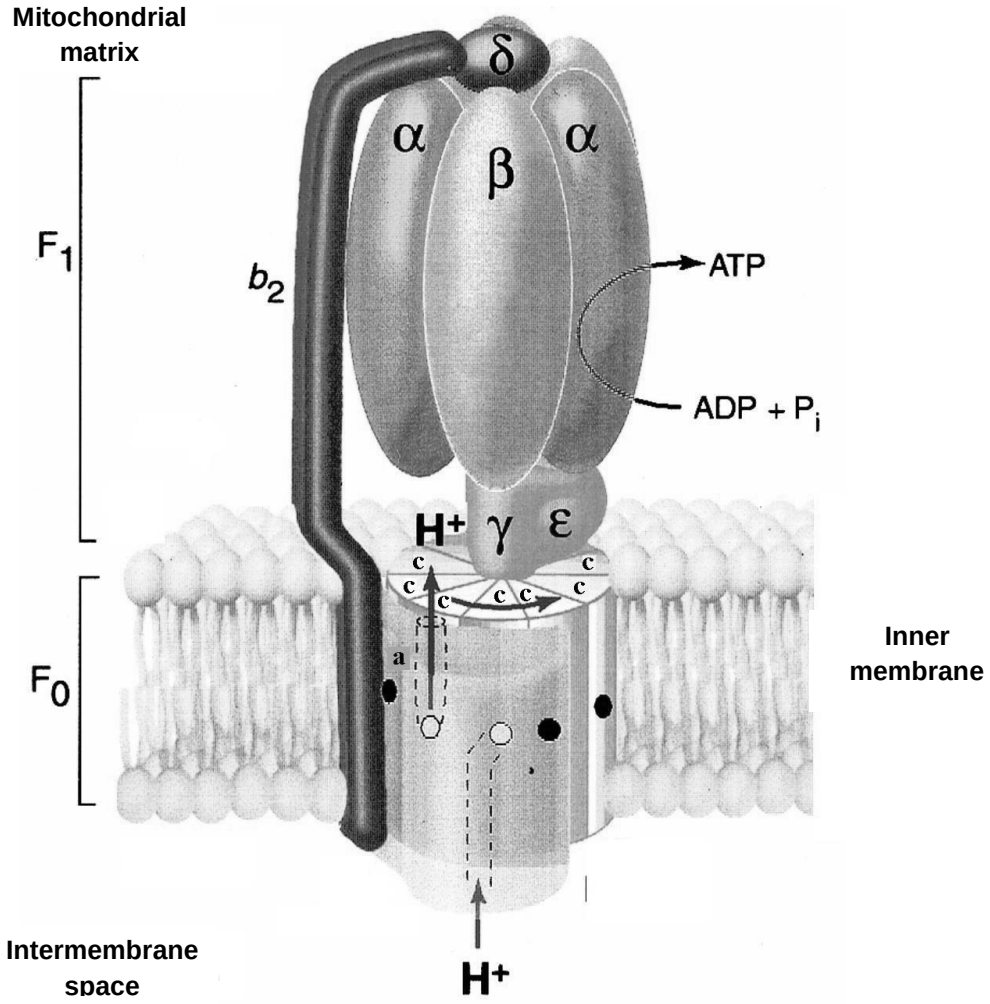
The energy released through the redox reaction in the respiratory chain is used for the active transport of H^+ from the mitochondrial matric into the intermembrane space, because the internal mitochondrial membrane is impermeable to H^+. Therefore, a pH gradient is created which generates an electrochemical potential between the mitochondrial matrix and the intermembrane space, with a corresponding electromotive force that will put in motion **Complex V (ATP synthase)**.

The mitochondrial **ATP synthase** is a type F ATP-ase with a similar structure to the ATP synthase found in bacteria and chloroplasts. It is composed of two distinct protein complexes, Fo (where "o" indicated that this complex is inhibited by the antibiotic **oligomycin**) which is integrated in the internal mitochondrial membrane and constitutes the effective ion channel through which the protons travel, and F_1 which is located immediately under the internal mitochondrial membrane, in the matrix, where the reaction of ATP synthesis from ADP is catalyzed.

The Fo complex consists of 3 types of subunits, a, b and c, with the following numeric ratio among them ab_2c_{10-12}. The F_1 complex contains 5 different types of subunits which make up a structure formed of 9 subunits total: $\alpha_3\beta_3\delta\varepsilon\gamma$.

The catalytic sites where ATP is synthesized are only located in the 3 β subunits which despite having the same aminoacid sequence are adopting a different conformation according to their association with a domain of the γ subunit. Only one β subunit can be associated to a γ subunit at once, this being called the **unoccupied β subunit**; the other two holding in their catalytic site an ADP (**β-ADP**), and an ATP (**β-ATP**) molecule, respectively. Each β subunit will pass successively through the three possible conformations, starting with the β-ADP followed by the β-ATP with ATP synthesis, releasing the ATP molecule corresponding to the unoccupied β subunit, and thus the cycle goes on and on. At a certain moment in time, each of the three β subunits will have a different conformation.

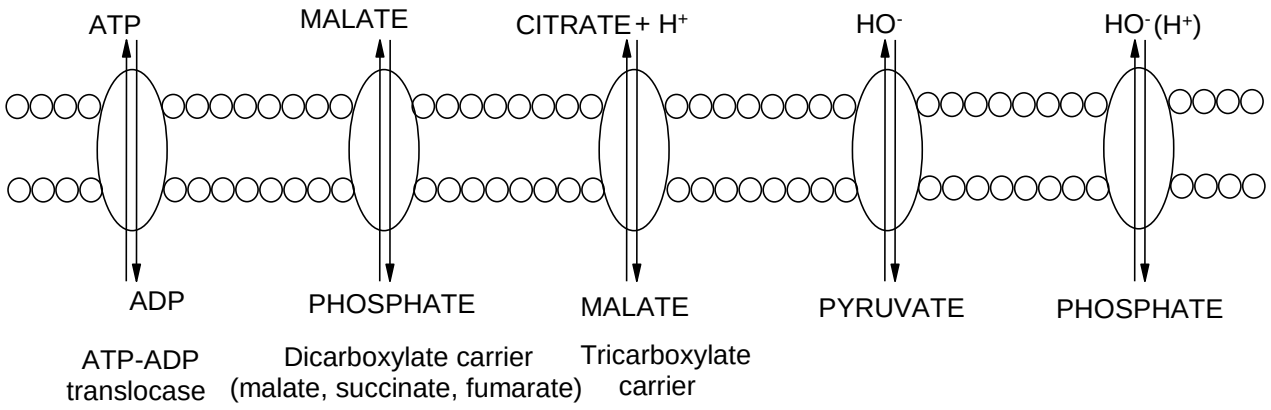
The cylinder shaped channel made of the c and γ attached subunits of the Fo complex will rotate when the flux of protons are passing through it. In addition, the γ subunit is the central axis of the spherical structure made of the α and β subunits of the F_1 complex, being stabilized on the internal mitochondrial membrane by the **b subunits** of the Fo complex. By spinning the γ axis within the αβ sphere, the γ subunit will be successively coupled with each of the β subunits that consecutively adopts the unoccupied β conformation, while the other two β subunits will adopt the β-ADP and β-ATP conformations. In this way, three ATP molecules will be synthesized for each complete rotation of the γ subunit. This catalysis mechanism is called **rotational catalysis**.



The structure and mechanism of the ATP synthase

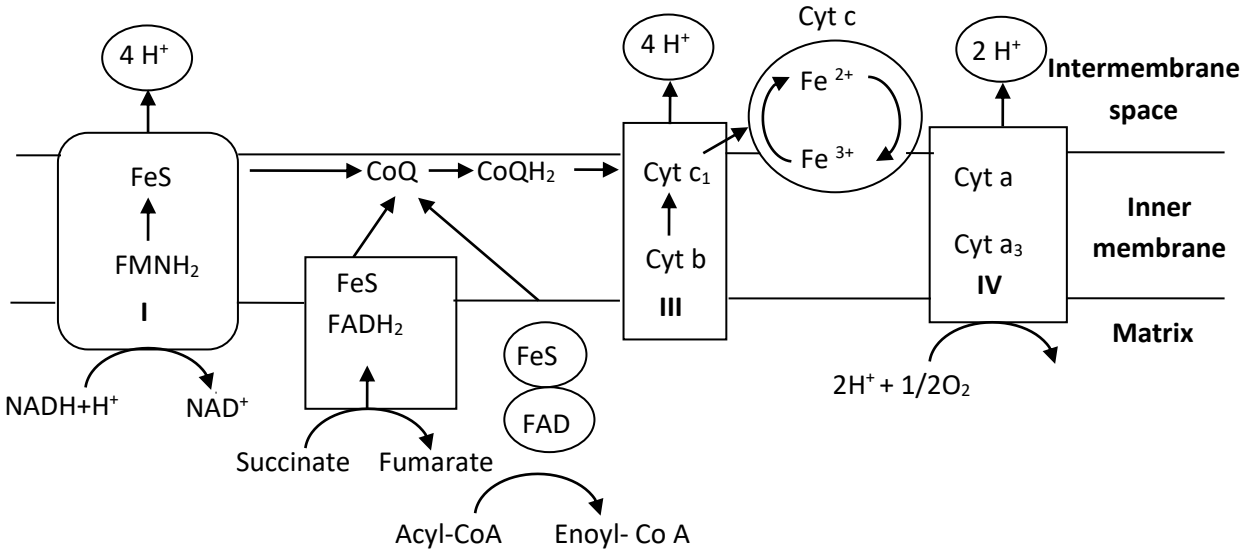
ATP and ADP cannot pass through the mitochondrial membrane and thus they will have to be transferred with the aid of a special protein called **ATP-ADP translocase**, which makes up to 14% of all proteins in the internal mitochondrial membrane. The two molecules can only be transported in a coupled fashion, when one ADP molecule enters the mitochondrial matric, one ATP molecule must exit and vice versa, thus automatically regulating and coupling the cell's energy needs with the energy production. All ADP molecules entering the mitochondrion will be converted into ATP.

Other transporters located along the mitochondrial membrane are presented in Figure 6. By oxidizing one $NADH+H^+$ molecule, $10H^+$ will pass from the matrix into the intermembrane space, while the oxidation of a $FADH_2$ molecule will produce the passing of only $6H^+$.

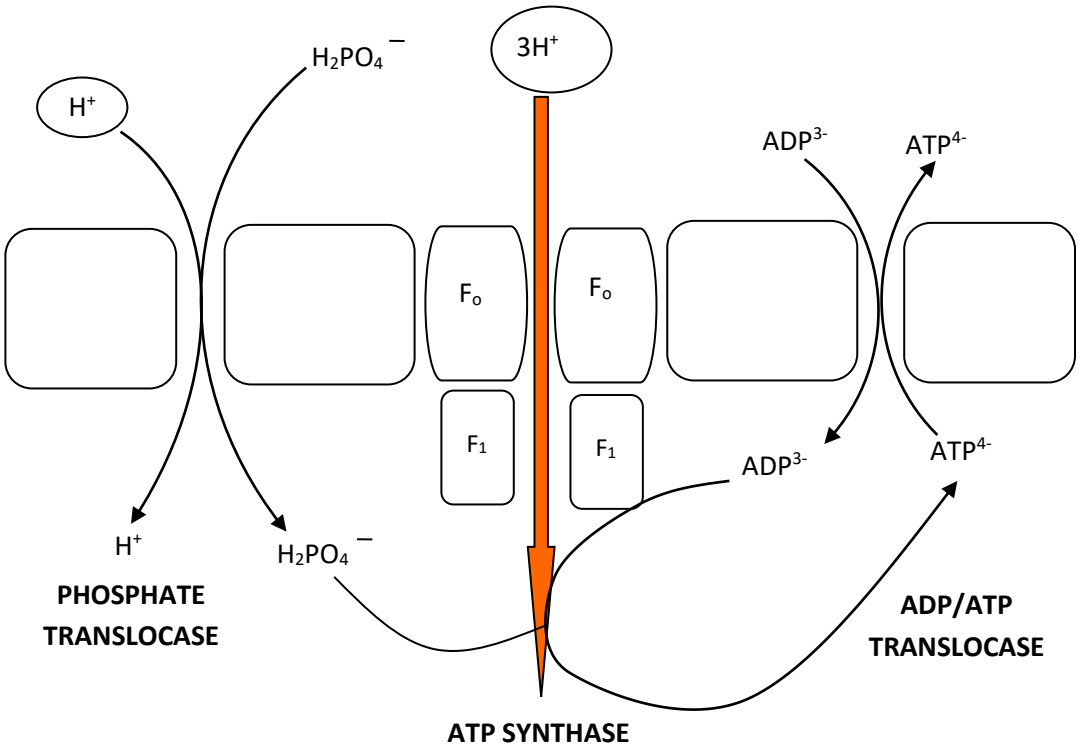


Transporters located in the mitochondrial membrane.

The excess of H^+ obtained via active transport in the intermembrane space will generate an electrical potential similar to the one found in an electric battery. This will be discharged at the level of the ATP-synthase which is an ionic channel in the internal mitochondrial membrane and will open up at a certain level of H^+ potential, when there is sufficient energy to sustain the ADP phosphorylation reaction in order to obtain ATP.



In order to obtain an ATP molecule, 4 H^+ are needed to pass through the internal mitochondrial membrane, of which one is for the translocation of a $H_2PO_4^-$ molecule and three are needed for the phosphorylation of ADP into ATP.



$$NADH+H^+ + \tfrac{1}{2} O_2 + 2{,}5\ ADP + 2{,}5\ P_i \rightarrow NAD^+ + 2{,}5ATP + H_2O$$

$10H^+$ transfer ↓ ; ↑ $2{,}5 \times 4H^+$

$$FADH_2 + \tfrac{1}{2} O_2 + 1{,}5\ ADP + 1{,}5\ P_i \rightarrow FAD + 1{,}5ATP + H_2O$$

$6\ H^+$ transfer ↓ ; ↑ $1{,}5 \times 4H^+$

In conclusion, there are 2.5 ATP molecules obtained by oxidizing one $NADH+H^+$ molecule through the respiratory chain, and 1.5 ATP molecules for each $FADH_2$ molecules oxidized. The flux of electrons in the respiratory chain generates energy, producing a flux of protons across the internal mitochondrial membrane, which in turn will generate an electrochemical potential used for producing the energy needed for the ADP phosphorylation into ATP. The oxygen consumption during the process of hydrogen oxidation with energy release is called cellular respiration.

There are several experimental facts supporting the electrochemical potential theory:

1. ATP will be generated by introducing protons in a suspension of mitochondria.
2. The oxidative phosphorylation does not occur in soluble systems where there is no membrane barrier. The existence of the impermeable mitochondrial membrane enforces an active transport across it through transporters.

3. The agents that uncouple the ADP phosphorylation from oxidation in the respiratory chain such dinitrophenol and valinomycin are ionophore substances which allow the transport of cations through membranes, thus impeding the formation of the electrochemical potential across the membrane.
4. Purified ATP synthase, when incorporated in the artificial membrane of some vesicles is capable of synthesizing ATP when there is an electrochemical potential across the membrane.
5. The purified Fo subunit of ATP synthase, when incorporated in an artificial membrane, **renders the membrane permeable to protons**.

Regulation of the phosphorylation in the respiratory chain

The regulation of cellular respiration intensity (coupled with ATP production) according to the cell's energy needs is called "**respiratory control**". Experimental data shows that if O_2 is available, the intensity of cellular respiration (quantified by O_2 consumption) varies according to the functional state and is correlated with the energy needs of the cell.

$$\mathbf{SH_2 + \frac{1}{2} O_2 + 2{,}5\ ADP + 2{,}5\ P_i \rightarrow S + 2{,}5\ ATP + 3{,}5\ H_2O}$$

From the above equation one can notice that any of the left side terms can be a limiting factor:

- SH_2 – organic substrate that is normally provided
- O_2 – limiting factor only in pathological conditions (hypoxia, anoxia)
- P_i – sufficient
- ADP – is directly proportional with the energy consumption and inversely proportional with the ATP concentration. When the energy needs are low, the ATP concentration will be higher than that of ADP, and vice versa during high energy needs. Given the fact that ADP is the substrate for ATP formation coupled with the respiratory chain, **ADP regulates the intensity of cellular respiration**.

Significance of ADP as regulator of cellular respiration

1. Meeting the energy needs of the cell is not done through ATP storage, but rather through mobilizing chemical energy from substrates (sugars, lipids, proteins).
2. The ATP, ADP, AMP quantity in the human body of approximately 50 grams (1-10 mM/L) is the linking belt through which energy is transmitted from the catabolic reactions that produce energy (e.g. $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$) to the anabolic synthesis reactions that consume energy. In a resting state, the human body synthesizes/hydrolyzes 40kg ATP/24h and during effort up to 0.5kg ATP/minute.

The difference in energy between the quantity obtained in the respiratory chain and the quantity contained in the macroergic bonds of ATP (there is an aprox. 40% efficacy) is released as heat, allowing the respiratory chain to be sufficiently exergonic so that the process is irreversible. In addition, the heat released contributes to maintaining body temperature.

ATP is not an energy deposit, nor energy source, but an element of energy transfer from processes generating energy (oxidation reaction of substrates) to processes that consume energy (movement, transport, syntheses, etc.). Therefore, the actual ATP quantity is very limited and could support the organism only for a few seconds.

Inhibitors of the oxidative phosphorylation in the respiratory chain

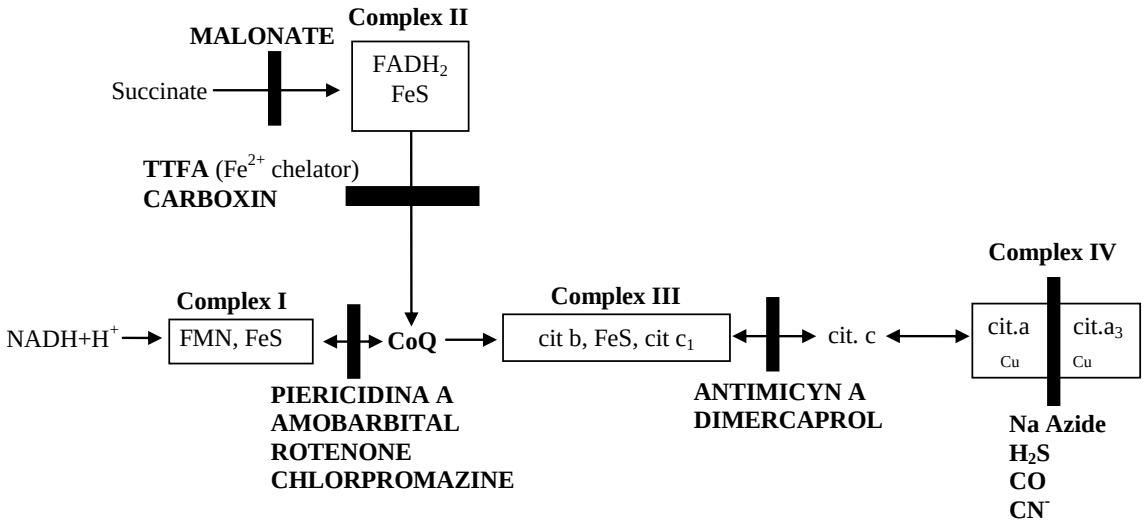
These are substances with inhibitory action on the oxidative phosphorylation in the respiratory chain, acting upon several levels of the process:

1. Uncoupling agents of the oxidation reaction from the ADP phosphorylation

Under the action of these agents, the respiratory chain is able to transport reducing equivalents, but does not generate ATP. The release of energy is associated with secondary lysis reactions, being independent of the ADP control. As a consequence, the substrates are rapidly oxidized, the energy being released as heat.

The uncoupling can be produced *in vitro* by inducing mitochondrial lesions in a suspension through mechanical agitation, low temperatures t^0=20-30^{o}C, hypoosmotic medium, chemical agents with dinitrocresol, DNF, valinomycin or hormones such as thyroxin in excess.

The uncoupling of oxidation from phosphorylation can also be a physiological adaptation such as in the brown adipose tissue of newborns or hibernating animals supporting thermogenesis. The uncoupling is due to a special protein called thermogenin (or uncoupling protein 1-UCP1) located in the internal mitochondrial membrane which makes the membrane permeable for protons, therefore bypassing the ATP synthase. In these conditions, the energy resulted from oxidation reactions will be released as heat, supporting the maintenance of body temperature for newborns or hibernating animals.



2. Respiratory chain inhibitors blocking the electrons transport and oxygen consumption. These have the following action sites:

a. agents that block the step NADH $\rightarrow$ NADH dehydrogenase $\rightarrow$ CoQ such as amobarbital, chlorpromazine, piericidin A, rotenone

b. agents that block the electron transfer between cyt. b $\rightarrow$ cyt. c such as antimycin A, dimercaprol

c. blockers of the step $FADH_2 \rightarrow CoQ$ such as carboxin, TTFA (Fe^{2+}chelator)

d. cytochrome oxidase inhibitors such as H_2S, CO, CN^-, aside.

3. Competitive inhibitors of succinate dehydrogenase such as malonate

4. **Inhibitors of phosphorylation and of oxidation (they block the H^+ transport in the Fo unit of ATP synthase)** such as oligomycin. They do not modify the P/O ratio.

5. Inhibitors of ADP transport in the matrix and of ATP transport in the cytosol such as atractyloside.