7

Bio chemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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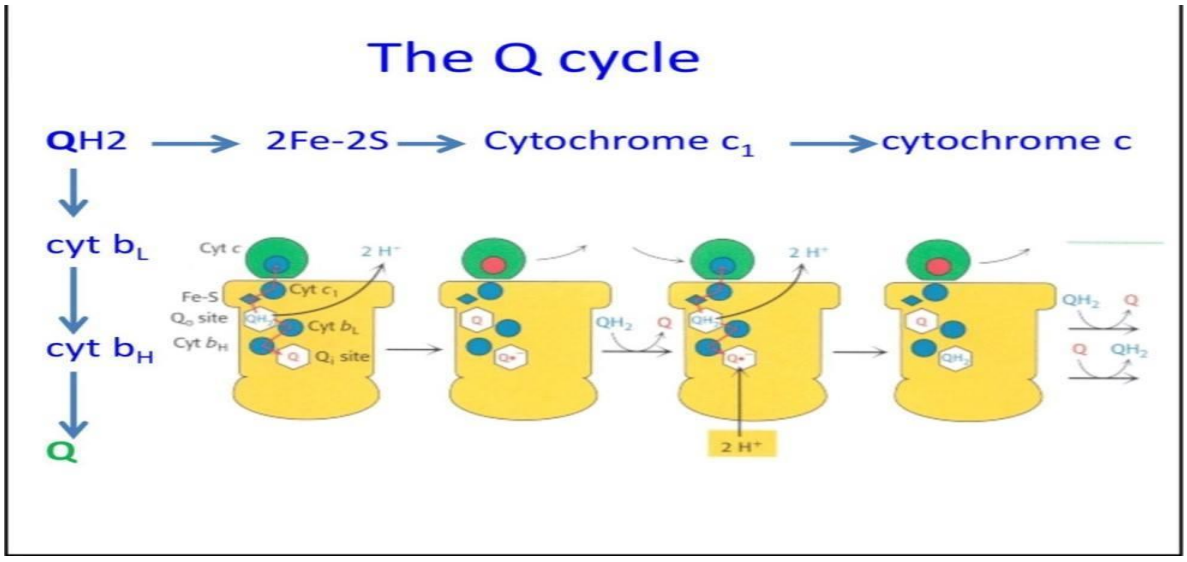
The complement of **cycle Q**

Cycle Q : the picture shows the flow of electrons from QH2 (which can donate 2 e in a form of 2 hydrogen)one to the accepter cytochrome c (which is a mobile carrier that is found in the outer part of inner membrane and accepts 1 e only) and the other to coenzyme Q (accepts 2 electrons) . SO, the 2 e of the QH2 will undergo tow routes :

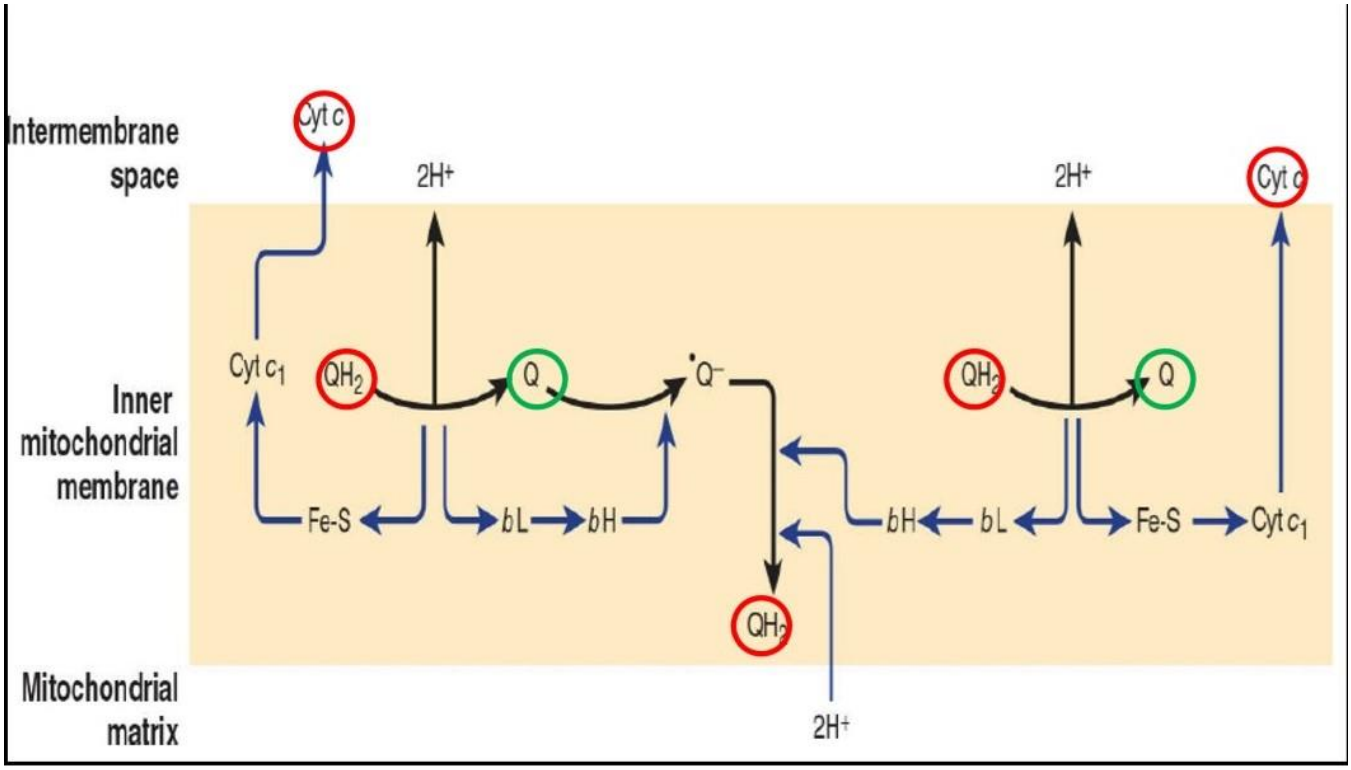
1) the first route is to pass to iron sulfur center then to cytochrome c1 then finally to cytochrome c .

2) the second e cannot pass the same route of the first one ; because of the difference in reduction potential , so it passes through cytochrome b(L) _LOW AFFINITY_ then to cytochrome b(H)_HIGH AFFINITY_ then finally to coenzyme Q .

What exactly happens?! : when one QH2 is used , we will get a reduced cytochrome c BUT **semi reduced** Q= (Q-) (partially reduced because Q need 2 e) so we will have Q-, BUT in order to fully reduce Q it needs 2 electrons so then a second QH2 is used and will undergo the same routes , we will have another cytochrome c and a **fully reduced** Q (accepted 2 electrons = Q-2) and in order for it to become QH2 it takes 2 H+ from the matrix . All of this happens with the help of complex 3 .



This is what's written in slides(The two electrons of a bound QH_2 are transferred, one to cytochrome *c* and the other to a bound Q to form the semiquinone $Q^{\bullet -}$. The newly formed Q dissociates and is replaced by a second QH_2, which also gives up its electrons, one to a second molecule of cytochrome *c* and the other to reduce $Q^{\bullet -}$ to QH_2. This second electron transfer results in the uptake of two protons from the matrix.



Prosthetic groups are shown in their oxidized forms in blue and in their reduced forms in red.)

Cytochrome oxidase (complex 4) : it is an enzyme that passes electrons from cytochrome c to oxygen, it is called oxidase because it binds to O2 and reduce it , thus it has an oxygen binding sites.

We know that cytochrome c is one electron carrier and oxygen require 4 electrons because it must accept 4 e to be reduced to two H2O molecules .

$$4Cytc_{red} + 4H^+ + O_2 \rightarrow 4Cytc_{ox} + 2H_2O$$

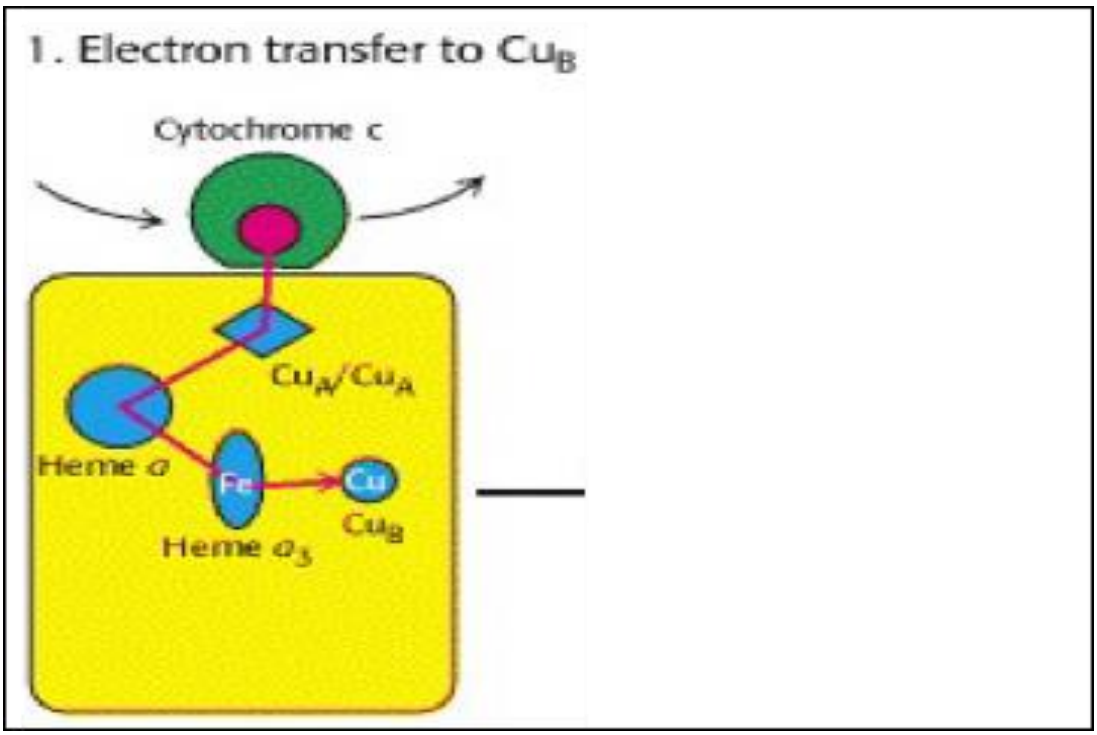
This is the whole reaction but it doesn't show the mechanism of it ,actually each ONE cytochrome c gives its electron one at a time , means that one e is passed at a time . When an electron comes to oxygen it is converted to super oxide molecule (free radical) and then to peroxide (also free radical) and they are strong oxidizing agent that should not be formed , SO partially reduction of o2 is hazardous(dangerous) because it can damage DNA and lipids and so on .

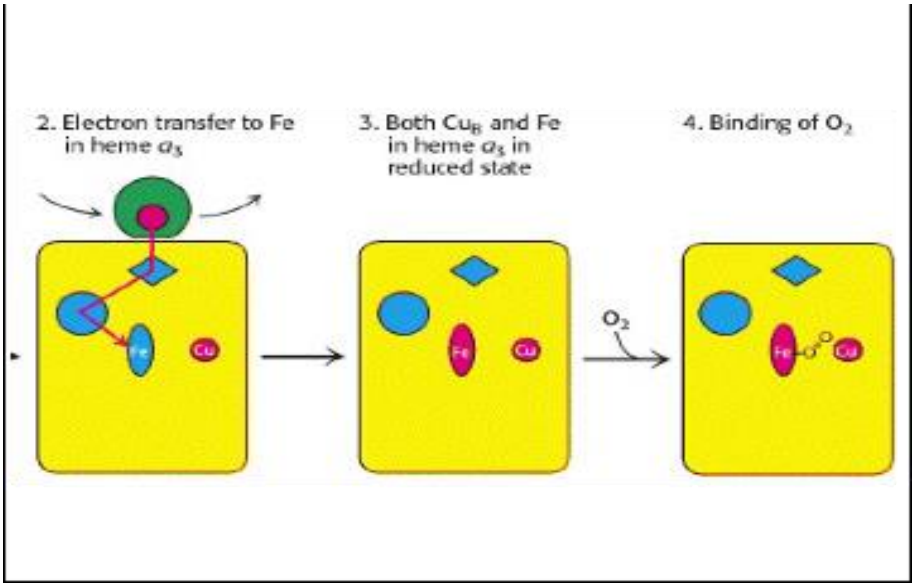
So how does it occur?!

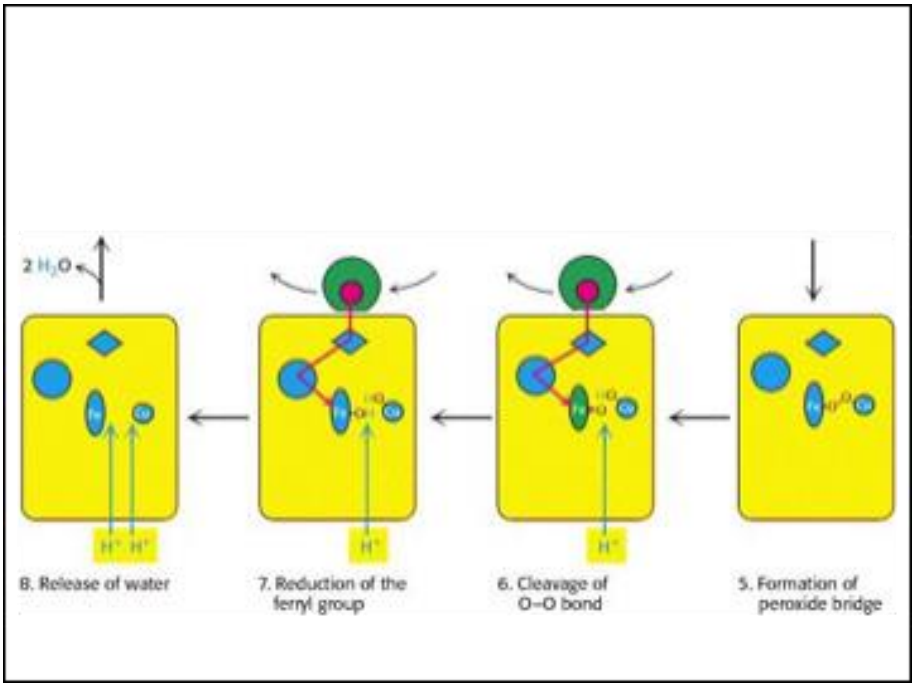
As we said cytochrome oxidase is protein complex , it contains 13 polypeptide chains , two cytochromes (a and a3) , three copper ions in two centers (A , B) ; center A contains two ions and B contains one ion – cuA / cuA cuB - , and oxygen binding sites .

Now , we want to pass electrons at a time without release of partially reduced oxygen .

This picture shows the content of the complex .







1) transfer of electron happens from the first cytochrome c to cu B and each complex reduces the next until we reach cuB

2) transfer of one electron from the second cytochrome c (second one) to the heme a3 and is also reduced

" oxygen cannot bind if the two : heme group a3 and copper are not reduced"

3) both cuB and heme a3 are reduced.

4) only after that o2 can bind , no way it can bind to enzyme until it accept 4 e .

5) now oxygen comes and bind to the binding site between heme a3 and cuB, heme a3 cannot bind oxygen in the oxidized form (if it is ferry , but if it become ferrous , o2 can bind) , peroxide will be formed as bridge because 2e are passed to o2 at the same time , one comes from heme a3 and other from copper , super oxide will not form but peroxide does .

6) the third cytochrome c comes and cleave the o-o bond.

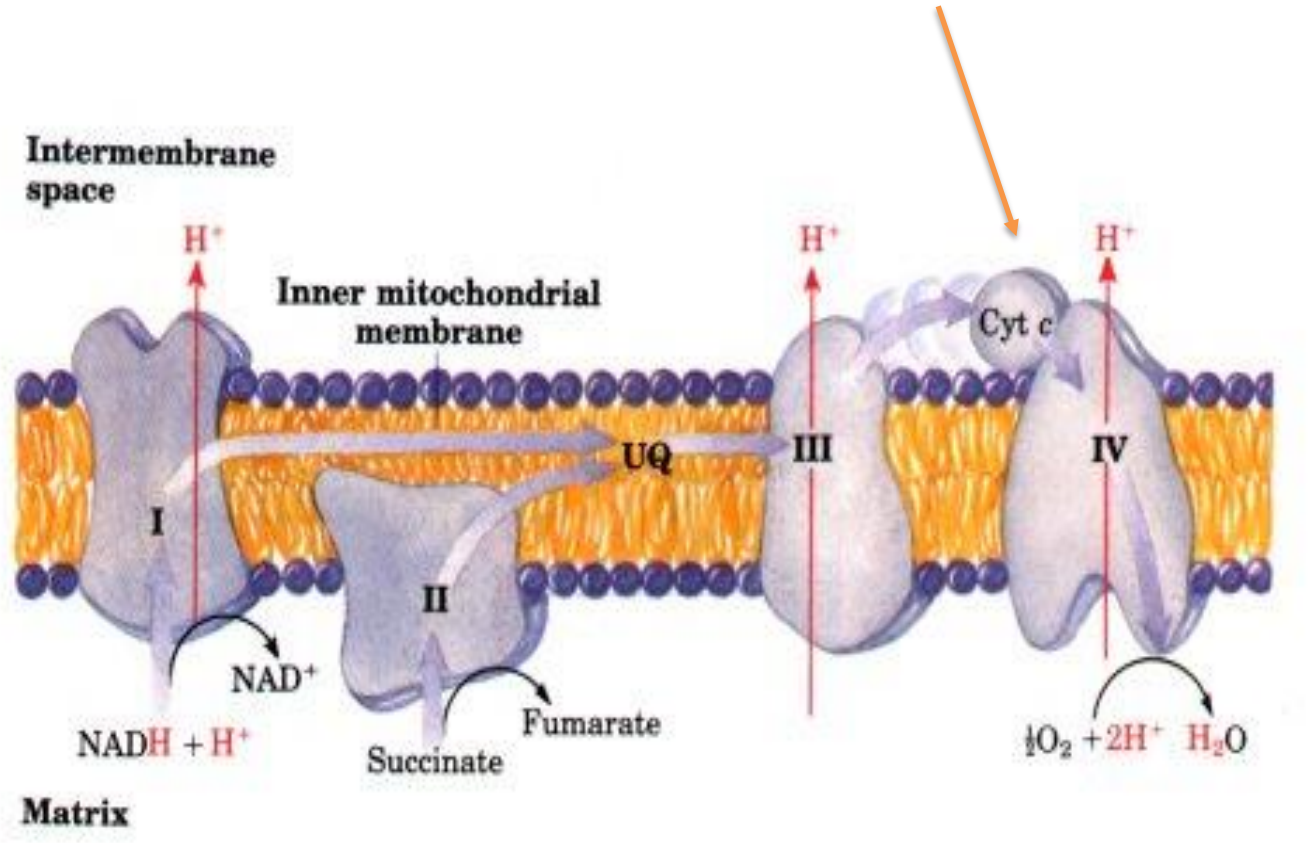
7) reduction of the ferryl group . The main idea here is to know that o2 is reduced by 4e without release of partially reduced o2 ; super oxide . And peroxide will not be released , it will be reduced by the 3rd and 4th cytochrome c .

We have talked about complex 1 ,3 and 4 , now we will talk about complex 2 .

Heme a was in the way and it helps in reducing both cuB and heme a3

Remember that complex 1 takes electrons from NADH and pass them to coenzyme Q , then coenzyme Q pass them to complex 3 then to complex 4 .

Look at cytochrome c , it is small protein ,peripheral part (not integral) and it is part of inner mitochondrial membrane (from the outer side; intermembrane space) so it can move easily over it .



Complex 2 : catalyze the oxidation of succinate to fumarate , so it is called **succinate dehydrogenase** (it takes hydrogen from substrate called succinate and convert it to fumarate).
It is one of the citric acid cycle enzymes.
NOTE: all citric acid cycle enzymes are found in the matrix of mitochondria except succinate dehydrogenase, it is attached to inner mitochondrial membrane.

During converting succinate to fumarate , the complex also reduces FAD to FADH2.

Be careful: that NADH is not formed here! Most of the reactions that involve dehydrogenases they convert NAD+ to NADH but this reaction is different .

Again complex 2 doesn't span the membrane it is just part of it , so it cant pump protons such as complex 1 , 3 and 4 .Thus electrons that are coming from succinate will

not cause pumping of protons as much as that come from NADH , for example , FADH2 will cause pumping of 6 protons whereas NADH will cause pumping of 10 protons . SO, the energy that is coming from oxidation of NADH is higher than the energy is coming from oxidation of FADH2 .

Oxidized + e^-	→Reduced	ΔE^o
Succinate	α ketoglutarate	- 0.67
Acetate	Acetaldehyde	- 0.60
NAD^+	NADH	- 0.32
Acetaldehyde	Ethanol	- 0.20
Pyruvate	Lactate	- 0.19
Fumarate	Succinate	+ 0.03
$Cytochrome^{+3}$	$Cytochrome^{+2}$	+ 0.22
oxygen	**water**	**+ 0.82**

Look at NADH it has higher tendency to give electron than succinate-fumarate .

NADH has more negative reduction potential , it can gives more energy ,if we convert NADH to NAD+ it will give 52 kcal but converting succinate to fumarate will give less than this . (it will not pass complex 1 so it produces less proton pump and less ATP.

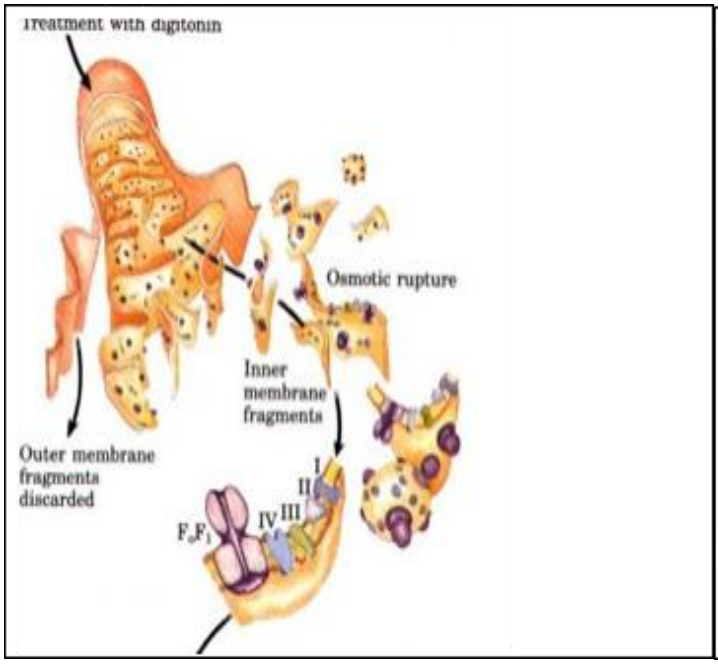
How do biochemists reach to these information ?

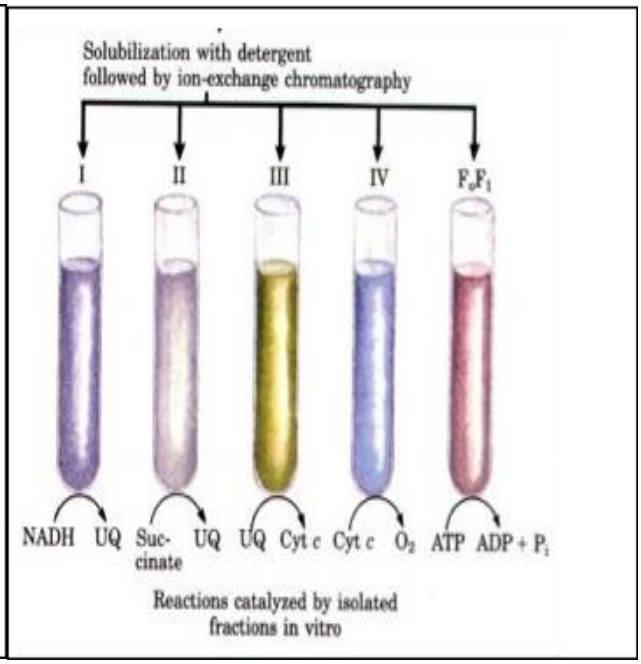
Usually they isolate and purify component of any process , for example , to understand any enzyme , it has to be obtained in pure form and use it to study enzyme´s structure and function alone . For example ,these complexes are part of the inner mitochondrial membrane , so they cannot be separated unless we use detergent to dissolve the membrane and to obtain them , by this mechanism the biochemist were able to rupture the mitochondria , get these 5 complexes and test their function as we mentioned them before .

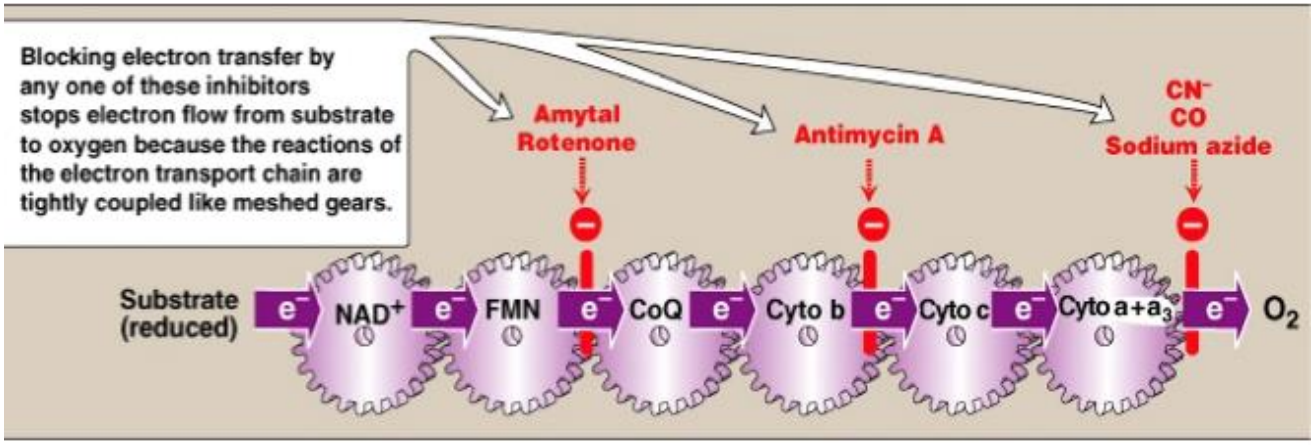
Complex 5 (FoF1) : it was found that this enzyme on its own catalyzes the hydrolysis of ATP into ADP and Pi. How come this happen while we said that it is an ATP synthase !!!

Well , by studying this complex on its own we uncoupled oxidation and phosphorylation , they have been coupled to each other by proton motive force , but here we separate enzymes so no more coupling is there. (In tube 5 so reaction goes toward ATP hydrolysis ; because its favorable and delta G is negative).

The picture shows each complex and their functions when they were separated :





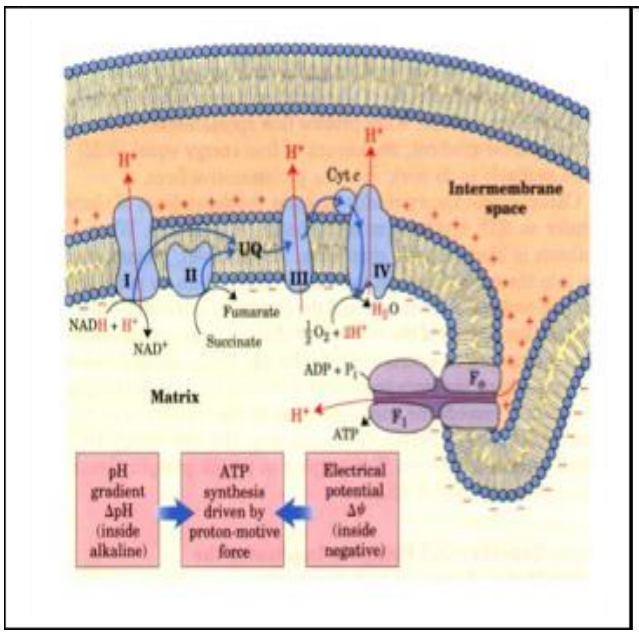


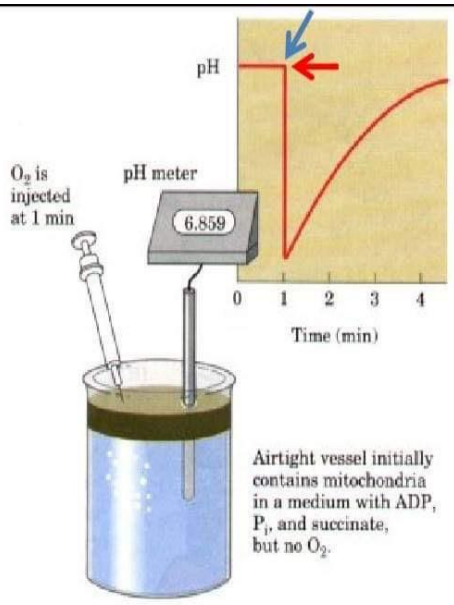
This picture shows the electron transport chain , through this chain there are some substances that inhibit the complexes ; for example, complex 1 is inhibited by Amytal and Rotenone (it is a fish poisoning is used when there is unwanted growth of certain kind of fish). Complex 3 is inhibited by Antimycin A and complex 4 is inhibited by CN- (cyanide ion), CO(carbon monoxide) and sodium azide.

These are inhibitors but WHAT will happen if we don't provide oxygen (if we isolate it and put sample in container without O2) ? the answer is that oxidation will not occur , actually every component will be reduced because there is no electron acceptor . And , if we use inhibitors , all carrier before inhibitor will be in a reduced form and any thing is located after inhibitor will be in an oxidized form . (all these are proved by experiment) . Blocking by any of the inhibitors stops the flow of electron from substrate to oxygen .

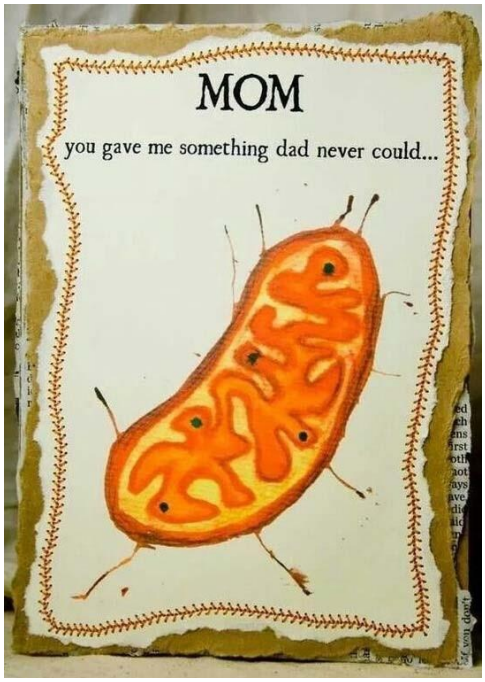
All these inhibitors do the same eventual effect which is prevention of O2 reduction thus preventing ATP synthesis

Remember Hanen and Hani´s story, their father gave them cyanide , and after 15 min they were dead because CN- is an inhibitor of e- transport chain ,thus the production of ATP is inhibited (no energy no life).





There is an experiment on vessel that contain mitochondria , they put the sample inside a container and is covered by a layer that prevent O2 to pass , all these are connected then with PH meter , after 1 min (when oxygen is injected) , PH goes down because the flow of protons ; this experiment shows that oxidative phosphorylation involves pumping of protons. Look at the above picture and study it carefully.



What did your mother give you that your father never could?

Mitochondria is inherited by the mother because ovum contains large number of mitochondria while sperm (gives the nucleus) contains one or two only . So, inherited mitochondrial disease come from mother (maternal) and they appear later on with age because there are many mitochondria not only one .

ATP synthase (complex 5) : it is a large multisubunit enzyme complex which is originally called (mitochondrial ATPáse)and it is called FoF1 ATPáse because it consists from two subunits ; the first one called Fo transmembrane and the second is F1.

Fo transmembrane : it is called (o) because it is inhibited by (oligomycin: it is antibiotic that completely block oxidation and phosphorylation by blocking the flow of protons through ATP synthase), is it a proton channel so it can inhibit the flow of proton , it consists of 2 subunits ; 1 a subunit and 12 c subunits.

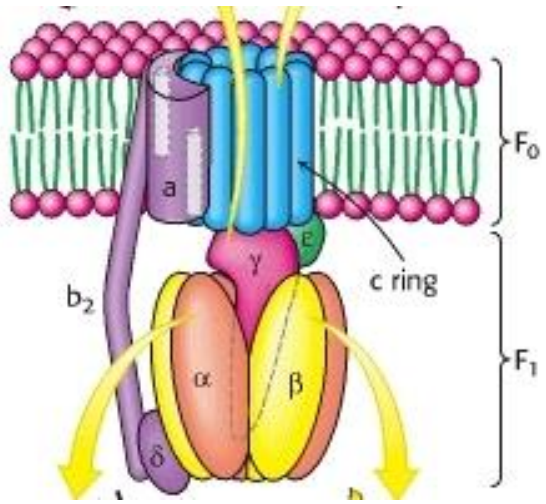
F1 : it is a head piece it has a catalytic activity , so it catalyzes the hydrolysis of ATP to ADP , it consists of 3α ,3β , γ ,δ ,ε . If we remove it , the hydrolysis will be inhibited .

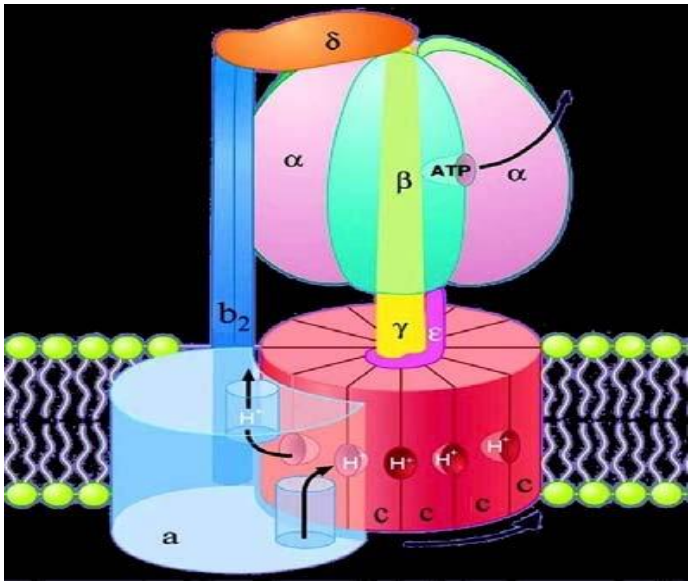
Protons enter Fo , and as they go through it , it will rotate 30° , as they enter from outside to the mitochondria , rotation of a spark occur . actually γ and ε will rotate but the other subunits change their conformation without rotating , Aβ dimers have three different conformations (T tight , L loose and O open).

What makes the protons move from outside to the mitochondria ? Two things :

1) difference in the concentration

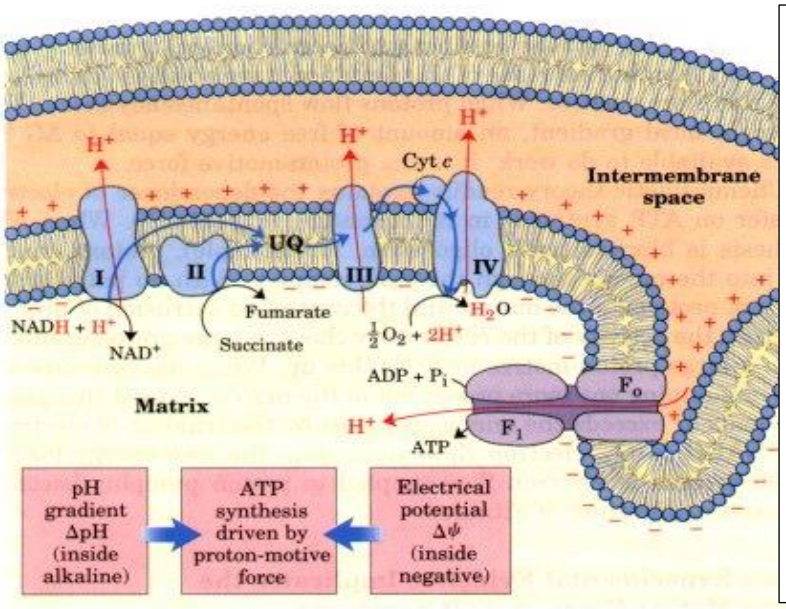
2) the channel (Fo) it is half-opened to the outside and mitochondria so they can pass easily .





The proton channel depends on both the **a** subunit and the **c** ring .The F_0 and F_1 subunits are connected in two ways:
- central γε stalk
- an exterior column

from the slide

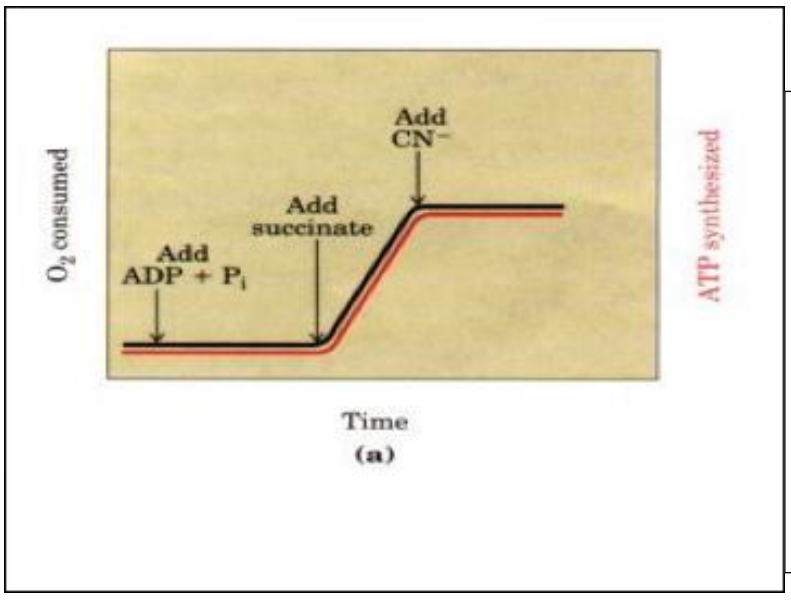


Oxidation and phosphorylation are tightly coupled and this will control oxidative phosphorylation .

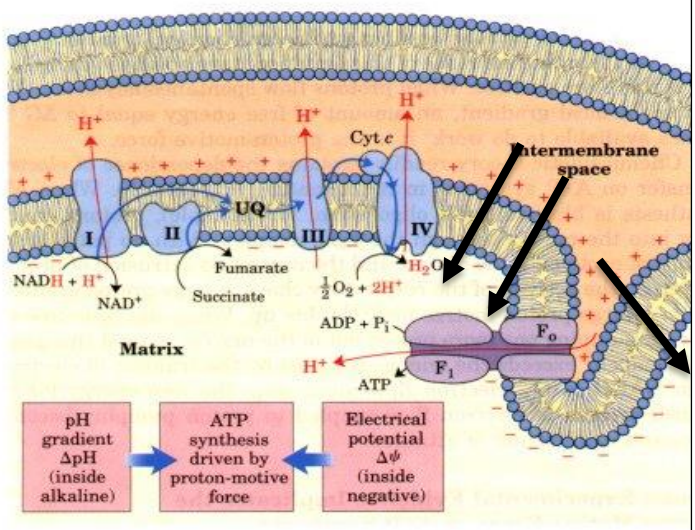
If cell doesn't use ATP , this will stop oxidation and stop all enzymes that produce NADH , because no more NAD+ to be converted . this is called respiratory control (by ATP)

Look at o2 consumption at the beginning; it is low but after we add ADP it is increased until all ADP are converted to ATP , then it will come back as beginning (horizontal line).

Oxidative phosphorylation requires (components) : source of electrons (NADH OR succinate) , ADP , Pi and O2 . If we remove any one of them the reaction will not occur .



As you see here if we add succinate it will give the same effect of ADP(increase consumption of o2) **but** adding CN- will stop the consumption .



What will happen if we allow the leakage of proton back into the matrix ?

We already know that oxidation and phosphorylation are coupled to each other but when protons leak(through pores); no phosphorylation will occur , but oxidation will because it is coupled with proton pumping , so when proton pumping is continued , oxidation is continued without phosphorylation (means coupling between oxidation and phosphorylation stops) .

SO, synthesizing of ATP require oxidation and the inner membrane to be intact , of there is any rupture for any reason ; oxidation will continue but phosphorylation will not because there is NO force generated by return of proton .

Chemical uncouplers of oxidative phosphorylation :

1) lipid soluble compounds (substances soluble in the inner mitochondrial membrane because it is hydrophobic and non-polar , at the same time they have an acidic or basic group and they are soluble in the inner mitochondrial membrane .

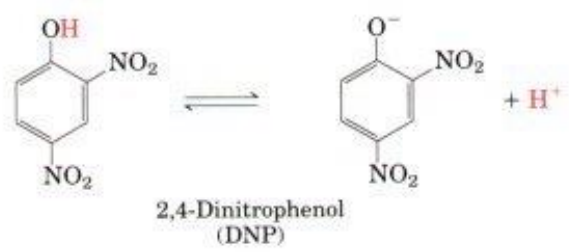
How does these uncouplers work ?!

For example : 2,4 dinitrophenol it is lipid soluble and it can alternate between HA and A- , this comes to the intermembrane space where proton concentration is high (the reaction goes in the reverse direction). And in the matrix the proton concentration is low so it goes in the forward direction.

So proton concentration in the matrix will be low and in the intermembrane space will be high ; so proton concentration determines the direction of reaction .

why is this considered as uncoupler ? because oxidation will occur but phosphorylation will not ;because there is no proton gradient .

$$HA \rightleftharpoons A^- + H^+$$



2,4-Dinitrophenol
(DNP)

When we put Dinitrophenol :

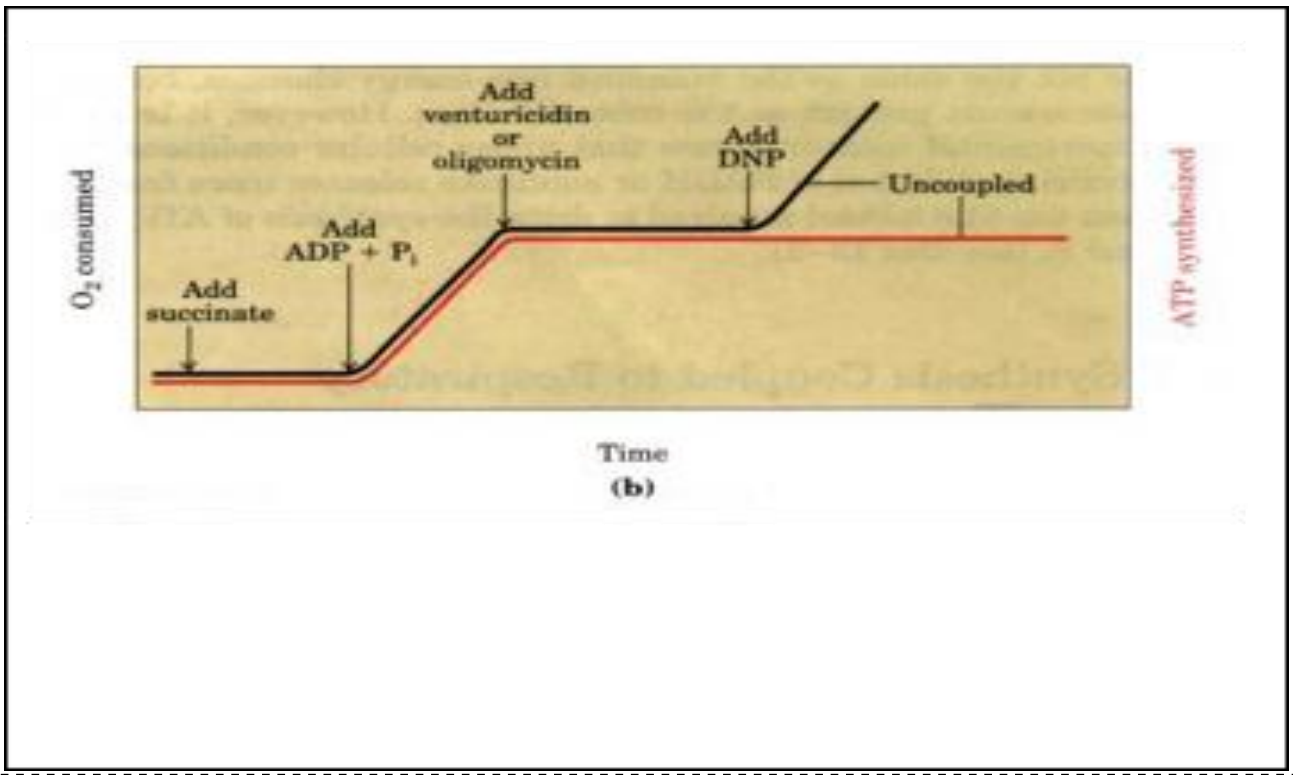
Oxidation occurs

Phosphorylation doesn´t occur

2) Pores : they can rapid the transport of protons across membrane .

Important note : as we said before oxidation and phosphorylation are coupled together and one cant happen without the other , thus it might be confusing how oxidation occur on its own when we do a pore in the membrane .

The answer is that they cannot happen without each other as long as they are coupled , once we uncouple them (as in rupturing membrane) oxidation happens on its own since it is already a favorable and it is coupled to proton pump .



In this graph it shows what happens to oxidation (blue line),phosphorylation (red line) ,we added succinate and ADP which both increase oxidation and phosphorylation , and we added oligomycin so it stops. When we add dinitrophenol (uncoupler) oxidation continues while phosphorylation stops .

Uncoupling protein and thermogenesis :

They are physiological proteins their function is to allow the uncoupling , thus oxidation to continue and phosphorylation to stop .

Where does the energy go ?

It will be released as heat

Also , they are channels in the inner membrane that conduct protons from the intermembrane space to the matrix .

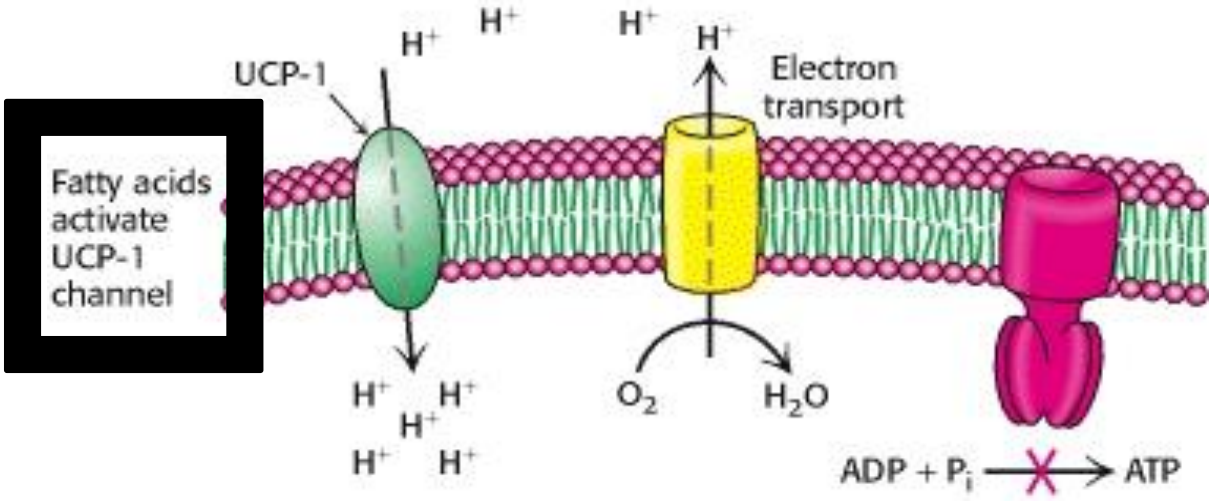
Types of them : UCP1 , UCP2 , UCP3 ,4 ,5 .

Example; UCP1 : thermogenin , it is heat generator , it´s especially needed in newborn (in brown adipose tissue which are rich in mitochondria and thermogenin).

UCP2,UCP3,4,5 are found in other tissues .

<u>Small note:</u> what gives the mitochondria it´s brown color ?

It´s the cytochrome (colored protein).



OxPhos Diseases

- DNA in mitochondria mtDNA encodes 13 subunits of comlexes I, III and IV
- High rate of mutations (10x nuclear DNA)
- Mutations → Defect in oxidative phosphorylation.
  - Tissues with highest ATP demands affected most
- Maternal inheritance
- Accumulation of somatic mutations with age
- Examples:- Leber's hereditary optic neuropathy
  -myoclonic epilepsy and ragged-red fiber disease (MERRF)

In this slide , the doctor said we should read it by ourselves

The end