A study material for M.Sc. Biochemistry (Semester: II) Students on the topic

(CC-6; Unit I)

Oxidative Phosphorylation

And

Electron Transport System

The use of Oxygen in Cells to produce ATP

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Phosphorylation is a biochemical process that involves the addition of phosphate to an organic compound. Examples include the addition of phosphate to Adenosine Di-phosphate (ADP) to form Adenosine Triphosphate (ATP).

Phosphorylation is of three types:

1. **Photo- Phosphorylation** – ATP formed through a series of sunlight-driven reactions in phototrophic organisms
2. **Oxidative Phosphorylation** - ATP formed through a series of redox reactions occurring during the final phase of the respiratory pathway
3. **Substrate Level Phosphorylation** – Transfer of Phosphate molecule directly from the substrate

Oxidative Phosphorylation is oxygen dependent phosphorylation. The site of Oxidative Phosphorylation is Mitochondria. The elements involved in this process are embedded in the Inner Mitochondrial membrane. Actually, several redox reactions take place during different metabolic pathways inside the cell and in those processes there is always an electron donor molecule and one electron acceptor molecule. Sometimes the electrons are donated by different substrates (during their oxidation) and in those cases different electron carriers (such as NAD^+/ $NADP^+$ or FAD^+) become reduced and they play the role of electron acceptors. They convert into:

NAD^+/ $NADP^+$ + $2e^-$ + $2H^+$ ----------→ $NADH+H^+$ / $NADPH+H^+$

$FAD^+ + 2e^- + 2H^+$ ----------→ $FADH_2$

Now, they have to oxidize again so that they can serve the purpose of electron acceptor in different metabolic pathways, so that the pathway may remain operative. This oxidation takes place inside the mitochondria with the help of oxygen. The reduced $NADH+H^+$ or $FADH_2$ transfers electrons to oxygen (O_2) to convert it into water molecule (H_2O). Since $NADH+H^+$ oxidizes at redox potential (E_0) of -0.32 V and O_2 reduces at redox potential of +0.82 V. This gap of +1.14 V is too much and hence $NADH+H^+$ or $FADH_2$ does not directly combine with O_2. To facilitate this transfer many intermediate electron carriers (having intermediate redox potentials) are arranged in a series which transport electrons from $NADH+H^+$ or $FADH_2$ to O_2. This sequence of electron carriers constitutes Electron Transport System (ETS).

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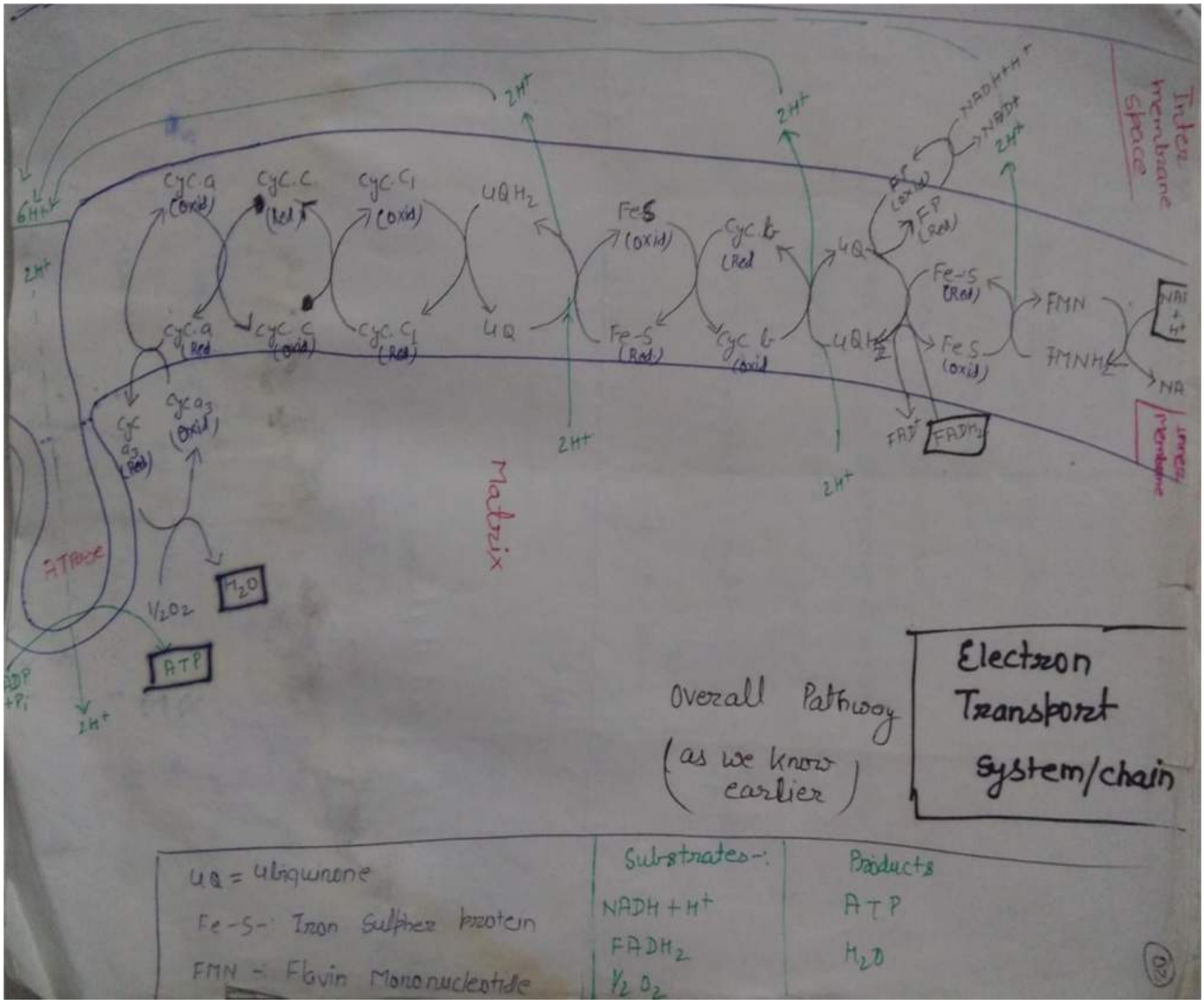
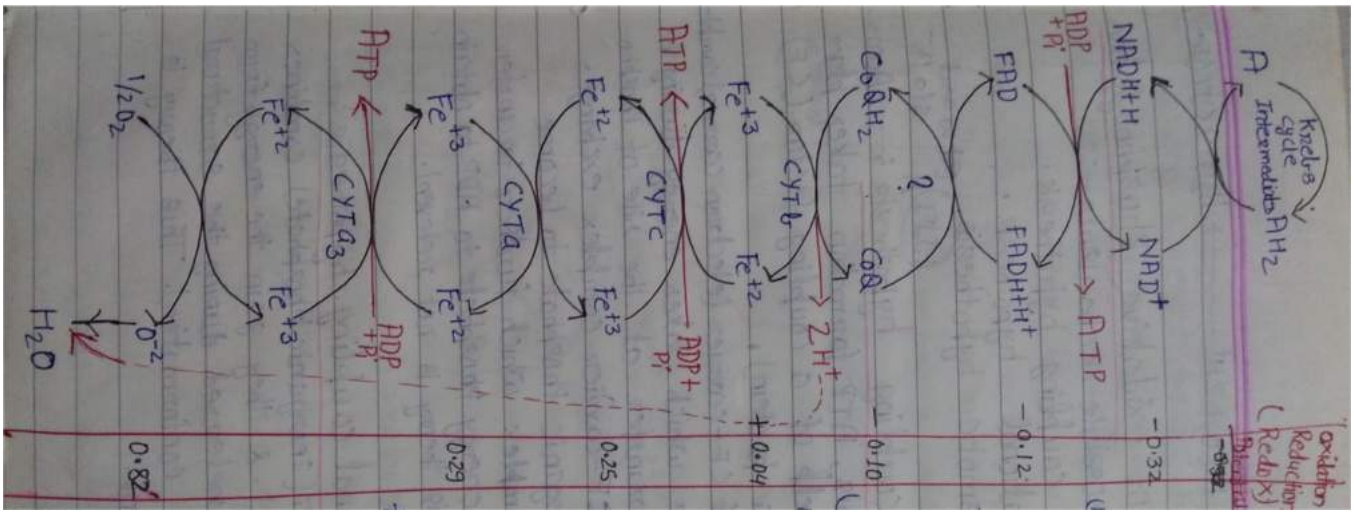
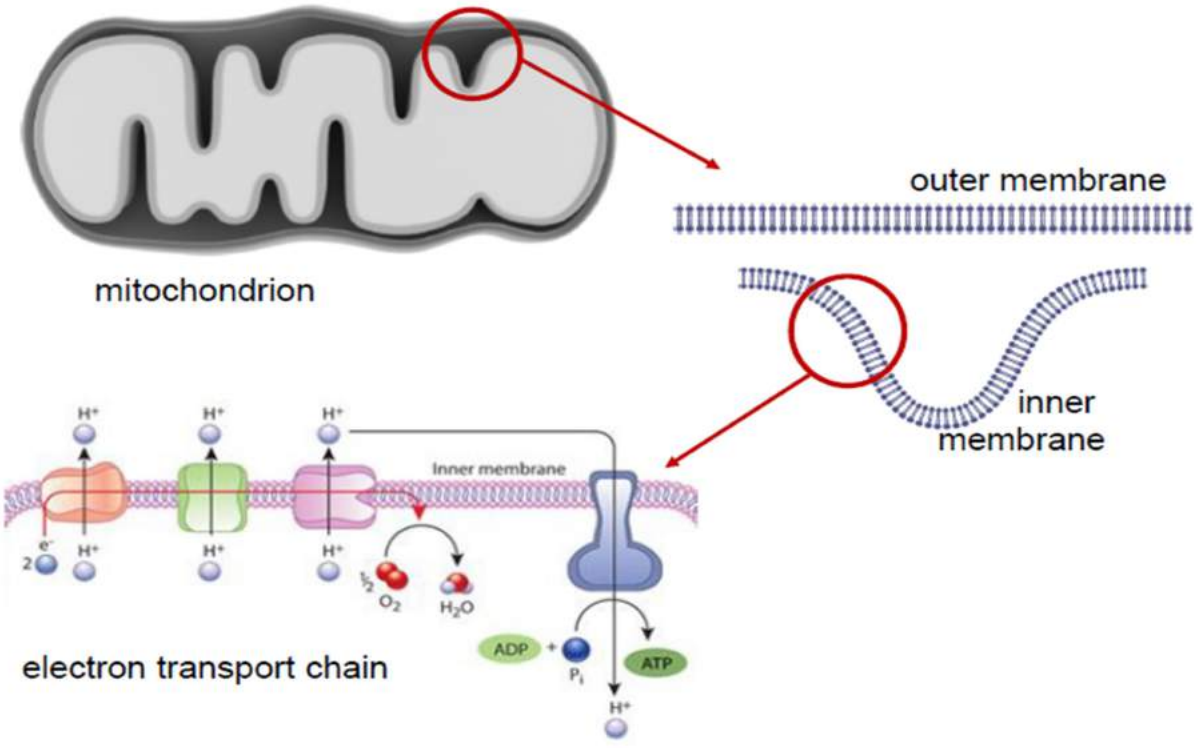




Redox potentials of different components of ETS.

Several thousand ETS are located in each mitochondrium in their inner mitochondrial membrane. You may remember from previous studies that the inner mitochondrial membrane is folded; it is just because to increase its surface area, so that it can accommodate much number of such ETS components. Electron transports from low redox potential carriers to high redox potential carriers along the ETS.



(Image Source: Google)

The electron transport down to the energy gradient through electron transport system leads to the formation of ATP from ADP and Pi (inorganic Phosphate). This generation of ATP is called Oxidative Phosphorylation.

Now, for this generation of ATP, the $NADH+H^+$ or $FADH_2$ have to gather inside the mitochondrial matrix. There are two separate pools of $NADH+H^+$ in the cell: Cytoplasmic pool and mitochondrial pool. This is because $NADH+H^+$ is a large molecule and it is unable to cross the inner mitochondrial membrane (IMM). There is also no transporter of $NADH+H^+$ in IMM. Hence it has to choose another way to transfer its electrons and protons inside the mitochondrial pool of $NADH+H^{+.}$ Malate aspartate shuttle serves this purpose:

ıalate- Aspartate shuttle for NADH+H⁺ Transport

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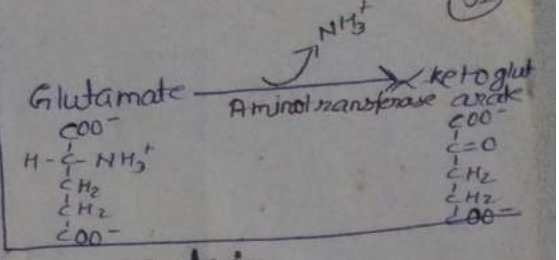
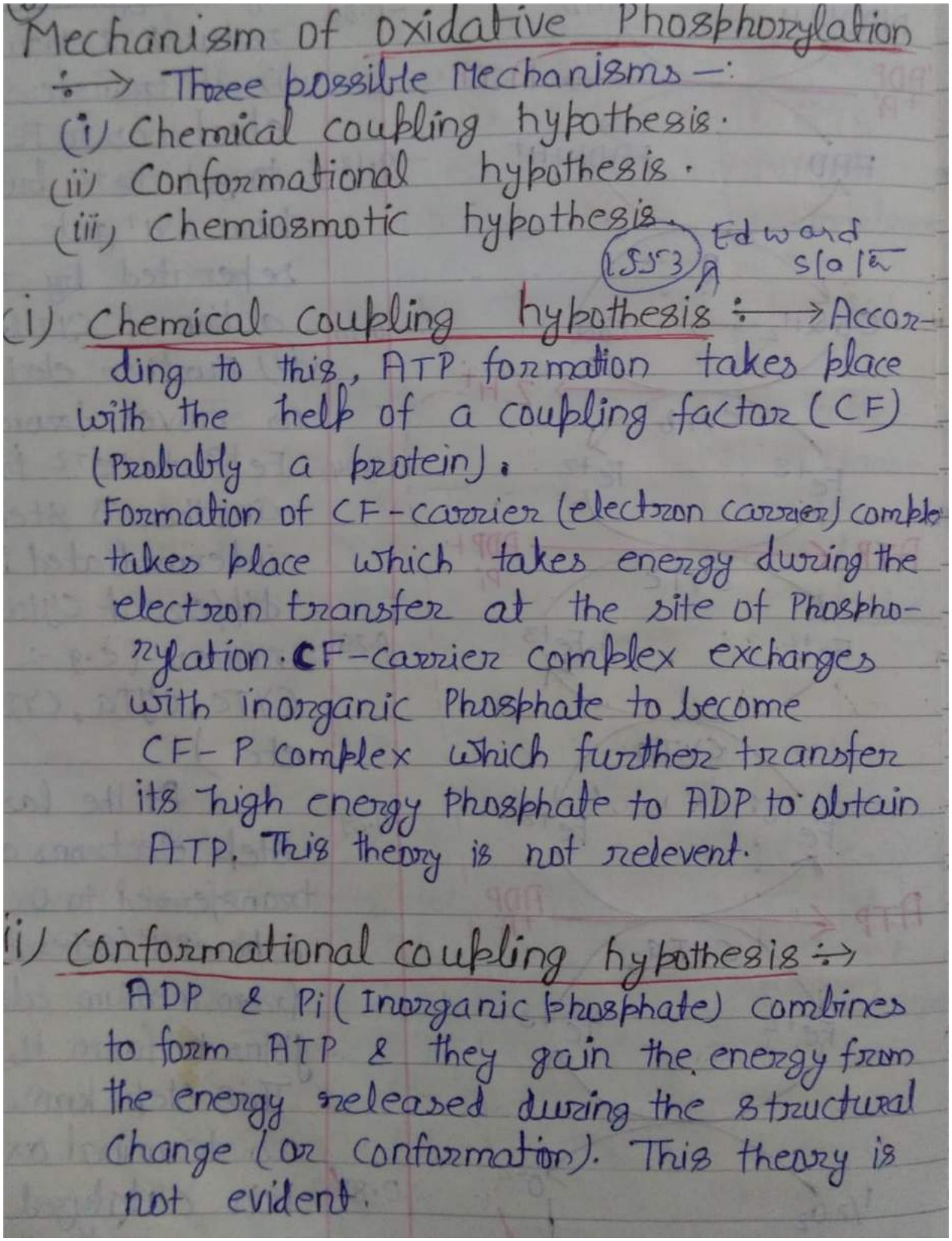


Note: The cell has two seperate pools of NADH+H⁺ (cytoplasmic Pool & Mitochondrial Pool). NADH+H⁺ is a larger molecule and it is unable to cross membrane (mitochondrial) But for ATP production and oxidation of NADH+H⁺ into NAD⁺ it has to cross the membrane

Hence for this purpose, NADH+H⁺ selects this shuttle pathway and transfer its electron & Proton inside the mitochondrial Matrix

Now, there are three separate hypothesis regarding Oxidative Phosphorylation. They are:



Mechanism of oxidative Phosphorylation

∴ → Three possible Mechanisms —:

(i) Chemical coupling hypothesis.

(ii) Conformational hypothesis.

(iii) Chemiosmotic hypothesis. (1953) Edward slater

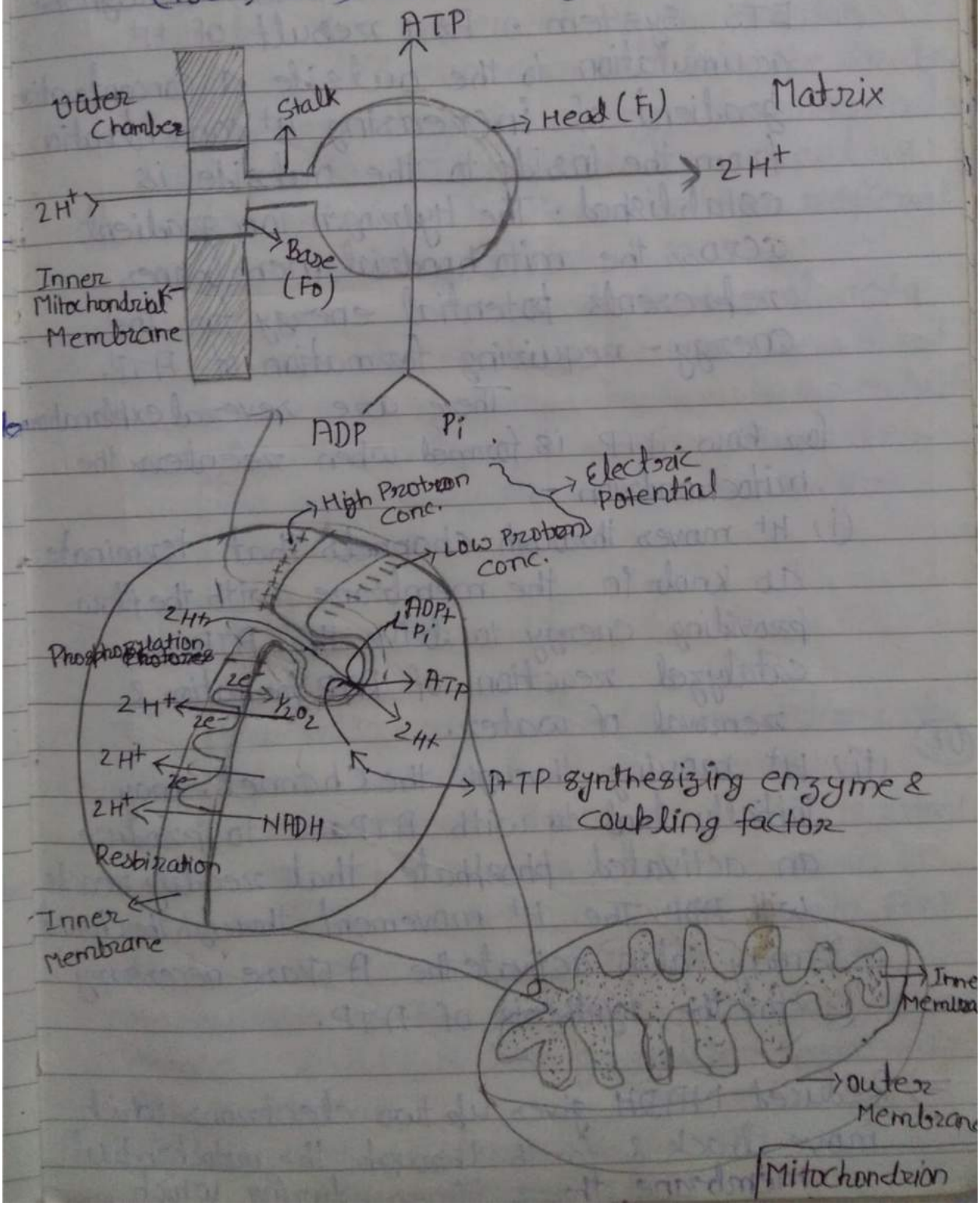
(i) Chemical coupling hypothesis ∴ → According to this, ATP formation takes place with the help of a coupling factor (CF) (Probably a protein).

Formation of CF-carrier (electron carrier) comple takes place which takes energy during the electron transfer at the site of Phosphorylation. CF-carrier complex exchanges with inorganic Phosphate to become CF-P complex which further transfer its high energy Phosphate to ADP to obtain ATP. This theory is not relevent.

(ii) Conformational coupling hypothesis ∴ →

ADP & Pi (Inorganic Phosphate) combines to form ATP & they gain the energy from the energy released during the structural change (or conformation). This theory is not evident.

(iii) Chemiosmotic Coupling Hypothesis :→
widely accepted theory proposed by a British biochemist Peter Mitchell (1961, 1966). According to it :-



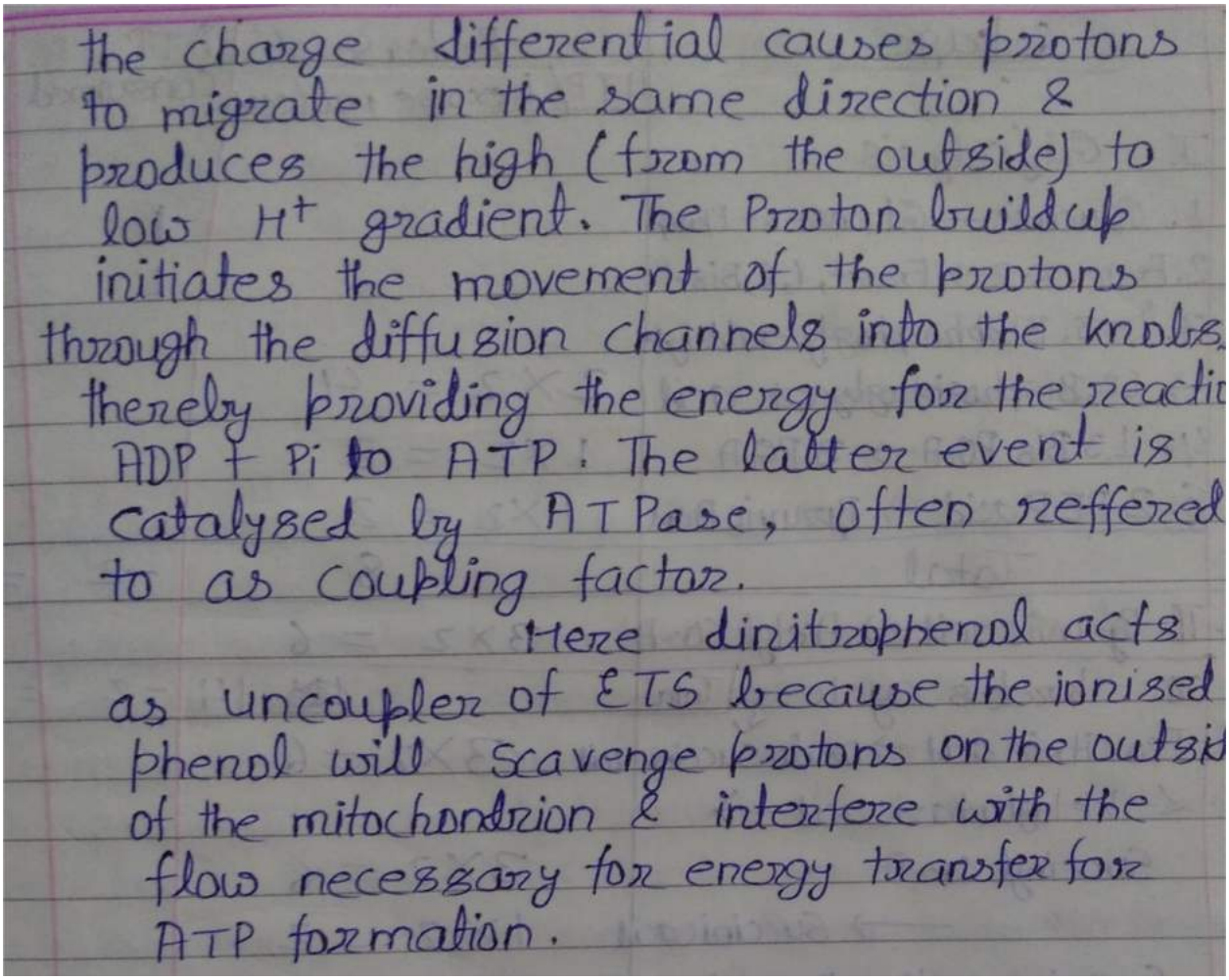
Active mitochondrion releases Hydrogen ions (H^+) to the outside & that the driving force for the proton displacement comes from the flow of electrons through the ETS system. As a result of H^+ accumulation to the outside a concentration gradient of increasing H^+ concentration from the inside to the outside is established. The Hydrogen ion gradient across the mitochondrial membranes represents potential energy for the energy-requiring formation of ATP.

There are several explanation for how ATP is formed when reenters the mitochondrion -:

(i) H^+ moves through channels that terminate as knob in the membrane with the flow providing energy to drive the ATPase catalyzed reaction of ATP formation & removal of water.

(ii) H^+ moving through the Channel, may initially bind ~~to~~ with ATPase to produce an activated phosphate that readily reacts with ADP. The H^+ movement through the channel may also activate the ATPase necessary for the synthesis of ATP.

⇒ Reduced NADH gives up two electrons which move back & forth through the mitochondrial membrane three times, during which

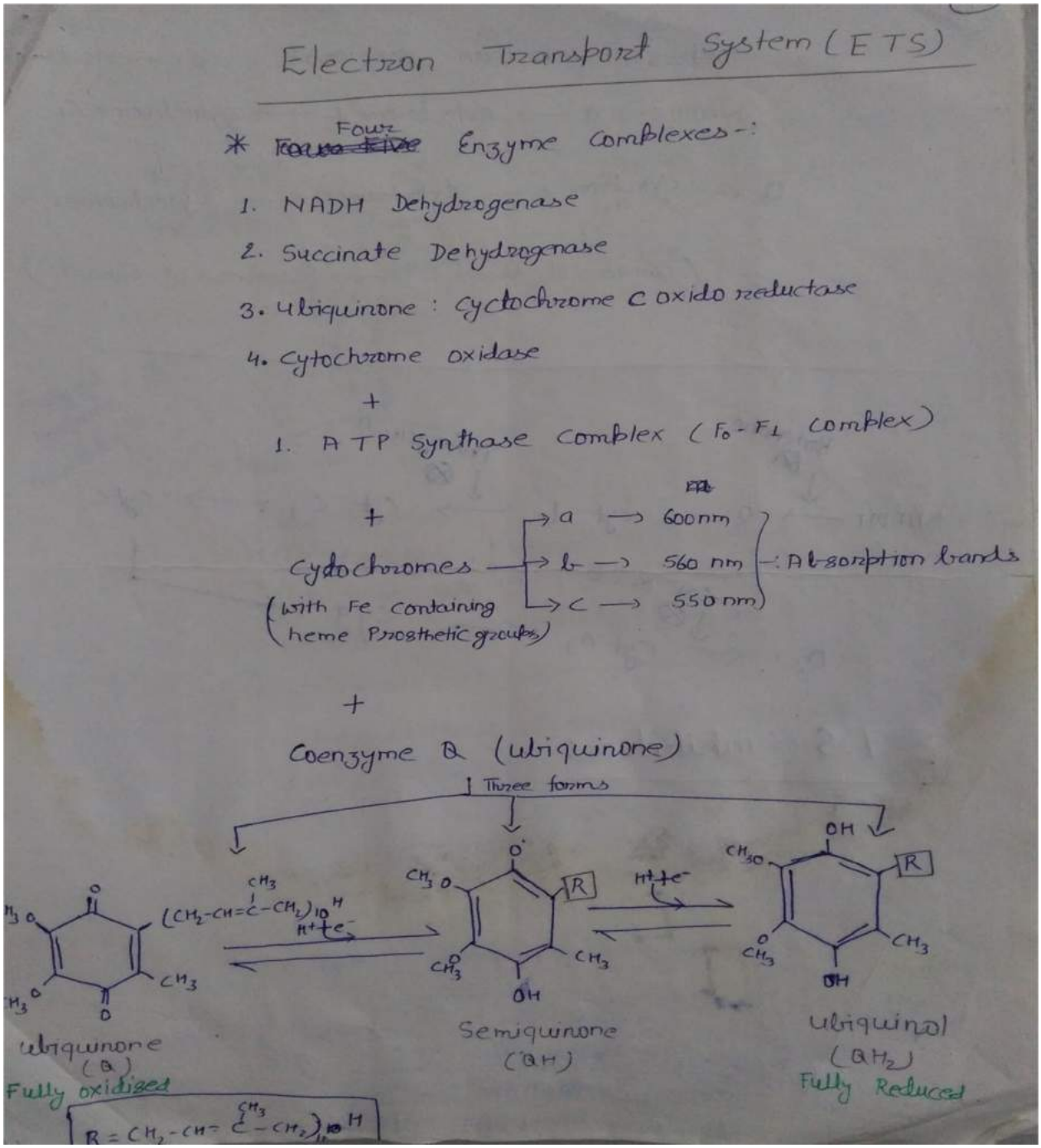


Components of Electron Transport System and their role in Oxidative Phosphorylation:

There are 5 different protein complexes are involved in Oxidative Phosphorylation. Four out of the five components (Complex I-IV) are involved in transfer of protons (H^+) from Matrix side to Inter-membrane Space (IMS) side. This transfer is against the concentration gradient. The required energy for this transfer is recovered from the energy liberated during the flow of electrons along with the components of ETS. Hence, both the electron transfer and Proton transport across the membrane are associated.

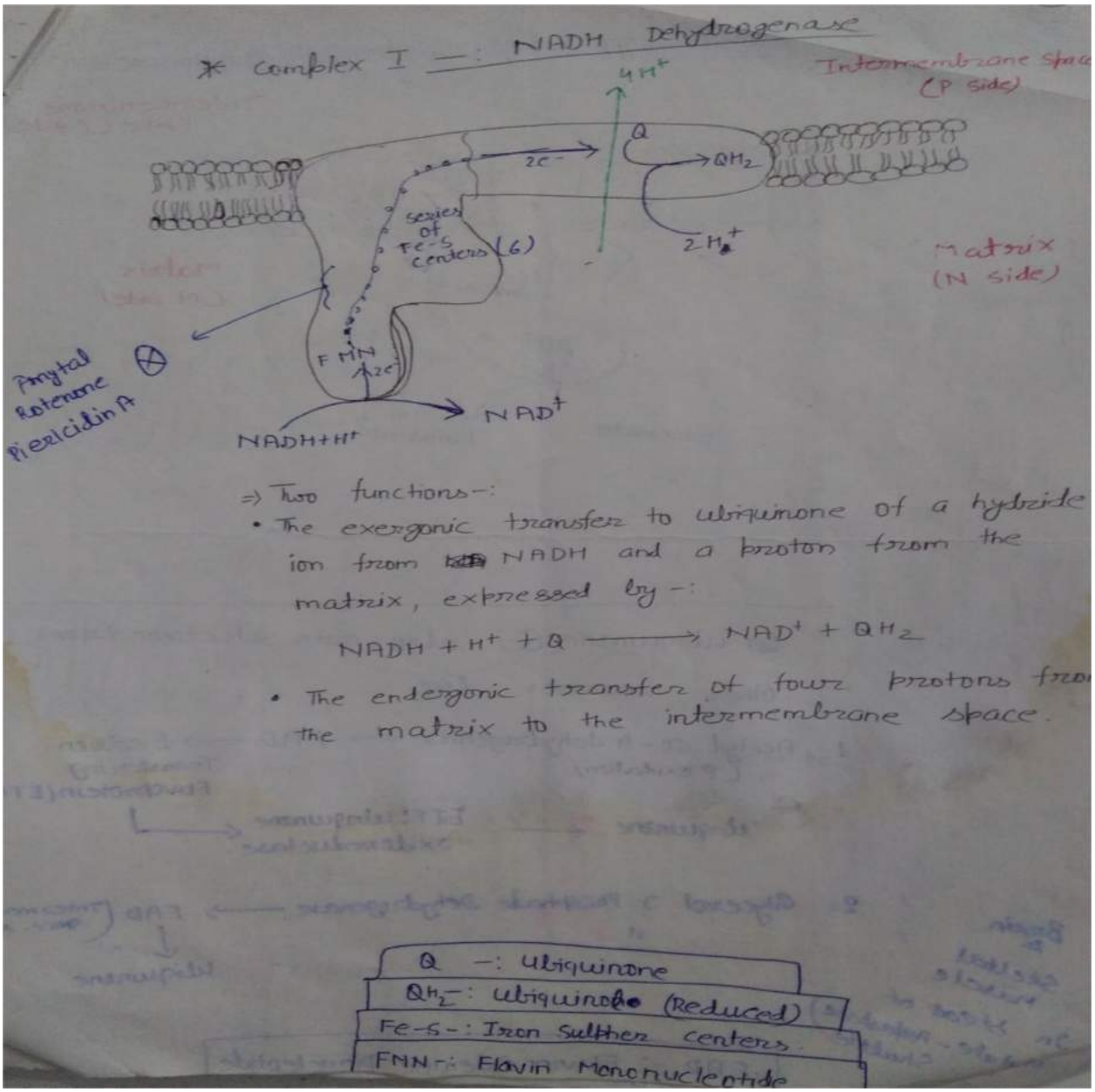
The electron transport system has different electron carriers. These electron carriers are basically Iron Sulphur (Fe-S) Centers. Some are cytochromes (cyt. a, cyt. b, cyt. c) which have Iron containing prosthetic groups. They have difference on the basis of their light absorption spectra. Complex IV has two Copper (Cu) centers too (Cu A and Cu B), they are more energy efficient.

There is a mobile electron carrier in the ETS too; it is Coenzyme - Q known as Ubiquinone. This is found in three different forms: Ubiquinone (Q), Semi-Quinone (QH) and Ubiquinol (QH_2). The first two complexes of ETS (i.e., Complex I and Complex II) convert Q into QH_2, while the QH_2 convers back into Q in the third complex (Complex III). Complex III which operates Q cycle also generates QH_2 from Q. but it is done in two different steps.

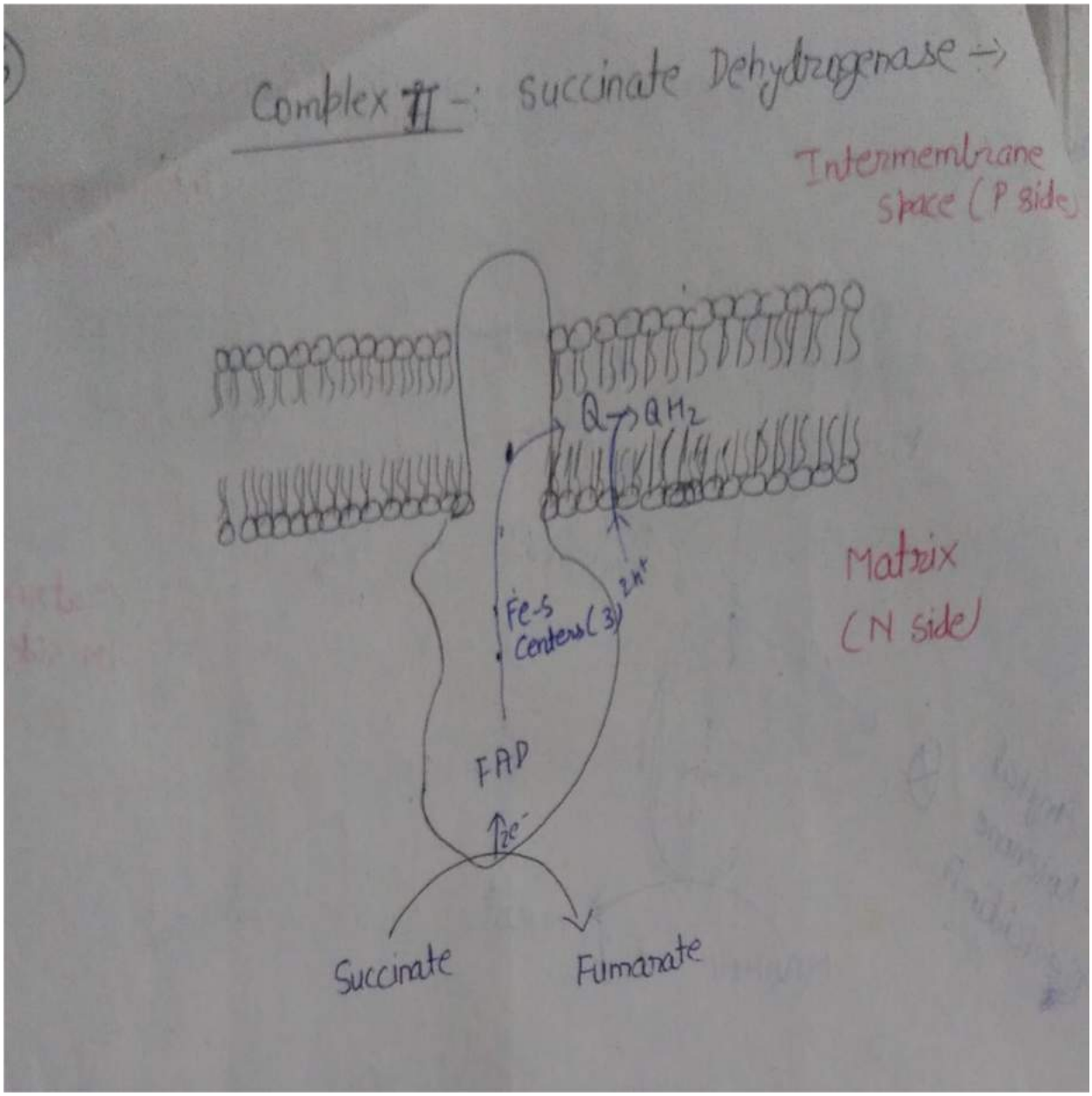


Now let us see different components separately:

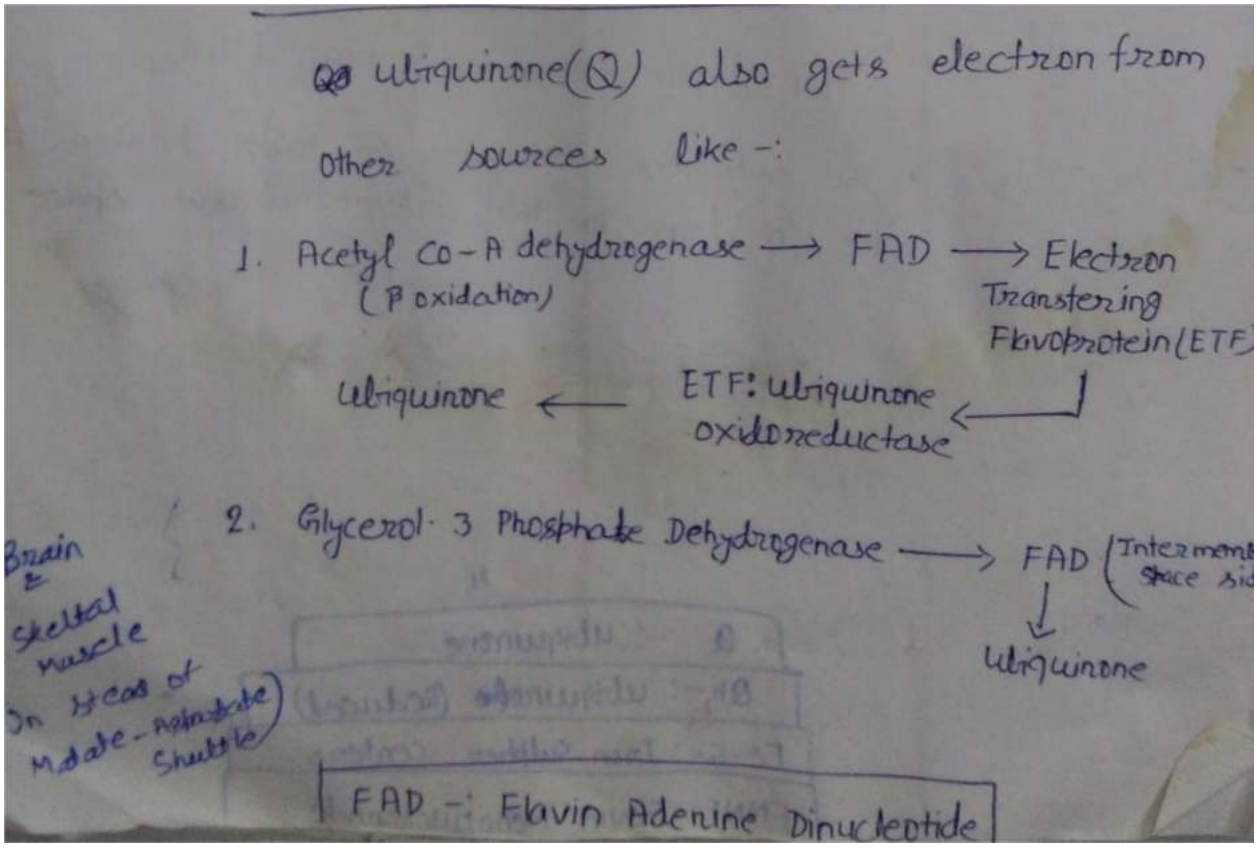
1. **Complex I: NADH dehydrogenase**-: $NADH+H^+$ donate its electrons to this complex. Protons of NADH are released in the matrix, while electrons are accepted by Flavin Adenine Mononucleotide (FMN). Now, these electrons are transferred to Q through a series of 6 Iron Sulphur Centers. Energy is released during this electron transfer who helps 4 proton ions to cross the Inner Mitochondrial Membrane through the Complex I (as the complex is a trans-membrane protein). The electrons are accepted by Q and it takes two protons from the matrix and it converts into QH_2. This QH_2 now travels to Complex III for its recycling into Q.



2. **Complex II: Succinate Dehydrogenase**:- This is embedded protein complex on the matrix side. It is not trans-membrane, hence it is unable to transport protons from matrix to IMS but it receives electrons from $FADH_2$ and converts Q into QH_2. $FADH_2$ donates its electrons to Flavin Adenine Dinucleotide (FAD) of Complex II and releases its protons to the matrix. The electrons are transferred to Q through a series of 3 Fe-S centers. The electrons are accepted by Q and it takes two protons from the matrix and it converts into QH_2. This QH_2 now travels to Complex III for its recycling into Q. This complex is also a part of Citric Acid Cycle. It is actually a link between TCA cycle and ETS.



In this way Complex I and Complex II have generated QH_2. But they are not the only sources to produce QH_2.



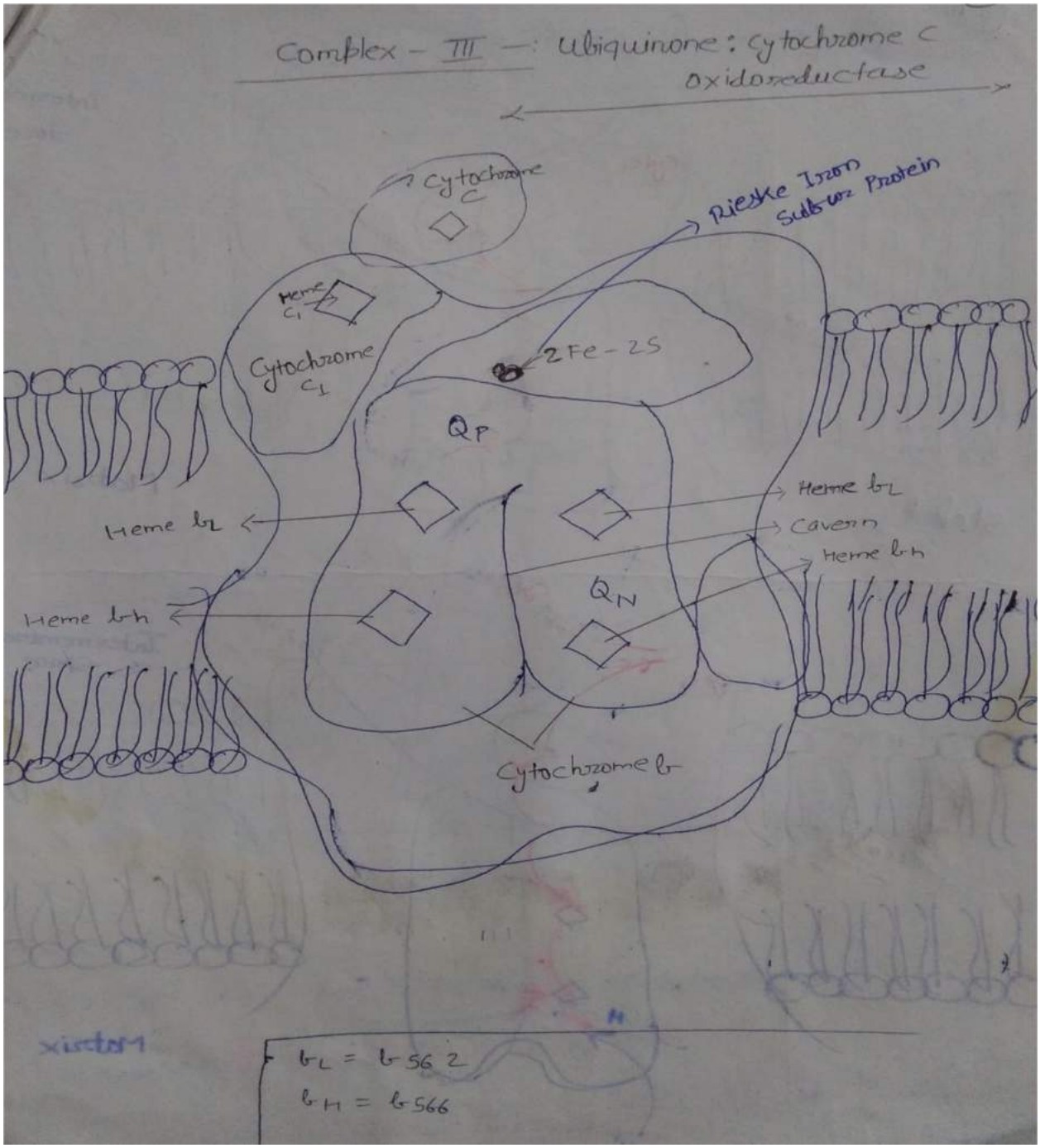
3. **Complex III: Ubiquinone: Cytochrome C oxidoreductase:-** It is the center of recycling of Ubiquinone (Q). This center consists of Cytochrome B, Cytochrome C and Cytochrome C_1. It has also two Iron Sulphur centers. Cytochrome B has two b_L (Cytochrome B having absorption spectra of 562 nm) and b_H (Cytochrome B having absorption spectra of 566 nm).

Now, when first QH_2 comes it donates its one electron to b_L and another to b_H and releases its two protons to the IMS side. In this way two protons crosses the Inner Mitochondrial Membrane. The electron gained by b_L passes through Fe-S centers to Cytochrome C_1 and then to Cytochrome C. while the electron gained by b_H is passes to another free Ubiquinone (Q), subsequently it takes one proton from the matrix and converts into Semi-Qninone (QH).

In the second stage, when another QH_2 arrives to Complex III, it again donates its one electron to b_L and another to b_H and two protons are again released to the IMS side.

The electron gained by b_L passes through Fe-S centers to Cytochrome C_1 and then to Cytochrome C. In this way now Cytochrome C has two electrons. The electron gained by b_H is passed to QH (prepared in the first stage). It again takes one proton from the matrix and converts into Ubiquinol (QH_2). In this way Q completes its cycle. We also see here that the breakdown of QH_2 generates another QH_2 in Complex III.

Now, let us see their structures:-



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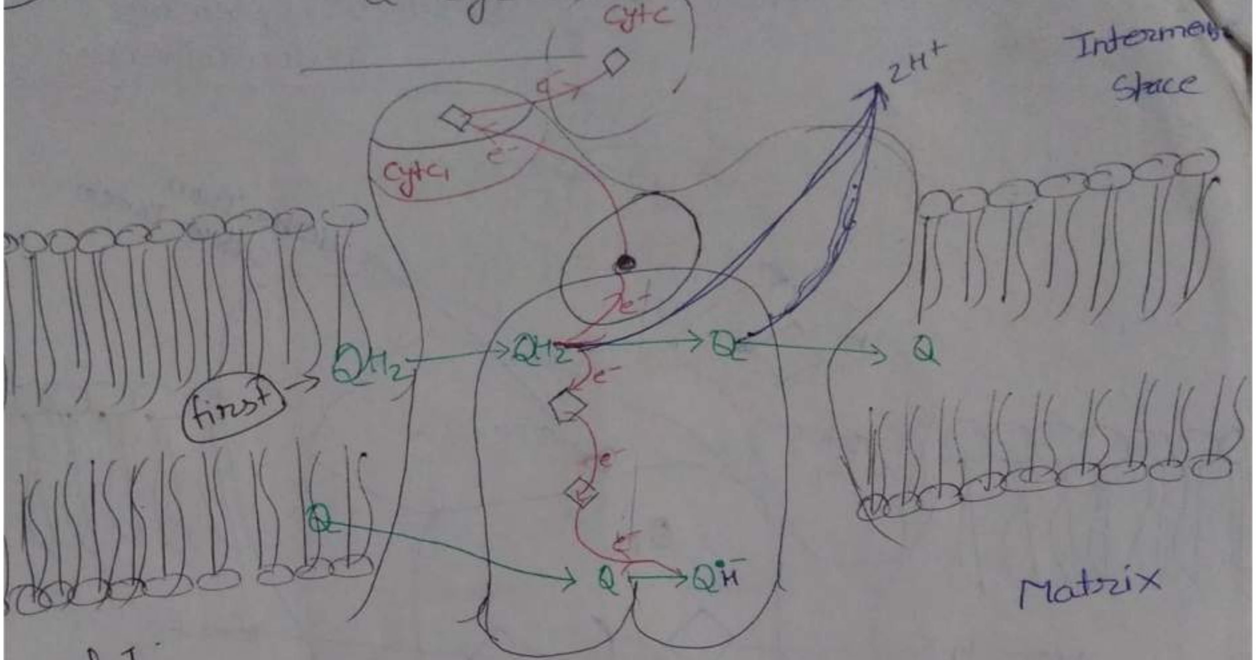
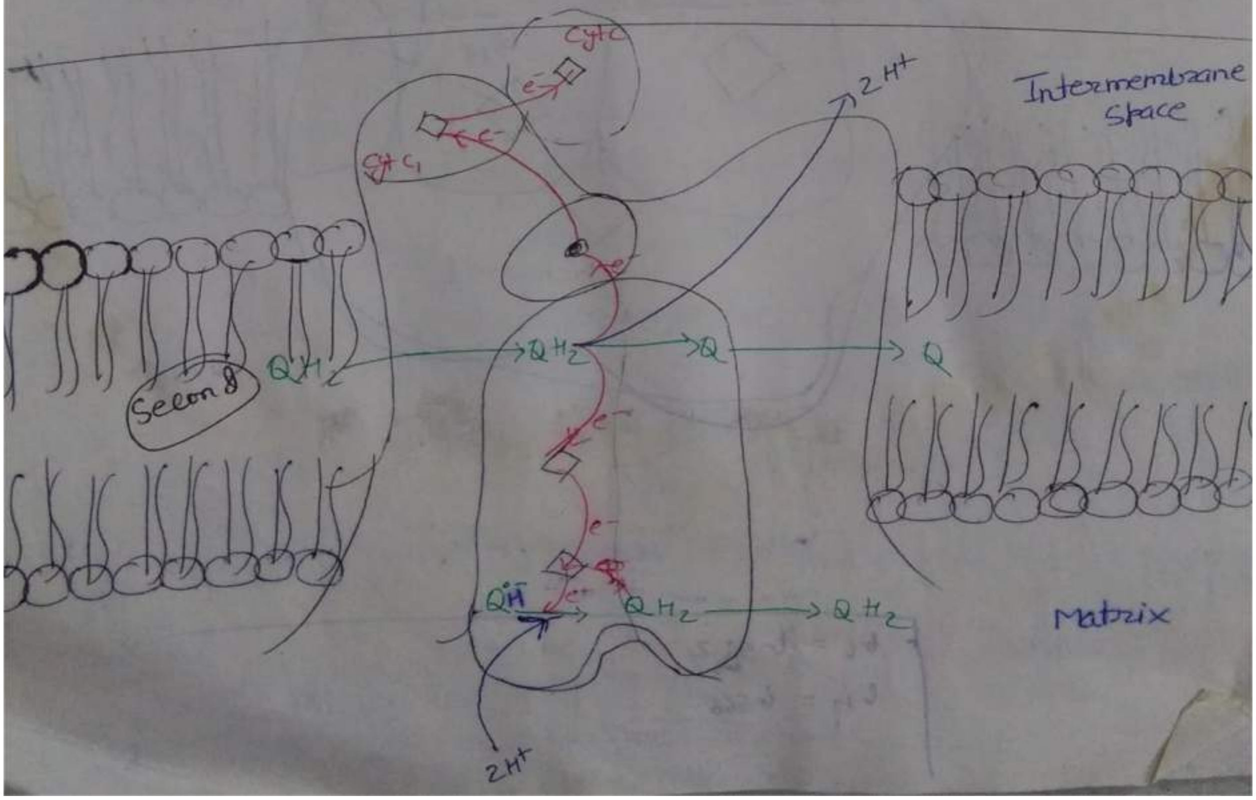
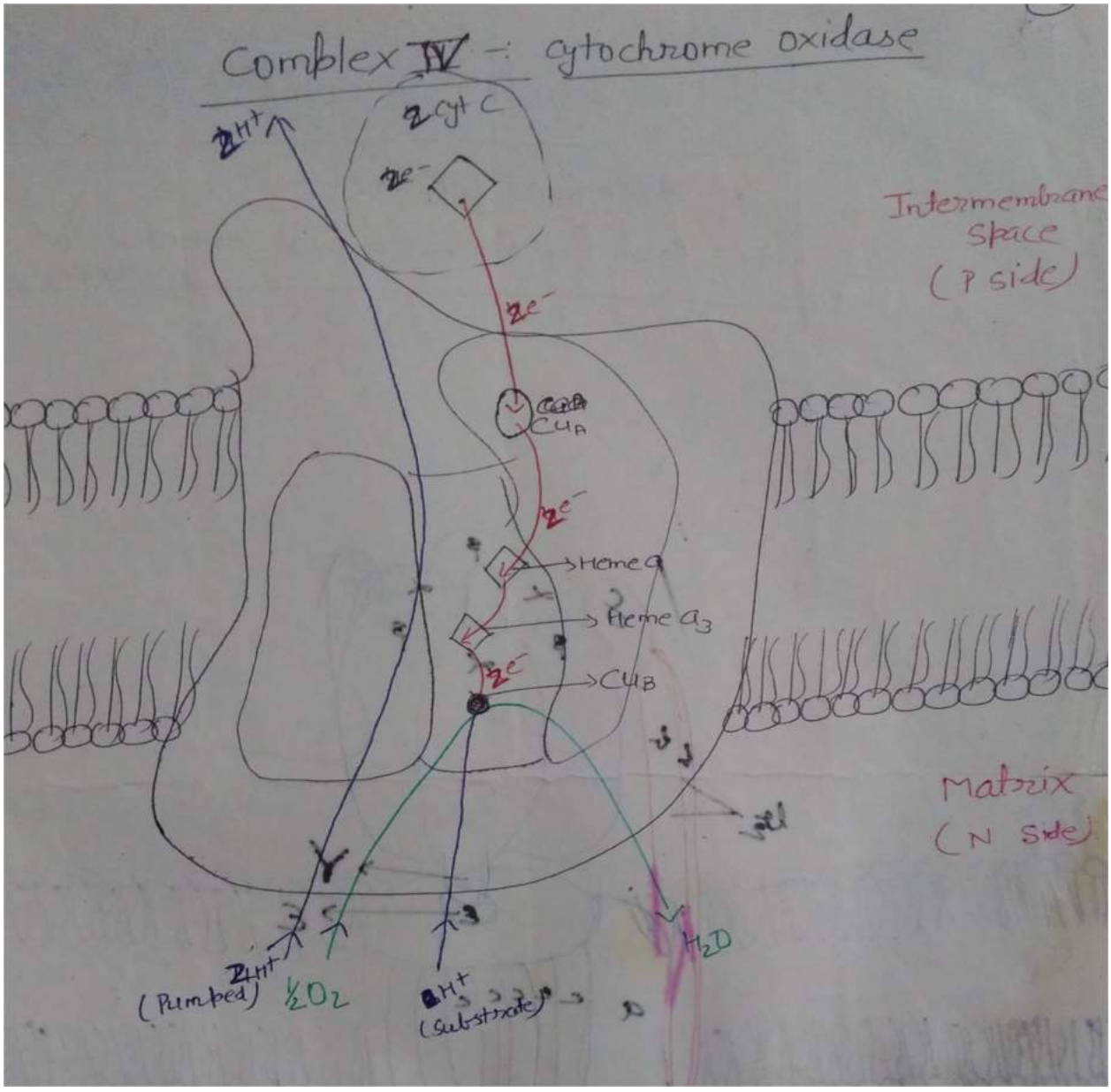
Q cycle



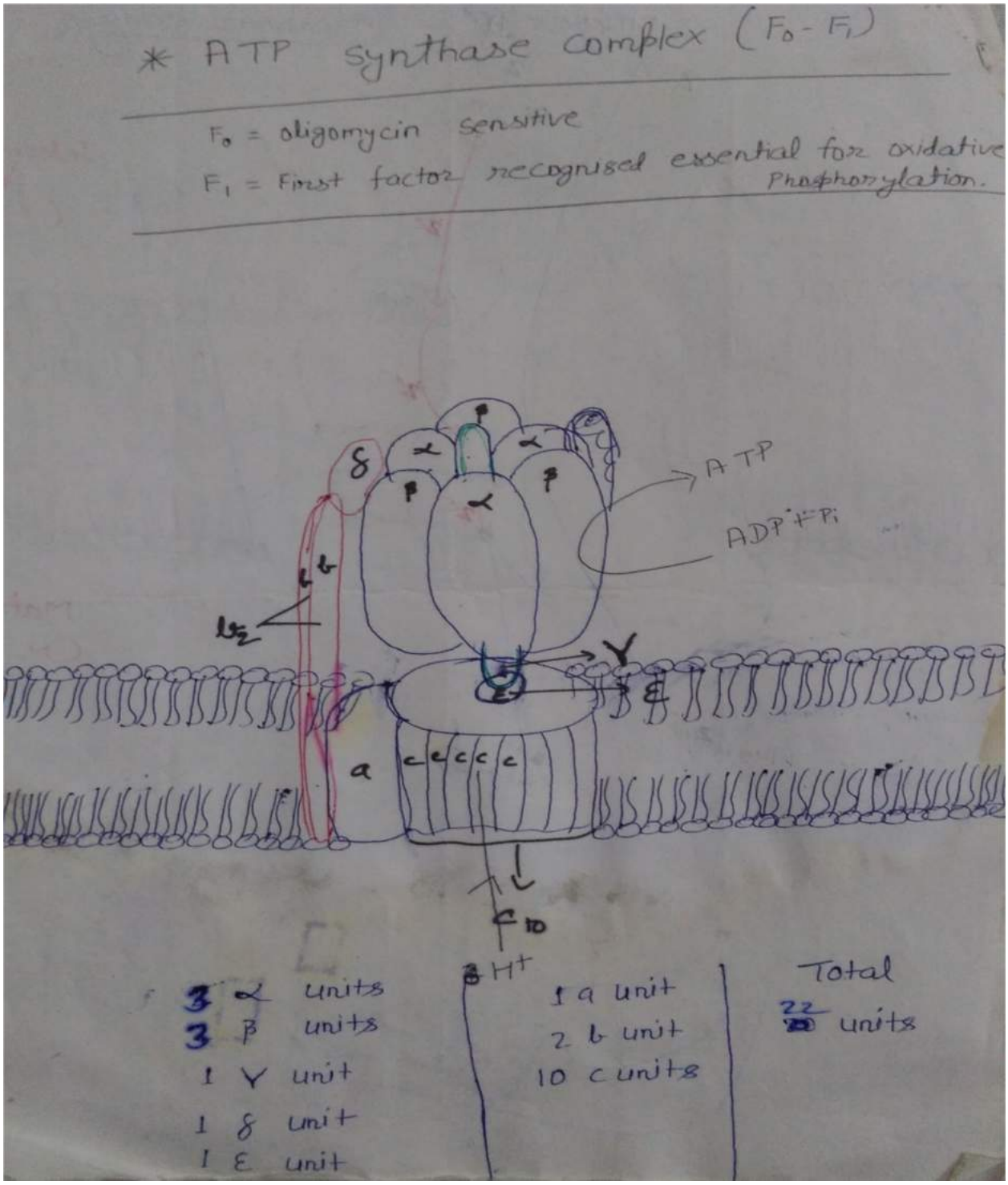
Step I



4. **Complex IV: Cytochrome Oxidase**:- This complex consists of two Cytochromes (Cytochrome A and Cytochrome A_3) and two copper centers (Cu_A and Cu_B). The two electrons gained by Cytochrome C (by Complex III) is transferred to Cu_A then it reaches to Cu_B through Cytochrome A and Cytochrome A_3. Cu_B passes the electron to its last electron acceptor Oxygen. Oxygen gains two electrons from ETS and two protons from matrix and converts into water molecule. In the meanwhile, two protons also crosses the Inner Mitochondrial membrane with the help of Complex IV (as it is also trans-membrane), the required energy comes from the energy released during the flow of electrons.



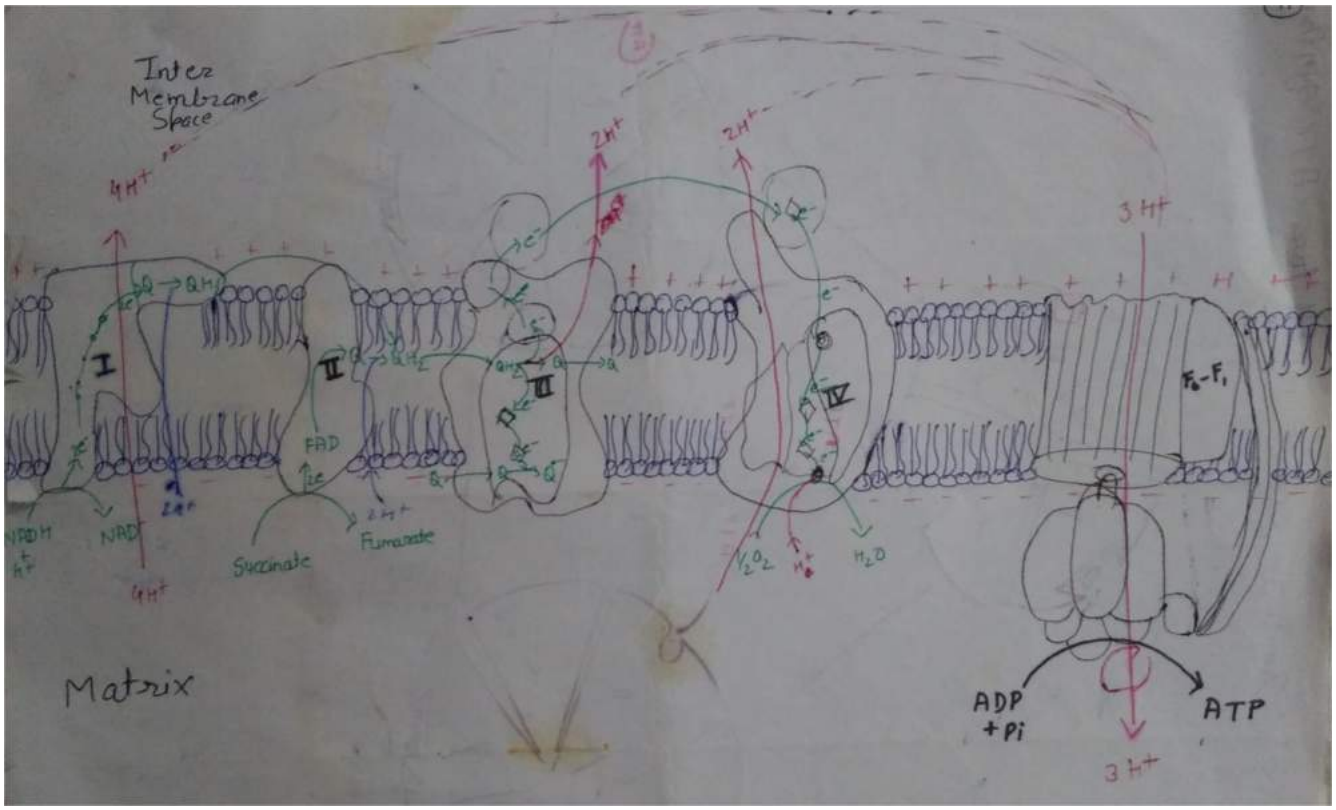
5. **Complex V: ATP synthase Complex**:- It is made up of 22 different units of proteins.



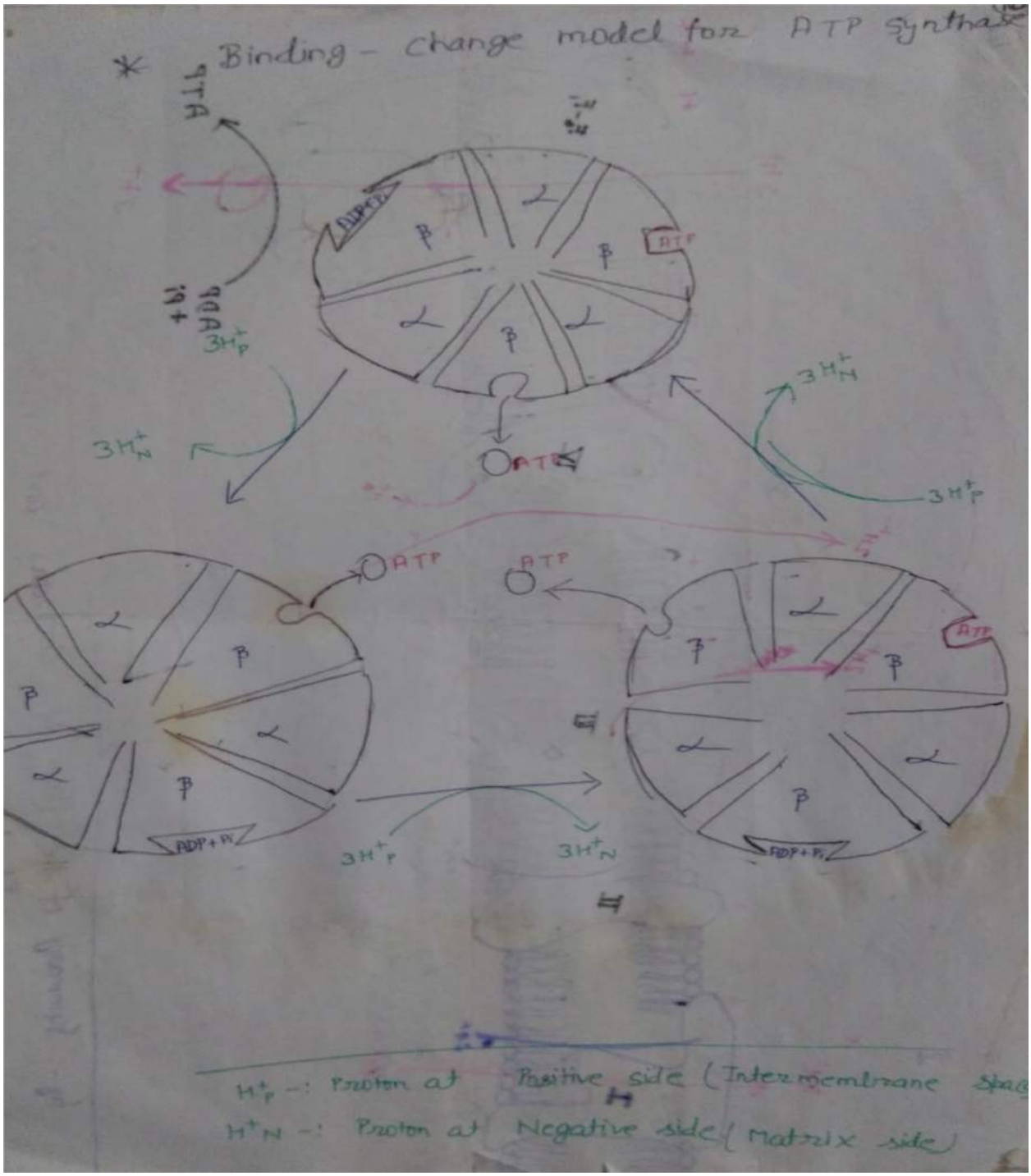
Now, As the Inner Mitochondrial Membrane (IMM) is completely impermeable for protons (H^+), hence the protons are accumulated in the IMS and they create an Electro-chemical gradient. There are three types of gradient created by protons transfer:

1. Electrical gradient: As protons have 1 positive charge, it creates a positive charged environment in IMS while the matrix side is negatively charge.
2. Chemical gradient: The presence of Proton (H^+) makes the environment acidic. Hence the IMS becomes more acidic as compared with the matrix side.
3. Osmotic gradient: As the protons are transferred from matrix to IMS, the solute particles in IMS increases and hence the solution of IMS becomes Hypertonic with respect to the solution of the matrix.

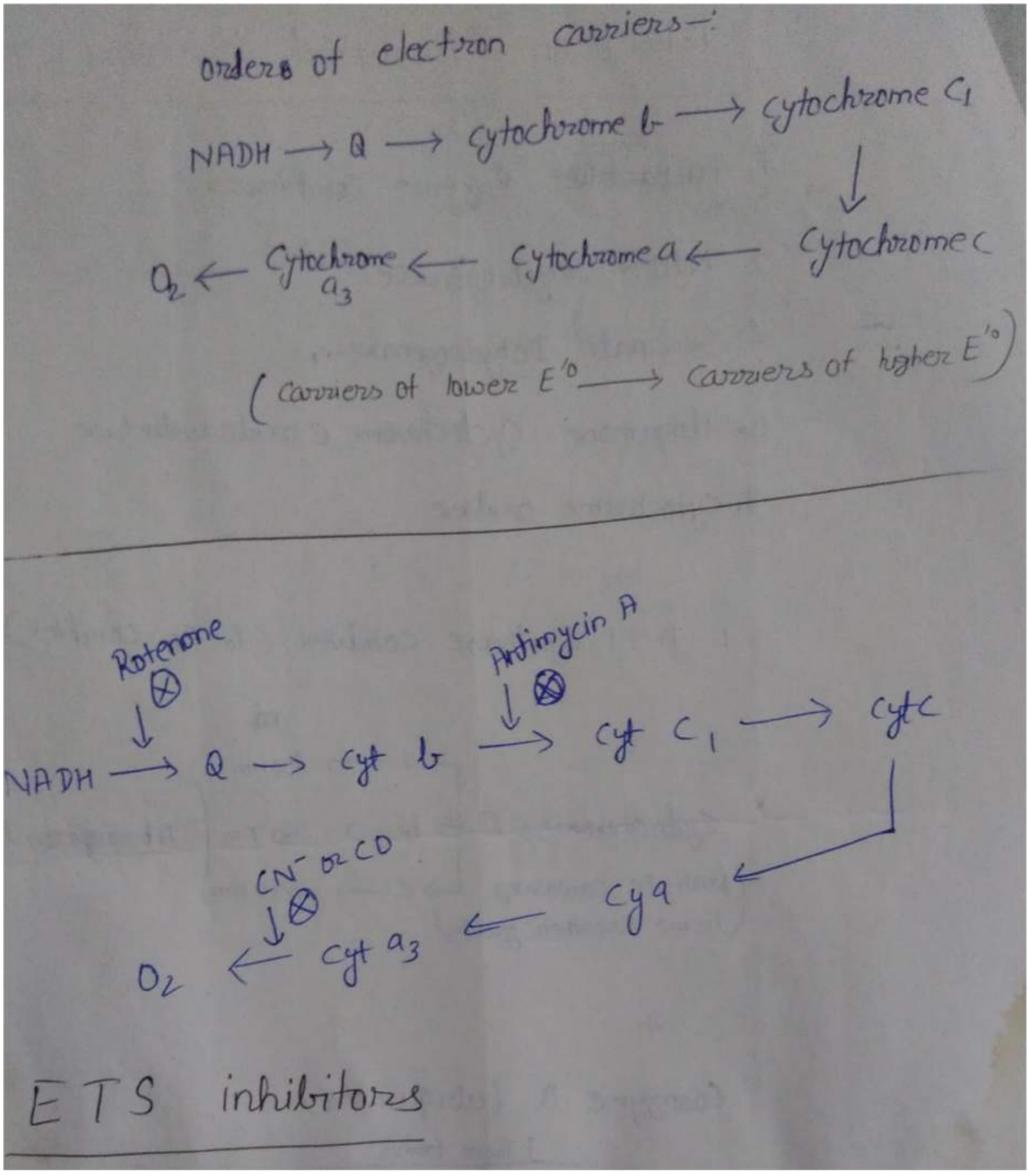
This gradient generates a force which is experienced by IMM. But the protons have only one way back, i.e., ATP synthase complex (the 5th Complex). Now, the proton motive force exerted by the flow of protons provides the required energy for the association of ADP and Pi to form ATP. The protons accumulated in IMS acts like water behind dam and the ATP synthase complex acts like turbine. As the water flows from the channels through the turbine, it rotates and generates electricity (Hydro-electric Power Plants), similarly the flow of proton through the ATP synthase complex make its rotation and during rotation, a conformational change takes place in the β- proteins of the ATP synthase complex. This conformational change converts ADP and Pi into ATP. This is the basic concept of Oxidative Phosphorylation.



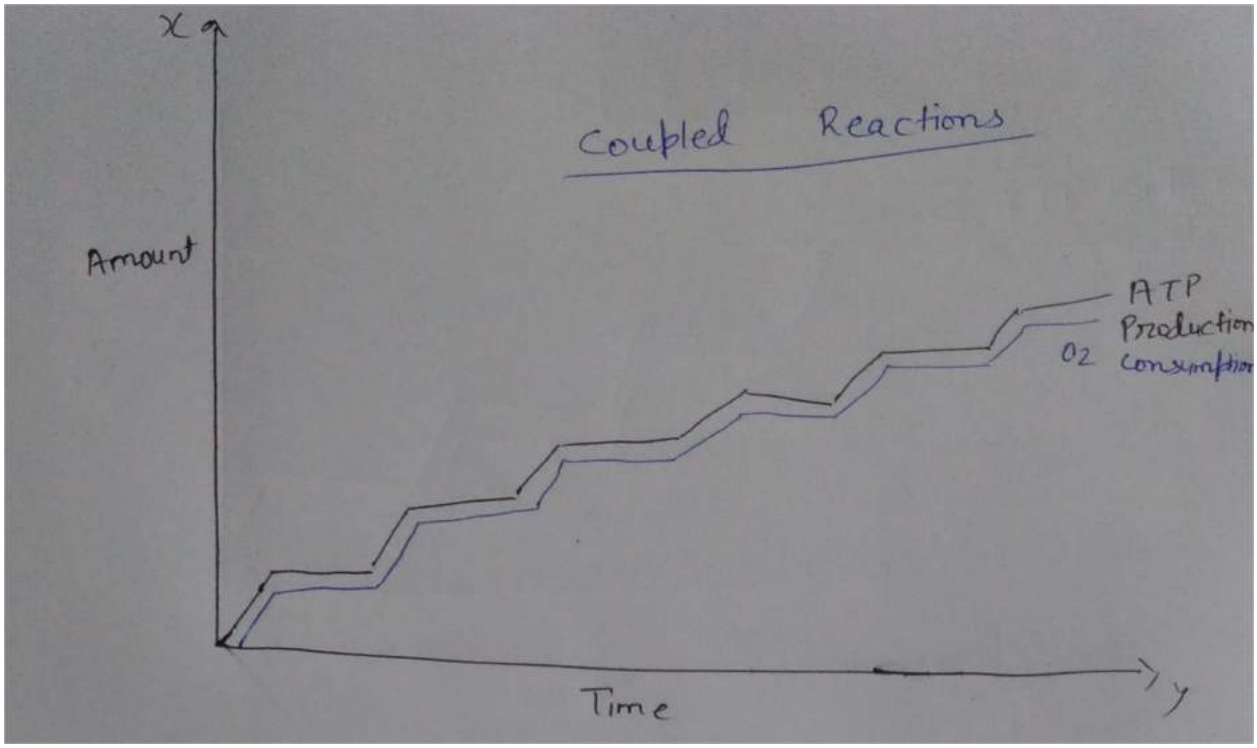
The formation of ATP through ATP synthase complex is explained by Binding Change Model. It explains that the β protein of the complex is found in three conformations and as the complex rotates because of the change in the flux of the internal tunnel due to the flow of protons; the β proteins change their conformation. It can be explained by the following figure:

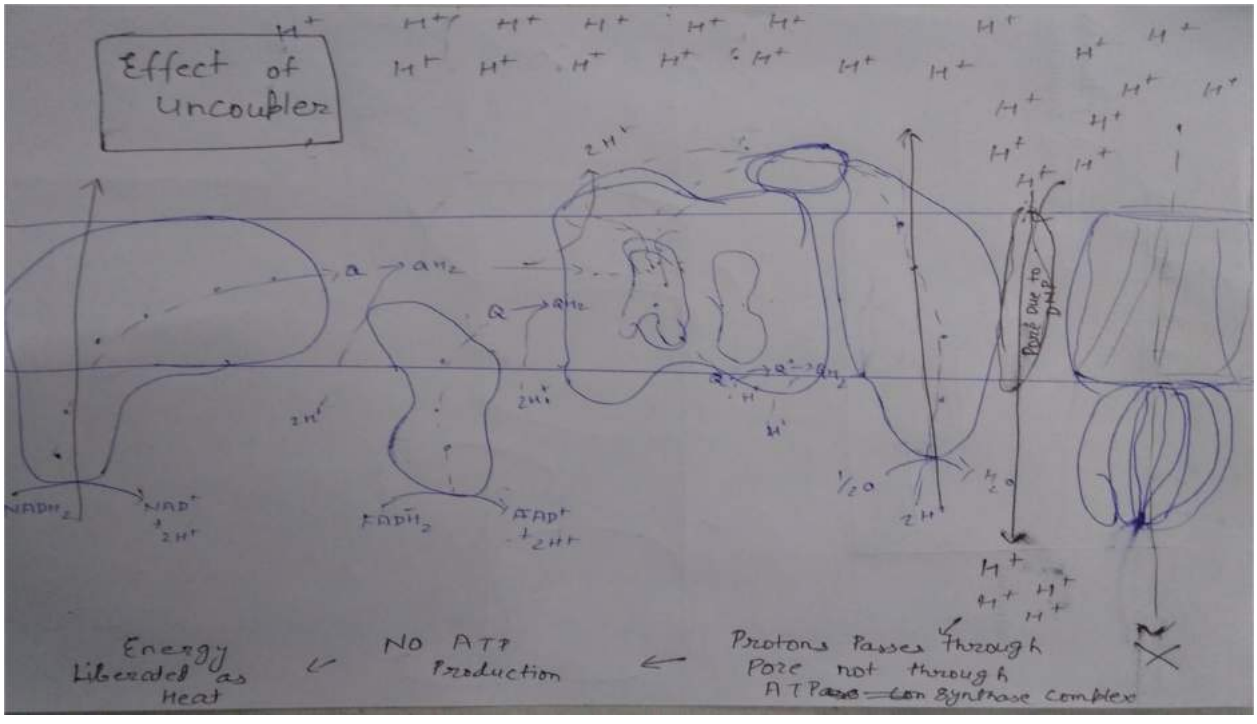


There are several chemicals which can stop this Electron Transport System. They acts as poison for us:



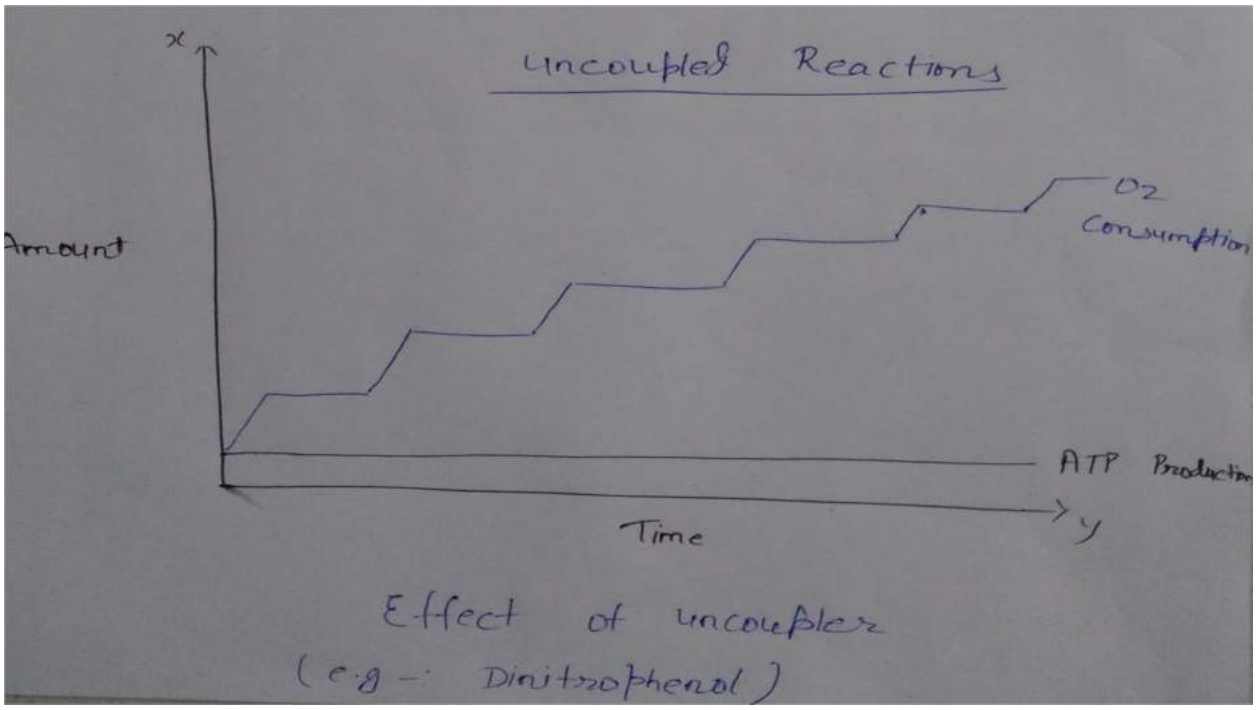
Uncouplers: In Oxidative Phosphorylation the amount of ATP production is coupled with the amount of oxygen consumed. As much as $NADH+H^+$ or $FADH_2$ transfers electrons to oxygen (O_2) through ETS, it will cause much more Proton Gradient and greater the proton gradient greater will be the ATP production. Hence we can say that the as much as oxygen is consumed so much will be tha amount of ATP produced. Their graph may be represented as follows:





Some chemicals which separates these two coupled reactions are known as uncouplers, such as Di-Nitrophenol (DNP). The protein “Thermogenine” (produced in fatty persons) also acts as uncouplers. They actually make pores in the Inner Mitochondrial Membrane. So, the protons present in IMS finds another way than the ATP synthase complex. They starts flowing through the pore and hence the proton motive force starts producing heat in the place of ATP. The ETS still works, Electrons are flowing, protons are transferred. Electrochemical gradient is produced. Oxygen is consumed but ATP is not produced, rather heat is produced.

The graph of un-coupled reactions converts into:



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