e-Learning

by Dr. M.G.R EDUCATIONAL AND RESEARCH INSTITUTE

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Biological Oxidation

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Oxidation, which occurs in living systems is called *biological oxidation*.

Biological oxidations are exergonic.

During biological oxidations, the reacting chemical systems move from a higher energy level to a lower one and therefore there is liberation of energy.

The energy released as heat is converted to chemical energy by formation of energy rich compound ATP.

The formation of ATP from ADP and Pi is termed **phosphorylation, as phosphorylation is coupled with** biological oxidation, the process is called ***biological oxidative phosphorylation.***

ENZYMES AND COENZYMES OF BIOLOGICAL OXIDATION

Biological oxidation is brought about with different **enzymes and *coenzymes.***

- The enzymes required for biological oxidation belong to the class of ***oxidoreductases, which includes:***

– Oxidases
– Dehydrogenases
– Hydroperoxidases
– Oxygenases.

Coenzymes involved in biological oxidation include:

- Nicotinamide adenine dinucleotide (NAD+)
- Nicotinamide adenine dinucleotide phosphate (NADP+)
- Flavin mononucleotide (FMN)
- Flavin adenine dinucleotide (FAD).

Oxidases

Oxidases catalyze the removal of hydrogen from a substrate in the form of H2O or H2O2 (hydrogen peroxide), using oxygen as a hydrogen acceptor, e.g.

- Cytochrome oxidase
- L-amino acid oxidases
- Xanthine oxidase.

Dehydrogenases

This group constitutes several enzymes. They all catalyze the removal of hydrogen from a substrate but are not able to use oxygen as a hydrogen acceptor.

These enzymes, therefore, require specific coenzymes as acceptors of hydrogen atoms. The coenzymes of dehydrogenases may be either:

– Nicotinamide coenzymes (NAD+ or NADP+)

– Flavin coenzymes (FMN or FAD).

Nicotinamide coenzymes (NAD+ or NADP+) linked dehydrogenases

Some dehydrogenases can use either nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+) coenzymes.

These coenzymes are derived from vitamin **niacin.**

- NAD+ or NADP+ linked dehydrogenases remove two hydrogen atoms from their substrate. One of these is transferred to the NAD+ or NADP+. The other appears as hydrogen ion (H+) in the medium.

- NAD linked dehydrogenases are involved in the oxidative pathways of metabolism like in glycolysis, TCA cycle and in the mitochondrial respiratory chain.
- NADP linked dehydrogenases, on the other hand, are involved in reductive biosynthetic reactions like fatty acid synthesis and cholesterol synthesis.

Flavin-coenzyme (FMN or FAD) linked dehydrogenases

- Flavin mononucleotide (FMN) and flavin adenide dinucleotide (FAD) are derived from vitamin **riboflavin.**

- Unlike NAD+, both hydrogen atoms from substrate are accepted by FMN or FAD. The general reaction can be written as:

$MH_2 + FAD/FMN \rightarrow M + FADH_2/FMNH_2$

Most of the FMN linked dehydrogenases are concerned with mitochondrial electron transport chain, e.g. NADH-dehydrogenase.

The examples of FAD linked dehydrogenases are **succinate dehydrogenase in TCA cycle, acyl-CoA dehydrogenase in β-oxidation of fatty acid, etc.**

Hydroperoxidases

Hydroperoxidases catalyze the reduction of H_2O_2 to H_2O.

There are two types of hydroperoxidases:

1. Peroxidases
2. Catalases.

Oxygenases

Oxygenases are a group of enzymes that catalyze the addition of one or both of the atoms of the O2 molecule into the substrate.

Oxygenases *are not concerned with energy production in the body. These are:*

Monooxygenase

- These enzymes incorporate one oxygen atom of O2 into the substrates in the form of hydroxyl group, while the other oxygen atom of O2 is reduced to H2O.

- Examples of monooxygenase is phenylalanine hydroxylase for the formation of tyrosine from phenylalanine.

Dioxygenases

- These catalyze the incorporation of both the atoms of O2 into the substrates. For example, the enzymes such as:

– Homogentisate oxidase of tyrosine metabolism.

– 3-Hydroxy anthranilate oxidase and L-tryptophan dioxygenase of tryptophan metabolism.

– Cycloxygenase involved in prostaglandin synthesis.

ELECTRON TRANSPORT CHAIN (ETC) OR RESPIRATORY CHAIN

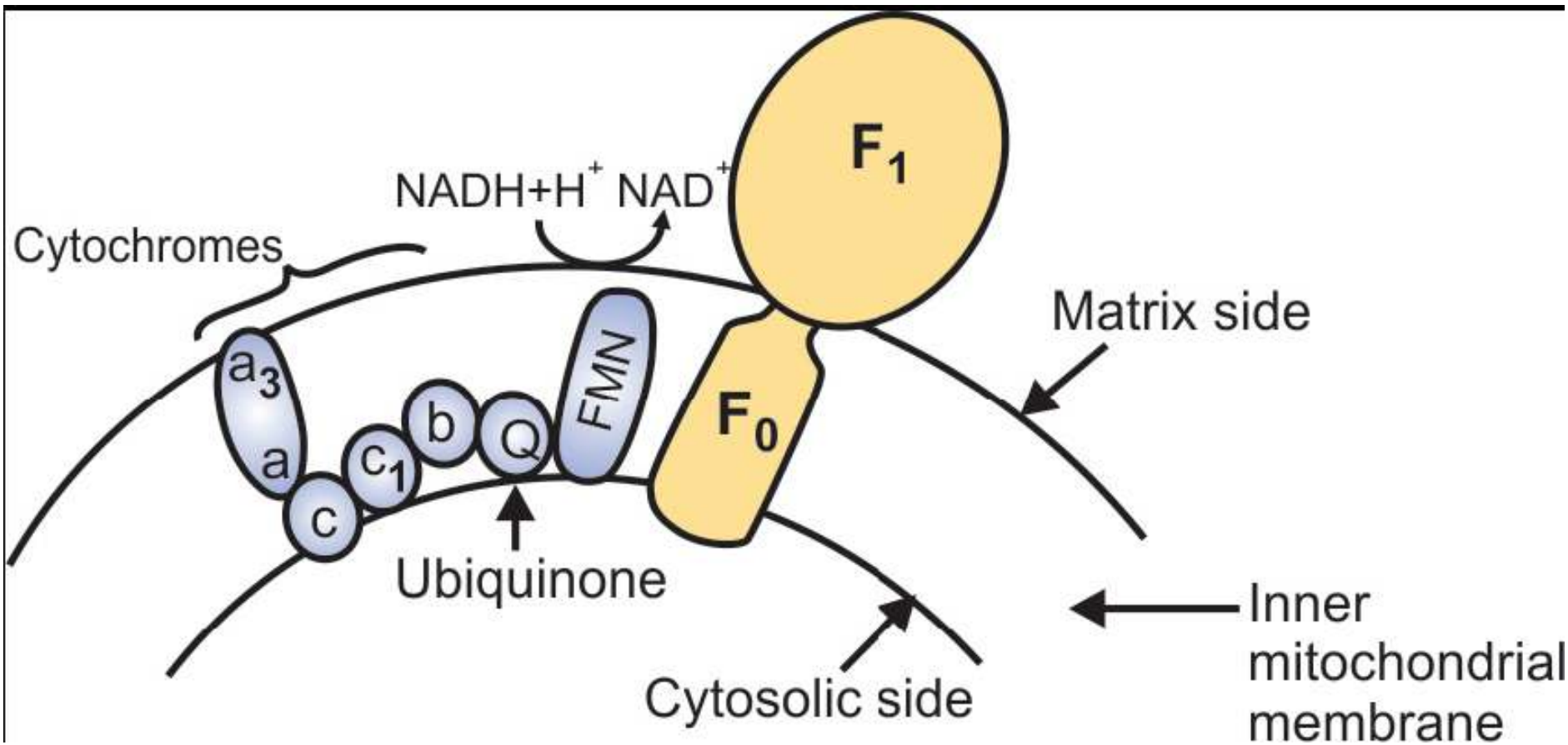
The final steps in the overall oxidation of food stuffs (carbohydrate, fat and amino acids) result in formation of NADH and FADH2.

The electron transport chain (ETC) oxidizes NADH and FADH2 by transferring electrons (reducing equivalents) by a series of oxidation reduction reactions to O2, the terminal electron acceptor. In the presence of O2, the ETC converts reducing equivalents into energy, (ATP) by oxidative phosphorylation

Localization of the Electron Transport Chain

The electron transport chain is present in the ***inner mitochondrial membrane (Figure 10.1).*** **The enzymes of the electron transport chain are embedded in the inner membrane.**

Figure 10.1: Structural organization of components of respiratory chain and FoF1 ATPase in the mitochondrial membrane



Components of the Electron Transport Chain

The major components of the electron transport chain include:

- Nicotinamide adenine dinucleotide (NAD+).

- Flavin mononucleotide (FMN) and Flavin adenine dinucleotide (FAD).

- Ubiquinone or coenzyme Q.

- The ***iron-sulfur (Fe-S) protein*** **associated with FMN and cytochrome b.**

- **Cytochromes (hemeproteins):**

  b, c1, c, a **and *a*3. Of these, only cytochrome c is water soluble and easily diffusible, whereas cytochromes b, c1, a and a3 are lipid soluble and therefore, are fixed components of** the membrane.

Cytochrome aa3 are also called **cytochrome oxidase;** and are **copper containing hemeproteins.**

Except coenzyme Q, all members of this chain are proteins. Coenzyme Q (CoQ) is a fat soluble quinone (ubiquinone) and is a constituent of mitochondrial lipids.

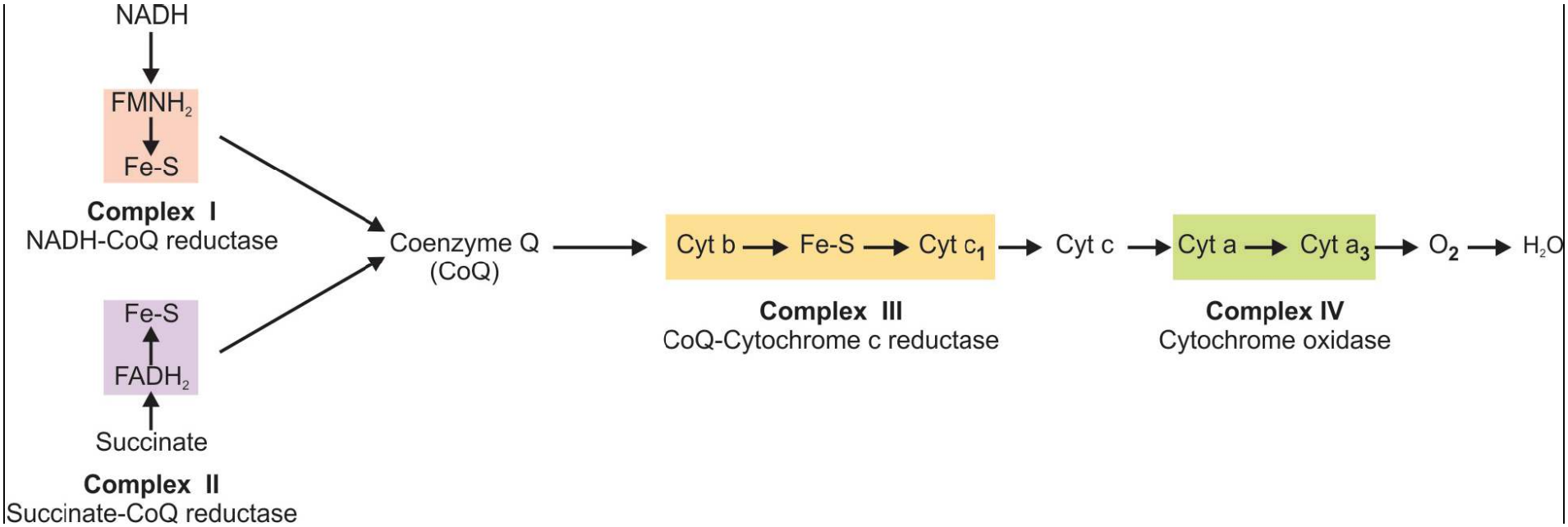
Structural Organization of Components of Electron Transport Chain

The mitochondrial electron carriers are organized into four complexes (complex I to IV) that catalyze oxidation-reduction reactions of the electron transport chain **(Figure 10.2).**

- **Complex I, NADH- CoQ reductase,** catalyzes the transfer of electrons from NADH to coenzyme Q (CoQ).

- **Complex II, Succinate-CoQ reductase,** transfers electrons from succinate to coenzyme Q.

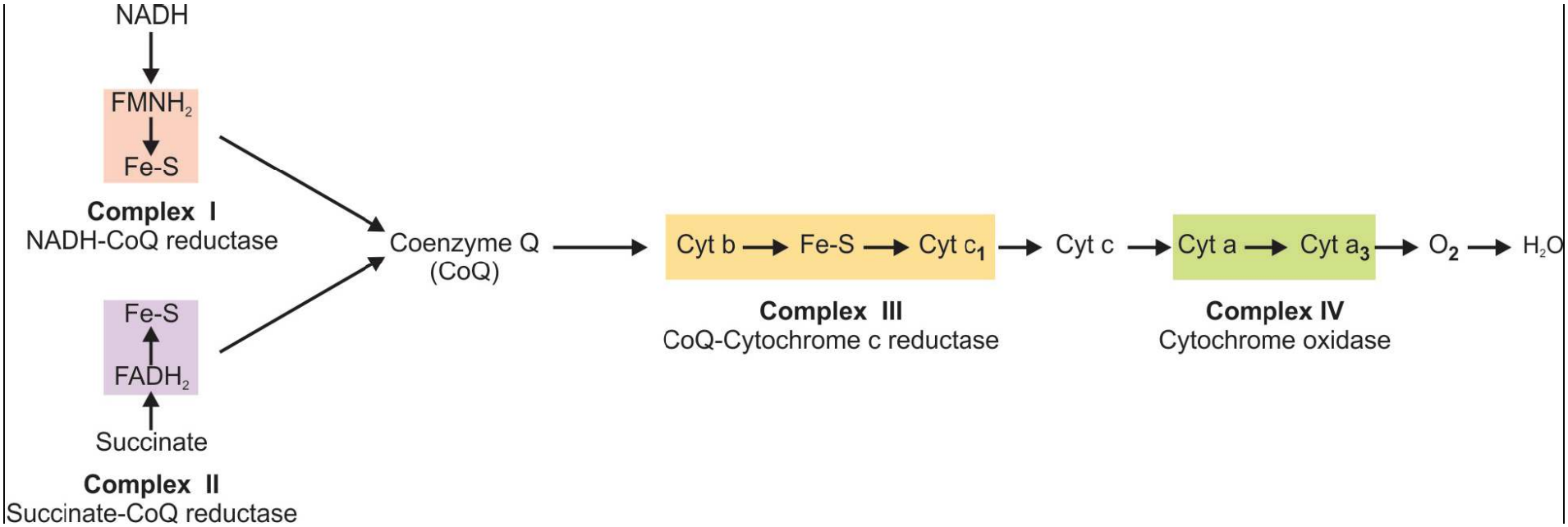
Figure 10.2: The electron transport complexes



- **Complex III, CoQ- Cytochrome c reductase,** transfers electrons from CoQ to cytochrome c.

- **Complex IV, Cyctochrome oxidase,** transfers electrons from cytochrome c to O2.

Figure 10.2: The electron transport complexes

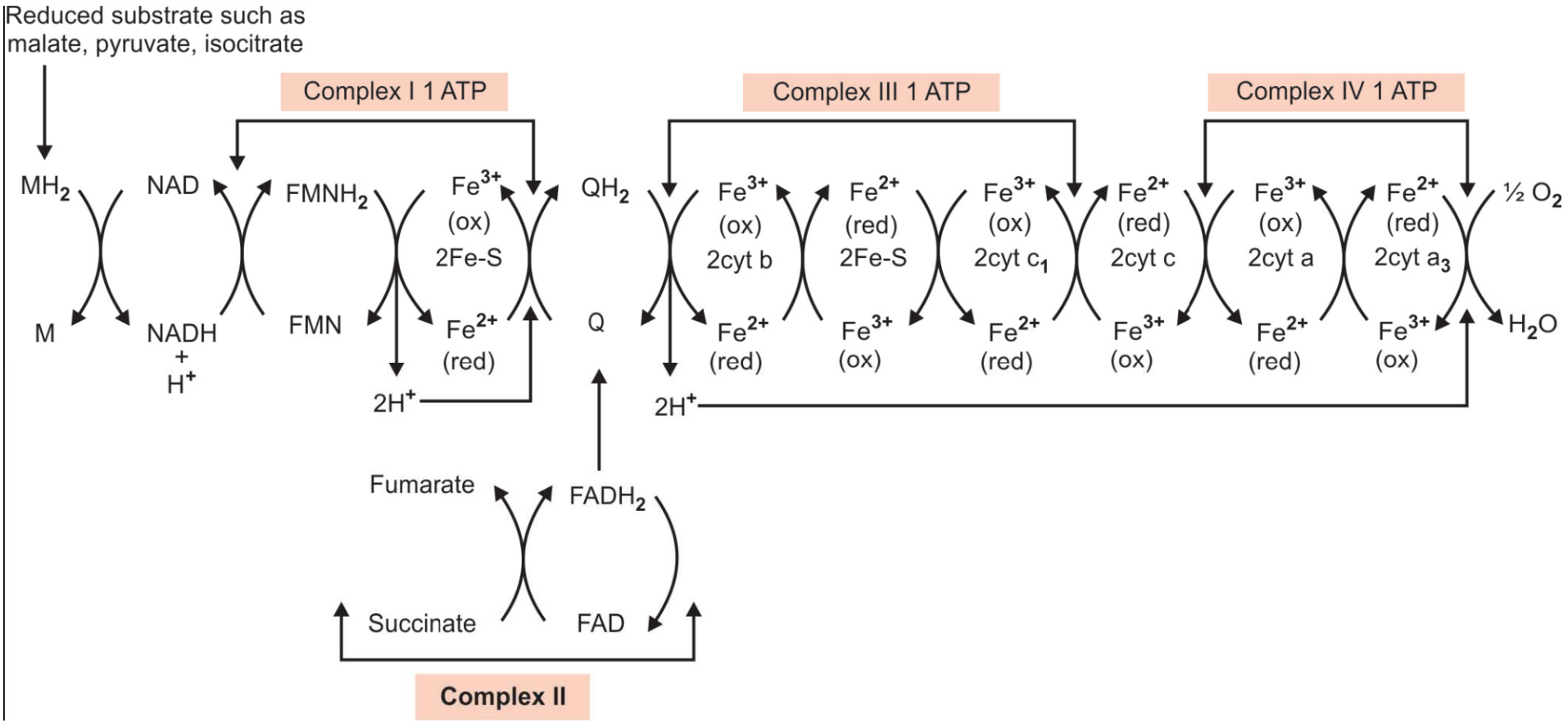


Reactions of Electron Transport Chain

The following sequence of reactions occurs in the transfer of electrons from substrate to the ultimate acceptor oxygen **(Figure 10.3).**

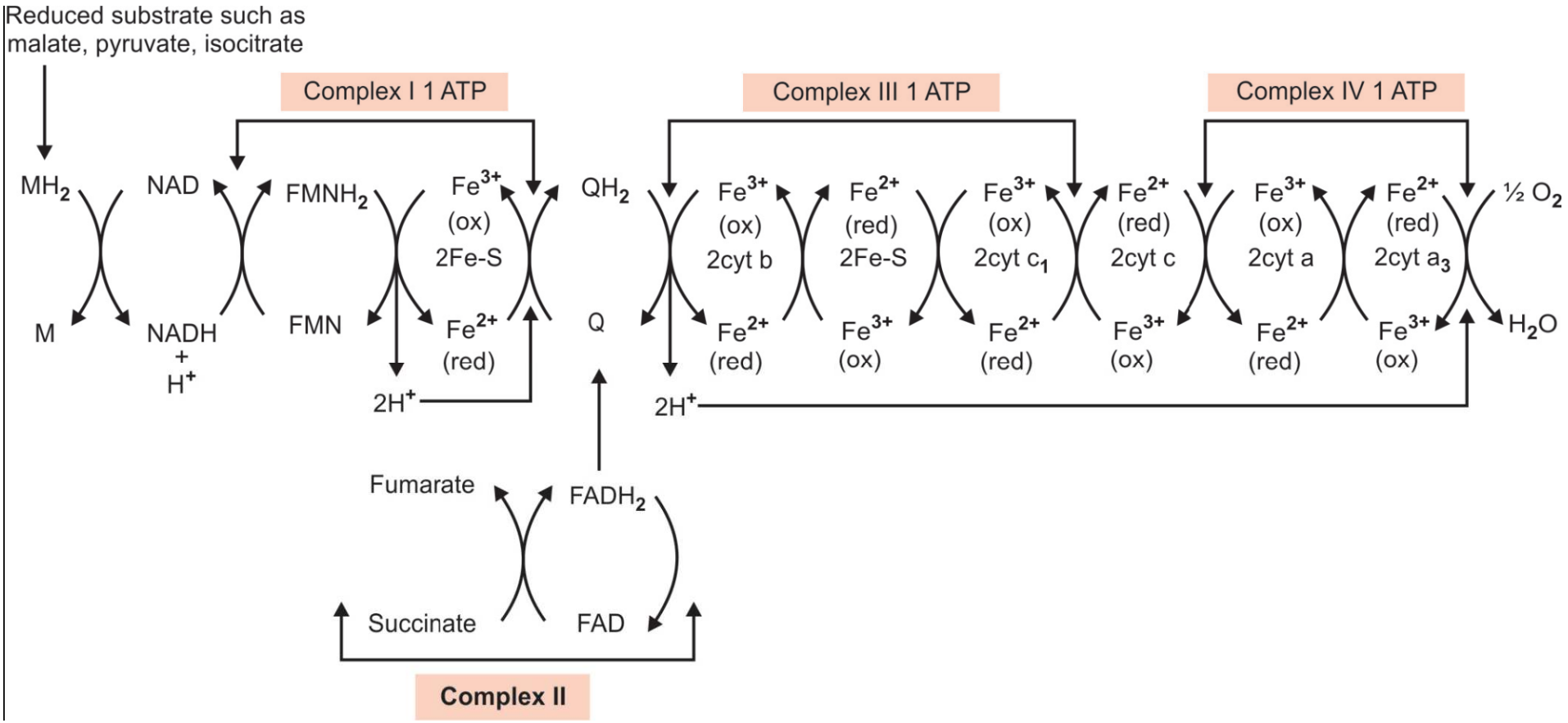
1. NAD+ is reduced to NADH by various dehydrogenases which remove two hydrogen atoms from their metabolite (MH2) and get oxidized to M. In this oxidation reduction reaction, one hydrogen atom is accepted by NAD+ to form NADH, while the second proton (H+) is released into the aqueous medium.

Figure 10.3: Electron transport chain



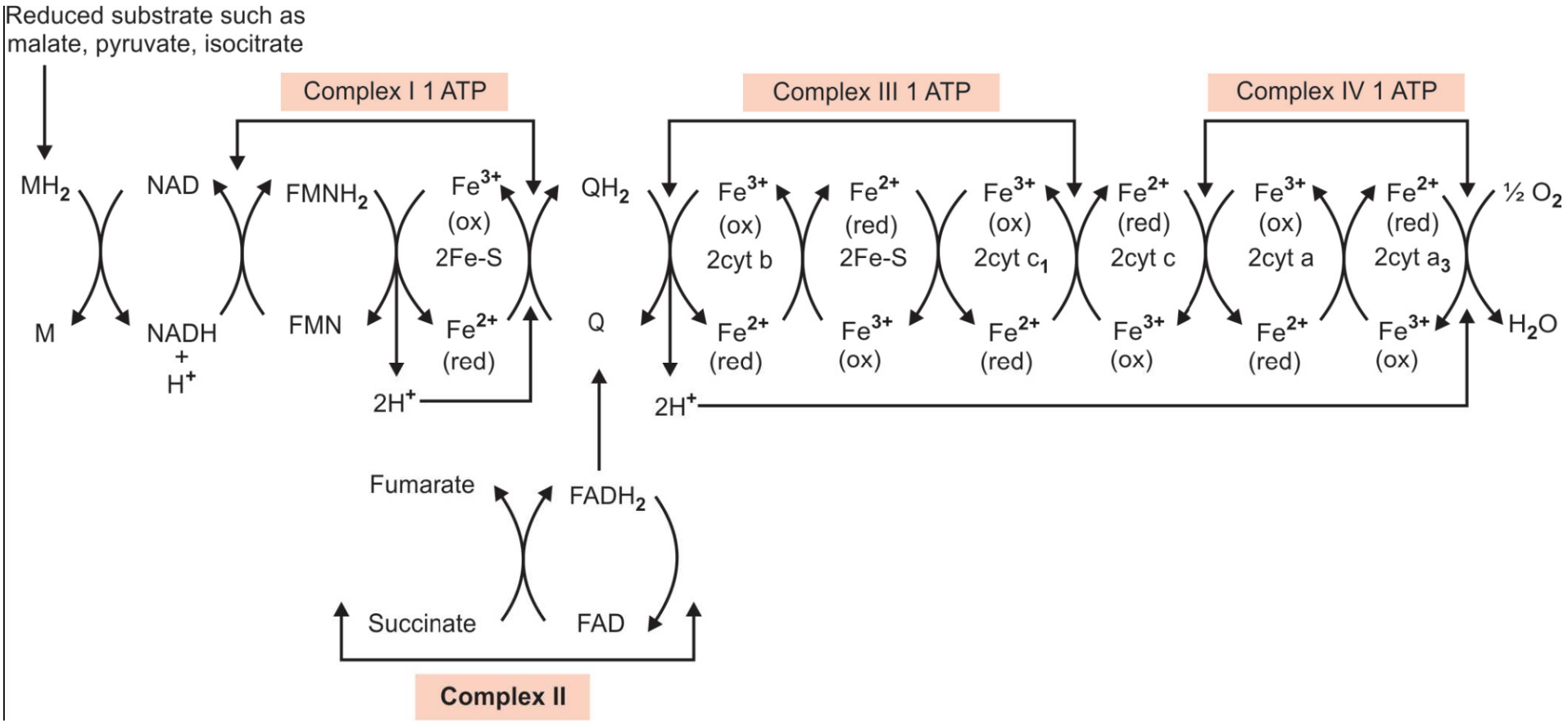
2. The reduced NADH is oxidized by an enzyme **NADH dehydrogenase.** This enzyme contains **coenzyme FMN.** The coenzyme FMN accepts two electrons (2e–) and a proton (H+) from NADH and a free H+ from the aqueous medium to form FMNH2.

Figure 10.3: Electron transport chain



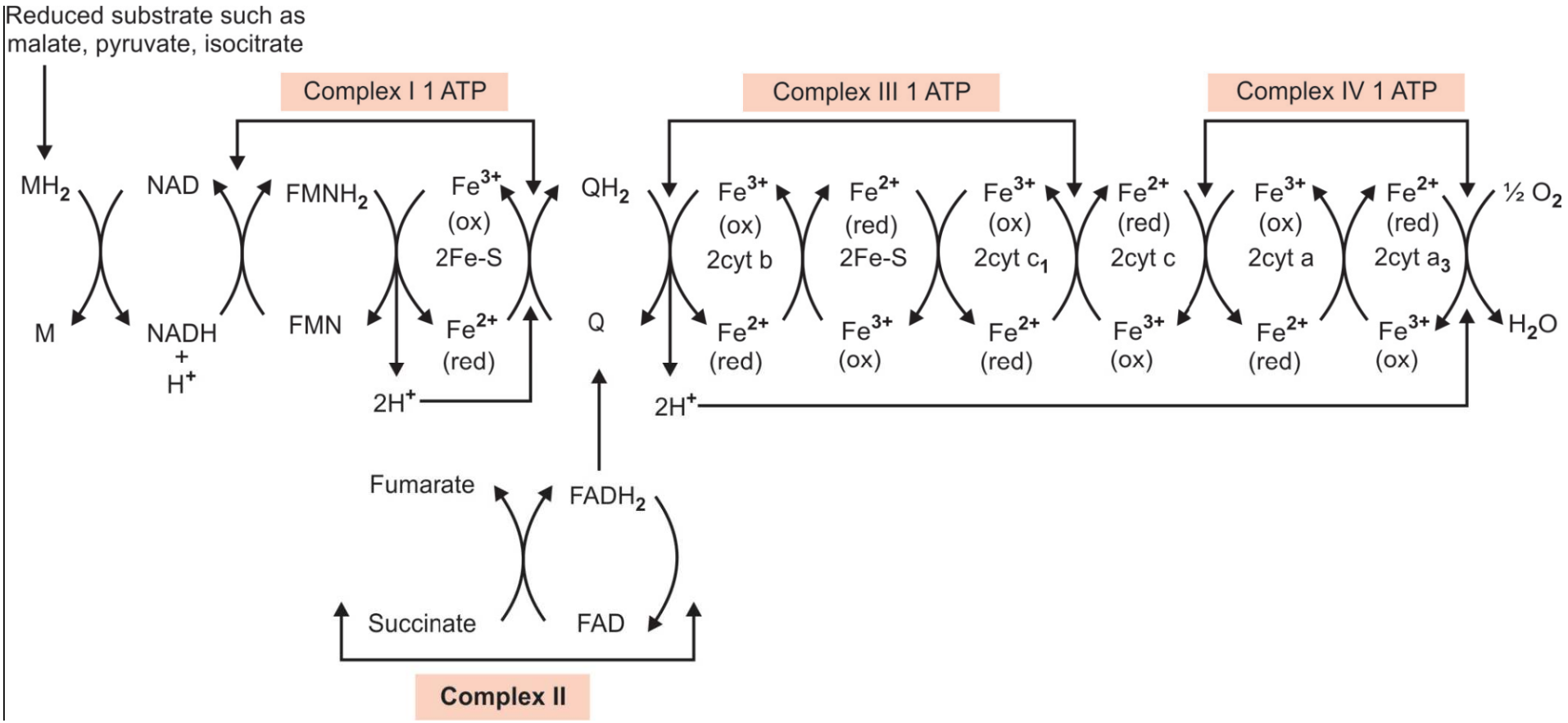
3. In addition to FMN, NADH dehydrogenase also consists of **Fe-S proteins,** which accepts only electron from FMNH2. Thus two Fe-S protein molecules accept two electrons from one FMNH2 molecule with release of two protons (2H+) into the medium and FMNH2 gets oxidized to FMN.

Figure 10.3: Electron transport chain



4. CoQ accepts two electrons from two Fe-S protein molecules and two protons (2H+) from the medium to get reduced to CoQH2. CoQ also collects reducing equivalents from FADH2 formed by FAD-linked dehydrogenases.

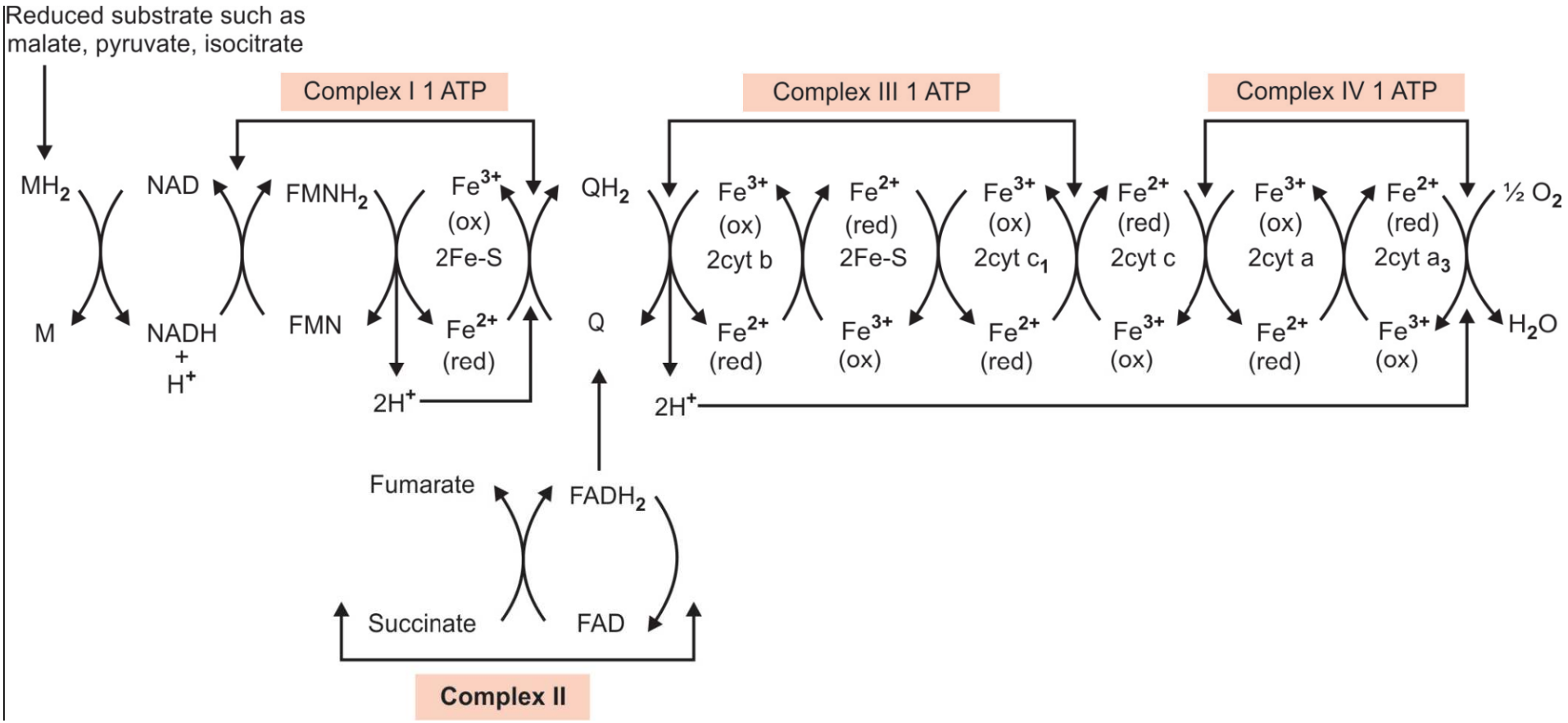
Figure 10.3: Electron transport chain



5. Beyond CoQ, oxidation reduction process occurs by removal of electrons with the help of **cytochromes.** Cytochromes accept only electrons from coenzyme QH2 with the release of 2H+ in the medium.

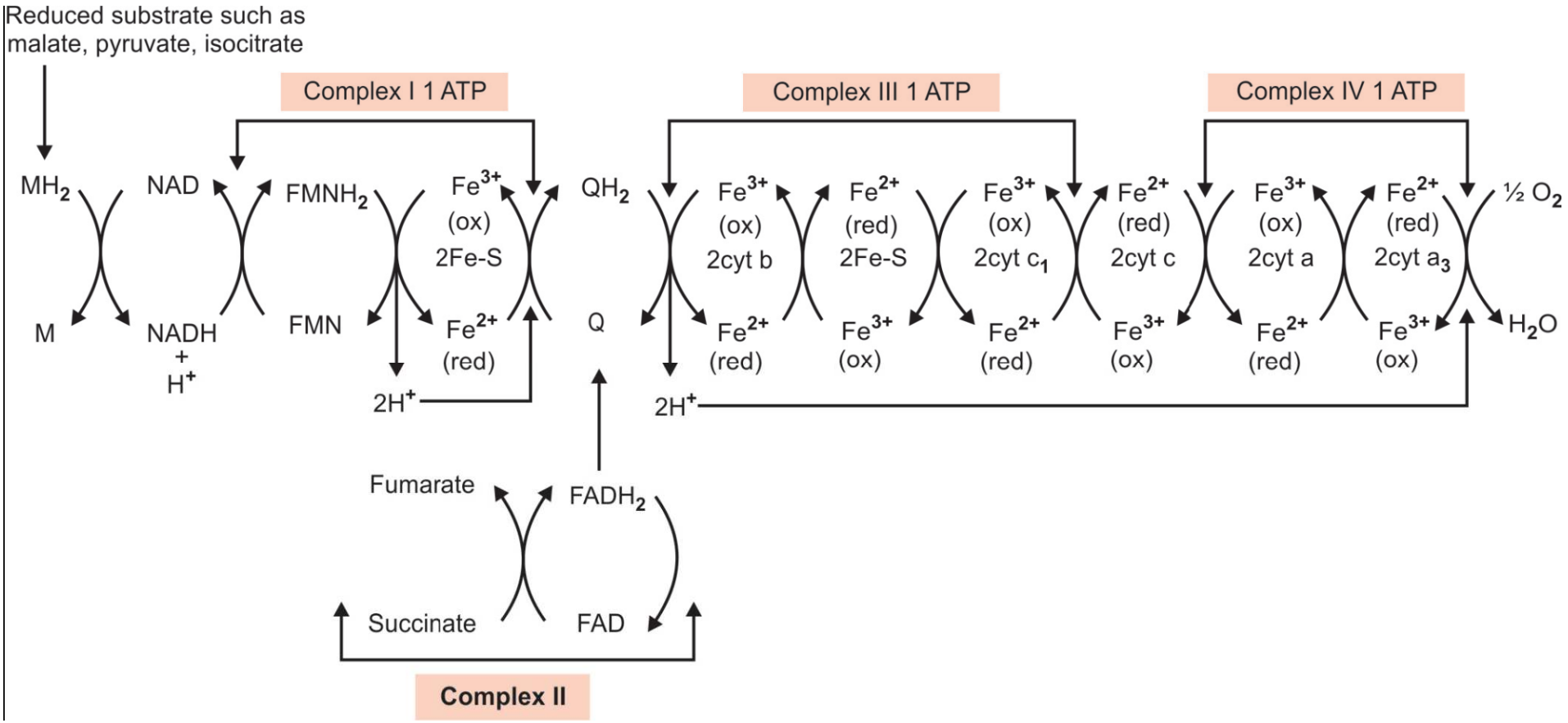
As a cytochrome can accept only one electron, CoQH2 transfers its two electrons to two molecules of cytochrome b, c1 c, a and a3 sequentially.

Figure 10.3: Electron transport chain



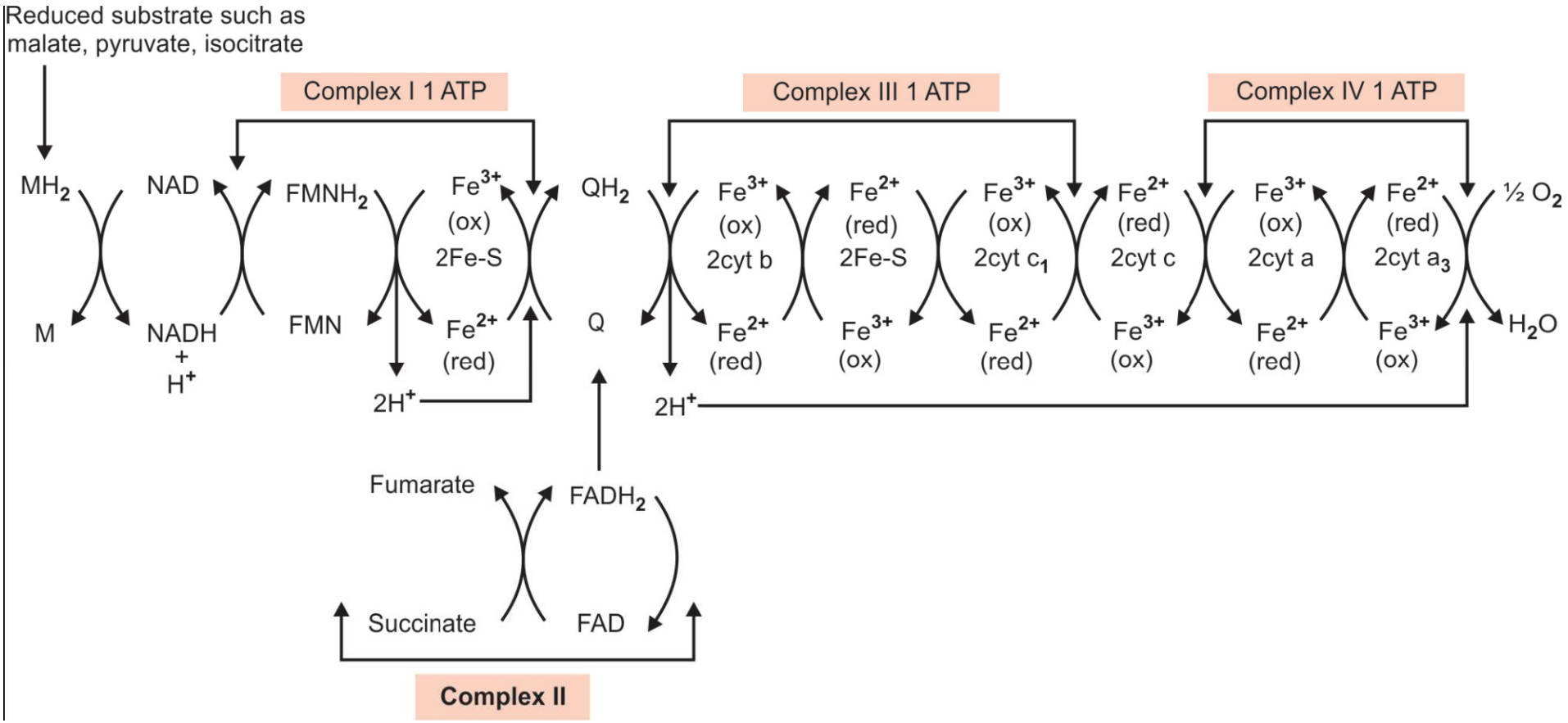
6. The last cytochrome complex is cytochrome oxidase (cyt aa3) which passes electrons from cytochrome c to molecular oxygen. Each oxygen atom accepts two electrons from cytochrome a3 and two protons from the medium and a molecule of water results.

Figure 10.3: Electron transport chain



- The reduction of O2 by cytochrome oxidase reaction accounts for the production of about **300 ml of water/ day.** This water is called ***metabolic water.***

Figure 10.3: Electron transport chain



Formation of ATP

During the transfer of electrons through the electron transport chain, energy is produced. This energy is coupled to the formation of ATP molecules by phosphorylation of ADP by an enzyme **F0 F1 ATPase.**

The formation of ATP from ADP and Pi is termed **phosphorylation,** as phosphorylation is coupled with biological oxidation, the process is called ***biological oxidative phosphorylation.***

Sites of ATP Synthesis

- There are three ATP synthesizing sites of the electron transport chain, these are **(Figure 10.4):**

1 Oxidation of FMNH2 by CoQ

2 Oxidation of cytochrome b by cytochrome c1

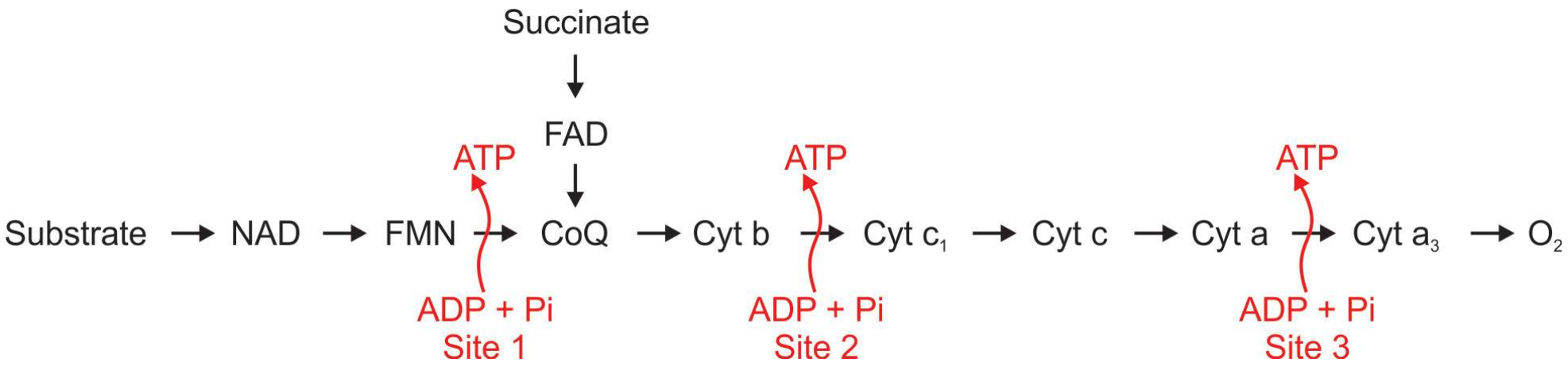
3 Cytochrome oxidase reaction (oxidation of cytochrome a by cytochrome a3.

These sites provide the energy required to make ATP from ADP and Pi by an enzyme F0 F1 ATPase.

- Electrons that enter the chain through NADH pass through all three ATP synthesizing sites and thus yield three ATPs.
- *However, electrons that enter the chain through*

FADH2 pass through only two ATP synthesizing sites, as they bypass site 1, they yield two ATPs.

Figure 10.4: ATP synthesizing sites of electron transport chain



INHIBITORS OF ELECTRON TRANSPORT CHAIN

Inhibitors of respiratory chain may be divided into three groups.

1. Inhibitors of the electron transport chain proper
2. Inhibitors of oxidative phosphorylation (F0F1 ATPase)
3. Uncouplers of oxidative phosphorylation.

Inhibitors of Electron Transport Chain Proper (Figure 10.5)

Inhibitors of electron transport chain proper include, inhibitors that inhibit the flow of electrons through the respiratory chain. These inhibitors block the respiratory chain at three sites:

1. Complex l (NADH to CoQ), inhibited by:

– Barbiturates such as amobarbital

– An antibiotic piericidin A

– The insecticide rotenone.

These inhibitors prevent the oxidation of substrates by blocking the transfer of reducing equivalents from Fe-S protein to CoQ.

Complex III (cytochrome b to cytochrome c1),

inhibited by:

– Dimercaprol

– Antimycin A (antibiotics)

– British antilewisite (BAL), an antidote used against war gas.

These inhibitors prevent the transfer of electrons from cytochrome b to cytochrome C1.

3. Complex IV (cytochrome oxidase), inhibited by:

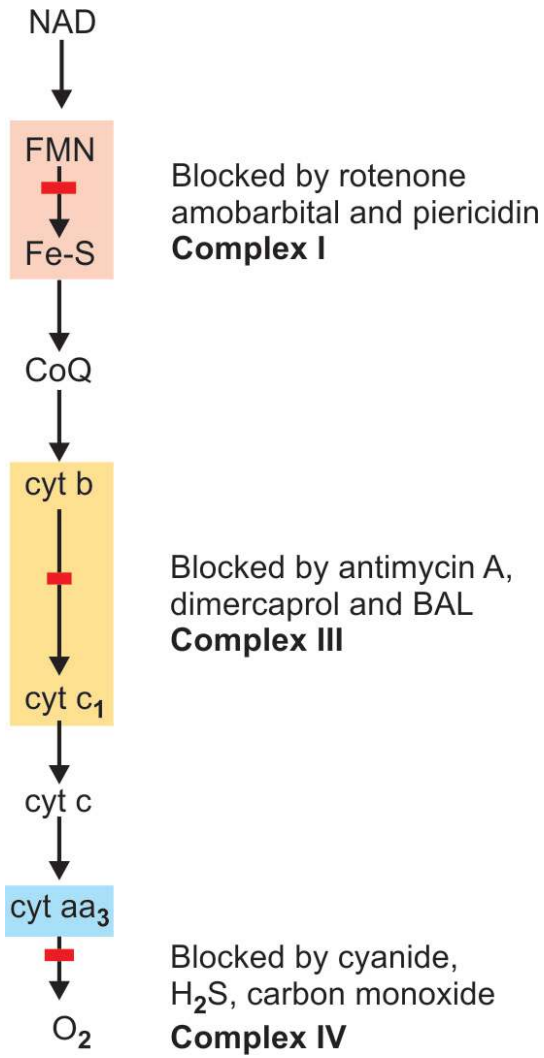
– Cyanide

– Carbon monoxide

– H_2S.

These inhibitors prevent transfer of electrons from cyt aa3 to molecular oxygen by inhibiting cytochrome oxidase and can therefore totally arrest respiration.

Figure 10.5: Sites of action of various inhibitors of electron transport chain



Inhibitors of Oxidative Phosphorylation (F0F1 ATPase)

Another set of inhibitors do not inhibit special complexes.

Instead, these compounds block phosphorylation directly by inhibiting ***F0F1 ATPase enzyme.***

For example, antibiotic ***oligomycin completely blocks oxidation and*** phosphorylation by inhibiting an enzyme F0F1 ATPase required for phosphorylation.

Uncouplers of Oxidative Phosphorylation

- Uncouplers are chemical substances that allow electron transport chain in mitochondria but prevent the phosphorylation of ADP to ATP by uncoupling the essential linkage between electron transport and phosphorylation for the synthesis of ATP.

- Uncoupling agents are lipophilic (lipid soluble) compounds, which readily diffuse through the mitochondrial membrane and are capable of binding H+ ion.

Uncouplers allow transport of H+ ion across the membrane towards the side with the lower H+ ion concentration, thus preventing the formation of proton gradient which is required for the formation of ATP.

- Thus, these compounds make the inner mitochondrial membrane abnormally permeable to protons.

The energy produced by the transport of electrons is released as heat rather than being used for synthesis of ATP.

Examples of uncouplers include:

– *2,4-Dinitrophenol (DNP)*

– Dicuomarol (an anticoagulant).

– Salicylate, a metabolite of aspirin.

Physiological uncouplers

Certain physiological substances act as uncouplers, e.g. thermogenin, thyroxine, bilirubin and free fatty acids.

However, these compounds normally are not present in mitochondria in concentrations high enough to act as uncouplers.

Ionophores

Ionophores means ion carrier molecules. They are lipid soluble substances, capable of binding and carrying specific cations (other than H+) through the mitochondrial membrane.

Oxidative phosphorylation can be prevented by certain ionophores.

They differ from uncoupling agents in that they promote the transport of cations other than H+ through the membrane and abolish the membrane potential and/or pH gradient across the membrane and phosphorylation is therefore completely inhibited.

For example, antibiotic **valinomycin** and **gramicidin.**

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Chemiosmotic Theory

The chemiosmotic coupling hypothesis is the most accepted theory. The chemiosmotic theory which was proposed by the ***British biochemist Peter Mitchell*** explains the mechanism of oxidative phosphorylation.

The theory states that the energy released from oxidation generates the ***electrochemical potential by the pumping*** of protons across the inner mitochondrial membrane and the energy in this electrochemical potential can be converted into ATP.

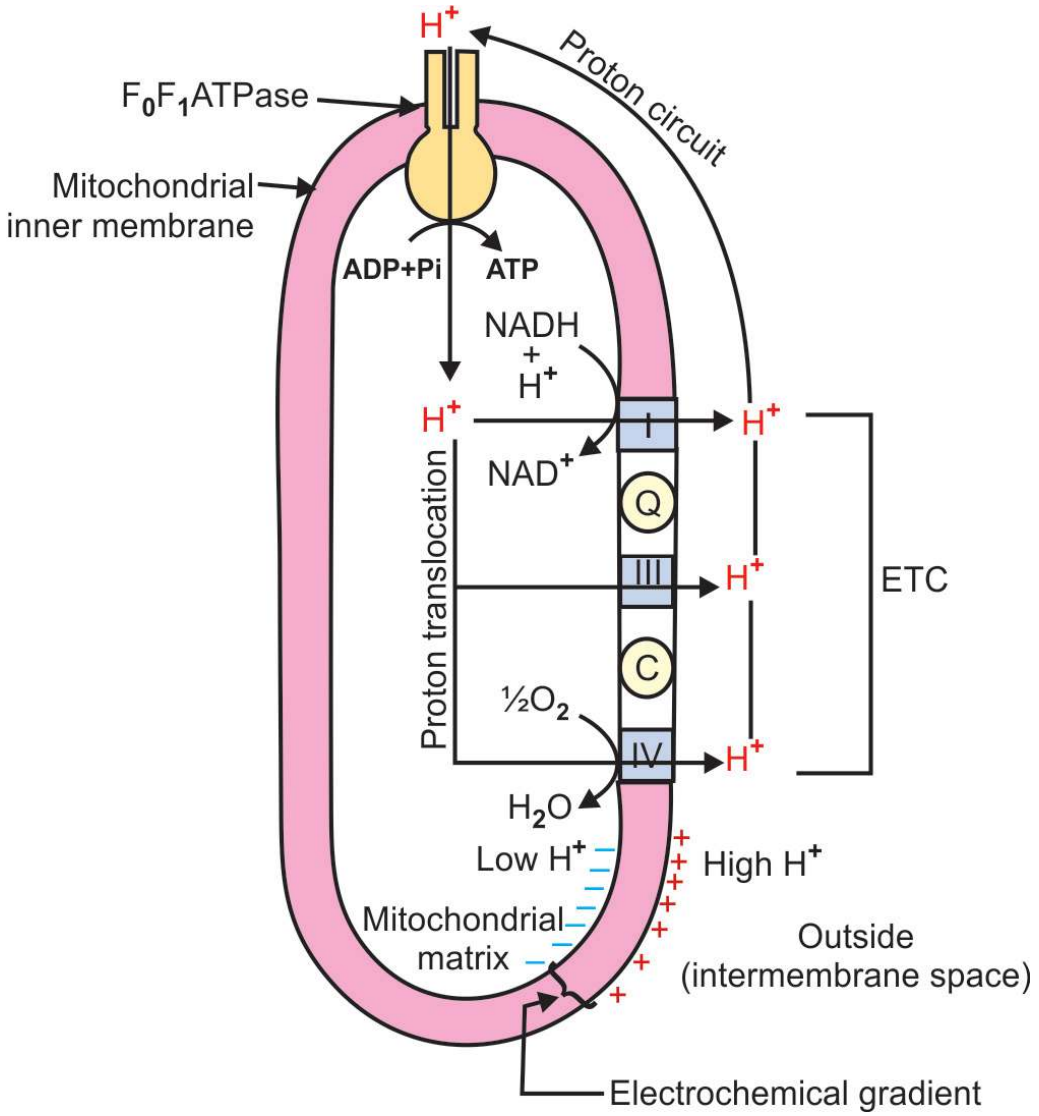
There are three basic principles of the theory.

1. The major electron carriers are organized into three complexes, complex I, III and IV **(Figure 10.6), which** span the inner mitochondrial membrane. The energy released during transport of electrons from one carrier to another allows proton to be pumped across the inner mitochondrial membrane from the matrix to the outside (intermembrane space).

2. The inner mitochondrial membrane is impermeable to protons, so that their pumping results in the generation of the ***electrochemical potential.***
3. Due to this electrochemical potential or proton-motive force, the H+ ions ejected out by electron transport flow back into the mitochondrial matrix down its electrochemical gradient through F0F1ATPase molecule **(Figure 10.6).**

The free energy is released as H+ ion flows back through the F0F1 ATPase into the released is coupled with the phosphorylation of ADP to ATP. The F0F1 ATPase catalyzes the addition of inorganic phosphate to ADP to form ATP.

Figure 10.6: Schematic diagram of chemiosmotic theory in which respiratory complexes, I, III and IV act as a proton pump and generate electrochemical gradient



P:O RATIO

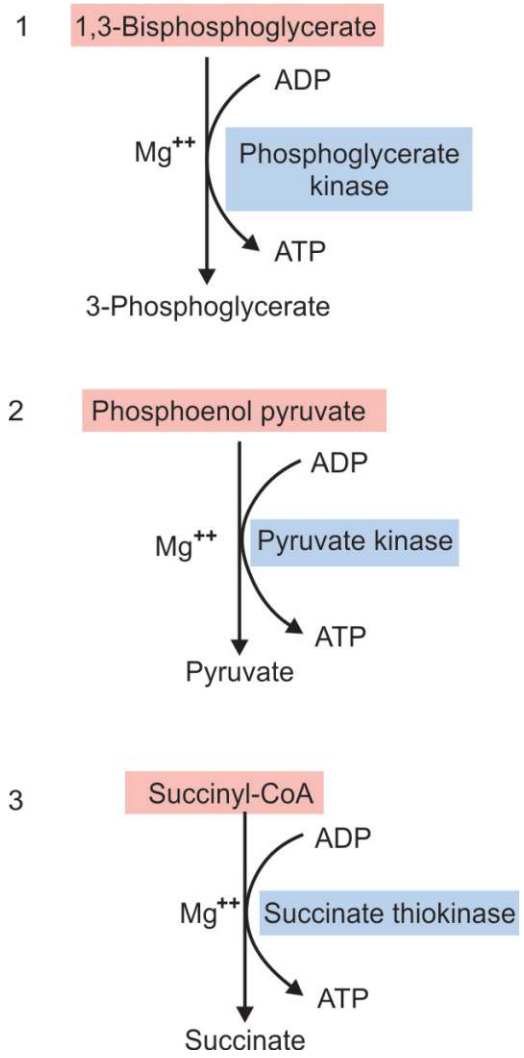
- The P:O ratio is a measure of the number of high energy phosphates (i.e. number of ATP molecules) synthesized per atom of oxygen consumed or per molecule of water produced.
- The P: O ratio for oxidation of metabolites that yield NADH is 3 and the ratio for those that yield FADH2 is 2

SUBSTRATE LEVEL PHOSPHORYLATION

The formation of ATP, directly coupled to metabolic process without involvement of electron transport chain and molecular O2, is called the production of ATP at the substrate level.

- Examples of substrate level phosphorylation processes are the conversion of:
  - Phosphoenolpyruvate to pyruvate
  - 1,3 bisphosphoglycerate to 3-phosphoglycerate
  - Succinyl-CoA to succinate **(Figure 10.7).**

Figure 10.7: Substrate level phosphorylation processes. Reaction 1 and 2 are of glycolysis and 3 occurs in TCA cycle



SHUTTLE SYSTEMS FOR OXIDATION OF EXTRAMITOCHONDRIAL NADH

Most of the NADH and FADH2 entering the mitochondrial electron transport chain arises from Kreb's cycle and β-oxidation of fatty acids, located in the mitochondria itself.

• Since, the inner mitochondrial membrane is not permeable to cytoplasmic NADH, how can the NADH generated by glycolysis, which take place outside of the mitochondria, be oxidized to NAD by respiratory chain located in mitochondria.

Special shuttle systems carry reducing equivalents from cytosolic NADH (rather than NADH itself) into the mitochondria by an indirect route.

Two such shuttle systems that can lead to the transport of reducing equivalent from the cytoplasm into mitochondria are:

- 1. The malate-aspartate shuttle
- 2. The glycerol phosphate shuttle.

The Malate-aspartate Shuttle System (Figure 10.8)

• The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate by cytosolic malate dehydrogenase.

• Malate, which carries the reducing equivalents, is transported across the inner membrane by a **dicarboxylate transport system.**

• The reducing equivalents carried by malate are then transferred to mitochondrial NAD+ by **mitochondrial malate dehydrogenase and malate itself gets** reoxidized to oxaloacetate.

- The resulting mitochondrial NADH is oxidized by the mitochondrial electron transport chain, leading to formation of 3 molecules of ATP.

- The oxaloacetate so formed cannot pass through the membrane from the mitochondrion back into cytosol,

so it is converted to **aspartate by transamination reaction which is transported to the cytosolic side** via amino acid transport system.

- In the cytosol, a reversal of the aspartate aminotransferase

reaction gives rise to oxaloacetate and glutamate, thereby completing the “shuttle like” process.

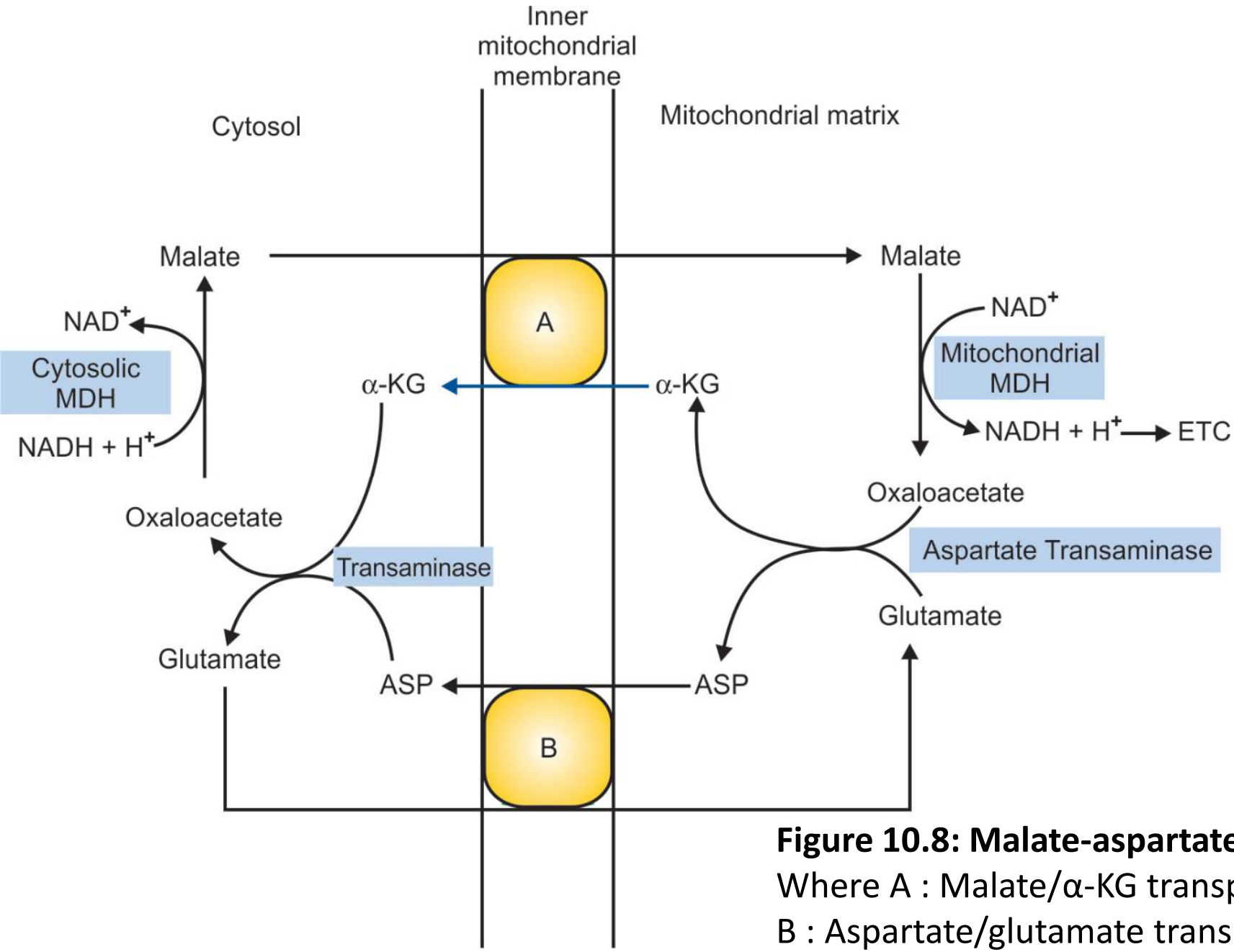


Figure 10.8: Malate-aspartate shuttle

Where A : Malate/α-KG transporter

B : Aspartate/glutamate transporter

α-KG : α-ketoglutarate

ASP : Aspartate

MDH : Malate dehydrogenase

ETC : Electron transport chain

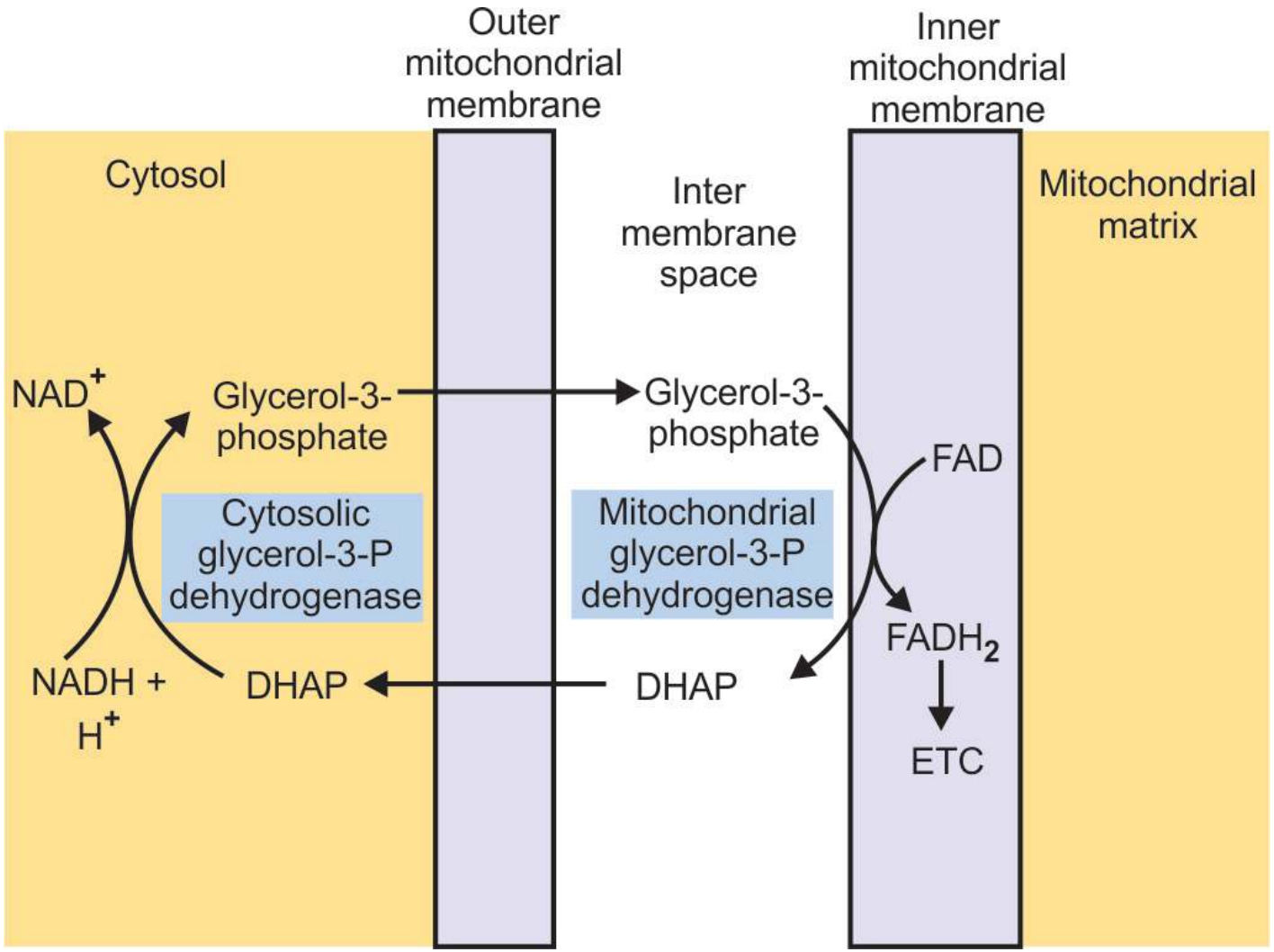
Glycerol Phosphate Shuttle (Figure 10.9)

- The first step in this shuttle is the transfer of a pair of electrons from cytosolic NADH to dihydroxyacetone phosphate to from glycerol-3-phosphate catalyzed by cytosolic glycerol-3-phosphate dehydrogenase enzyme.
- Glycerol-3-phosphate in turn diffuses through the outer mitochondrial membrane into the intermembrane space of the mitochondria.

- Here glycerol-3-phosphate is reoxidized to dihydroxyacetone phosphate on the outer surface of the inner mitochondrial membrane by a membrane bound FAD containing isoenzyme of glycerol-3-phosphate dehydrogenase.
- An electron pair from glycerol-3-phosphate is transferred to an FAD of the enzyme to form FADH2. FADH2 gets oxidized via ETC to generate 2 ATP.
- The dihydroxyacetone phosphate returns to the cytosol and can be reused for reduction of glycerol-3-phosphate.

Figure 10.9: Glycerol phosphate shuttle

where, DHAP: Dihydroxyacetone phosphate



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