BIOCHEMISTRY

Fourth Class-First Couse

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Oxidative Phosphorylation

We begin our study of oxidative phosphorylation by examining the oxidation–reduction reactions that allow the flow of electrons from NADH and $FADH_2$ to oxygen. The electron flow takes place in four large protein complexes that are embedded in the inner mitochondrial membrane, together called the ***respiratory chain* or the *electron-transport chain*.**

$$NADH + H^+ + 3\,ADP + 3\,Pi + 1/2\,O_2 \longrightarrow NAD^+ + 3\,ATP + H_2O$$

$$FADH_2 + 2\,ADP + 2\,Pi + 1/2\,O_2 \longrightarrow FAD + 2\,ATP + H_2O$$

The overall reaction is exergonic. Importantly, three of the complexes of the electron-transport chain use the energy released by the electron flow to pump protons from the mitochondrial matrix into the cytoplasm. The resulting unequal distribution of protons generates a pH gradient and a transmembrane electrical potential that creates a ***proton-motive force***. ATP is synthesized when protons flow back to the mitochondrial matrix through an enzyme complex.

$$ATP + Pi + H^+ \longrightarrow ATP + H_2O$$

Thus, *the oxidation of fuels and the phosphorylation of ADP are coupled by a proton gradient across the inner mitochondrial membrane* (Figure 22). Collectively, the generation of high-transfer-potential electrons by the citric acid cycle, their flow through the respiratory chain, and the accompanying synthesis of ATP is called *respiration* or *cellular respiration*.

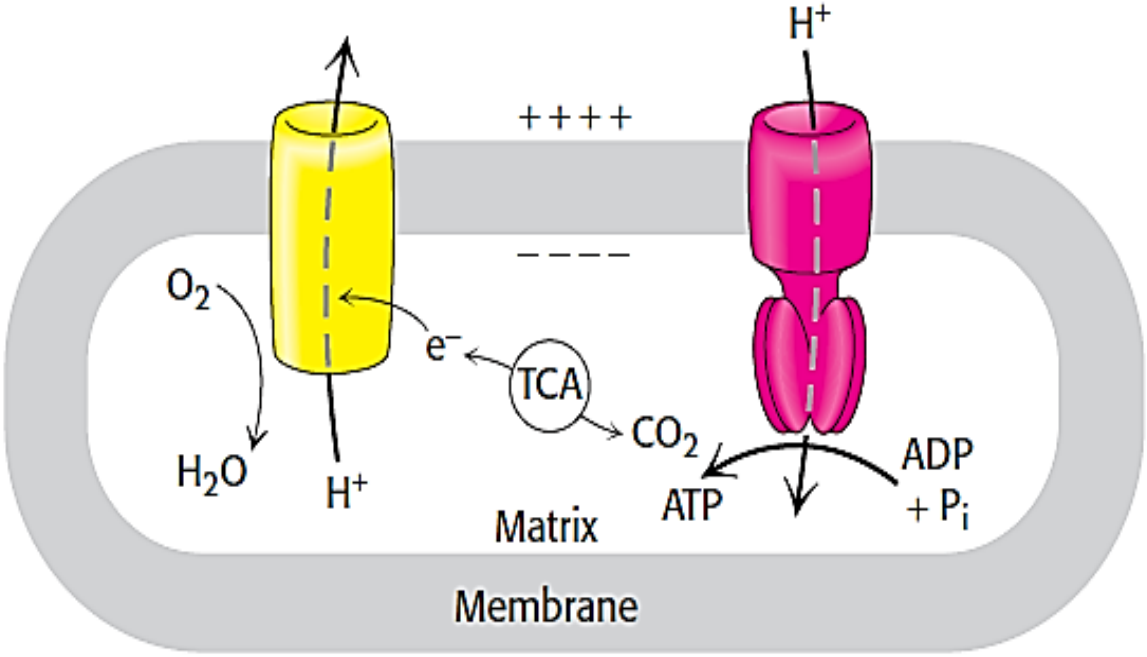
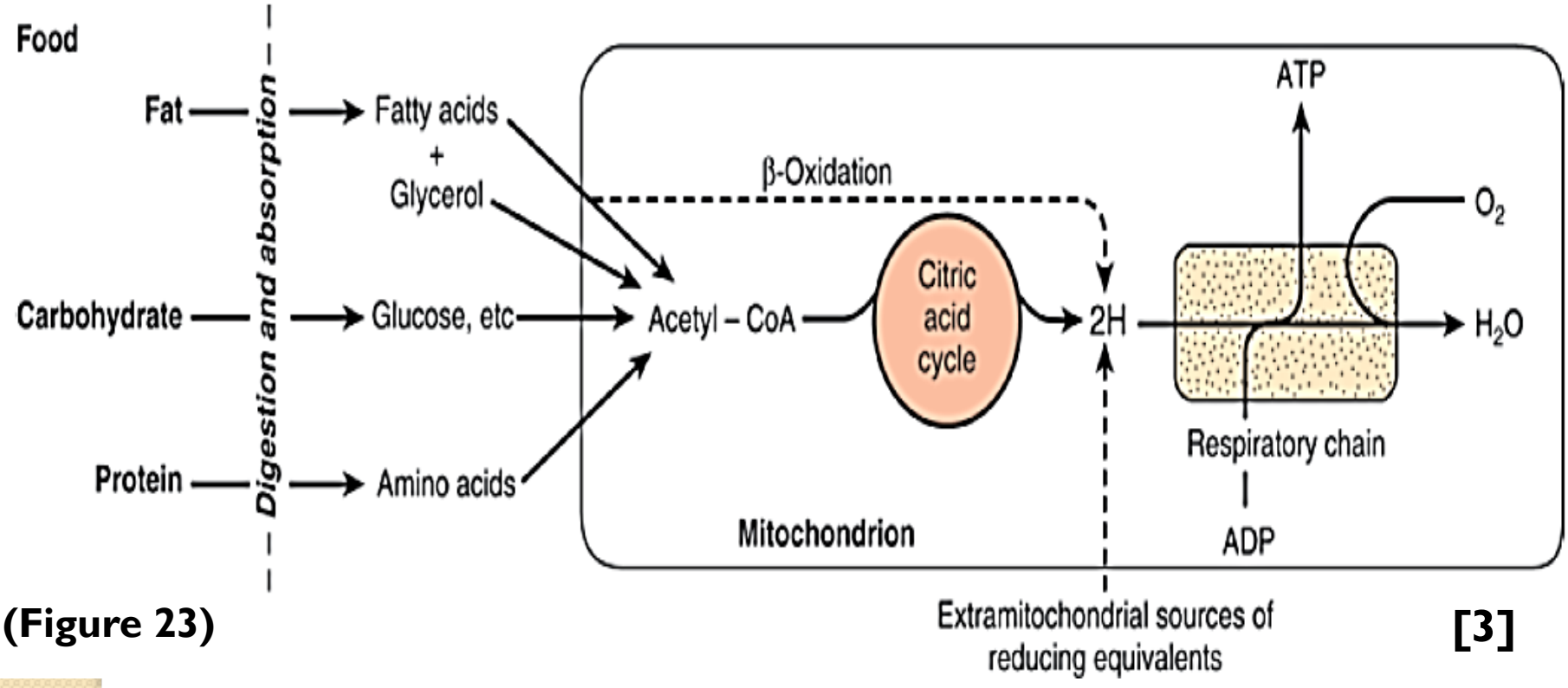


Figure 22: Overview of oxidative phosphorylation. Oxidation and ATP synthesis are coupled by transmembrane proton fluxes. Electrons flow from NADH and $FADH_2$ through four protein complexes to reduce oxygen to water. Three of the complexes pump protons from the mitochondrial matrix to the exterior of the mitochondria. The protons return to the matrix by flowing through another protein complex, ATP synthase, powering the synthesis of ATP.

[2]

THE RESPIRATORY CHAIN OXIDIZES REDUCING EQUIVALENTS & ACTS AS A PROTON PUMP

Most of the energy liberated during the oxidation of carbohydrate, fatty acids, glycerol and amino acids is made available within mitochondria as reducing equivalents (—H or electrons from coenzyme (NADH or $FADH_2$) (Figure 23). Note that the enzymes of the citric acid cycle and β-oxidation are contained in mitochondria, together with the respiratory chain, which collects and transports reducing equivalents, directing them to their final reaction with oxygen to form water, and the machinery for oxidative phosphorylation, the process by which the liberated free energy is trapped as ATP.



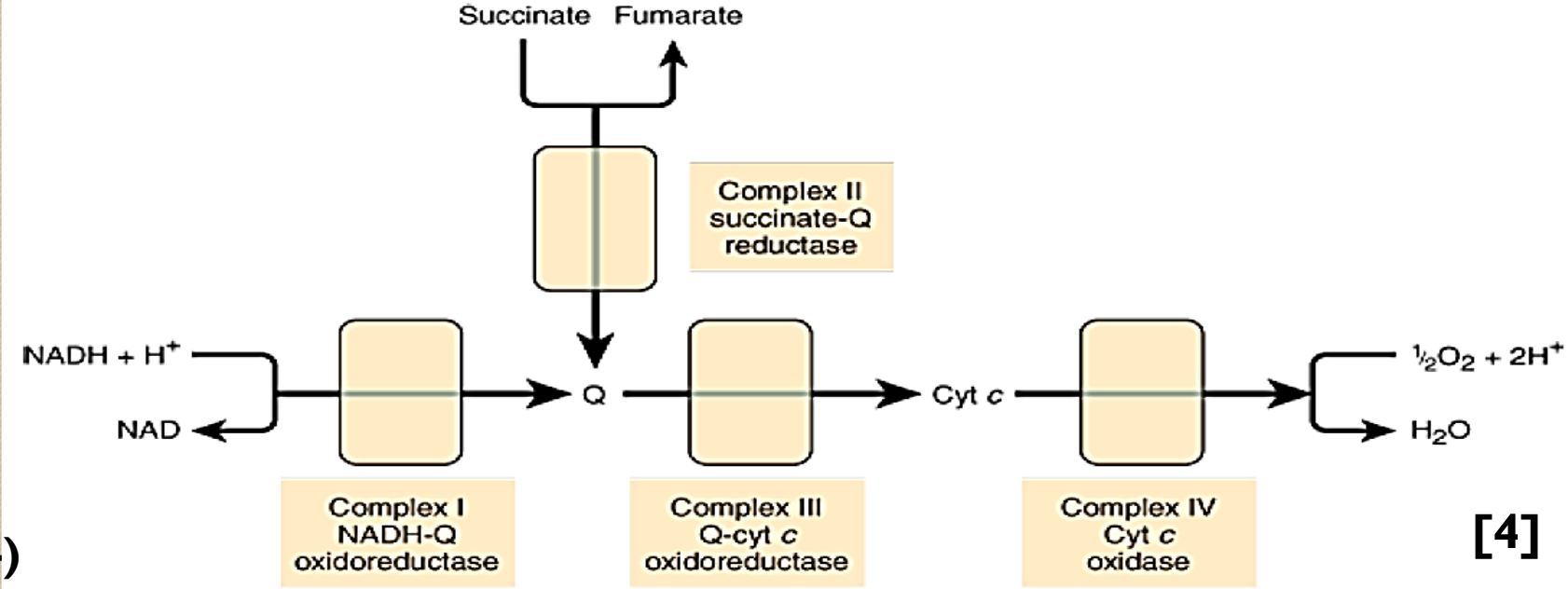
(Figure 23)

[3]

Components of the Respiratory Chain Are Contained in Four Large Protein Complexes Embedded in the Inner Mitochondrial Membrane

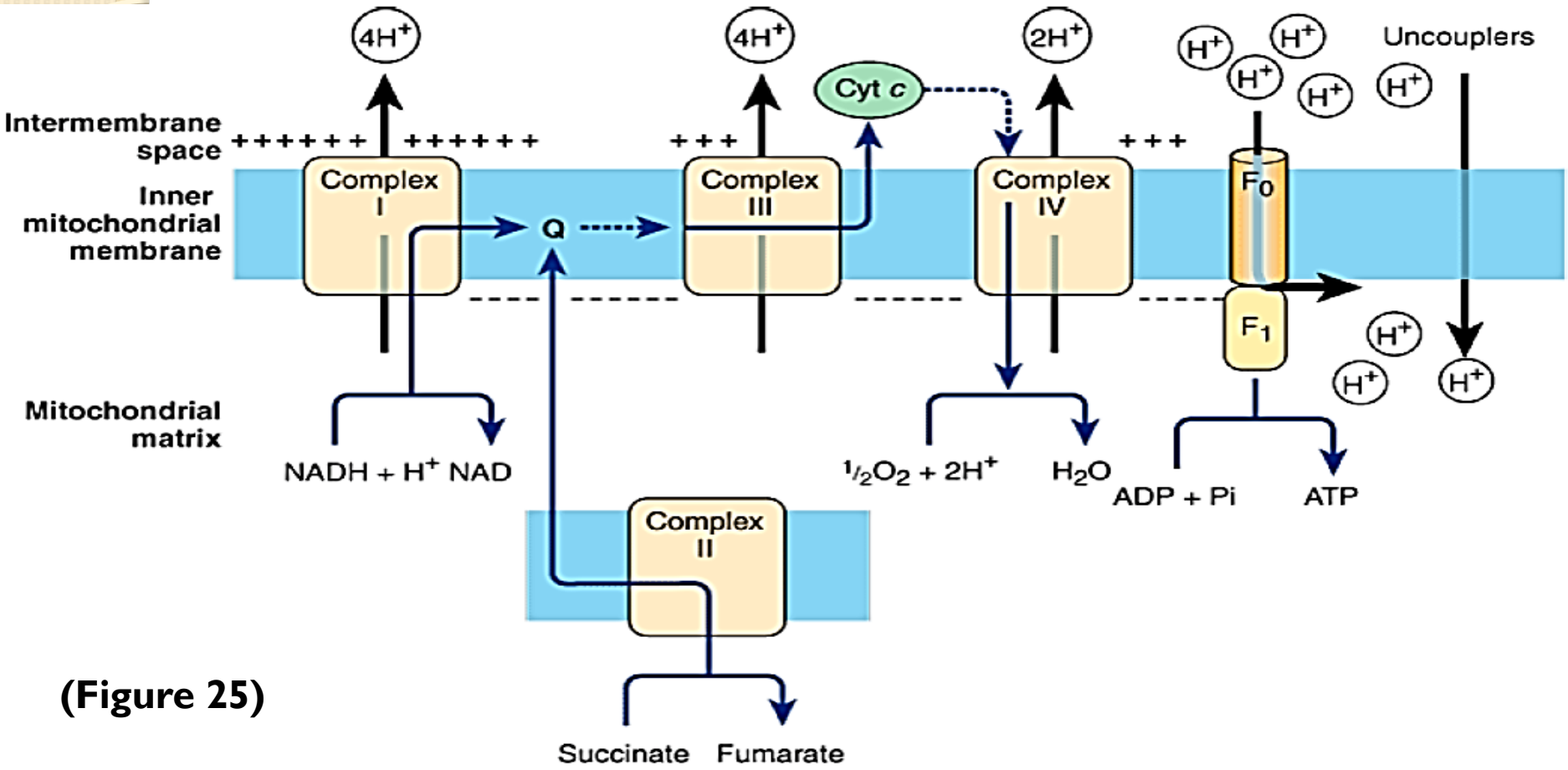
Electrons flow through the respiratory chain through three large protein complexes: NADH-Q oxidoreductase **(Complex I**), where electrons are transferred from NADH to coenzyme Q (Q) (also called ubiquinone); Q-cytochrome *c oxidoreductase* **(Complex III)**, which passes the electrons on to cytochrome *c; and cytochrome c oxidase* **(Complex IV)**, which completes the chain, passing the electrons to O_2 and causing it to be reduced to H_2O (Figure 24) . Some substrates with more positive redox potentials than NAD+ /NADH (eg, succinate) pass electrons to Q via a fourth complex, succinate-Q reductase **(Complex II),** rather than Complex I.

The four complexes are embedded in the inner mitochondrial membrane, but Q and cytochrome *c are mobile. Q diffuses rapidly within the membrane, while* cytochrome *c is a soluble protein. The flow of electrons through Complexes I, III, and IV results in the pumping of* protons from the matrix across the inner mitochondrial membrane into the intermembrane space.



(Figure 24)

[4]



(Figure 25)

The chemiosmotic theory of oxidative phosphorylation. Complexes I, III, and IV act as proton pumps creating a proton gradient across the membrane, which is negative on the matrix side. The proton motive force generated drives the synthesis of ATP as the protons flow back into the matrix through the ATP synthase enzyme (see Figure 25). Uncouplers increase the permeability of the membrane to ions, collapsing (يخفق) the proton gradient by allowing the H^+ to pass across without going through the ATP synthase, and thus uncouple electron flow through the respiratory complexes from ATP synthesis. (Q, coenzyme Q or uinone; cyt, cytochrome.)

[5]

Uncouplers dissociate oxidation in the respiratory chain from phosphorylation (Figure 26). These compounds are toxic in vivo, causing respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or Pi . The uncoupler that has been used most frequently is 2,4-dinitrophenol, but other compounds act in a similar manner.

Malonate inhibition for complex II but, antimycine A inhibition complex III and H_2S, CO, and CN inhibition for complex IV.

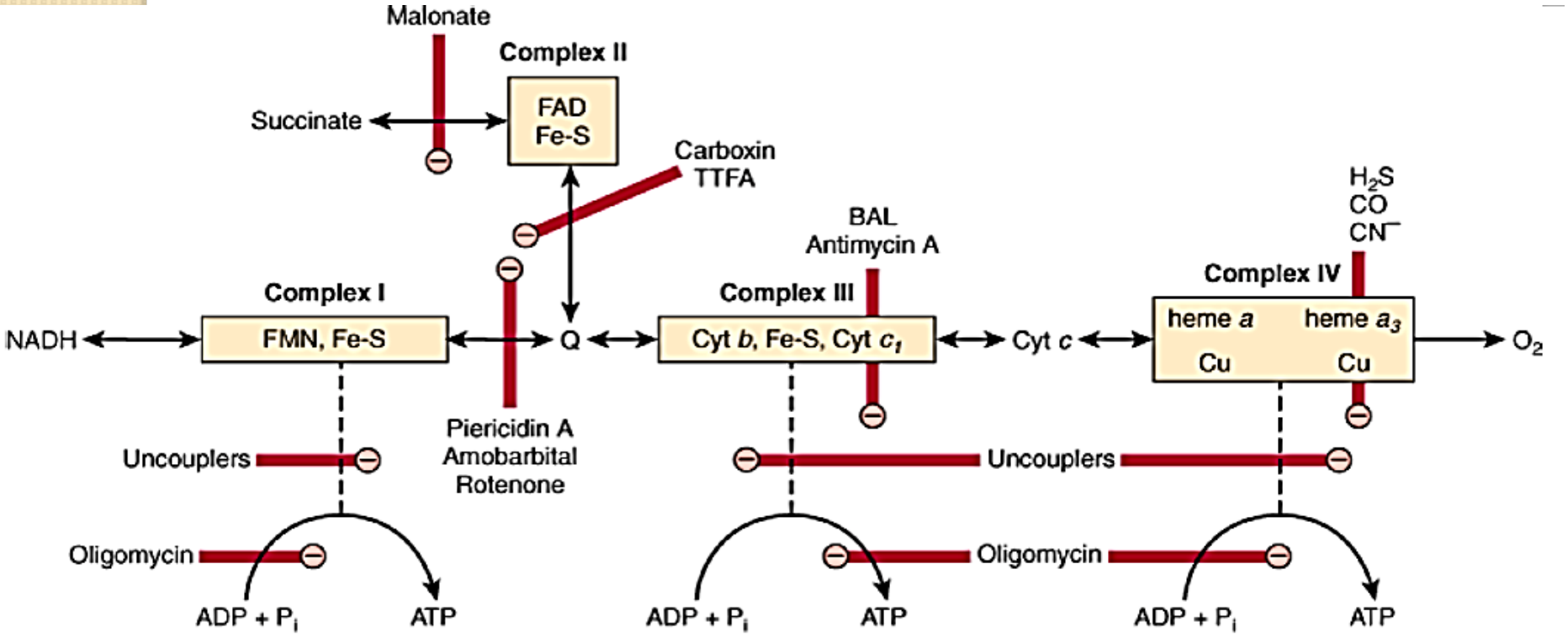


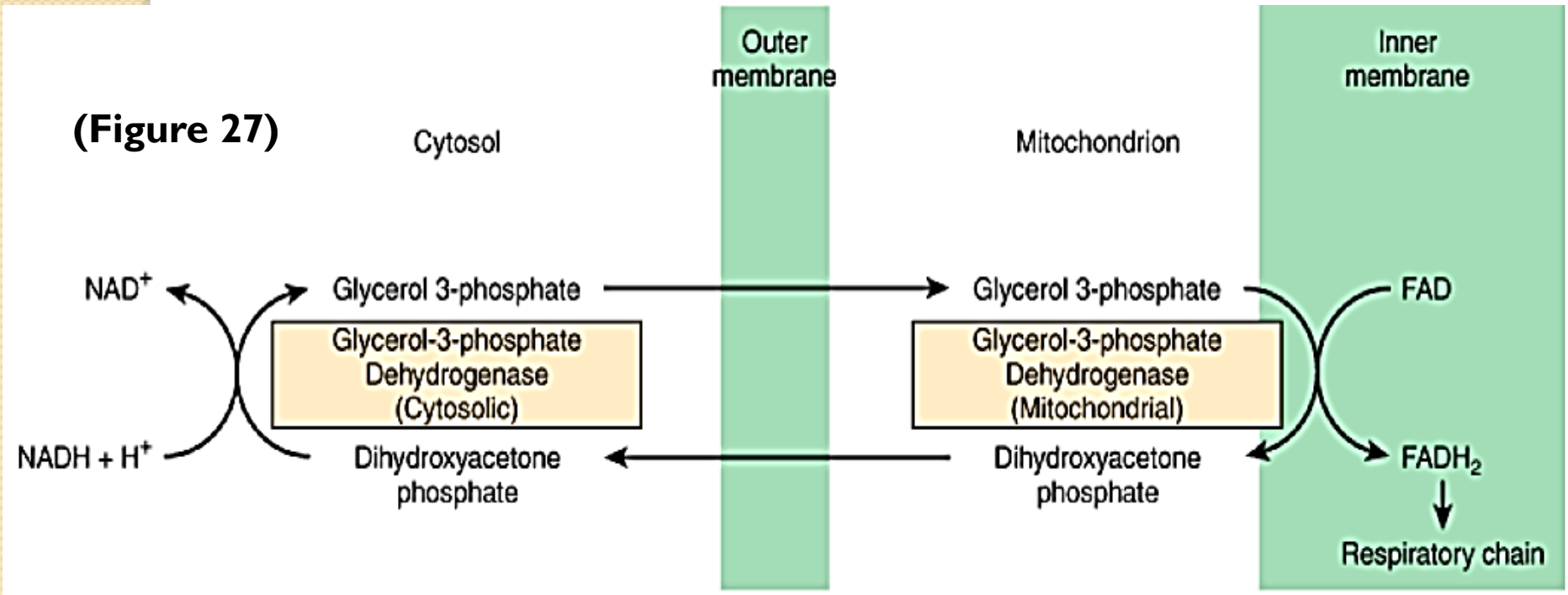
Figure 26 : Sites of inhibition (-) of the respiratory chain by specific drugs, chemicals, and antibiotics. (BAL, dimercaprol; TTFA, an Fechelating agent. (For see only)

[6]

Oxidation of Extramitochondrial NADH Is Mediated by Substrate Shuttles

NADH cannot penetrate the mitochondrial membrane and extramitochondrial NADH does not accumulate and is presumed to be oxidized by the respiratory chain in mitochondria. The transfer of reducing equivalents through the mitochondrial membrane requires substrate pairs, linked by suitable dehydrogenases on each side of the mitochondrial membrane. The mechanism of transfer using the glycerophosphate shuttle is shown in Figure 27. Since the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein rather than NAD. Although this shuttle is present in some tissues (eg, brain, white muscle), in others (eg, heart muscle) it is deficient. It is therefore believed that the malate shuttle system is of more universal utility. The complexity of this system is due to the impermeability of the mitochondrial membrane to oxaloacetate, which must react with glutamate to form aspartate and α-ketoglutarate by transamination before transport through the mitochondrial membrane and reconstitution to oxaloacetate in the cytosol**(Figure 28)**

(Figure 27)



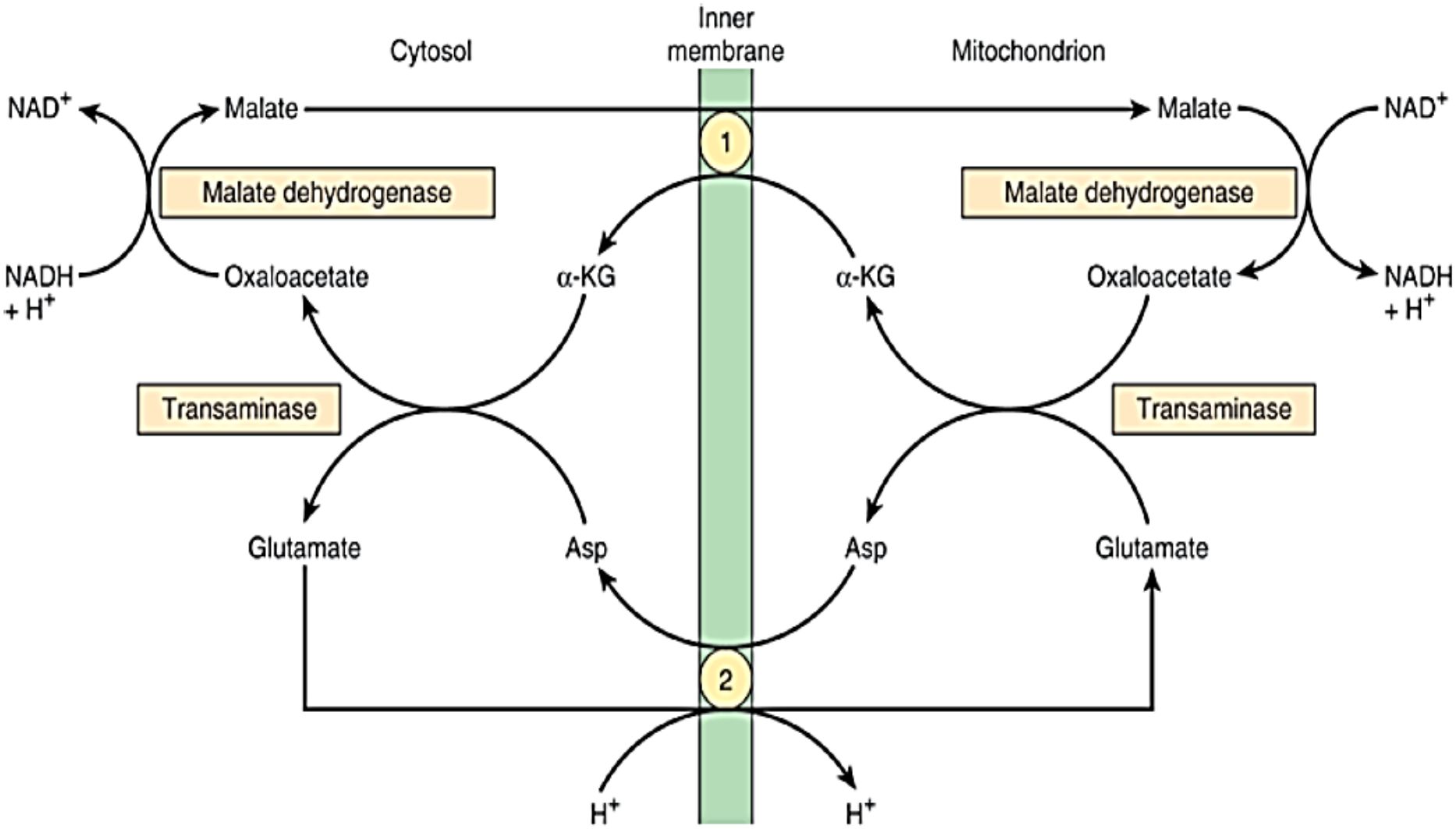


Figure 28 : Malate shuttle for transfer of reducing equivalents from the cytosol into the mitochondrion.α-Ketoglutarate transporter, glutamate/aspartate transporter (note the proton symport with glutamate). **[8]**