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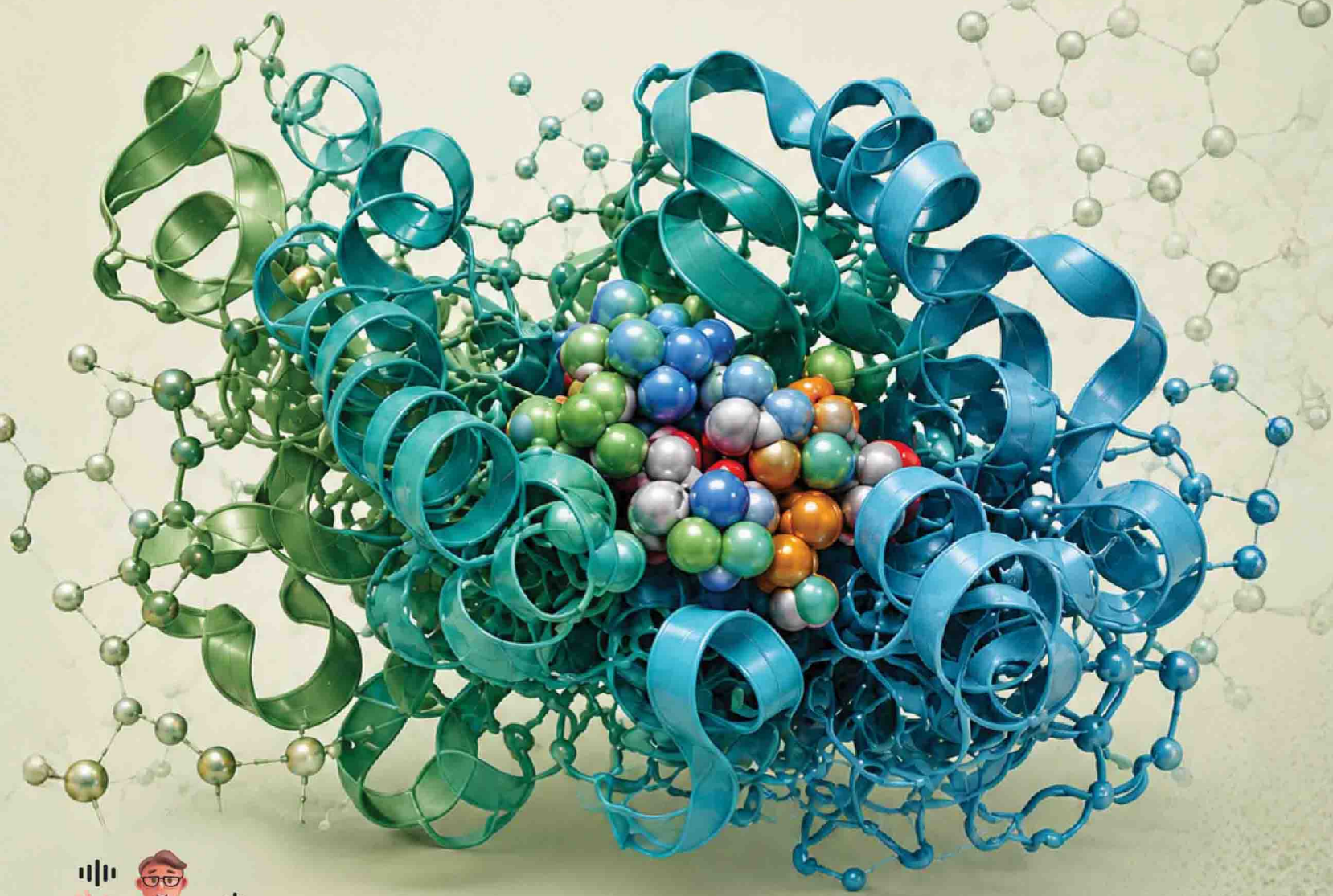



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Pankaja Naik

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CHAPTER

10

Biological Oxidation

Learning Objectives

On completion of the chapter, the students should be able to:

1. Describe electron transport chain.
2. Describe oxidative phosphorylation.
3. Describe substrate level phosphorylation.
4. Describe inhibitors of electron transport chain.

INTRODUCTION

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**.

ELECTRON TRANSPORT CHAIN (ETC)

Electron transport chain or **respiratory chain** is the pathway in aerobic cells by which electrons derived from various substrates are transferred to oxygen. The **ETC** is the final common pathway in aerobic cells by which electrons derived from various substrates are transferred to oxygen. Electron transport chain is a series of highly organized oxidation reduction **enzymes**, **coenzymes** and **electron carrier cytochromes**.

Location of ETC

The **electron transport chain** or **respiratory chain is** present in the **inner mitochondrial membrane**. The enzymes of the electron transport chain are embedded in the inner membrane in association with the enzymes of oxidative phosphorylation.

Components of the Electron Transport Chain

The major components of the respiratory chain include **coenzymes** and **cytochromes**.

Coenzymes

- Nicotinamide adenine dinucleotide (NAD^+) of various dehydrogenases.
- Flavin mononucleotide (FMN) of NADH dehydrogenase and Flavin adenine dinucleotide (FAD). The iron-containing protein called, iron-**sulfur (Fe-S) protein** is associated with **FMN** and **FAD**.
- Ubiquinone or coenzyme Q. Except for coenzyme Q, all members of this chain are proteins. Coenzyme Q (Co. Q) is a fat-soluble quinone and is a constituent of mitochondrial lipids.

Cytochromes

- The **cytochromes** are iron-containing electron transferring **hemoproteins.**
- There are three classes of cytochromes **a, b** and **c** having differences in their light absorption spectra.
- Several cytochromes occurring in the respiratory chain are **cytochrome b**, **cytochrome** $\mathbf{c_1}$, **cytochrome c** and **cytochrome** $\mathbf{aa_3}$.

Structural Organization of Components of Respiratory Chain

The components of the ETC are organized into four complexes, Complex I, Complex II, Complex III, and Complex IV **(Fig. 10.1)**.

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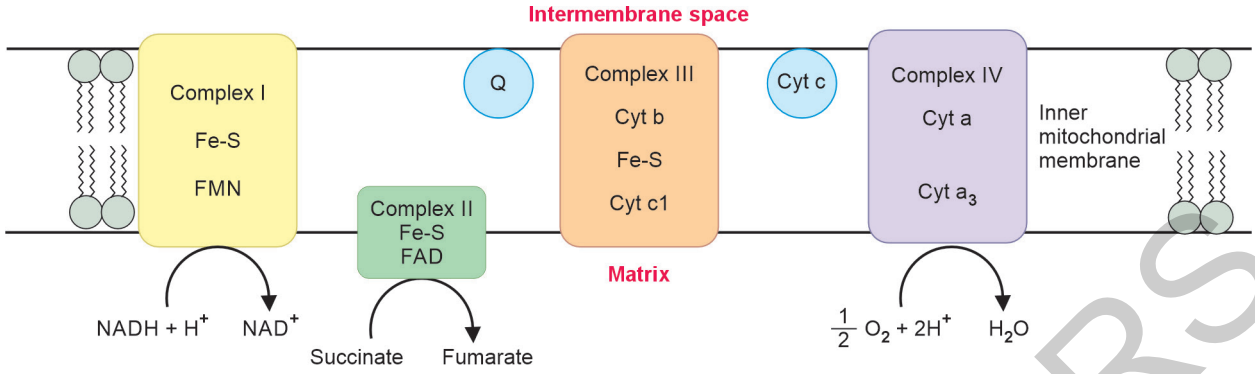

FIG. 10.1: Components of electron transport chain.
(Fe-s: Iron-sulfur protein, Q: coenzyme Q (ubiquinone) b, c_1, c, a, a_3: cytochromes)

Complex I

- Complex I also called **NADH: Ubiquinone oxidoreductase** or **NADH dehydrogenase**.
- It contains enzyme **NADH dehydrogenase**, **FMN**, and **iron-sulfur (Fe-S) proteins**.
- It transfers electrons from NADH to Q.

Complex II

- Complex II is also called **Succinate-Q reductase**.
- It consists of FAD and Fe-S protein.
- It transfers electrons from Succinate to Q.

Complex III

- Complex III; also called **Ubiquinone (Co.Q): Cytochrome c oxidoreductase**.
- It consists of Cyt b and Cyt c1 and **Fe-S protein**.
- It transfers electrons from **Q** to cytochrome **c**.

Complex IV

- Complex IV also called **Cytochrome oxidase**
- It consists of Cyt a and Cyt a_3
- It transfers electrons from cytochrome c to molecular oxygen and gets reduced to H_2O.

REACTIONS OF ELECTRON TRANSPORT CHAIN

The energy liberated during oxidation of carbohydrates, fatty acids and amino acids is made available within the mitochondria as reducing equivalents (H^+ or e^-). The respiratory chain (located in mitochondria) transports these reducing equivalents and hand them over to their final acceptor, oxygen to form water. Liberated free energy is trapped as high-energy phosphate **ATP** in the mitochondria by **oxidative phosphorylation**. The reactions of ETC are as follows **(Fig. 10.2)**:

1. Complex I oxidize mitochondrial NADH and transfers electrons through FMN and iron–sulfur (Fe-S) complexes to ubiquinone, providing enough energy to pump **four protons** ($4H^+$) from the matrix to the intermembrane space in the reaction. Coenzyme Q is reduced to QH_2.
2. In complex-II, $FADH_2$ is formed during the conversion of **succinate** to **fumarate** in the citric acid cycle and electrons are then passed through Fe-S complex to coenzyme Q to form QH_2. Complex II in contrast with complex I **does not pump protons** across the membrane. Consequently, no ATP is formed from at this complex. Thus, the oxidation of $FADH_2$ produces less ATP than from NADH.
3. Complex III catalyse the transfer of electrons from QH_2 to cytochrome c, with **transfer of four protons ($4H^+$) from the matrix to the intermembrane space.**
4. Complex IV (**cytochrome oxidase)** is the terminal component of the respiratory chain that transports electrons from cytochrome c to molecular oxygen, reducing it to **H_2O**. In this reaction **$2H^+$ are pumped** across the membrane. The O_2 remains tightly bound to complex IV until it is fully reduced to water, and this minimizes the release of incompletely reduced potentially damaging intermediates such as hydrogen peroxide, or hydroxyl free radicals.

Oxidative Phosphorylation

- During the transport of electrons through respiratory chain liberated free energy is conserved in the form of **ATP** through phosphorylation of ADP to ATP by an enzyme **F_0F_1-ATPase**, the overall coupled process is known as **oxidative phosphorylation**.
- There are **three energy liberating** sites of the electron transport chain, these are **complex I**, **complex III** and **complex IV (Fig. 10.2)** that provides the energy required

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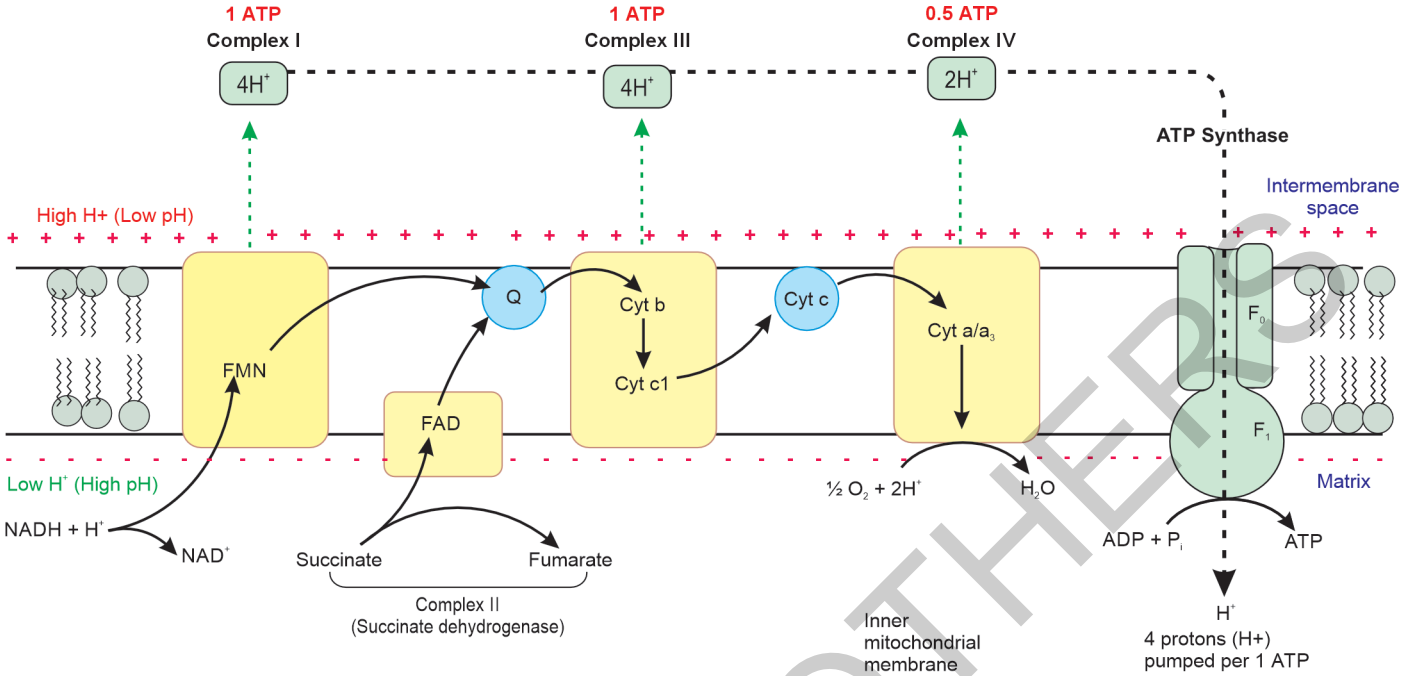

FIG. 10.2: Electron transport chain and oxidative phosphorylation.

to make ATP from ADP and Pi by an enzyme **F^0F_1-ATPase** in the process of oxidative phosphorylation.

- Electrons that enter the chain through NADH (complex I) pass through all three energy liberating sites and thus yield **2.5 ATPs**.
- However, electrons that enter the chain through FADH2 (complex II) pass through only two energy liberating sites, as they bypass complex I, they yield **1.5 ATPs (Fig. 10.2)**.

F_oF_1-ATPase.

- The enzyme complex F_oF_1-ATPase that synthesizes ATP is also called ATP synthase. 'F' is for protein factor. F_oF_1-ATPase is composed of two protein subunits, F_o and F_1. F_o and F_1 are embedded in the inner membrane and extend across it **(Fig. 10.3)**.
- The subscript 'O' is not zero, but the letter 'O' to denote that it is the portion of ATP synthase that binds the toxic antibiotic oligomycin, an inhibitor of this enzyme and thus oxidative phosphorylation. F_o unit forms a channel or path, through which hydrogen ions may pass across the membrane.
- F_1 is tightly bound to F_o and protruding into the matrix from the inner mitochondrial membrane. It contains the catalytic site for ATP synthesis.

SUBSTRATE LEVEL PHOSPHORYLATION

- The formation of ATP, directly coupled to metabolic processes without the involvement of the electron transport chain and molecular O_2, is called substrate-level phosphorylation.

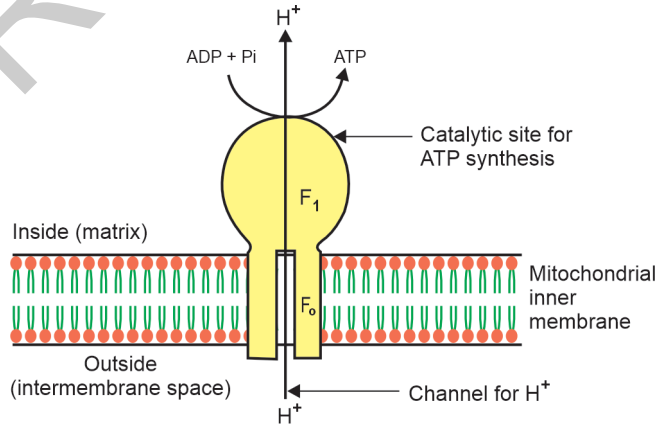

FIG. 10.3: F_oF_1-ATPase (ATP synthase) showing catalytic site for ATP synthesis and H^+ channel.

- Examples of substrate level phosphorylation processes are the conversion of:
  - Phosphoenolpyruvate to pyruvate
  - 1,3 bisphosphoglycerate to 3-phosphoglycerate
  - Succinyl-CoA to succinate **(Fig. 10.4)**.

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 (F_oF_1-ATPase)
3. Uncouplers of oxidative phosphorylation.

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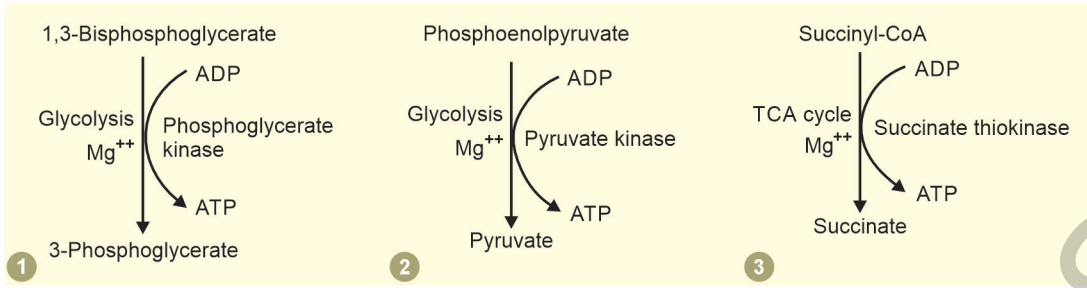

FIG. 10.4: Substrate level phosphorylation processes. Reaction 1 and 2 are of glycolysis and 3 occur in TCA cycle.

Inhibitors of Electron Transport Chain Proper

Inhibitors of the ETC are site specific and block the ETC at 4 sites: **Complex-I, complex-II, complex-III and complex-IV** and interrupt the flow of electron through the chain and therefore block the overall process of oxidative phosphorylation. Many poisons inhibit the electron transport chain. Some of these are **(Fig. 10.5)**:

- **Complex I:** Amytal (a barbiturate drug), rotenone (a plant product used as an insecticide) piericidin-A (an antibiotic) inhibit electron flow from **Fe-S** of complex I to **ubiquinone (Q)**.
- **Complex II** is inhibited by malonate, carboxin fungicide. Malonate is a competitive inhibitor of **succinate dehydrogenase**.
- **Complex III:** Dimercaprol, antimycin A, and British anti-lewisite (BAL) block electron transfer from **cytochrome b** to **cytochrome c_1**.

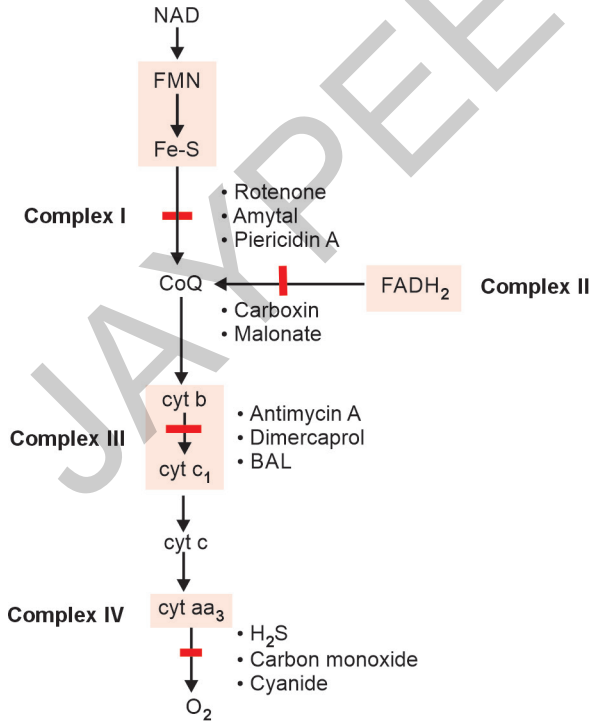

FIG. 10.5: Inhibitors of electron transport chain.

- **Complex IV** is inhibited by **Cyanide**, **Carbon monoxide** and **H_2S** by inhibiting cytochrome oxidase which blocks the transfer of electrons from cyt aa_3 to molecular oxygen and therefore totally arrest respiration.

Inhibitors of Oxidative Phosphorylation (F_oF_1-ATPase)

Another set of inhibitors do not inhibit special complexes. Instead, these compounds block phosphorylation directly by inhibiting **F_oF_1-ATPase** enzyme. For example, antibiotic **oligomycin** inhibits F_oF_1.

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^+ ions 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).
  - Dicumarol (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.

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EXAM QUESTIONS

LONG ANSWER QUESTION

1. Explain the electron transport chain in detail. Mention the ATP-synthesizing sites, the inhibitors and uncouplers of oxidative phosphorylation.

SHORT NOTES

1. Electron transport chain
2. Oxidative phosphorylation
3. Enzymes, coenzymes and electron carriers of biological oxidation
4. Substrate level phosphorylation
5. Inhibitors of ETC
6. Ionophores
7. Uncouplers of oxidative phosphorylation.

MULTIPLE CHOICE QUESTIONS

1. **All of the following are true regarding mitochondrial cytochromes, *except:***
   a. They all contain heme groups
   b. All are bound to protein components
   c. Iron must remain in the ferrous state to function in electron transport
   d. They accept or donate one electron at a time
2. **Which one of the following respiratory chain components reacts directly with molecular oxygen?**
   a. Coenzyme Q
   b. Cytochrome b
   c. Cytochrome aa_3
   d. Cytochrome C_1
3. **In oxidative phosphorylation, the oxidation of one molecule of $FADH_2$ produces:**
   a. 3 ATP molecules
   b. 1.5 ATP molecules
   c. 1 ATP molecule
   d. No ATP at all
4. **All of the following statements are true regarding ETC, *except:***
   a. Located in inner mitochondrial membrane
   b. Components are organized in decreasing order of redox potential
   c. Involved with ATP synthesis
   d. Cyanide inhibits electron flow
5. **The oxidation and phosphorylation of intact mitochondria are blocked by:**
   a. Puromycin
   b. Oligomycin
   c. Streptomycin
   d. Erythromycin
6. **Respiratory chain is found in:**
   a. Mitochondria
   b. Cytoplasm
   c. Nucleus
   d. Endoplasmic reticulum
7. **In oxidative phosphorylation, the oxidation of one molecule of NADH produces:**
   a. 2 ATP molecules
   b. 2.5 ATP molecules
   c. 4 ATP molecules
   d. 1 ATP molecule
8. **Which of the following vitamins is involved in ETC?**
   a. Thiamine
   b. Folic acid
   c. Riboflavin
   d. Cobalamin
9. **Which of the following trace elements has role in ETC?**
   a. Iron
   b. Iodine
   c. Zinc
   d. Fluoride
10. **An uncoupler of oxidative phosphorylation:**
    a. Inhibits electron transport and ATP synthesis
    b. Allows electron transport to occur without ATP formation
    c. Inhibits electron transport without impairment of ATP synthesis
    d. Inhibits transfer of electrons from cytochrome aa_3 to molecular oxygen
11. **Cyanide inhibits the electron transport chain by preventing transfer of electrons from:**
    a. Cyt aa_3 to molecular O_2
    b. Cyt b to $Cytc_1$
    c. From $FMNH_2$ to ubiquinone
    d. From $FADH_2$ to ubiquinone
12. **The enzyme that synthesizes ATP in oxidative phosphorylation is:**
    a. Hexokinase
    b. NADH dehydrogenase
    c. Cytochrome oxidase
    d. F_oF_1 ATPase
13. **A naturally occurring uncoupler is:**
    a. Dicumarol
    b. 2,4-DNP
    c. Thermogenin
    d. Salicylate

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14. A biochemical substance that can act as an uncoupler is:
a. Bilirubin
b. Free fatty acid
c. Thyroxine
d. All of the above

15. Which of the following is an ionophore?
a. 2,4-dinitrophenol
b. Valinomycin
c. Thermogenin
d. Oligomycin

16. Which of the following is not an ATP synthesizing site in respiratory chain?
a. Complex I
b. Complex II
c. Complex III
d. Complex IV

ANSWERS

1. c	**2.** c	**3.** b	**4.** b	**5.** b	**6.** a	**7.** b	**8.** c	**9.** a	**10.** b
11. a	**12.** d	**13.** c	**14.** d	**15.** b	**16.** b				

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