Notes

9

BIOLOGICAL OXIDATION, ELECTRON TRANSFER CHAIN AND OXIDATIVE PHOSPHORYLATION

9.1 INTRODUCTION

Chemically, oxidation is defined as the removal of electrons and reduction as the gain of electrons. Thus, oxidation is always accompanied by reduction of an electron acceptor. This principle of oxidation-reduction applies equally to biochemical systems and is an important concept underlying understanding of the nature of biologic oxidation. Many biologic oxidations can take place without the participation of molecular oxygen, eg, dehydrogenations. The life of higher animals is absolutely dependent upon a supply of oxygen for respiration, the process by which cells derive energy in the form of ATP from the controlled reaction of hydrogen with oxygen to form water. In addition, molecular oxygen is incorporated into a variety of substrates by enzymes designated as oxygenases; many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized by enzymes of this class, known as the cytochrome P450 system. Administration of oxygen can be lifesaving in the treatment of patients with respiratory or circulatory failure.

OBJECTIVES

After reading this lesson, you will be able to

- describe biological oxidation
- explain Electron transfer chain
- describe oxidation phosphorylation

9.2 BIOLOGICAL OXIDATION-REDUCTION

9.2.1 Redox potential – free energy changes

In reactions involving oxidation and reduction, the free energy change is proportionate to the tendency of reactants to donate or accept electrons. Free energy change expressed as oxidation-reduction or redox potential. The redox potential of a system is usually compared with the potential of the hydrogen electrode (0.0 volts at pH 0.0). However, for biologic systems, the redox potential is normally expressed at pH 7.0, at which pH the electrode potential of the hydrogen electrode is -0.42 volts. Enzymes involved in oxidation and reduction are called oxidoreductases and are classified into four groups: oxidases, dehydrogenases, hydroperoxidases, and oxygenases. Oxidases use oxygen as a hydrogen acceptor. Oxidases catalyze the removal of hydrogen from a substrate using oxygen as a hydrogen acceptor and form water or hydrogen peroxide as a reaction product.

9.2.2 Some oxidases contain copper

Cytochrome oxidase is a hemoprotein widely distributed in many tissues, having the typical heme prosthetic group present in myoglobin, hemoglobin, and other cytochromes. It is the terminal component of the chain of respiratory carriers found in mitochondria and transfers electrons resulting from the oxidation of substrate molecules by dehydrogenases to their final acceptor, oxygen. The enzyme is poisoned by carbon monoxide, cyanide, and hydrogen sulfide. It has also been termed cytochrome *a*3. It is now known that cytochromes *a* and *a*3 are combined in a single protein, and the complex is known as cytochrome *aa*3. It contains two molecules of heme, each having one Fe atom that oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Furthermore, two atoms of Cu are present, each associated with a heme unit.

9.2.3 Other oxidases are Flavoproteins

Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin. FMN and FAD are usually tightly – but not covalently – bound to their respective apoenzyme proteins. Metalloflavoproteins contain one or more metals as essential cofactors. Examples of flavoprotein enzymes include L-amino acid oxidase, an FMN-linked enzyme found in kidney with general specificity for the oxidative deamination of the naturally occurring L-amino acids.

9.2.4 Dehydrogenases cannot use oxygen as a hydrogen acceptor

There are a large number of enzymes in this class. They perform two main functions:

1. Transfer of hydrogen from one substrate to another in a coupled oxidation-reduction reaction. These dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+ (Figure 9.1). Since the reactions are reversible, these properties enable reducing equivalents to be freely transferred within the cell. This type of reaction, which enables one substrate to be oxidized at the expense of another, is particularly useful in enabling oxidative processes to occur in the absence of oxygen, such as during the anaerobic phase of glycolysis.

$$NAD^+ + AH_2 \longleftrightarrow NADH + H^+ + A$$

Fig. 9.1: NAD acting as a hydrogen carrier in the dehydrogenase reaction.

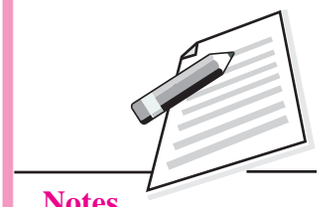
2. As components in the respiratory chain of electron transport from substrate to oxygen.

9.2.5 Many dehydrogenases depend on Nicotinamide Coenzymes

These dehydrogenases use nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+)—or both—and are formed in the body from the vitamin niacin. These coenzymes are reduced by the specific substrate of the dehydrogenase and reoxidized by a suitable electron acceptor. They may freely and reversibly dissociate from their respective apoenzymes. Generally, NAD-linked dehydrogenases catalyze oxidoreduction reactions in the oxidative pathways of metabolism, particularly in glycolysis, in the citric acid cycle, and in the respiratory chain of mitochondria. NADP-linked dehydrogenases are found characteristically in reductive syntheses, as in the extramitochondrial pathway of fatty acid synthesis and steroid synthesis— and also in the pentose phosphate pathway.

9.2.6 Other dehydrogenases depend on Riboflavin

The flavin groups associated with these dehydrogenases are similar to FMN and FAD occurring in oxidases. They are generally more tightly bound to their apoenzymes than are the nicotinamide coenzymes. Most of the riboflavin-linked dehydrogenases are concerned with electron transport in (or to) the respiratory chain. NADH dehydrogenase acts as a carrier of electrons between NADH and the components of higher redox potential. Other dehydrogenases such as succinate dehydrogenase, acyl-CoA dehydrogenase, and mitochondrial glycerol-3-phosphate dehydrogenase transfer reducing equivalents directly from the substrate to the respiratory chain. Another role of the flavin-dependent dehydrogenases is in the dehydrogenation of reduced lipoate, an intermediate in the oxidative decarboxylation of pyruvate and α-ketoglutarate. The electron-



transferring flavoprotein is an intermediary carrier of electrons between acyl-CoA dehydrogenase and the respiratory chain.

9.2.7 Cytochromes may also be regarded as dehydrogenases

The cytochromes are iron-containing hemoproteins in which the iron atom oscillates between Fe^{3+} and Fe^{2+} during oxidation and reduction. Except for cytochrome oxidase, they are classified as dehydrogenases. In the respiratory chain, they are involved as carriers of electrons from flavoproteins on the one hand to cytochrome oxidase on the other. Several identifiable cytochromes occur in the respiratory chain, ie, cytochromes b, c1, c, a, and a3 (cytochrome oxidase). Cytochromes are also found in other locations, eg, the endoplasmic reticulum (cytochromes P450 and b5), and in plant cells, bacteria, and yeasts.

9.2.8 Hydroperoxidases use hydrogen peroxide or organic peroxide as substrate

Two type of enzymes found both in animals and plants fall into this category: peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides. Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and perhaps cause cancer and atherosclerosis.

9.2.9 Peroxidases reduce peroxides using various electron acceptors

Peroxidases are found in milk and in leukocytes, platelets, and other tissues involved in eicosanoid metabolism. The prosthetic group is protoheme. In the reaction catalyzed by peroxidase, hydrogen peroxide is reduced at the expense of several substances that will act as electron acceptors, such as ascorbate, quinones, and cytochrome c. The reaction catalyzed by peroxidase is complex, but the overall reaction is as follows: In erythrocytes and other tissues, the enzyme glutathione peroxidase, containing selenium as a prosthetic group, catalyzes the destruction of H2O2 and lipid hydroperoxides by reduced glutathione, protecting membrane lipids and hemoglobin against oxidation by peroxides.

9.2.10 Catalase uses hydrogen peroxide as electron donor and electron acceptor

Catalase is a hemoprotein containing four heme groups. In addition to possessing peroxidase activity, it is able to use one molecule of H_2O_2 as a substrate electron donor and another molecule of H_2O_2 as an oxidant or electron acceptor (Figure 9.2). Under most conditions in vivo, the peroxidase activity of catalase seems to be favored. Catalase is found in blood, bone marrow, mucous membranes,

kidney, and liver. Its function is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases. Peroxisomes are found in many tissues, including liver. They are rich in oxidases and in catalase, Thus, the enzymes that produce H_2O_2 are grouped with the enzyme that destroys it. However, mitochondrial and microsomal electron transport systems as well as xanthine oxidase must be considered as additional sources of H_2O_2.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Fig. 9.2: Catalase uses hydrogen peroxide as electron donor and electron acceptor.

9.2.11 Cytochromes P450 are monooxygenases important for the detoxification of many drugs

Cytochromes P450 are an important superfamily of heme-containing monooxgenases, and more than 1000 such enzymes are known. Both NADH and NADPH donate reducing equivalents for the reduction of these cytochromes, which in turn are oxidized by substrates in a series of enzymatic reactions collectively known as the hydroxylase cycle (Figure 9.3). In liver microsomes, cytochromes P450 are found together with cytochrome *b*5 and have an important role in detoxification. Benzpyrene, aminopyrine, aniline, morphine, and benzphetamine are hydroxylated, increasing their solubility and aiding their excretion. Many drugs such as Phenobarbital have the ability to induce the formation of microsomal enzymes and of cytochromes P450. Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol.

$$\text{Reduced cytochrome P450} \longrightarrow \text{Oxidized cytochrome P450}$$

$$RH + O_2 \longrightarrow R - OH + H_2O$$

Fig. 9.3: Reduction and oxidation of cytochrome.

9.2.12 Superoxide dismutase protects aerobic organisms against oxygen toxicity

Transfer of a single electron to O_2 generates the potentially damaging superoxide anion free radical (O2"-) (Figure 9.4), the destructive effects of which are amplified by its giving rise to free radical chain reactions. The ease with which superoxide can be formed from oxygen in tissues and the occurrence of superoxide dismutase, the enzyme responsible for its removal in all aerobic organisms (although not in obligate anaerobes) indicate that the potential toxicity of oxygen is due to its conversion to superoxide. Superoxide is formed when reduced flavins – present, for example, in xanthine oxidase – are reoxidized univalently by molecular oxygen.

$$O_2^- + O_2^- + 2H^+ \xrightarrow{\text{Superoxide Dismutase}} H_2O + O_2$$

Fig. 9.4: Removal of superoxide anion free radical by superoxide dismutase enzyme.

9.3 THE RESPIRATORY CHAIN AND OXIDATIVE PHOSPHORYLATION

Aerobic organisms are able to capture a far greater proportion of the available free energy of respiratory substrates than anaerobic organisms. Most of this takes place inside mitochondria, which have been termed the "powerhouses" of the cell. Respiration is coupled to the generation of the high-energy intermediate, ATP, by oxidative phosphorylation, and the chemiosmotic theory offers insight into how this is accomplished. A number of drugs (eg, amobarbital) and poisons (eg, cyanide, carbon monoxide) inhibit oxidative phosphorylation, usually with fatal consequences. Several inherited defects of mitochondria involving components of the respiratory chain and oxidative phosphorylation have been reported. Patients present with myopathy and encephalopathy and often have lactic acidosis.

9.3.1 Specific enzymes act as markers

Mitochondria have an outer membrane that is permeable to most metabolites, an inner membrane that is selectively permeable, and a matrix within. The outer membrane is characterized by the presence of various enzymes, including acyl-CoA synthetase and glycerolphosphate acyltransferase. Adenylyl kinase and creatine kinase are found in the intermembrane space. The phospholipid cardiolipin is concentrated in the inner membrane together with the enzymes of the respiratory chain.

9.3.2 The respiratory chain collects and oxidizes reducing equivalents

Most of the energy liberated during the oxidation of carbohydrate, fatty acids, and amino acids is made available within mitochondria as reducing equivalents (H+ or electrons). Mitochondria contain the respiratory chain, which collects and transports reducing equivalents directing them to their final reaction with oxygen to form water, the machinery for trapping the liberated free energy as high-energy phosphate, and the enzymes of β-oxidation and of the citric acid cycle that produce most of the reducing equivalents.

9.3.3 Components of the respiratory chain are arranged in order of increasing redox potential

The respiratory chain consists of a number of redox carriers that proceed from the NAD-linked dehydrogenase systems, through flavoproteins and cytochromes,

to molecular oxygen. Not all substrates are linked to the respiratory chain through NAD-specific dehydrogenases; some, because their redox potentials are more positive (eg, fumarate/succinate; are linked directly to flavoprotein dehydrogenases, which in turn are linked to the cytochromes of the respiratory chain.

9.3.4 Ubiquinone or Q (coenzyme Q)

Coenzyme Q links the flavoproteins to cytochrome b, the member of the cytochrome chain of lowest redox potential. Q exists in the oxidized quinone or reduced quinol form under aerobic or anaerobic conditions, respectively. The structure of Q is very similar to that of vitamin K and vitamin E and of plastoquinone, found in chloroplasts. Q acts as a mobile component of the respiratory chain that collects reducing equivalents from the more fixed flavoprotein complexes and passes them on to the cytochromes. An additional component is the iron-sulfur protein (FeS; nonheme iron). It is associated with the flavoproteins (metalloflavoproteins) and with cytochrome b. The sulfur and iron are thought to take part in the oxidoreduction mechanism between flavin and Q, which involves only a single e-change, the iron atom undergoing oxidoreduction between Fe^{2+} and Fe^{3+}.

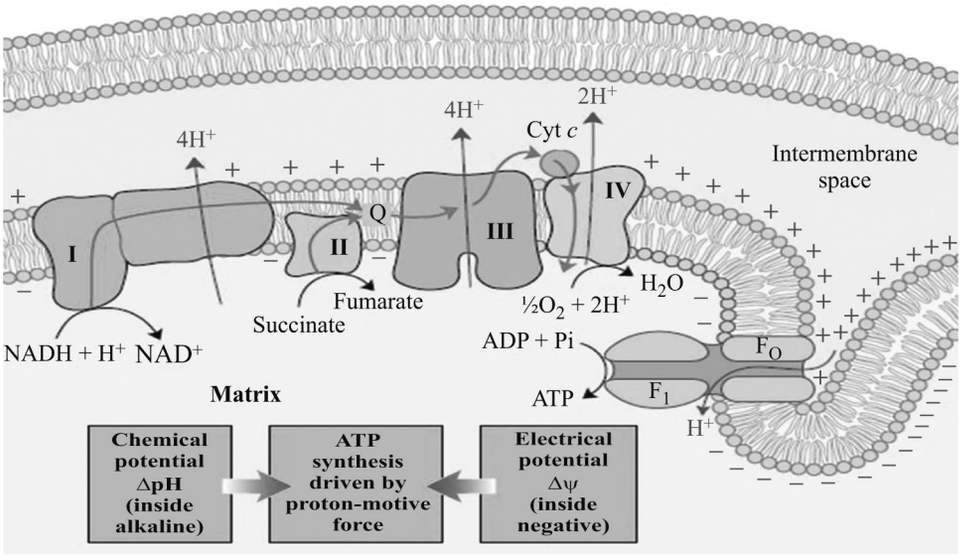
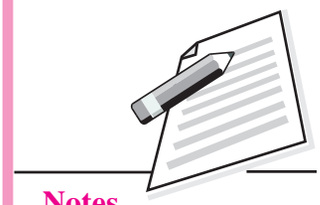


Fig. 9.5: In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient (Δψ). The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (Fo). The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F1 complex associated with Fo.



Pyruvate and α-ketoglutarate dehydrogenase have complex systems involving lipoate and FAD prior to the passage of electrons to NAD, while electron trans fers from other dehydrogenases, e.g., L(+)-3-hydroxyacyl- CoA dehydrogenase, couple directly with NAD. The reduced NADH of the respiratory chain is in turn oxidized by a metalloflavoprotein enzyme – NADH dehydrogenase. This enzyme contains FeS and FMN, is tightly bound to the respiratory chain, and passes reducing equivalents on to Q. Electrons flow from Q through the series of cytochromes in order of increasing redox potential to molecular oxygen. The terminal cytochrome aa3 (cytochrome oxidase), responsible for the final combination of reducing equivalents with molecular oxygen, has a very high affinity for oxygen, allowing the respiratory chain to function at maximum rate until the tissue has become depleted of O_2. Since this is an irreversible reaction (the only one in the chain), it gives direction to the movement of reducing equivalents and to the production of ATP, to which it is coupled. Functionally and structurally, the components of the respiratory chain are present in the inner mitochondrial membrane as four protein-lipid respiratory chain complexes that span the membrane. Cytochrome c is the only soluble cytochrome and, together with Q, seems to be a more mobile component of the respiratory chain connecting the fixed complexes. The overall reaction is given in the figure 9.5.

9.3.5 The respiratory chain provides most of the energy captured during catabolism

ADP captures, in the form of high-energy phosphate, a significant proportion of the free energy released by catabolic processes. The resulting ATP has been called the energy "currency" of the cell because it passes on this free energy to drive those processes requiring energy. There is a net direct capture of two high-energy phosphate groups in the glycolytic reactions, equivalent to approximately 103.2 kJ/mol of glucose. (In vivo, ΔG for the synthesis of ATP from ADP has been calculated as approximately 51.6 kJ/mol. (It is greater than ΔG0' for the hydrolysis of ATP, which is obtained under standard concentrations of 1.0 mol/ L.) Since 1 mol of glucose yields approximately 2870 kJ on complete combustion, the energy captured by phosphorylation in glycolysis is small. Two more high-energy phosphates per mole of glucose are captured in the citric acid cycle during the conversion of succinyl CoA to succinate.

All of these phosphorylations occur at the substrate level. When substrates are oxidized via an NAD-linked dehydrogenase and the respiratory chain, approximately 3 mol of inorganic phosphate are incorporated into 3 mol of ADP to form 3 mol of ATP per half mol of O_2 consumed; ie, the P:O ratio = 3. On the other hand, when a substrate is oxidized via a flavoprotein- linked dehydrogenase, only 2 mol of ATP are formed; ie, P:O = 2. These reactions are

known as oxidative phosphorylation at the respiratory chain level. Such dehydrogenations plus phosphorylations at the substrate level can now account for 68% of the free energy resulting from the combustion of glucose, captured in the form of high-energy phosphate. It is evident that the respiratory chain is responsible for a large proportion of total ATP formation.

9.3.6 Respiratory control ensures a constant supply of ATP

The rate of respiration of mitochondria can be controlled by the availability of ADP. This is because oxidation and phosphorylation are tightly coupled; ie, oxidation cannot proceed via the respiratory chain without concomitant phosphorylation of ADP. When work is performed, ATP is converted to ADP, allowing more respiration to occur, which in turn replenishes the store of ATP. Under certain conditions, the concentration of inorganic phosphate can also affect the rate of functioning of the respiratory chain. There is also the possibility that the ADP/ATP transporter, which facilitates entry of cytosolic ADP into and ATP out of the mitochondrion, becomes rate limiting. Thus, the manner in which biologic oxidative processes allow the free energy resulting from the oxidation of foodstuffs to become available and to be captured is stepwise, efficient (approximately 68%), and controlled – rather than explosive, inefficient, and uncontrolled, as in many nonbiologic processes. The remaining free energy that is not captured as high-energy phosphate is liberated as heat. This need not be considered "wasted," since it ensures that the respiratory system as a whole is sufficiently exergonic to be removed from equilibrium, allowing continuous unidirectional flow and constant provision of ATP. It also contributes to maintenance of body temperature.

9.3.7 Many poisons inhibit the respiratory chain

Much information about the respiratory chain has been obtained by the use of inhibitors, and, conversely, this has provided knowledge about the mechanism of action of several poisons (Figure 9.6). They may be classified as inhibitors of the respiratory chain, inhibitors of oxidative phosphorylation, and uncouplers of oxidative phosphorylation. Barbiturates such as amobarbital inhibit NAD linked dehydrogenases by blocking the transfer from FeS to Q. At sufficient dosage, they are fatal in vivo. Antimycin A and dimercaprol inhibit the respiratory chain between cytochrome b and cytochrome c. The classic poisons H2S, carbon monoxide, and cyanide inhibit cytochrome oxidase and can therefore totally arrest respiration. Malonate is a competitive inhibitor of succinate dehydrogenase. Atractyloside inhibits oxidative phosphorylation by inhibiting the transporter of ADP into and ATP out of the mitochondrion. The

action of uncouplers is to dissociate oxidation in the respiratory chain from phosphorylation. 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. The antibiotic oligomycin completely blocks oxidation and phosphorylation by acting on a step in phosphorylation.

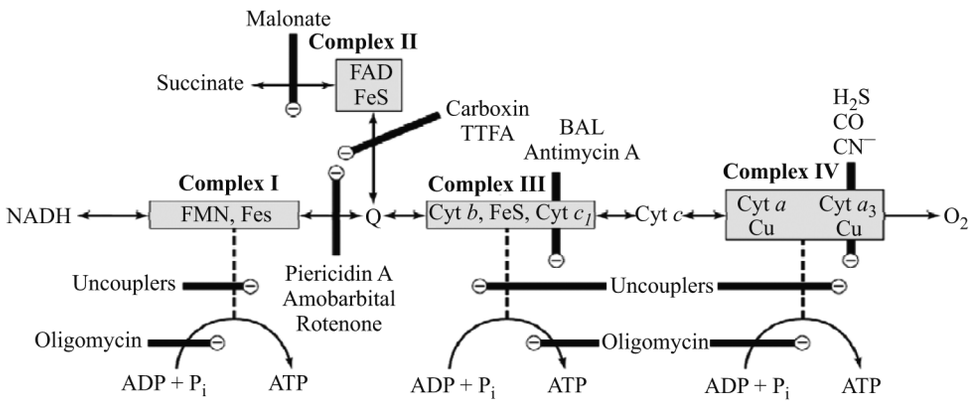


Fig. 9.6: Proposed sites of inhibition (--) of the respiratory chain by specific drugs, chemicals, and antibiotics. The sites that appear to support phosphorylation are indicated. BAL, dimercaprol. TTFA, an Fe-chelating agent. Complex I, NADH:ubiquinone oxidoreductase; complex II, succinate:ubiquinone oxidoreductase; complex III, ubiquinol:ferricytochrome c oxidoreductase; complex IV, ferrocytochrome c:oxygen oxidoreductase.

9.3.8 The chemiosmotic theory explains the mechanism of oxidative phosphorylation

Mitchell's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions (protons, H+) from the inside to the outside of the inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP (Figure 9.7).

9.3.9. The respiratory chain is a proton pump

Each of the respiratory chain complexes I, III, and IV act as a proton pump. The inner membrane is impermeable to ions in general but particularly to protons, which accumulate outside the membrane, creating an electrochemical potential difference across the membrane. This consists of a chemical potential (difference in pH) and an electrical potential.

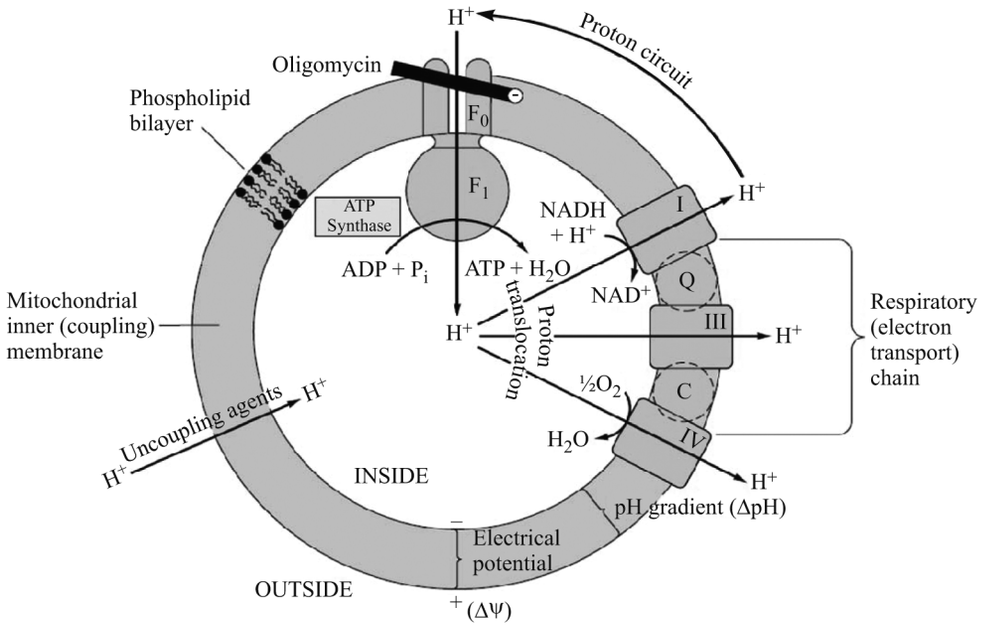
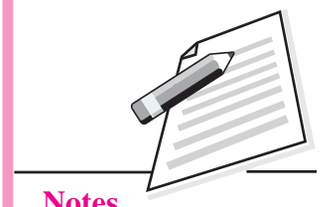


Fig. 9.7: Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the inside to the outside of the membrane, driven by the respiratory chain complexes I, III, and IV, each of which acts as a proton pump. Q, ubiquinone; C, cytochrome c; F1, F0, protein subunits which utilize energy from the proton gradient to promote phosphorylation. Uncoupling agents such as dinitrophenol allow leakage of H+ across the membrane, thus collapsing the electrochemical proton gradient. Oligomycin specifically blocks conduction of H^+ through F0.

9.3.10 A membrane-located ATP synthase functions as a rotary motor to form ATP

The electrochemical potential difference is used to drive a membrane-located ATP synthase which in the presence of Pi + ADP forms ATP. Scattered over the surface of the inner membrane are the phosphorylating complexes, ATP synthase, responsible for the production of ATP. These consist of several protein subunits, collectively known as F1, which project into the matrix and which contain the phosphorylation mechanism. These subunits are attached to a membrane protein complex known as F0, which also consists of several protein subunits. F0 spans the membrane and forms the proton channel. The flow of protons through F0 causes it to rotate, driving the production of ATP in the F1 complex. Estimates suggest that for each NADH oxidized, complex I translocates four protons and complexes III and IV translocate 6 between them. As four protons are taken into the mitochondrion for each ATP exported, the P:O ratio would not necessarily be a complete integer, ie, 3, but possibly 2.5. However, for simplicity, a value of 3 for the oxidation of NADH + H+ and 2 for the oxidation of FADH2 will continue to be used throughout this text.



9.3.11 The chemiosmotic theory can account for respiratory control and the action of uncouplers

The electrochemical potential difference across the membrane, once established as a result of proton translocation, inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back translocation of protons across the membrane through the vectorial ATP synthase. This in turn depends on availability of ADP and Pi. Uncouplers (eg, dinitrophenol) are amphipathic and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.

9.3.12 Impermeability of the inner mitochondrial membrane

The relative impermeability of the inner mitochondrial membrane necessitates exchange transporters. Exchange diffusion systems are present in the membrane for exchange of anions against OH- ions and cations against H+ ions. Such systems are necessary for uptake and output of ionized metabolites while preserving electrical and osmotic equilibrium. The inner bilipoid mitochondrial membrane is freely permeable to uncharged small molecules, such as oxygen, water, CO2, and NH3, and to monocarboxylic acids, such as 3-hydroxybutyric, acetoacetic, and acetic. Long-chain fatty acids are transported into mitochondria via the carnitine system, and there is also a special carrier for pyruvate involving a symport that utilizes the H+ gradient from outside to inside the mitochondrion. However, dicarboxylate and tri- carboxylate anions and amino acids require specific transporter or carrier systems to facilitate their passage across the membrane. Monocarboxylic acids penetrate more readily in their undissociated and more lipid-soluble form. The transport of di- and tricarboxylate anions is closely linked to that of inorganic phosphate, which penetrates readily as the $H_2PO_4^-$ ion in exchange for OH^-.

9.3.13. Ionophores permit specific cations to penetrate membranes

Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes, eg, valinomycin (K^+). The classic uncouplers such as dinitrophenol are, in fact, proton ionophores.

9.3.14 A proton-translocating transhydrogenase is a source of intramitochon-drial NADPH

Energy-linked transhydrogenase, a protein in the inner mitochondrial membrane, couples the passage of protons down the electrochemical gradient from outside

Notes

to inside the mitochondrion with the transfer of H from intramitochondrial NADH to NADPH for intramitochondrial enzymes such as glutamate dehydrogenase and hydroxylases involved in steroid synthesis.

9.3.15 Oxidation of extramitochondrial NADH is mediated by substrate shuttles

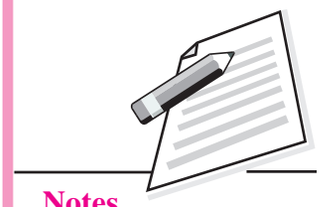
NADH cannot penetrate the mitochondrial membrane, but it is produced continuously in the cytosol by 3-phosphoglyceraldehyde dehydrogenase, an enzyme in the glycolysis sequence. However, under aerobic conditions, extramitochondrial NADH does not accumulate and is presumed to be oxidized by the respiratory 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 uses glycerophosphate shuttle. Since the mitochondrial enzyme is linked to the respiratory chain via a flavoprotein rather than NAD, only 2 mol rather than 3 mol of ATP are formed per atom of oxygen consumed. 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 and transaminate to aspartate and á-ketoglutarate before transport through the mitochondrial membrane and reconstitution to oxaloacetate in the cytosol.

9.3.16. Ion transport in mitochondria is energy-linked

Mitochondria maintain or accumulate cations such as K+, Na+, Ca2+, and Mg2+, and Pi. It is assumed that a primary proton pump drives cation exchange.

9.3.17 The creatine phosphate shuttle facilitates transport of high-energy phosphate from mitochondria

The creatine phosphate shuttle augments the functions of creatine phosphate as an energy buffer by acting as a dynamic system for transfer of high-energy phosphate from mitochondria in active tissues such as heart and skeletal muscle. An isoenzyme of creatine kinase is found in the mitochondrial intermembrane space, catalyzing the transfer of high-energy phosphate to creatine from ATP emerging from the adenine nucleotide transporter. In turn, the creatine phosphate is transported into the cytosol via protein pores in the outer mitochondrial membrane, becoming available for generation of extramitochondrial ATP.



INTEXT QUESTIONS 9.1

I. Choose the best answer

1. Chemically, the removal and the gain of electrons is defined respectively as

(a) Oxidation and reduction

(b) Reduction and oxidation

(c) Oxidation and dehydrogenase

(d) Reduction and dehydrogenase

2. Chemical carcinogens (xenobiotics) are metabolized by the enzymes system known as

(a) Cytochrome P450 (b) Xanthine oxidase

(c) Succinate dehydrogenase (d) Hydroperoxides

3. Oxidative phosphorylation is inhibited usually with fatal consequences by

(a) Quinalones (b) Cyanide

(c) Anacin (d) Amoxycillin

4. The action of uncouplers is to dissociate oxidation in the respiratory chain from

(a) Gluconeogenesis (b) Glycolysis

(c) TCA cycle (d) Phosphorylation

5. This antibiotic completely blocks oxidation and phosphorylation by acting on a step in phosphorylation.

(a) Dinitrophenol (b) Benzpyrene

(c) Oligomycin (d) Morphine

II. Fill in the blanks

6. Flavoprotein enzymes contain ..... or ..... as prosthetic groups.

7. Generally, NAD-linked dehydrogenases catalyze ..... reactions in the oxidative pathways of metabolism.

8. ..... protect the body against harmful peroxides.

9. The function of ..... is assumed to be the destruction of hydrogen peroxide formed by the action of oxidases.

10. Cytochromes P450 are an important superfamily of heme-containing .....

III. Match the following

11.	Mitochondria	(a)	Energy currency of the cell
12.	Cardiolipin	(b)	Uncouplers
13.	ATP	(c)	Powerhouses of the cell
14.	Valinomycin	(d)	Phospholipid
15.	Dinitrophenol	(e)	Ionophores

WHAT HAVE YOU LEARNT

- Oxidation is defined as the removal of electrons and reduction as the gain of electrons.
- Many drugs, pollutants, and chemical carcinogens (xenobiotics) are metabolized oxygenases known as cytochrome P450 system.
- Enzymes involved in oxidation and reduction are called oxidoreductases and are classified into four groups: oxidases, dehydrogenases, hydroperoxidases, and oxygenases.
- Flavoprotein enzymes contain flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as prosthetic groups. FMN and FAD are formed in the body from the vitamin riboflavin.
- Oxidation-reduction reactions carried out by dehydrogenases are specific for their substrates but often utilize common coenzymes or hydrogen carriers, eg, NAD+.
- The cytochromes are iron-containing hemoproteins in which the iron atom oscillates between Fe3+ and Fe2+ during oxidation and reduction.
- Cytochromes are also found in the endoplasmic reticulum (cytochromes P450 and b5), and in plant cells, bacteria, and yeasts.
- Two type of enzymes found both in animals and plants are peroxidases and catalase. Hydroperoxidases protect the body against harmful peroxides.
- Accumulation of peroxides can lead to generation of free radicals, which in turn can disrupt membranes and perhaps cause cancer and atherosclerosis.
- Mitochondrial cytochrome P450 systems are found in steroidogenic tissues such as adrenal cortex, testis, ovary, and placenta and are concerned with the biosynthesis of steroid hormones from cholesterol.
- The potential toxicity of oxygen is due to its conversion to superoxide in tissues and the enzyme superoxide dismutase is responsible for its removal.
- Mitochondria are termed as the “powerhouses” of the cell. Respiration is coupled to the generation of the high-energy intermediate, ATP, by oxidative

phosphorylation, and the chemiosmotic theory offers insight into how this is accomplished.

- A number of drugs (eg, amobarbital) and poisons (eg, cyanide, carbon monoxide) inhibit oxidative phosphorylation, usually with fatal consequences.
- Mitochondria contain the respiratory chain, which collects and transports reducing equivalents directing them to their final reaction with oxygen to form water, the machinery for trapping the liberated free energy as high-energy phosphate, and the enzymes of β-oxidation and of the citric acid cycle that produce most of the reducing equivalents.
- Mitchell's chemiosmotic theory postulates that the energy from oxidation of components in the respiratory chain is coupled to the translocation of hydrogen ions (protons, H+) from the inside to the outside of the inner mitochondrial membrane. The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ions is used to drive the mechanism responsible for the formation of ATP.
- Uncouplers (eg, dinitrophenol) are amphipathic and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.
- Ionophores are lipophilic molecules that complex specific cations and facilitate their transport through biologic membranes, eg, valinomycin (K+).

TERMINAL QUESTIONS

1. Write short note on electron transfer chain.
2. Write short note on oxidative phosphorylation.

ANSWERS TO INTEXT QUESTIONS

I. 1. (a) 2. (a) 3. (b) 4. (d) 5. (c)

II. 6. Flavin mononucleotide (FMN) or Flavin adenine dinucleotide (FAD)
7. Oxidoreduction
8. Hydroperoxidases
9. Catalase
10. Monooxgenases

III. 11. (c) 12. (d) 13. (a) 14. (e) 15. (b)