B.Sc. Zoology
(Honours)

M.B.B. University
(CBCS)

SEMESTER-IV
CORE COURSE - PAPER –X
BIOCHEMISTRY OF METABOLIC PROCES

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SEMESTER-IV
CORE COURSE - PAPER –X
BIOCHEMISTRY OF METABOLIC PROCES
UNIT – IV

(INTERMEDIARY METABOLISM & OXIDATIVE PHOSPHORYLATION)

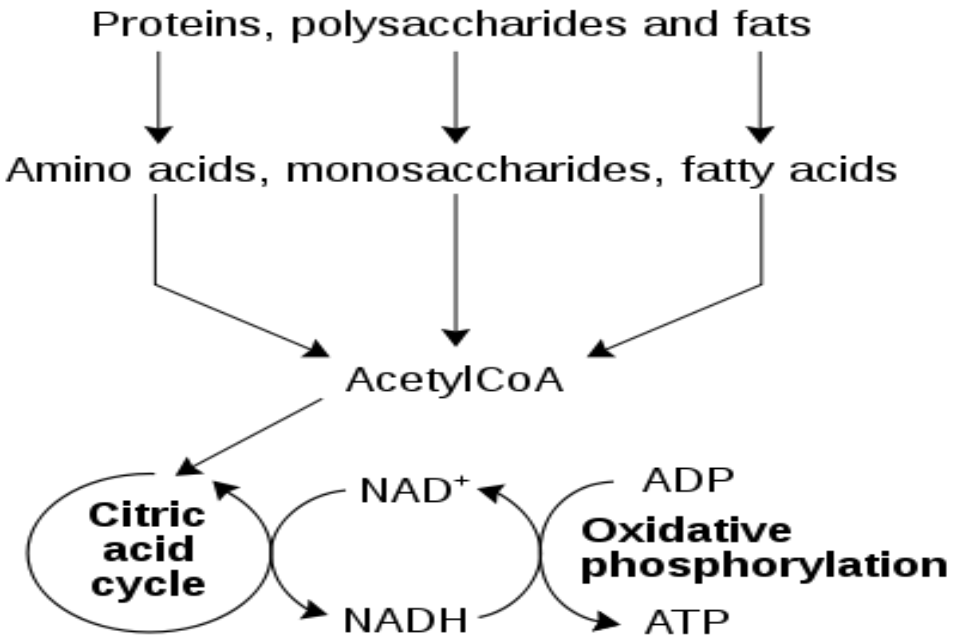
Syllabus/ Learning Highlights:

a) Inter-relationship of carbohydrates, lipid and protein metabolism.
b) Oxidative phosphorylation and ETS.
c) Role of ATP synthase, Inhibitors and Uncouplers.

A) INTER-RELATIONSHIP OF CARBOHYDRATES, LIPID AND PROTEIN METABOLISM

We know that catabolism of glucose, which provides energy to living cells. But living things consume more than glucose for food. How does a turkey sandwich end up as ATP in your cells? This happens because all of the catabolic pathways for carbohydrates, proteins, and lipids eventually connect into glycolysis and the citric acid cycle pathways. Metabolic pathways should be thought of as porous—that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways.

Metabolic pathways should be thought of as porous; that is, substances enter from other pathways, and intermediates leave for other pathways. These pathways are not closed systems. Many of the substrates, intermediates, and products in a particular pathway are reactants in other pathways. Like sugars and amino acids, the catabolic pathways of lipids are also connected to the glucose catabolism pathways.



Connections of Other Sugars to Glucose Metabolism:

Glycogen, a polymer of glucose, is an energy storage molecule in animals. When there is adequate ATP present, excess glucose is shunted into glycogen for storage. Glycogen is made and stored in both liver and muscle. The glycogen will be hydrolyzed into glucose monomers (G-1-P) if blood sugar levels drop. The presence of glycogen as a source of glucose allows ATP to be produced for a longer period of time during exercise. Glycogen is broken down into G-1-P and converted into G-6-P in both muscle and liver cells, and this product enters the glycolytic pathway.

Sucrose is a disaccharide with a molecule of glucose and a molecule of fructose bonded together with a glycosidic linkage. Fructose is one of the three dietary monosaccharides, along with glucose and galactose (which is part of the milk sugar, the disaccharide lactose), which are absorbed directly into the bloodstream during digestion. The catabolism of both fructose and galactose produces the same number of ATP molecules as glucose.

Glycogen, a polymer of glucose, is an energy-storage molecule in animals. When there is adequate ATP present, excess glucose is shunted into glycogen for storage. Glycogen is made and stored in both the liver and muscles. The glycogen is hydrolyzed into the glucose monomer, glucose-1-phosphate (G-1-P), if blood sugar levels drop. The presence of glycogen as a source of glucose allows ATP to be produced for a longer period of time during exercise. Glycogen is broken down into G-1-P and converted into glucose-6-phosphate (G-6-P) in both muscle and liver cells; this product enters the glycolytic pathway.

Connections of Proteins to Glucose Metabolism:

Proteins are hydrolyzed by a variety of enzymes in cells. Most of the time, the amino acids are recycled into the synthesis of new proteins. If there are excess amino acids, however, or if the body is in a state of starvation, some amino acids will be shunted into the pathways of glucose catabolism. Each amino acid must have its amino group removed prior to entry into these pathways. The amino group is converted into ammonia. In mammals, the liver synthesizes urea from two ammonia molecules and a carbon dioxide molecule. Thus, urea is the principal waste product in mammals produced from the nitrogen originating in amino acids, and it leaves the body in urine.
The carbon skeletons of certain amino acids (indicated in boxes) derived from proteins can feed into the citric acid cycle.

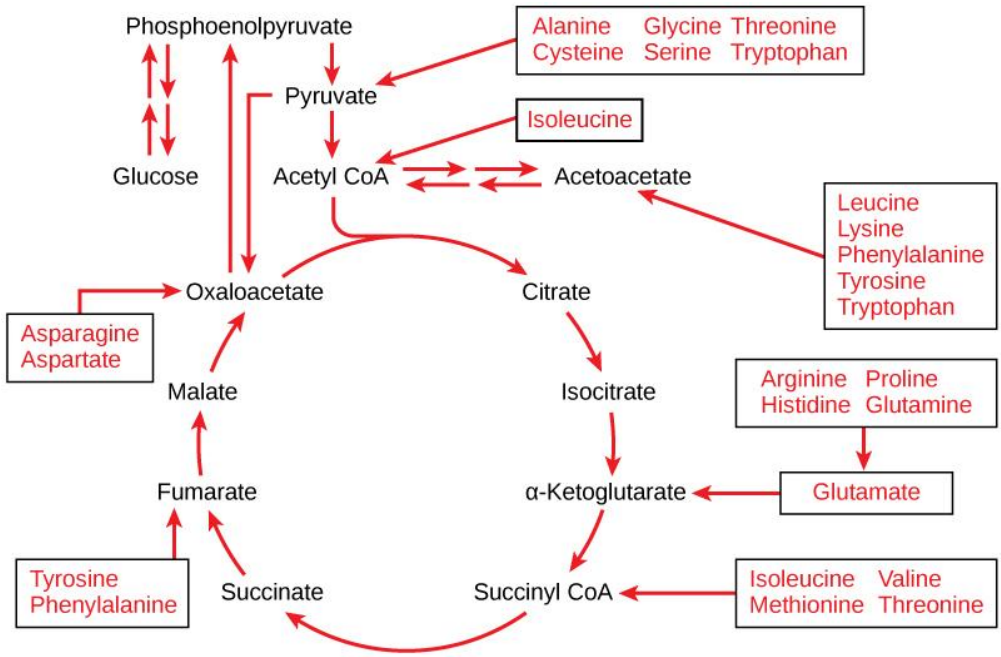


Figure: Connections of Proteins to metabolic pathways of Glucose

Connections of Lipid and Glucose Metabolisms:

The lipids that are connected to the glucose pathways are cholesterol and triglycerides. Cholesterol is a lipid that contributes to cell membrane flexibility and is a precursor of steroid hormones. The synthesis of cholesterol starts with acetyl groups and proceeds in only one direction. The process cannot be reversed.

Triglycerides are a form of long-term energy storage in animals. Triglycerides are made of glycerol and three fatty acids. Animals can make most of the fatty acids they need. Triglycerides can be both made and broken down through parts of the glucose catabolism pathways. Glycerol can be phosphorylated to glycerol-3-phosphate, which continues through glycolysis. Fatty acids are catabolized in a process called beta-oxidation that takes place in the matrix of the mitochondria and converts their fatty acid chains into two carbon units of acetyl groups. The acetyl groups are picked up by CoA to form acetyl CoA that proceeds into the citric acid cycle.

Glycogen from the liver and muscles, hydrolyzed into glucose-1-phosphate, together with fats and proteins, can feed into the catabolic pathways for carbohydrates.

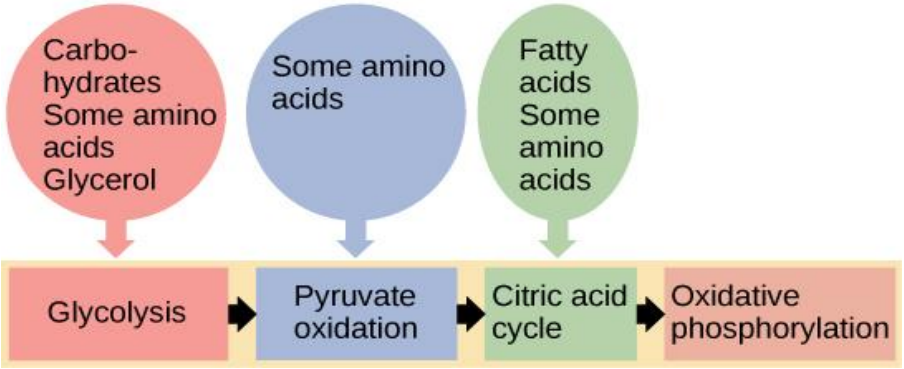


Figure: Common intermediately & coinciding pathways of metabolism

Conclusion: The breakdown and synthesis of carbohydrates, proteins, and lipids connect with the pathways of glucose catabolism. The simple sugars are galactose, fructose, glycogen, and pentose. These are catabolized during glycolysis. The amino acids from proteins connect with glucose catabolism through pyruvate, acetyl CoA, and components of the citric acid cycle. Cholesterol synthesis starts with acetyl groups, and the components of triglycerides come from glycerol-3-phosphate from glycolysis and acetyl groups produced in the mitochondria from pyruvate.

B) OXIDATIVE PHOSPHORYLATION AND ETS

The mitochondrial electron transport chain is composed of three main membrane-associated electron carriers flavoproteins (FMN, FAD), cytochromes, and quinones (coenzyme Q, also known as ubiquinone because it is a ubiquitous quinone in biological systems).

All these electron carriers reside within the inner membrane of the mitochondria and operate together to transfer electrons from donors, like NADH and $FADH_2$, to acceptors, such as O_2. The, electrons flow from carriers with more negative reduction potentials to those with more positive reduction potentials and eventually combine with O_2 and H to form water.

However, the mitochondrial electron transport system is arranged into four enzyme complexes of carriers, each capable of transporting electrons part of the way to O_2 (Fig. 24.5). Coenzyme Q and cytochrome c connect the complexes with each other.

The four enzyme complexes of carriers are: NADH-Q oxidoreductase, succinate-Q-reductase, Q-cytochrome c oxidoreductase, and cytochrome c oxidase. These complexes are the enzyme complex and each of them consists of different prosthetic groups (Table 24.2).

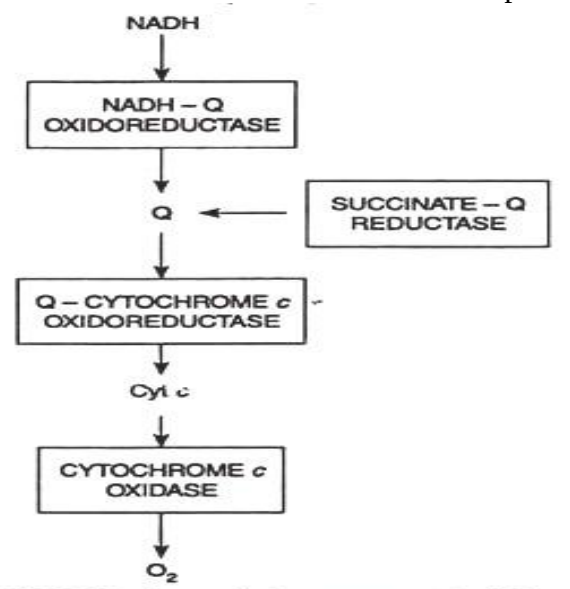


FIG. 24.5. Sequential arrangement of four complexes of electron carriers in mitochondrial electron transport chain.

TABLE 24.2. Four enzyme complexes of mitochondrial electron transport chain

	Enzyme complex	*Mass (KDa)*	*Number of subunits*[1]	*Prosthetic groups*
Complex I :	NADH-Q oxidoreductase (NADH dehydrogenase)	880	42(14)	FMN, FeS
Complex II:	Succinate-Q reductase (succinate dehydrogenase)	140	4	FAD, FeS
Complex III:	Q-cytochrome *c* oxidoreductase	250	10	Cyt b_H (Heme b_H), Cyt b_L (Heme b_L), FeS, Cyt c_L (Heme c_L)
Complex IV :	Cytochrome *c* oxidase	160	10 (3-4)	Cyt *a* (Heme *a*), Cyt a_3 (Heme a_3), Cu_A, Cu_B

The process of mitochondrial electron transport chain is summarized in Figure 24.6, which shows the flow of electrons and protons through the four enzyme complexes of the transport chain.

The whole process of mitochondrial electron transport can be represented in brief in the following manner:

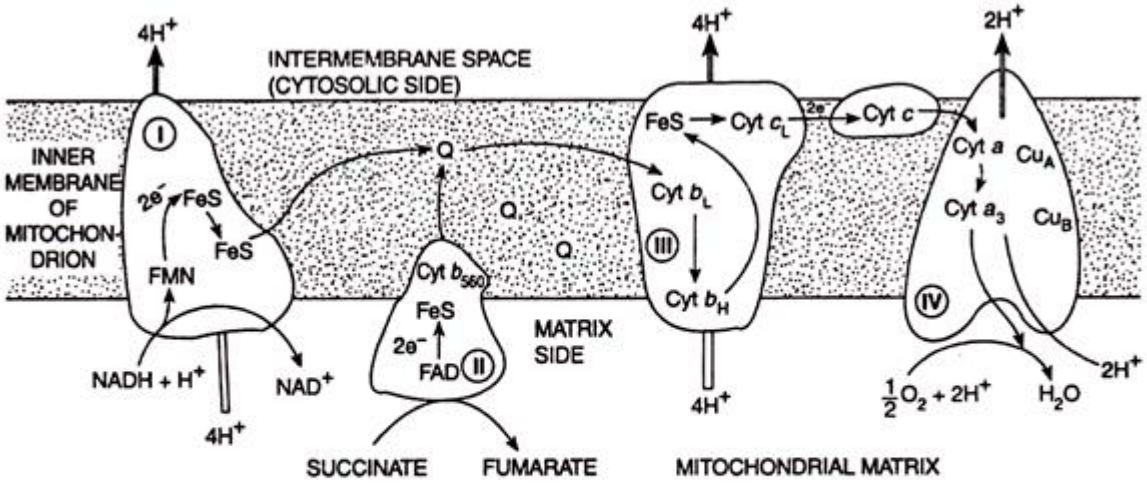
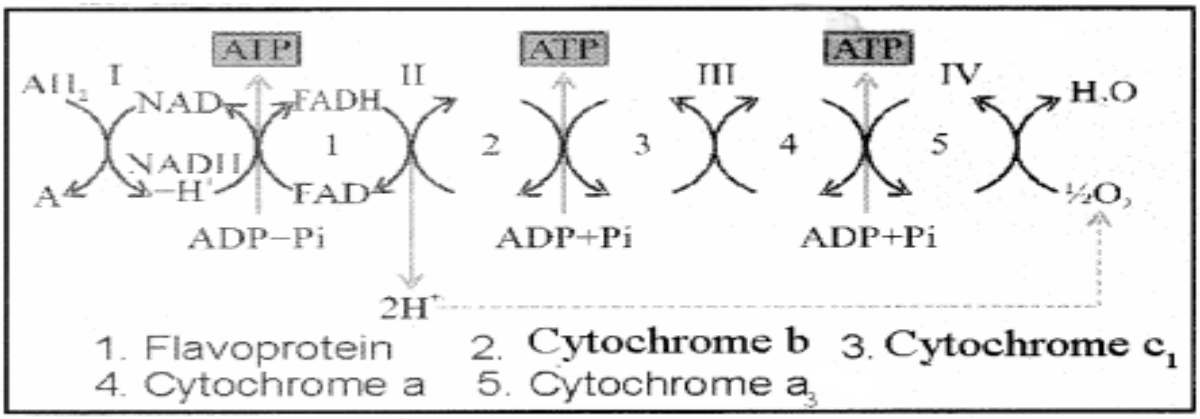


FIG. 24.6. Summary of mitochondrial electron transport chain showing the flow of electrons and protons (H^+) through the four enzyme complexes of the transport chain. Electrons reach quinone (Q) through complexs I and II. Q serves as a mobile carrier of electrons and passes them to complex III, which then passes them to cytochrome *c*, another mobile connecting link. Complex IV then transfers electrons from reduced cytochrome *c* to O_2. Electron flow through complexes I, III and IV is accompanied by proton flow from the mitochondrial matrix to the intermembrane space (cytosolic side)

1. Electrons donated by NADH enter the chain at complex I (NADH-Q-oxidoreductase) and pass through a flavoprotein (FMN) to a series of iron-sulphur-proteins (FeS) and then to ubiquinone (Q).
2. Electrons donated by succinate enter the chain at Complex II (succinate-Q-reductase) and pass through a flavoprotein (FAD) and FeS centres and then to ubiquinone (Q).
3. Ubiquinone (Q) serves as a mobile carrier of electrons received from complexes I and II and passes them to complex III (Q-cytochrome c oxidoreductase).
4. Complex III called Q-cytochrome c oxidoreductase or cytochrome bc_1 complex passes the electrons through its prosthetic groups Cyt b_L (Heme b_L), Cyt b_H (heme b_H), FeS, and Cyt c_L (Heme c_L) to cytochrome c.
5. Cytochrome c (Cyt c), a mobile connecting link between complex III and IV, passes electrons to complex IV (cytochrome c oxidase). The latter carries electrons through its prosthetic groups Cyt a (Heme a), Cyt a_3 (Heme a_3) Cu_A and Cu_B and transfers them to molecular oxygen, reducing it to H_2O.
6. Electron flow through complexes I, III and IV is accompanied by proton flow from the mitochondrial matrix (which becomes negatively charged) to inter membrane space or

cytosolic side (which becomes positively charged). The number of protons (H^+) moved across the membrane at each site per pair of electrons transported is still somewhat uncertain; the current consensus is that at least 10 protons move outward during NADH oxidation.



Electron Transport System

Figure: Simplified version of electron Transport system

Key Points about the Electron Transport Chain

The ETC is located in the mitochondrial inner membrane and contains several different kinds of electron carriers: FMN, iron-sulfur proteins, coenzyme Q, heme-containing cytochromes, and copper ions.

Three large multiprotein complexes serve as proton pumps by harnessing the energy from electron flow through the ETC to oxygen; in turn, the chemiosmotic energy in the proton gradient that is created by the pumps is coupled to the synthesis of ATP by the (F_1-ATPase) complex.

ATP regulates its own synthesis and the flow of electrons through respiratory control; if ATP synthesis slows down, electron transport slows down and vice versa.

Cytosolic NADH cannot pass through the mitochondrial membrane, so it shuttles its electrons through the glycerol phosphate shuttle and the malate-aspartate shuttle.

ATP and ADP are transported in exchange for each other by the ATP/ADP translocase.

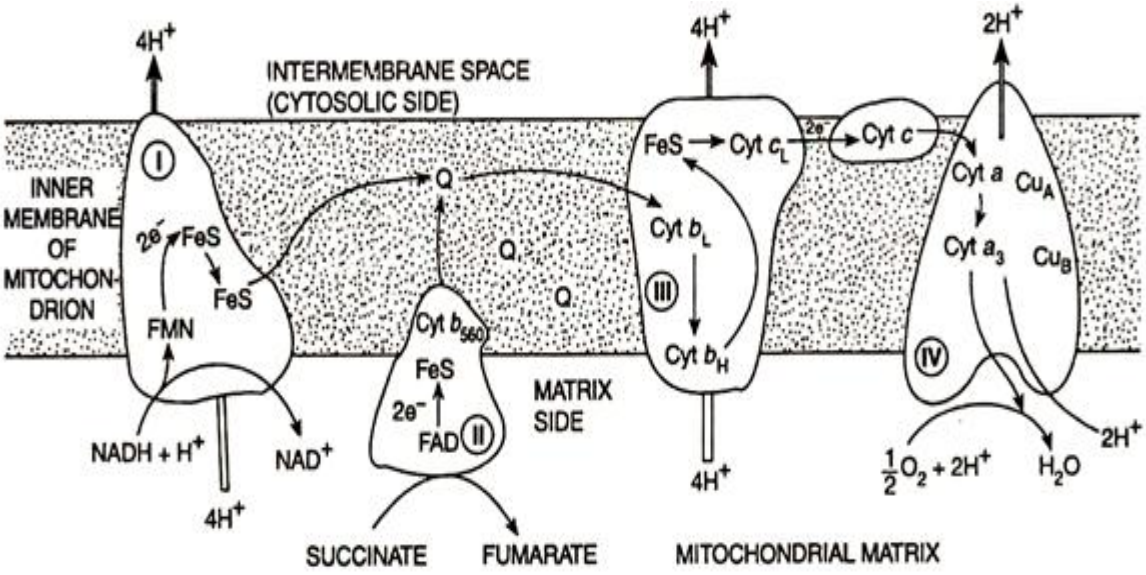
OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation is the process by which energy from electron transport chain (respiratory chain) is used to make ATP, and is the culmination of energy yielding metabolism in aerobic organisms. Oxidative phosphorylation involves the reduction of O_2 to H_2O with electrons donated by NADH and $FADH_2$, and equally occurs in light or darkness.
Our current understanding of ATP synthesis is based on chemiosmotic hypothesis first formulated in 1961 by Peter Mitchell, a British biochemist who later received the Nobel Prize for this important contribution. Chemiosmotic hypothesis has been accepted as one of the great unifying principles of 20th century biology.

It provides insight into not only the processes of photophosphorylation and oxidative phosphorylation but also the processes of disparate energy transductions as active transport across membranes and the rotation of bacterial flagella.

Chemiosmotic Hypothesis and Oxidative Phosphorylation:

According to chemiosmotic hypothesis the electron transport chain is organized so that protons move outward from the mitochondrial matrix to inter-membrane space (in eukaryotes; Fig. 24.6) and from cytoplasm to periplasmic space passing across the plasma membrane (in prokaryotes; Fig. 24.7).



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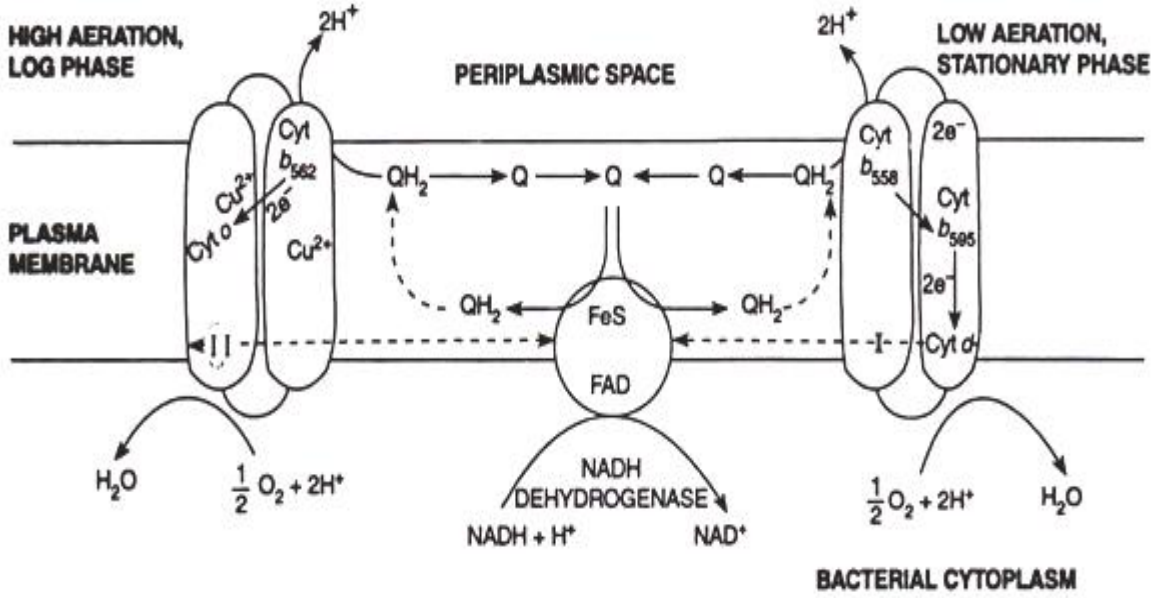

FIG. 24.7. Electron transport chain of *E. coli* that operates in aerobic conditions. NADH is the electron donor. Ubiquinone (Q) is the connecting link between NADH dehydrogenase with two terminal oxidase systems of the two branches, cytochrome *d* branch (shown as I) and cytochrome *o* branch (shown as II).

Proton movement may result either from different complexes or from the action of special proton pumps that derive their energy from electron transport resulting in proton motive force (PMF) composed of a gradient of protons and a membrane potential due to the unequal distribution of charges.

1. Generation of Proton Motive Force (PMF):

When O_2 is reduced to H_2O after accepting electrons transferred from electron transport chain, it requires proton (H^+) from the cytoplasm to complete the reaction.

These protons originate from the dissociation of water into H^+ and OH^-. The use of H^+ in the reduction of O_2 to H_2O and the extrusion of H^+ outside the membrane during electron transport chain (Fig. 24.8) cause a net accumulation of OH^- on the inside of the membrane.

Despite their small size, because they are charged, neither H^+ nor OH^- freely passes through the membrane, and so equilibrium cannot be spontaneously restored on both sides of membrane.

This non-equilibrium state of H^+ and OH^- on opposite sides of the membrane results in the generation of a pH gradient and an electrochemical potential across the membrane, with the inside of the membrane (cytoplasm side) electrically negative and alkaline, and the outside of the membrane electrically positive and acidic.

This pH gradient and electrochemical potential cause the membrane to be energised. The energised state of a membrane, which is referred to as proton motive force (PMF) and is expressed in volts, is used directly to drive the formation of ATP, ion transport, flagellar rotation, and other useful work.

2. Proton Motive Force and ATP Synthesis:

Proton motive force-derived ATP synthesis involves a catalyst, which is a large membrane enzyme complex called ATP synthase or ATPase for short (Fig. 24.9).

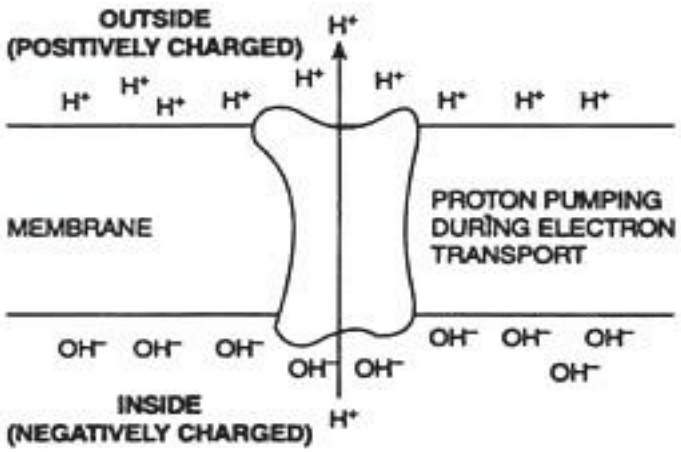

FIG. 24.8. Proton motive force. The protons originate from the dissociation of H_2O into H^+ and OH^-. They are used in reduction of O_2 to H_2O as well as are extruded outside the memberance. Contrary to it, OH^- accumulate on the inside of membrane. This nonequilibrium state generates proton motive force (PMF).

The ATPase contains two major parts:

(1) A multi-subunit head piece called F_1 located on mitochondrial matrix side (in eukaryotes) and on cytoplasmic side (in prokaryotes) and

(2) A proton conducting channel called F_0 that resides in the inner membrane of mitochondrion (in eukaryotes) and in plasma membrane (in prokaryotes) and spans the membrane.

The ATP synthesis takes place at the F_1/F_0 ATPase, which is the smallest known biological motor. F_1, is the catalytic complex responsible for the inter conversion of ADP + Pi (inorganic phosphate) and ATP, and consists of five different polypeptides present as an $\alpha_3\ \beta_3\ \Upsilon\varepsilon\delta$ complex. F_0 is integrated in the membrane and consists of three polypeptides in an $ab_2\ c_{12}$ complex. 3, 3, 2 and 12 denote the numerical numbers of α, β, b and c, respectively.

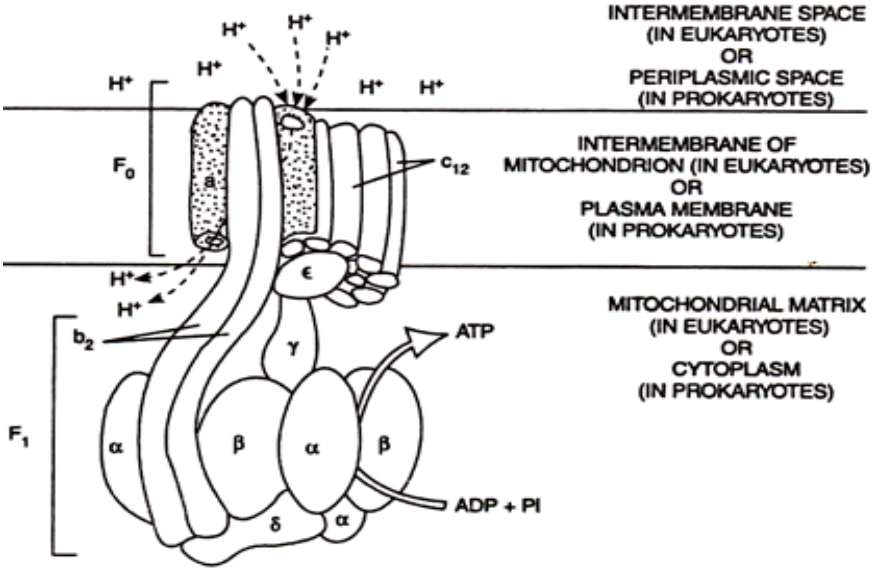

FIG. 24.9. Structure and function of ATP synthase (ATPase). See details in text.

According to the current model of how the ATPase functions in Escherichia coli (Fig. 24.9), subunit 'a' is responsible for channeling protons (H^+) across the membrane while subunit b protrudes outside the membrane and forms, along with b_2 and δ subunit, the stator. Protein movement through 'a' submit of F_0 drives rotation of the c proteins generating a torque that is transmitted to F_1, by the ϒε subunits.
As a result, energy is transferred to F_1through coupled rotation of yε subunits causing conformational changes in the β subunits. The conformational changes in the β subunits allow for binding of ADP + Pi and these are converted to ATP when the β subunits return to their original conformation.

Role of ATPsynthase (Adenosine triphosphate synthase complex) in ETS and Oxidative Phosphorylation

The ATP synthase complex (F_oF_1-ATP synthase) allows protons to flow back into the matrix and uses the free energy change from this process to synthesize ATP from ADP and inorganic phosphate P_i. It is located in knob-shaped structures embedded in the cristae (invaginations of the inner mitochondrial membrane) and extending into the matrix.
The F_o protein (the "o" in F_o refers to its sensitivity to oligomycin, a poison that blocks the flow of protons) extends through the inner mitochondrial membrane and serves as the proton channel between the intermembrane space and the matrix.
The ATP synthase (F_1-ATPase) is attached to the F_o protein on the inside of the matrix. F_1-ATPase uses the protons flowing into the matrix to bind ADP and P_i and release ATP. The F_1-ATPase is named by the reverse reaction it catalyzes when it is isolated from mitochondria and thus uncoupled from the proton gradient.

C) INHIBITORS AND UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

Many chemicals inhibit the synthesis of ATP and can even kill cells to sufficiently high concentrations. Two such classes of chemicals are known **inhibitors and un-couplers**. Inhibitors directly block electron transport chain.
The antibiotic piericidin competes with coenzyme Q; the antibiotic antimycin. A blocks electron transport between cytochromes b, and c, and both carbon monoxide (CO) and cyanide (CN^-) bind tightly to certain cytochromes and prevent their functioning.
The un-couplers, in contrast, prevent ATP synthesis without affecting electron transport chain itself. Normally the electron transport chain is tightly coupled with oxidative phosphorylation, and the un-couplers disconnect oxidative phosphorylation from electron transport chain.
Therefore, the energy released by the chain is given off as heat rather than as ATP. Many lipid soluble un-couplers (e.g., dinitrophenol, dicumarol, valinomycin) make membranes leaky allowing free passage of protons through the membrane without activating F_1/F_0 ATPase. In this way they destroy the proton motive force and its ability to drive ATP synthesis.

The difference between an inhibitor and an uncoupler is that an inhibitor actually STOPS either electron transport or ATP synthesis by interfering with one of the enzymes, but an uncoupler just unlinks electron transport and ATP synthesis by breaking down the hydrogen ion gradient. This effectively stops ATP synthesis, but it does not interfere with electron transport. DNP is able to do this because it can move freely through membranes, and when it moves across the inner mitochondrial membrane it transfers a hydrogen ion with it, breaking down the gradient so there is no power for the ATP synthase pump. As an aside, if there were

some way to artificially INCREASE the hydrogen ion gradient, this would cause an INCREASE in the rate of oxidative phosphorylation because you would have more power for the pump, but it would actuallycause a DECREASE in electron transport and oxygen consumption because it would have to work harder against the gradient.

Another good example of an uncoupler is thermogenin, a naturally occurring protein that is found in brown adipose tissue. Recall from histology that brown adipose tissue is found in baby humans, other young mammals, and animals that hibernate. The reason forthis is that thermogenin is able to use the energy from the hydrogen ion gradient to generate heat energy, instead of using it for ATP synthesis. So thermogenin, as the name implies, GENERATES HEAT. This is good for small mammals like rabbits because they do not have to shiver to generate heat in the winter time, so they will not be easily spotted by other animals that would eat them.

Inhibitors and Uncouplers

Table 1. Inhibitors of Respiration and Oxidative Phosphory

Site-Specific	Target Complex
Carbon monoxide	IV
Cyanide	IV
Sodium Azide	IV
Rotenone	I
Antimycin A	III
Amytal	I
Phosphorylation	
Oligomycin	F_o
Uncouplers	
2,4-Dinitrophenol (DNP)	Proton gradient
Trifluorocarbonylcyanide Phenylhydrazone (FCCP)	Proton gradient

Any compound that stops electron transport will stop respiration…this means you stop breathing

Electron transport can be stopped by inhibiting ATP synthesis

An uncoupler breaks the connection between ATP synthesis and electron transport

Expected Questions:

1. *Explain ETC with a diagram and show the tansport of e- in it (5)*
2. *What is proton motive force? (2)*
3. *What are uncouplors in ETC give example (2)*
4. *Name some inhibitors of ETS(2)*
5. *Differentiate between oxidative and substrate level phosphorylation(4)*
6. *Mention the Role of ATP Synthase in production of ATP (3)*
7. *With a schematic diagram explain common intermediately & coinciding pathways of metabolism (5)*
8. *Explain how proteins are metabolically connected to the pathways of Glucose (5)*
9. *AcetylCoA is the metabolic link of all pathways Justify the statement and show the metabolic link by flow diagram (2+3)*
10. *Explain Chemiosmotic hypothesis (5)*

References:

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