2.3 Respiration

Living cells require an input of free energy. Energy is required for the maintenance of highly organized structures, synthesis of cellular components, movement, generation of electrical currents and for many other processes. Cells acquire free energy from the oxidation of organic compounds that are rich in potential energy.

Respiration is an oxidative process, in which free energy released from organic compounds is used in the formation of ATP. The compounds that are oxidized during the process of respiration are known as *respiratory substrates*, which may be carbohydrates, fats, proteins or organic acids. Carbohydrates are most commonly used as respiratory substrates.

During oxidation within a cell, all the energy contained in respiratory substrates is not released free in a single step. Free energy is released in multiple steps in a controlled manner and used to synthesise ATP, which is broken down whenever (and wherever) energy is needed. Hence, ATP acts as the energy currency of the cell.

During cellular respiration, respiratory substrates such as glucose may undergo *complete or incomplete* oxidation. The complete oxidation of substrates occurs in the presence of oxygen, which releases CO_2, water and a large amount of energy present in the substrate. A complete oxidation of respiratory substrates in the presence of oxygen is termed as *aerobic respiration*.

Although carbohydrates, fats and proteins can all be oxidized as fuel, but here processes have been described by taking glucose as a *respiratory substrate.* Oxidation of glucose is an exergonic process. An exergonic reaction proceeds with a net release of free energy. When one mole of glucose (180 g) is completely oxidized into CO_2 and water, approximately 2870 kJ or 686 kcal energy is liberated. Part of this energy is used for synthesis of ATP. For each molecule of glucose degraded to carbon dioxide and water by respiration, the cell makes up to about 30 or 32 ATP molecules, each with 7.3 kcal/mol of free energy.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy (ATP + Heat)}$$

The incomplete oxidation of respiratory substrates occurs under anaerobic conditions i.e. in the absence of oxygen. As the substrate is never totally oxidized, the energy generated through this type of respiration is lesser than that during aerobic respiration.

2.3.1 Aerobic respiration

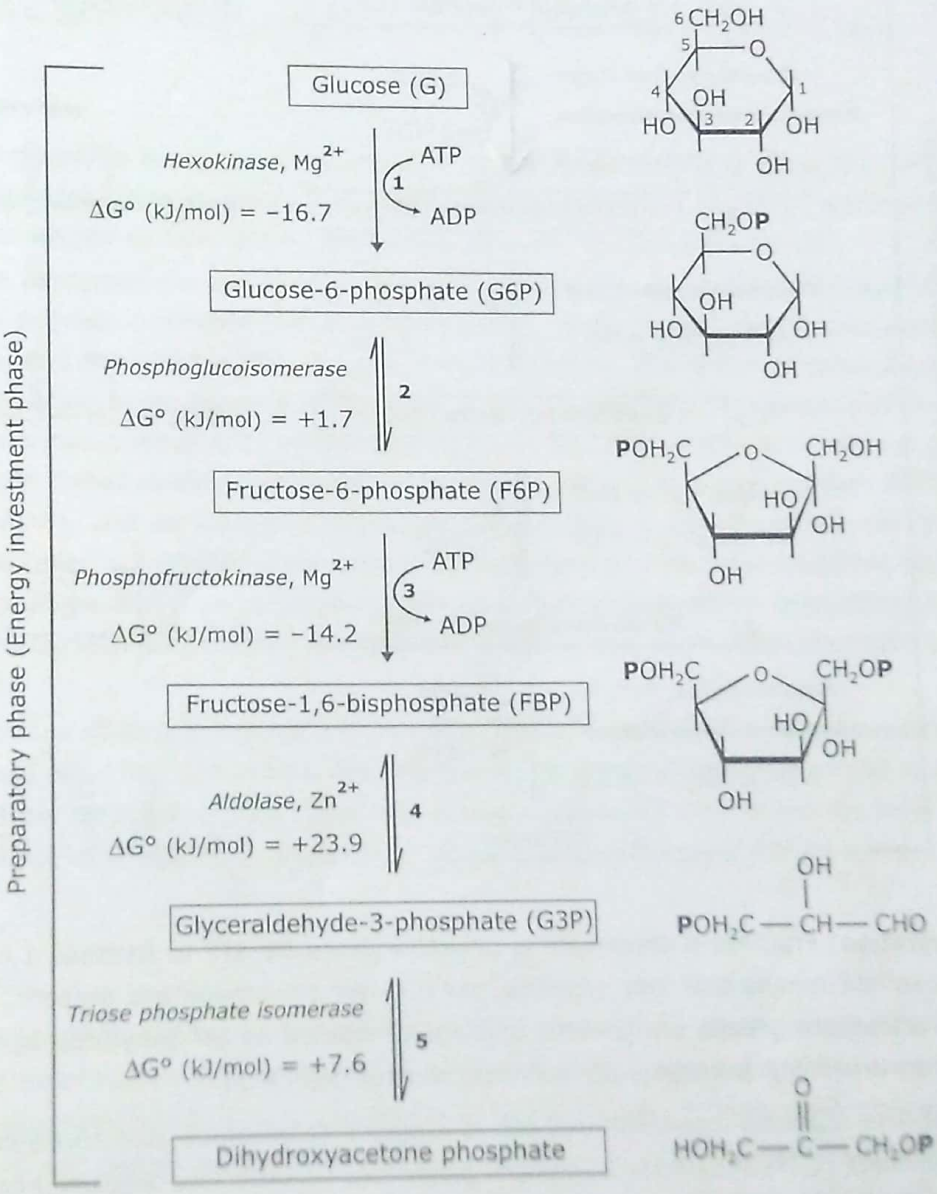
Enzyme catalyzed reactions during aerobic respiration can be grouped into three major processes: glycolysis, citric acid cycle and oxidative phosphorylation. Glycolysis takes place in the cytosol of cells in all living organisms. The citric acid cycle takes place within the mitochondrial matrix of eukaryotic cells and in the cytosol of prokaryotic cells. The oxidative phosphorylation takes place in the inner mitochondrial membrane. However, in prokaryotes, oxidative phosphorylation takes place in the plasma membrane.

Table 2.3 Intracellular location of major processes of aerobic respiration

In eukaryotes,		
Glycolysis	–	Cytosol
Citric acid cycle	–	Mitochondrial matrix
Oxidative phosphorylation	–	Inner mitochondrial membrane
In prokaryotes,		
Glycolysis	–	Cytosol
Citric acid cycle	–	Cytosol
Oxidative phosphorylation	–	Plasma membrane

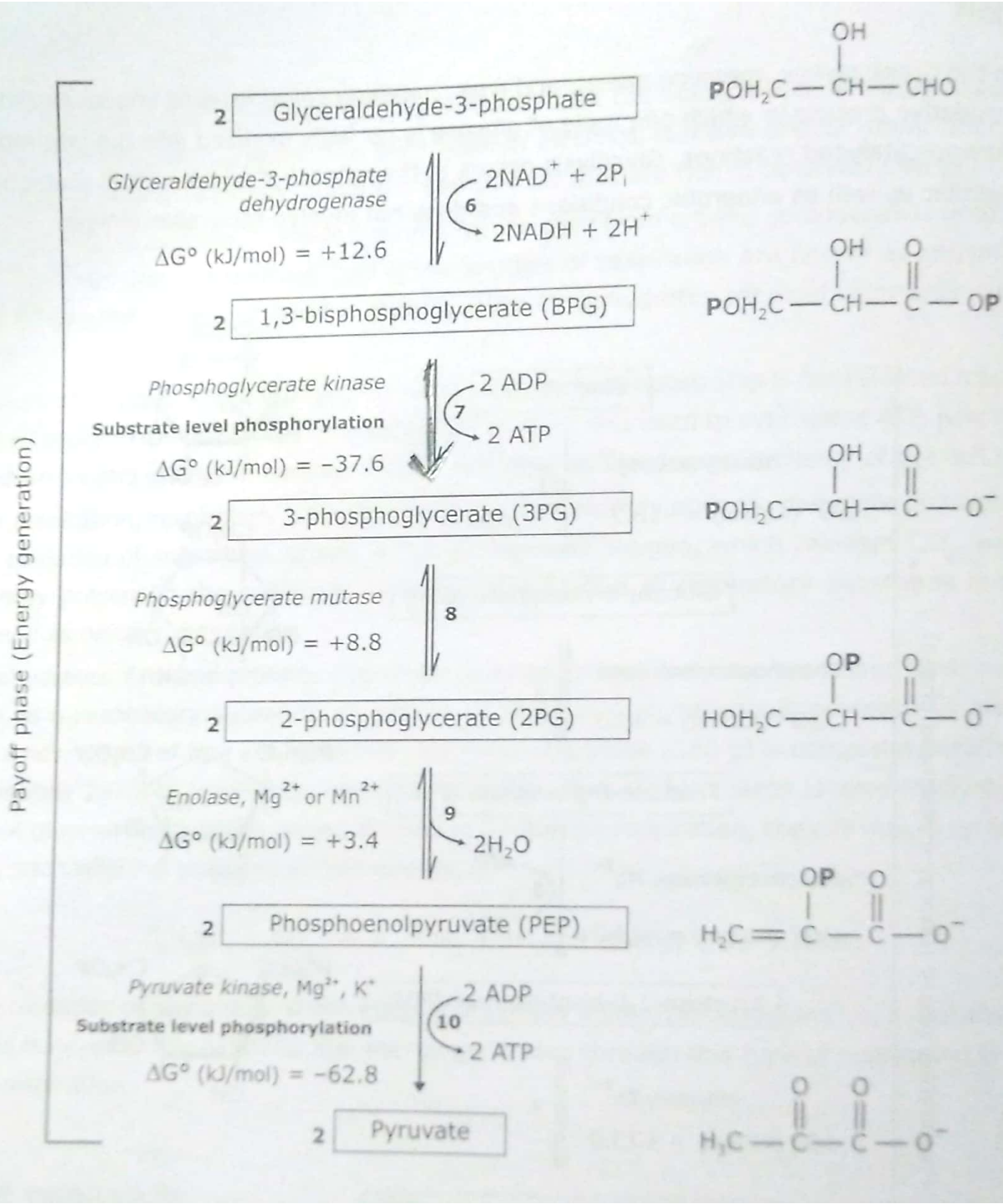
2.3.2 Glycolysis

Glycolysis (from the Greek *glykys*, meaning *sweet*, and *lysis*, meaning *splitting*) also known as *Embden-Meyerhof pathway*, is an oxidative process in which one mole of glucose is partially oxidized into the two moles of pyruvate in a series of enzyme-catalyzed reactions. Glycolysis occurs in the cytosol of all cells. It is a unique pathway that occurs in both aerobic as well as anaerobic conditions and does not involve molecular oxygen.



Step 1 : (**Phosphorylation**) Glucose is phosphorylated by ATP to form a glucose 6-phosphate. The negative charge of the phosphate prevents the passage of the glucose 6-phosphate through the plasma membrane, trapping glucose inside the cell. This *irreversible* reaction is catalyzed by *hexokinase*. Hexokinase is present in all cells of all organisms. Hexokinase requires divalent metal ions such as Mg^{2+} or Mn^{2+} for activity. Hepatocytes and β-cells of the pancreas also contain a form of hexokinase called **glucokinase** (hexokinase D). Hexokinase and glucokinase are isozymes. Glucokinase is present in liver and beta-cells of the pancreas and has a high K_m and V_{max} as compared to hexokinase.

Step 2 : (**Isomerization**) A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. Thus, the isomerization of glucose 6-phosphate to fructose 6-phosphate is a conversion of an aldose into a ketose.



Step 3 : (**Phosphorylation**) Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate. The prefix *bis-* in bisphosphate means that two separate monophosphate groups are present, whereas the prefix *di-* means that two phosphate groups are present and are connected by an anhydride bond. This *irreversible* reaction is catalyzed by an allosteric enzyme *phosphofructokinase-1* (PFK-1).

Step 4 : (**Cleavage**) The fructose 1,6-bisphosphate is cleaved to produce two three-carbon molecules - *glyceraldehyde 3-phosphate* (G3P) and *dihydroxyacetone phosphate* (DHAP). This reaction is catalyzed by *aldolase*.

Step 5 : (**Isomerization**) Dihydroxyacetone phosphate is isomerized to form glyceraldehyde 3-phosphate. The isomerization of these three-carbon phosphorylated sugars is catalyzed by *triose phosphate isomerase*.

Step 6 : The two molecules of glyceraldehyde 3-phosphate are oxidized. Enzyme *glyceraldehyde 3-phosphate dehydrogenase* catalyzes the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG). The reaction occurs in two steps: first the *oxidation* of the aldehyde to a carboxylic acid by NAD^+ and second the *joining* of the carboxylic acid and orthophosphate to form the acyl-phosphate product. **Iodoacetate** is a potent inhibitor of *glyceraldehyde-3 phosphate dehydrogenase* because it forms a covalent derivative of the essential –SH group of the enzyme active site, rendering it inactive.

Step 7 : In this step high-energy phosphate group is transferred from 1,3-bisphosphoglycerate to ADP. The formation of ATP is referred to as *substrate-level phosphorylation* because the phosphate donor, 1,3-bisphosphoglycerate, is a substrate with high phosphoryl-transfer potential. This step is catalyzed by enzyme *phosphoglycerate kinase*.

Step 8 : The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.

Step 9 : The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage. The enzyme catalyzing this step, *enolase*, is inhibited by *fluoride*.

Step 10 : The transfer of the high-energy phosphate group that was generated in step 9 to ADP forms ATP. This last step in glycolysis is the irreversible transfer of the phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by *pyruvate kinase*. Pyruvate kinase requires either K^+ or Mg^{2+}.

Net reaction : Glucose + $2NAD^+$ + 2ADP + $2H_2PO_4^-$ $\longrightarrow$ 2Pyruvate + 2NADH + 2ATP + $2H_2O$

Glycolysis : An overview

All of the enzymes of glycolysis are found in the cytosol. Certain trypanosomes carry out the first seven reactions of glycolysis in an organized cytoplasmic organelles called *glycosome*. Three glycolytic reactions are irreversible. These reactions are catalyzed by hexokinase, phosphofructokinase and pyruvate kinase.

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps. The first five steps of this pathway constitute the *preparatory phase*. In this phase, energy is consumed as glucose is phosphorylated twice, and converted to FBP. For both phosphorylations, ATP is the phosphoryl group donor. FBP is split to yield two three-carbon molecules. One of the products, dihydroxyacetone phosphate, is immediately converted to G3P. This yields two molecules of G3P, which are then converted to pyruvate in a five-step process of payoff phase. The energy gain comes in the *payoff phase* of glycolysis. First, G3P is oxidized with NAD^+ as the electron acceptor (to form NADH), and an inorganic phosphate (not by ATP) is simultaneously incorporated to give a high-energy molecule called 1,3-bisphosphoglycerate. The high-energy phosphate on carbon one is subsequently donated to ADP to produce ATP. This synthesis of ATP is called *substrate-level phosphorylation* because ADP phosphorylation is coupled with the exergonic breakdown of a high-energy bond. Thus, the payoff phase of glycolysis yields ATP and NADH.

The energy yield from one molecule of glucose in glycolysis is 2 ATP and 2 NADH. In the presence of oxygen, one NADH reoxidize to yield about 2.5 ATP. Hence, glycolysis in the presence of oxygen (termed as aerobic glycolysis) yields 7 ATP (5 ATP from oxidation of two molecules of NADH and 2 ATP from substrate level phosphorylation). Glycolysis in the absence of oxygen (i.e. anaerobic glycolysis) produces only 2 ATP as a result of substrate-level phosphorylation.

Regulation of glycolysis

The rate at which the glycolytic pathway operates is controlled primarily by allosteric regulation of three enzymes: *hexokinase*, *phosphofructokinase-1* and *pyruvate kinase*. The reactions catalyzed by these enzymes are *irreversible*. Allosteric effector glucose-6-phosphate inhibits the hexokinases. A high AMP concentration activates phosphofructokinase-1 and pyruvate kinase. In contrast, a high ATP concentration inhibits both enzymes. Citrate and acetyl-CoA, which indicate that alternative energy sources are available, inhibit phosphofructokinase-1 and pyruvate kinase, respectively. Finally, fructose-2,6-bisphosphate stimulates glycolysis by activating phosphofructokinase-1 and fructose-1,6-bisphosphate activates pyruvate kinase.

Enzyme	*Activator*	*Inhibitor*
Hexokinase	----------	Glucose-6-phosphate
Phosphofructokinase-1	Fructose-2,6-bisphosphate, AMP	Citrate, ATP
Pyruvate kinase	Fructose-1,6-bisphosphate, AMP	Acetyl-CoA, ATP

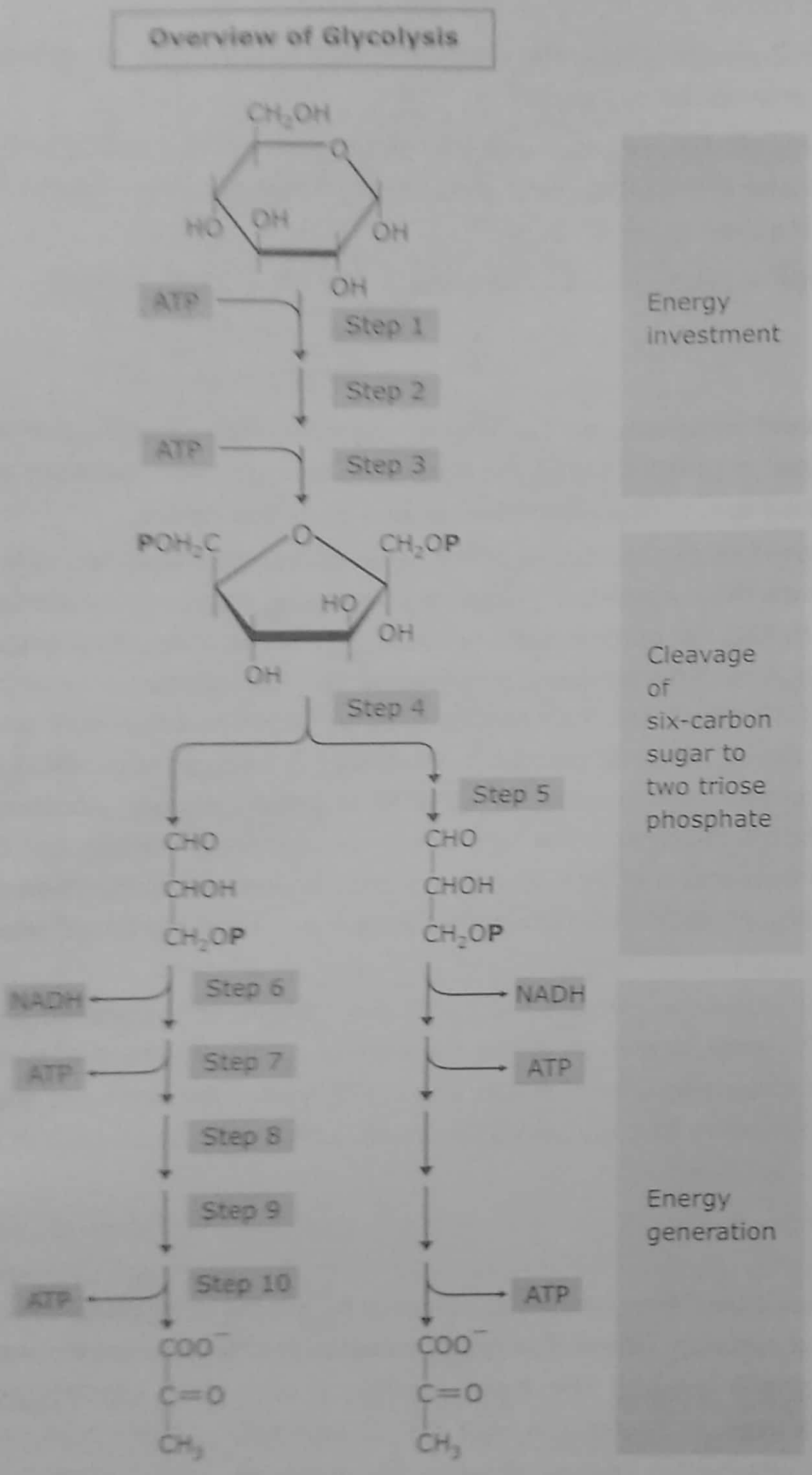


Figure 2.1
An outline of glycolysis.
Adapted and modified from Albert et al, Molecular Biology of Cell, Garland Science.

2.3.3 Pyruvate oxidation

In the presence of oxygen, further oxidation of pyruvate occurs in the mitochondrial matrix (cytosol in case of prokaryotes). In the mitochondrial matrix, the pyruvate first oxidizes into acetyl-CoA.

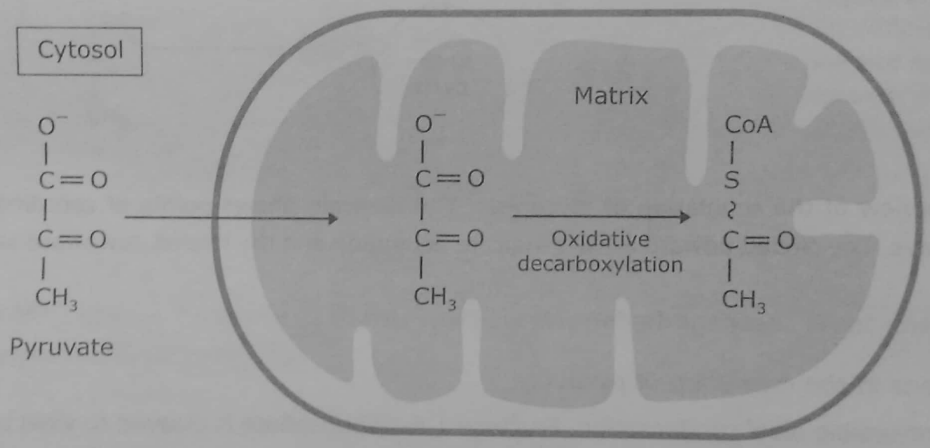
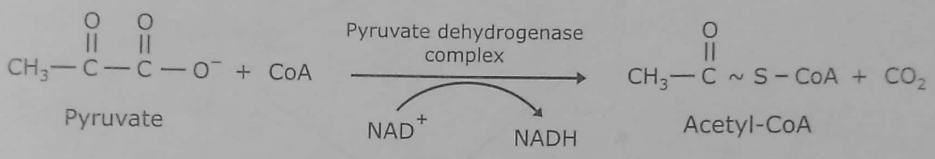


Figure 2.4 Conversion of pyruvate to acetyl-CoA. It is the junction between glycolysis and the citric acid cycle. Pyruvate is a charged molecule, so in eukaryotic cells, it must enter the mitochondrion with the help of a transport protein. Next, the pyruvate dehydrogenase complex catalyzes the process of oxidative decarboxylation.

The conversion of pyruvate to acetyl-CoA, catalyzed by highly organized multienzyme *pyruvate dehydrogenase complex*, is an oxidative decarboxylation process. In the overall reaction, the carboxyl group of pyruvate is lost as CO_2, while the remaining two carbons form the acetyl moiety of acetyl-CoA. The reaction is highly exergonic and essentially irreversible *in vivo*.



Pyruvate dehydrogenase complex is an assembly of three individual enzymes: *Pyruvate dehydrogenase* (E_1), *Dihydrolipoyl transacetylase* (E_2) and *Dihydrolipoyl dehydrogenase* (E_3). The oxidation of pyruvate to acetyl-CoA involves the coenzymes thiamine pyrophosphate (TPP), lipoic acid, FAD, NAD^+ and co-enzyme A (CoA), acting in association with E_1, E_2 and E_3 in the pyruvate dehydrogenase complex. In eukaryotes, this enzyme complex also contains small amounts of two regulatory enzymes as well–a *kinase* that phosphorylates serine residues and a *phosphatase* that removes those phosphates. Arsinite and mercuric ions inhibit the activity of pyruvate dehydrogenase.

Table 2.4 *E. coli* pyruvate dehydrogenase complex

Enzyme	*Function*	*No. of polypeptide*	*Cofactors*
Pyruvate dehydrogenase (E_1)	Decarboxylation and oxidation of pyruvate	24	TPP
Dihydrolipoyl transacetylase (E_2)	Catalyzes transfer of acetyl group to CoA	24	Lipoic acid, CoA
Dihydrolipoyl dehydrogenase (E_3)	Reoxidizes dihydrolipoamide	12	NAD^+, FAD

Mechanism of action of the pyruvate dehydrogenase complex

E_1 first catalyzes the decarboxylation of pyruvate, producing hydroxyethyl-TPP, and then the oxidation of the hydroxyethyl group to an acetyl group. The electrons from this oxidation reduce the disulfide of lipoate bound to E_2, and the acetyl group is transferred into thioester linkage with on –SH group of reduced lipoate. E_2 catalyzes the transfer of the acetyl group to coenzyme A, forming acetyl-CoA. E_3 catalyzes the regeneration of the disulfide (oxidized) form of lipoate; electrons pass first to FAD and then to NAD^+.

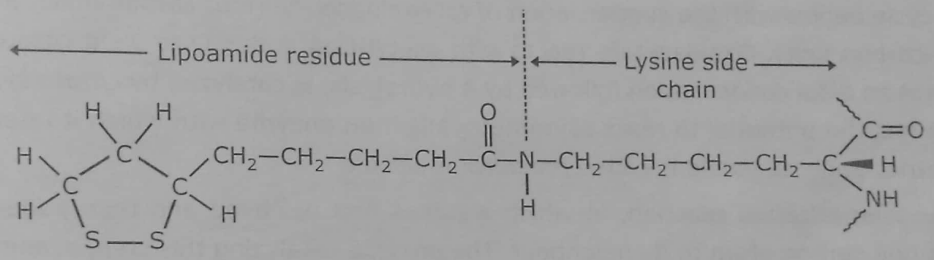


Figure 2.5 Structure of dihydrolipoamide.

Pyruvate dehydrogenase complex is regulated by two processes – *allosteric regulation* and *covalent modification*. Allosteric inhibition is mediated by NADH and acetyl-CoA. Covalent modification, which occurs only in eukaryotes, is mediated by phosphorylation/dephosphorylation. E_2 is inhibited by the acetyl-CoA and activated by CoA while E_3 is inhibited by NADH and activated by NAD^+. E_1 subunit of enzyme complex undergoes reversible phosphorylation/ dephosphorylation.

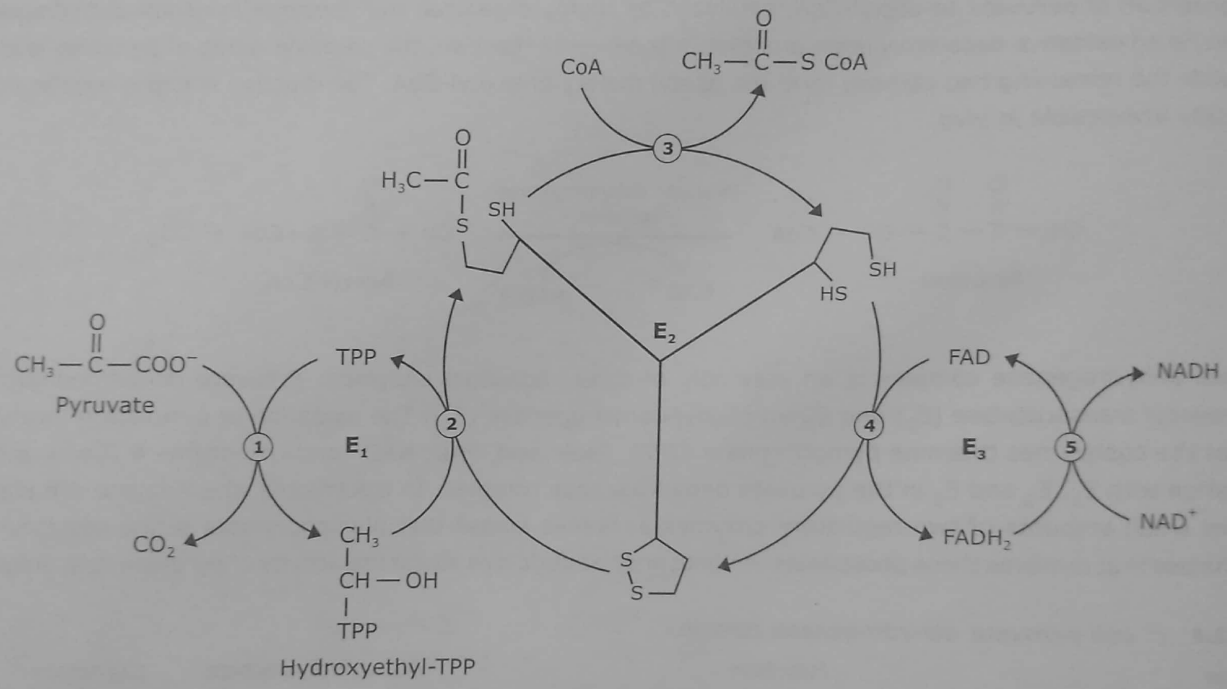


Figure 2.6 Structure of pyruvate dehydrogenase and its catalytic activities. Catalytic activities occur in four steps:

Step 1 : Decarboxylation of pyruvate occurs with formation of hydroxy ethyl – TPP.

Step 2 : Transfer of the two carbon unit to lipoic acid.

Step 3 : Formation of acetyl-CoA.

Step 4 : Lipoic acid is re-oxidized.

2.3.4 Krebs cycle

Krebs cycle (also known as the citric acid cycle or tricarboxylic acid cycle) was discovered by H. A. Kreb, a German born British Biochemist, who received the Nobel prize in 1953. This cycle occurs in the matrix of mitochondria (cytosol in prokaryotes). The whole cycle is explained in the following figure. The net result of Kreb cycle is that for each acetyl group entering the cycle as acetyl-CoA, two molecules of CO_2 are produced.

Step 1 : The Krebs cycle begins with the condensation of an oxaloacetate (four carbon unit), and the acetyl group of acetyl-CoA (two-carbon unit). Oxaloacetate reacts with acetyl-CoA and H_2O to yield citrate and coenzyme A. This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by *citrate synthase.* Citrate has no chiral center but has the potential to react asymmetrically if an enzyme with which it interacts has an active site that is asymmetric. Such molecule is called *prochiral molecule*.

Step 2a and 2b : An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbour. The enzyme catalyzing this step, *aconitase* (nonheme iron protein), is the target site for the toxic compound **fluoroacetate** (used as a pesticide). Fluoroacetate blocks the citric acid cycle by its metabolic conversion of *fluorocitrate*, which is a potent inhibitor of aconitase.

Step 3 : Isocitrate is oxidized and decarboxylated to α-ketoglutarate (also called oxoglutarate). In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing CO_2 while still bound to the enzyme. The oxidative decarboxylation of isocitrate is catalyzed by *isocitrate dehydrogenase.*

Step 4 : A second oxidative decarboxylation reaction results in the formation of succinyl-CoA from α-ketoglutarate. *α-ketoglutarate dehydrogenase* catalyzes this oxidative step and produces NADH, CO_2, and a high-energy thioester bond to coenzyme A.

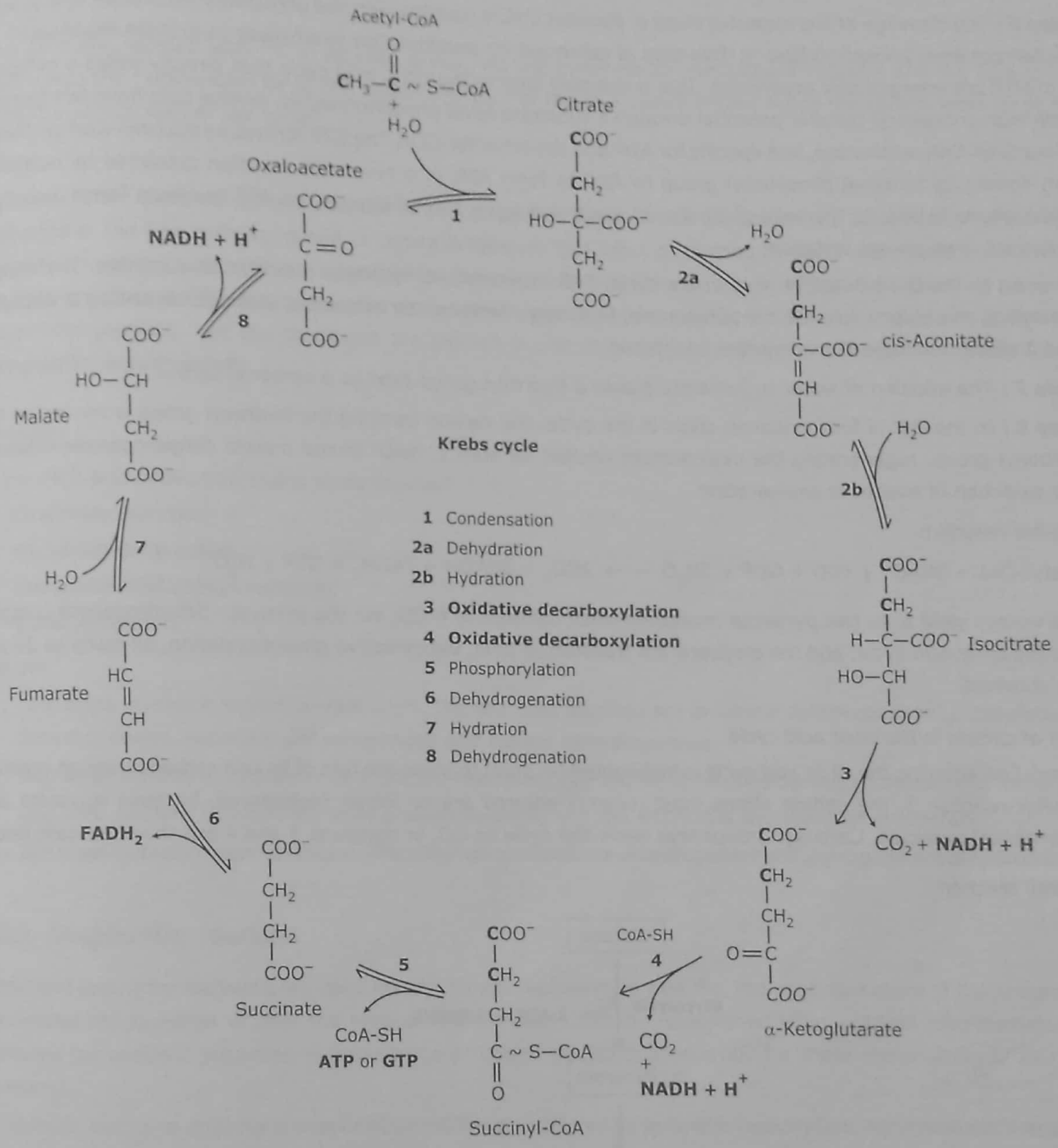


Figure 2.7 Krebs cycle. Acetyl-CoA is the fuel for the Krebs cycle. A four-carbon compound (oxaloacetate)

1. Citrate synthase
2a. Aconitase
2b. Aconitase
3. Isocitrate dehydrogenase
4. α-Ketoglutarate dehydrogenase
5. Succinate thiokinase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase

Figure 2.7 Krebs cycle. Acetyl-CoA is the fuel for the Krebs cycle. A four-carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid (citrate). An isomer of citrate is then oxidatively decarboxylated. The resulting five-carbon compound (α-ketoglutarate) also is oxidatively decarboxylated to yield a four carbon compound (succinate). Oxaloacetate is then regenerated from succinate. During oxidative decarboxylation, two carbon atoms come out as CO_2 from the oxidation of isocitrate and α-ketoglutarate. The energy released during this cycle is used in the reduction of three NAD^+ and one FAD and the production of one ATP or GTP. Note that the two carbon atoms appearing as CO_2 are not the same two carbons that entered in the form of the acetyi group; additional turns around the cycle are required to release these carbons as CO_2.

Step 5 : The cleavage of the thioester bond of succinyl CoA is coupled with the phosphorylation of an ADP or a GDP (*substrate level phosphorylation*). This step is catalyzed by *succinyl CoA synthetase* (succinate thiokinase). ATP and GTP are energetically equivalent. This is the only step in the citric acid cycle that directly yields a compound with high phosphoryl transfer potential through a substrate-level phosphorylation. Animal cells have two isozymes of succinyl-CoA synthetase, one specific for ADP and the other for GDP. The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by *nucleoside diphosphate kinase*. In the cells of plants, bacteria and some animal tissues, an ATP molecule forms directly by substrate-level phosphorylation.

Step 6 : In the third oxidation step in the cycle, FAD removes two hydrogen atoms from succinate. The enzyme catalyzing this step, succinate dehydrogenase, is strongly inhibited by **malonate**, a structural analog of succinate and a classic example of a competitive inhibitor.

Step 7 : The addition of water to fumarate places a hydroxyl group next to a carbonyl carbon.

Step 8 : In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1. NAD^+ linked *malate dehydrogenase* catalyzes the oxidation of malate to oxaloacetate.

Overall reaction:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + 3H_2O \longrightarrow 2CO_2 + 3NADH + FADH_2 + GTP + H_2O$$

The energy yield from two pyruvate molecules when oxidized to 6 CO_2 via the pyruvate dehydrogenase complex and the citric acid cycle, and the electrons are transferred to O_2 via oxidative phosphorylation, as many as 25 ATP are obtained.

Fate of carbon in the citric acid cycle

Acetyl-CoA entering the citric acid cycle is highlighted (in bold) to show the fate of its two carbons through reaction 4. After reaction 5, the carbon atoms most recently entered are no longer highlighted, because succinate is a symmetrical molecule. Carboxyl groups that leave the cycle as CO_2 in reactions 3 and 4 are shown in *italic* letter.

Overall reaction:

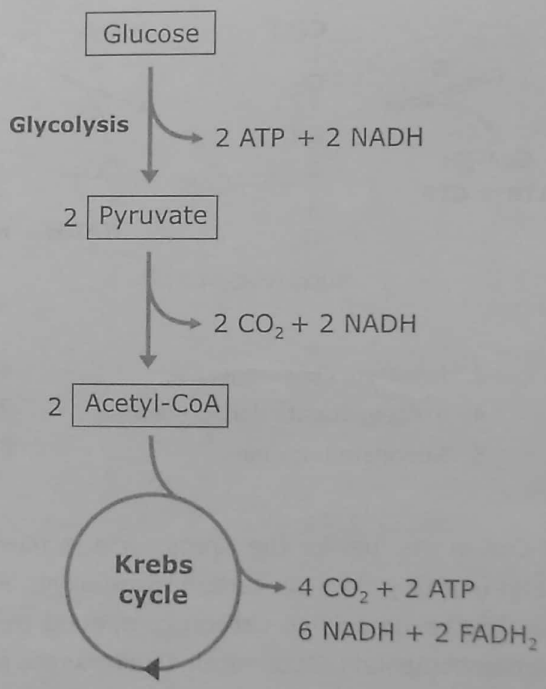


Figure 2.8 Glycolysis and the Krebs cycle, produce only 4 ATP molecules per glucose molecule, all by substrate level phosphorylation: 2 net ATP from glycolysis and 2 ATP from the Krebs cycle. At this point, molecules of NADH (and $FADH_2$) account for most of the energy extracted from the glucose. These electron escorts link glycolysis and the Krebs cycle to the machinery of oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis.

Problem

Using pyruvate, labeled with ^{14}C in its keto group, via the pyruvate dehydrogenase reaction and the TCA cycle, where would the carbon label be at the end of one turn of the TCA cycle? Where would the carbon label be at the end of the second turn of the cycle?

Solution

The labeled keto carbon of pyruvate becomes the labeled carboxyl carbon of acetyl–CoA. After condensation with oxaloacetate, the first carboxyl group of citrate is labeled. This label is retained through subsequent reactions to succinate. However, succinate is a symmetrical compound to the enzyme, so, in effect, both carboxyl group of succinate are labeled. This means that the oxaloacetate regenerated is labeled in both carboxyl groups at the end of one turn (actually, half the molecules are labeled in one carboxyl and half in the other) but this cannot be distinguished experimentally.

Problem

In the citric acid cycle, how many steps involve?

P. Oxidation-reduction

Q. Hydration-dehydration

R. Substrate-level phosphorylation

S. Decarboxylation

Solution

P. Four steps involve in oxidation-reduction. The enzymes involved are *isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase, succinate dehydrogenase* and *malate dehydrogenase*.

Q. Two steps involve in hydration-dehydration reactions. The enzymes responsible are *aconitase* and *fumarase*.

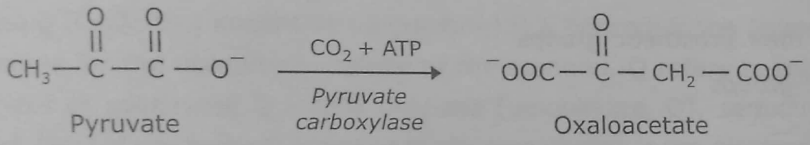
R. One step involves in substrate-level phosphorylation, and the enzyme is *succinyl-CoA synthetase*.

S. Two steps involve in decarboxylation. The enzymes are *isocitrate dehydrogenase* and *2-oxoglutarate dehydrogenase*.

2.3.5 Anaplerotic reaction

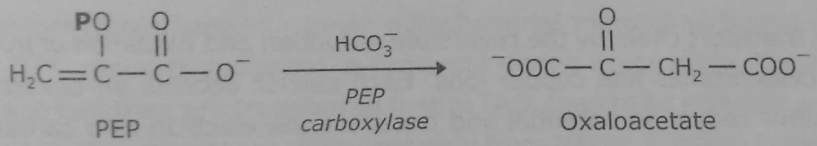
Citric acid cycle intermediates are used in biosynthetic pathways as well. So, the anabolic nature of the Krebs cycle has raised the question of how the level of intermediates can be replenished when, certain intermediates are removed for anabolic purposes. Kornberg has proposed the term *anaplerotic* for these replenishing or *filling up* reactions.

In animals, the most important anaplerotic reaction is catalyzed by *pyruvate carboxylase*, a mitochondrial enzyme, which converts pyruvate to oxaloacetic acid.



Pyruvate — Oxaloacetate

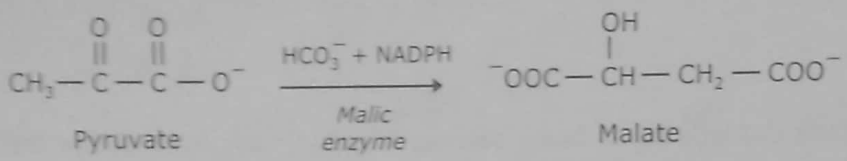
In plants and bacteria, an alternative route leads directly from phosphoenolpyruvate (PEP) to oxaloacetate. This reaction is catalyzed by *phosphoenolpyruvate carboxylase*.



PEP — Oxaloacetate

A related enzyme, phosphoenolpyruvate carboxykinase, also converts phosphoenolpyruvate to oxaloacetate.

In addition to pyruvate carboxylase and phosphoenolpyruvate carboxylase, a third anaplerotic process is provided by an enzyme commonly known as *malic enzyme*, but officially as *malate dehydrogenase*. The malic enzyme catalyzes the reductive carboxylation of pyruvate to give malate.



2.3.6 Oxidative phosphorylation

Most of the free energy released during the oxidation of glucose to CO_2 is retained in the reduced coenzymes NADH and $FADH_2$, generated during glycolysis and TCA cycle. Electrons are released from NADH and $FADH_2$ and eventually transferred to O_2, forming H_2O.

$$NADH + H^+ + 1/2\ O_2 \longrightarrow NAD^+ + H_2O$$

$$FADH_2 + 1/2\ O_2 \longrightarrow FAD + H_2O$$

The free energy change for these exergonic reactions are –52.6 kcal/mol (NADH) and –43.14 kcal/mol ($FADH_2$). The large amount of free energy released during the oxidation of NADH and $FADH_2$ is used in the formation of ATP. For this reason, the term *oxidative phosphorylation* is used to describe this energy conversion process.

Electrons are transferred from NADH/$FADH_2$ to O_2 through a series of electron carriers present on the inner mitochondrial membrane. The process of electron transport begins when the hydride ion is removed from NADH and is converted into a proton and two electrons. Most of the proteins (electron carriers) involved are grouped into four large *respiratory enzyme complexes*, each containing transmembrane proteins that hold the complex. The electrons start with very high energy and gradually lose it as they pass along the chain. Each complex in the chain has a greater affinity for electrons than its predecessor and electrons pass sequentially from one complex to another until they are finally transferred to oxygen, which has the highest affinity for electrons. The four major respiratory enzyme complexes of electron transport chain in the inner mitochondrial membrane are:

Complex I, II and III appear to be associated in a supramolecular complex termed the *respirasome*. All the four multiprotein enzyme complexes which act as electron carriers comprise prosthetic groups, such as flavins, heme, Fe-S clusters and copper. The table below lists the prosthetic group in each multiprotein enzyme complex.

Table 2.5 Name of enzyme complex and their prosthetic groups

Enzyme complex	*Prosthetic groups*
Complex I (46 subunits)	FMN, FeS
Complex II (4 subunits)	FAD, FeS
Complex III (11 subunits)	Heme, FeS
Complex IV (13 subunits)	Heme, Cu^{+}

Electrons are transferred along the electron transport chain by the reversible reduction and oxidation of iron-sulfur clusters, coenzyme Q (abbreviated CoQ), cytochromes and copper ions. Each carrier accepts an electron or an electron pair from a carrier with a less positive reduction potential and transfers the electron to a carrier with a more positive reduction potential. Thus, reduction potentials of electron carriers favour unidirectional electron flow from NADH and $FADH_2$ to O_2.

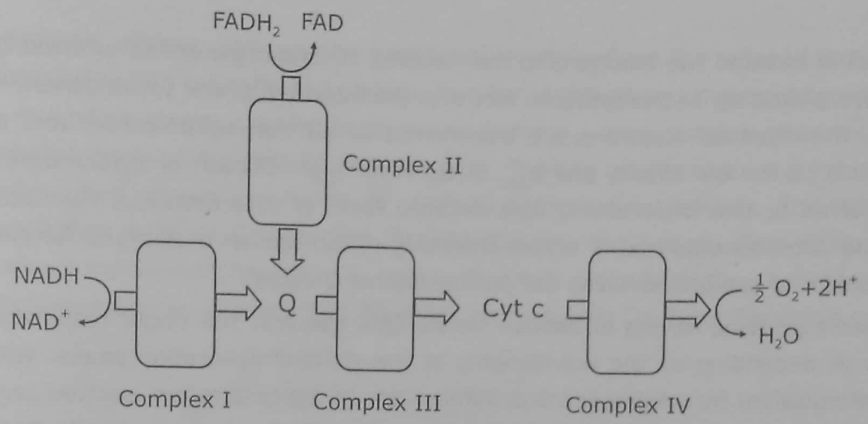


Figure 2.9 Overview of electron flow through the respiratory chain (Q, co-enzyme Q; Cyt, cytochrome).

Complex I

Complex I is a large, multisubunit complex with about 40 polypeptide chains passes electron from NADH to coenzyme Q. It contains one molecule of flavin mononucleotide (FMN) and six to seven iron-sulfur clusters that participate in the electron-transport process. During transport of each pair of electron from NADH to coenzyme Q, complex I pumps four protons across the inner mitochondrial membrane.

Iron-sulfur proteins consist of non-heme iron complexed to sulfur. There are two very common types of iron-sulfur proteins: designated [2Fe–2S] and [4Fe–4S]. These iron-sulfur centers consist of equal numbers of iron and sulfide ions and are both coordinated to four *Cys* sulfhydryl groups.

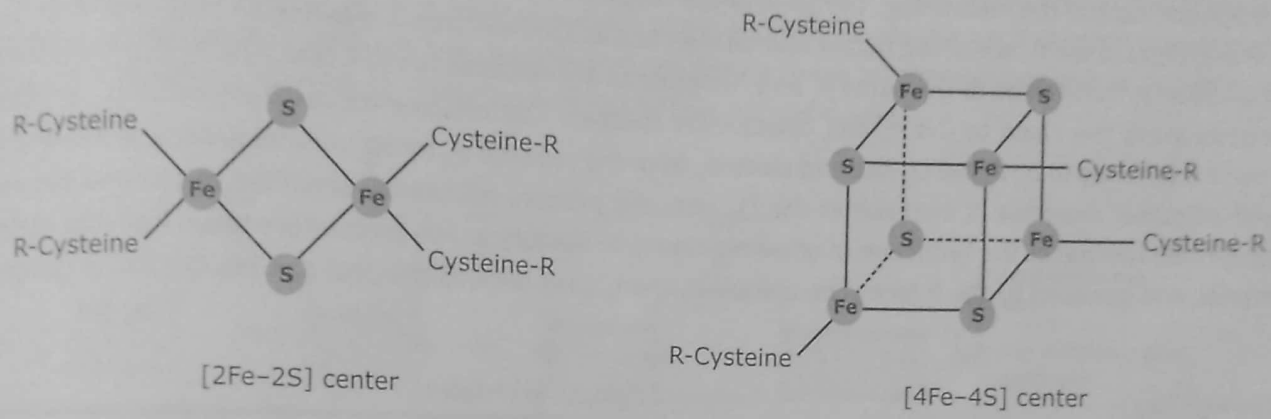


Figure 2.10 Arrangement of the iron-sulfur centers of non-heme iron-sulfur proteins. The cysteine linkages are from the protein portion of the molecule. Iron-sulfur proteins typically carry electrons only.

Coenzyme Q (CoQ, also known as *ubiquinone*) is a benzoquinone linked to a number of isoprene units. The name ubiquinone is for the ubiquitous nature of the quinone. Q refers to the quinone chemical group. There are three redox states of coenzyme Q - fully oxidized (ubiquinone, Q), semiquinone (*ubisemiquinone*), and fully reduced (ubiquinol, QH_2). *Coenzyme Q is the only electron carrier in the electron transport chain that is not a protein-bound prosthetic group.* It is a carrier of hydrogen atoms, that is protons plus electrons.

Complex II

Succinate dehydrogenase, an inner mitochondrial membrane-bound enzyme, is an integral component of the complex II. It converts succinate to fumarate during Krebs cycle. The two electrons released in the conversion of succinate to fumarate are transferred first to FAD, then to an iron-sulfur center and finally to CoQ. Thus, CoQ draws electrons into the respiratory chain, not only from NADH, but also from $FADH_2$. Complex II does not pump protons during transport of electrons across the inner mitochondrial membrane.

Complex III

Complex I or complex II donates two electrons to the complex III and regenerates oxidized CoQ. Concomitantly, it releases two protons picked up on the cytosolic face into the intermembrane space generating proton gradient. Within complex III, the released electrons are transferred to an iron-sulfur center and then to two *b*-type cytochromes (b_{566} or b_L, L for low affinity and b_{562} or b_H, H for high affinity) or cytochrome c_1. Finally, the two electrons are transferred to two molecules of the oxidized form of cytochrome c. Two additional protons are translocated from the mitochondrial matrix across the inner mitochondrial membrane for each pair of electrons transferred. This transfer of protons involves the proton-motive **Q cycle**.

Cytochromes are heme proteins having distinctive visible-light spectra. The major respiratory cytochromes are classified as *b*, *c* or *a*, depending on the wavelengths of the spectral absorption peaks. Within each class, the cytochromes are distinguished by smaller spectral differences. In the respiratory electron carriers, there are two *b*-type cytochromes (b_{566} and b_{562}), two *c*-type cytochromes (c and c_1), and two *a*-type cytochromes (a and a_3). The heme prosthetic groups of a and b cytochromes are tightly, but not covalently, bound to their associated proteins; whereas heme groups of c-type cytochromes are covalently attached through Cys residues. Cytochrome c is present in all aerobic organisms. It is a peripheral protein of the inner mitochondrial membrane and binds via electrostatic interactions to acidic phospholipids in intermembrane space. It is composed of a single polypeptide chain of 104 amino acid residues covalently bound to the heme group. The degree of sequence homology in cytochrome c among species has been used as a measure of the evolutionary distances that separate species.

Q-cycle

The mechanism of the participation of ubiquinone in the electron transport process was proposed by Peter Mitchell and termed as a proton motive *Q-cycle*. Ubiquinones are hydrophobic and uncharged, and hence can migrate along the hydrophobic core of the membrane. Diffusion of one ubiquinol takes place to the Q_p binding site adjacent to the iron-sulfur protein at the P face of the mitochondrial membrane. One electron is transferred to Fe-S protein and the second electron is transferred to the heme b_L and two protons are released to the P face. The Fe-S protein transfers the electron along the chain to Cyt c_1 and cytochrome oxidase. The electron moves from heme b_L to heme b_H. Ubiquinone then binds to b_H at the Q_n site and electron from the reduced b_H forms ubisemiquinone at this site. Now, a second ubiquinol molecule is oxidized at the Q_P site, the process follows as described above and the second electron formed completes the reduction of ubisemiquinone to ubiquinol. Two protons are taken from the matrix for this purpose and released to the P face. The ubiquinol, then, goes back to the pool and the Q-cycle is completed.

$2H^+$

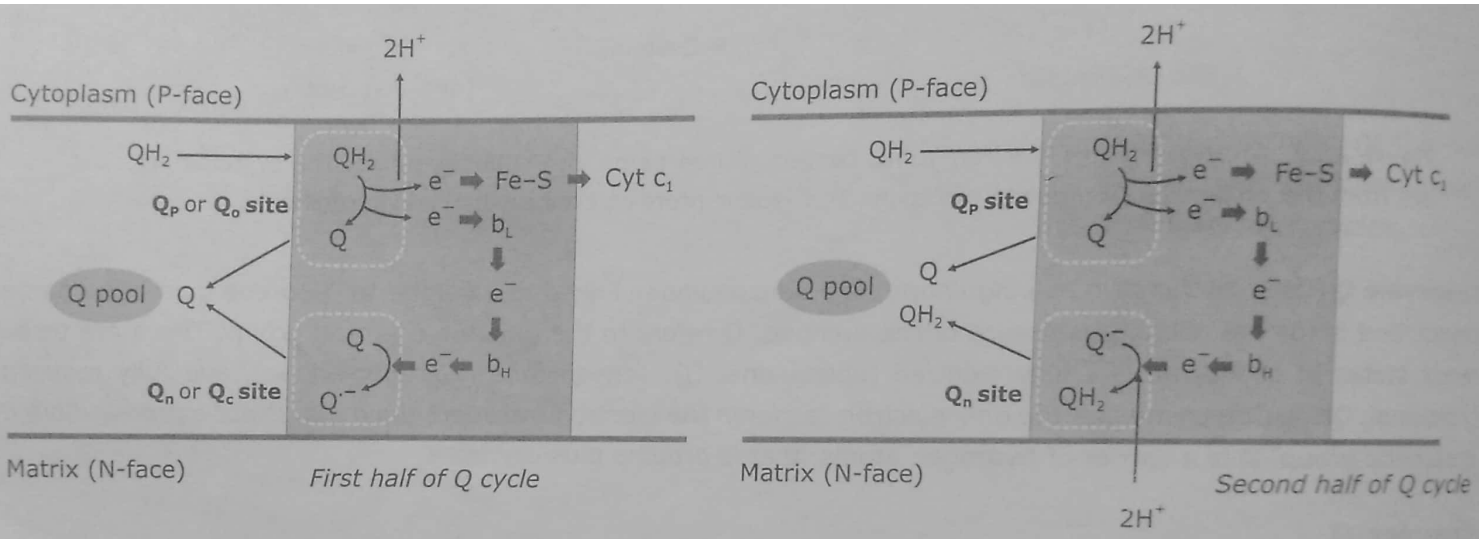


Figure 2.11 A simplified outline of Q-cycle in mitochondria. In the first half of the cycle, two electrons of a bound QH_2 are transferred, one to cytochrome c_1 and the other to a bound Q in a second binding site to form the ubisemiquinone. The newly formed Q dissociates and enters the Q pool. In the second half of the cycle, a second QH_2 also gives up its electrons to complex III, one to a molecule of cytochrome c_1 and the other to reduce $Q^{\bullet -}$ to QH_2. The second electron transfer results in the uptake of two protons from the matrix. Ubiquinol – QH_2, Ubisemiquinone – $Q^{\bullet -}$ and Ubiquinone – Q.

Complex IV

Complex IV or *cytochrome c oxidase* catalyzes the transfer of electrons from the reduced form of cytochrome c to molecular oxygen. It consists of 13 subunits and contains two heme groups and three copper ions, arranged as two copper centers. The two heme groups termed *heme* a and *heme* a_3, have distinct properties because they are located in different environments within cytochrome *c* oxidase. The two copper centers are designated as *a* and *b*. One center, Cu_a, contains two copper ions linked by two bridging cysteine residues. The second center, Cu_b, is coordinated by three histidine residues. Cytochrome *c* transports electrons, one at a time, to the complex IV. Within this complex, electrons are transferred, first to a Cu_a center, then to Cyt a, next to Cu_b center and Cyt a_3, and finally to O_2, the ultimate electron acceptor, yielding H_2O. Together, heme a_3 and Cu_b form the active center at which O_2 is reduced to H_2O.

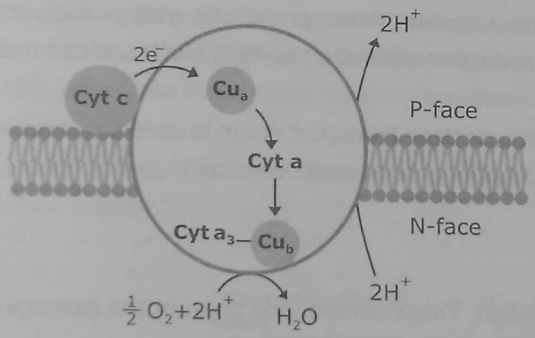


Figure 2.12
The electron transfer pathway for cytochrome oxidase.

($Cyt\ c \rightarrow Cu_a \rightarrow Cyt\ a \rightarrow Cu_b$. $Cyt\ a_3 \rightarrow O_2$)

Cytochrome c (a peripheral protein) binds on the P face of inner mitochondrial membrane, transferring electrons through the copper and heme centers to reduce O_2 on the matrix side of the membrane.

Two electrons, sequentially released from two molecules of reduced cytochrome *c* together with two protons from the matrix, combine with one O atom to form one water molecule. Additionally, for each electron transferred from cytochrome *c* to oxygen, one proton is transported from the matrix to the intermembrane space, or a total of four electrons are transferred for each O_2 molecule reduced to two H_2O molecules.

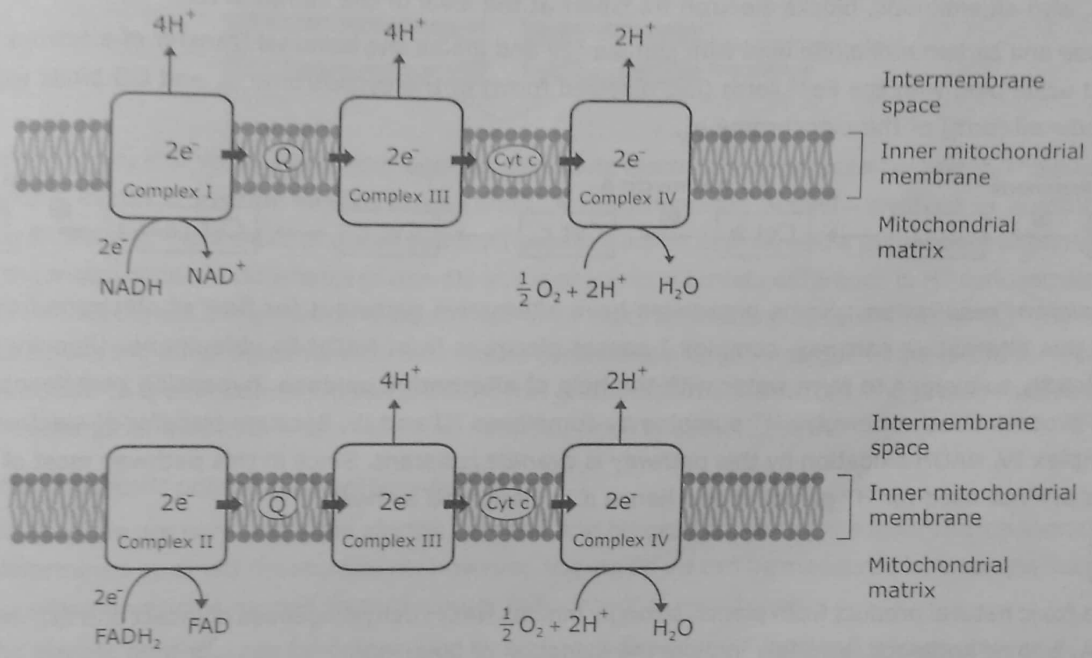


Figure 2.13 Flow of electrons through the respiratory chain complexes, showing the entry points for reducing equivalents. Q and cyt c are mobile components of the system.

Problem

Each of the cytochromes in the mitochondria contains a heme prosthetic group, which permits these proteins to transport electrons in the electron transport system.

a. Explain how heme prosthetic groups function as electron carriers?

b. What property of the cytochromes assures unidirectional electron flow along the electron transport chain?

c. As electrons flow through the electron transport chain, they lose energy. How much of this energy is utilized?

Solution

a. Each of the heme prosthetic groups present in cytochromes contains an iron atom that accepts an electron as it is reduced and releases an electron as it is oxidized. Because the heme ring has numerous resonance forms, the second electron is delocalized to the heme carbon and nitrogen atoms.

b. The various cytochromes in the electron transport chain contain heme prosthetic groups with different axial ligands. As a result, each cytochrome has a different reduction potential, so that electrons can move only in a single order through the electron carriers.

c. Much of the energy lost by electrons moving through the electron transport chain is used to pump protons from the matrix to the intermembrane space, thus generating the proton-motive force.

2.3.7 Inhibitors of electron transport

Site-specific inhibitors of electron transport have been identified. These compounds prevent the passage of electrons by binding to a component of the chain, blocking the oxidation/reduction reaction.

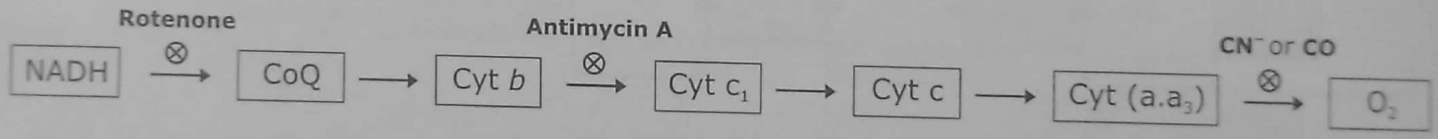
Rotenone, a plant product, inhibits the transfer of electrons through complex I. It is used as a fish poison and as an insecticide.

Amobarbital (Amytal) also acts at the same site and inhibits electron transport through complex I.

Piericidin A, an antibiotic, blocks the transfer of electrons at complex I by competing with Q. The electrons from the complex are transferred to piericidin A instead of Q.

Antimycin A, also an antibiotic, blocks electron transport at the level of the complex III.

Cyanide, azide and *carbon monoxide* bind with complex IV and inhibit the terminal transfer of electrons to oxygen. Cyanide and azide bind with the Fe^{3+} form (i.e. oxidized form) of the cytochrome a_3 and CO binds with the Fe^{2+} form (i.e. reduced form) of the cytochrome a_3.



Cyanide-resistant respiration : Some organisms have alternative pathways for flow of electrons from NADH to oxygen. In this alternative pathway, complex I passes electrons from NADH to ubiquinone. Ubiquinone donates electrons directly to oxygen to form water with the help of **alternative oxidase**, bypassing complexes III and IV. Hence, this process does not involve H^+ pumping by complexes III and IV. Because transfer of electrons does not involve complex IV, NADH oxidation by this pathway is *cyanide resistant*. Since in this pathway most of the energy of electrons are not used for H^+ pumping and hence it is dissipated as heat.

Problem

Rotenone, a toxic natural product from plants, strongly inhibits NADH dehydrogenase of insect and fish mitochondria. Antimycin A, a toxic antibiotic, strongly inhibits the oxidation of ubiquinol.

a. Explain why rotenone ingestion is lethal to some insects and fish species?

b. Explain why antimycin A is a poison?

c. Given that rotenone and antimycin A are equally effective in blocking their respective sites in the electron transport chain, which would be a more potent poison? Explain.

Solution

a. Inhibition of NADH dehydrogenase by rotenone decreases the rate of electron flow through the respiratory chain, which in turn decreases the rate of ATP production. If this reduced rate is unable to meet the organism's ATP requirements, the organism dies.

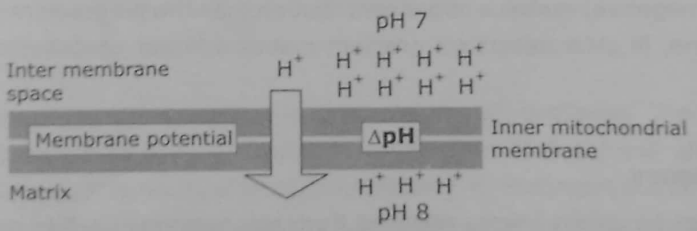
b. Antimycin A strongly inhibits the oxidation of Q in the respiratory chain, reducing the rate of electron transfer and leading to the consequences described in (a).

c. Because antimycin A blocks all electron flow to oxygen, it is a more potent poison than rotenone, which blocks electron flow from NADH, but not from $FADH_2$.

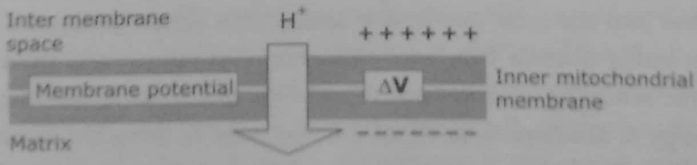
2.3.8 Electrochemical proton gradient

Transfer of electrons through the electron transport chain is accompanied by pumping of protons across inner mitochondrial membrane, from the mitochondrial matrix to inter membrane space. A total of $10H^+$ ions are translocated from the matrix across the inner mitochondrial membrane per electron pair flowing from NADH to O_2. This movement of H^+ generates:

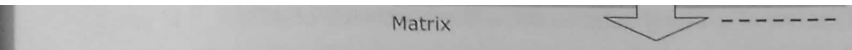
a. *pH gradient* across the inner mitochondrial membrane (with the pH higher in the matrix than in the inter membrane space).



b. *Voltage gradient* (membrane potential) across the inner mitochondrial membrane (with the inside negative and outside positive).



The pH gradient for



The pH gradient (ΔpH) and voltage gradient together constitute *electrochemical proton gradient.* The electrochemical proton gradient exerts a **proton motive force** (pmf). A mitochondrion actively involved in aerobic respiration typically has a membrane potential of about 160 mV (negative inside matrix) and a pH gradient of about 1.0 pH unit (higher on the matrix side). A difference of one pH unit represents a tenfold difference in H^+ concentration and a pH gradient of one unit across a membrane is equivalent to an electric potential of 59 mV (at 20°C). The total proton-motive force across the inner mitochondrial membrane consists of a large force due to the membrane potential and a smaller force due to the H^+ concentration gradient (pH gradient). In a typical cell, the proton motive force across the inner mitochondrial membrane of a respiring mitochondrion is about 220 mV.

Determination of electric potential and pH gradient

Because mitochondria are very small, the electric potential and pH gradient across the inner mitochondrial membrane cannot be determined by direct measurement. However, the inside pH can be measured by trapping fluorescent pH-sensitive dyes inside vesicles formed from the inner mitochondrial membrane.

Similarly, the electric potential can be determined by adding radioactive K^+ ions and a trace amount of valinomycin to a suspension of respiring mitochondria. Valinomycin is an *ionophore.* Although the inner membrane is normally impermeable to K^+ ions, but valinomycin selectively binds K^+ ions in its hydrophilic interior and carries it across through the impermeable membranes. In the presence of valinomycin, K^+ ions equilibrates across the inner membrane of isolated mitochondria in accordance with the electric potential; the more negative the matrix side of the membrane,

the more K^+ ions will accumulate in the matrix. At equilibrium, the value of electric potential can be calculated from the Nernst equation.

Calculation of proton motive force

Proton motive force (pmf) is the electrochemical potential difference between protons in the aqueous phases on different sides of any membrane. The sum of the membrane potential and the pH gradient together constitute the pmf. This indicates the total potential energy stored in the transmembrane gradients, which is available to drive protons back into the matrix space, and provide the power for biologically useful processes. Proton motive force has two components - an electrical term and a concentration term. These are related by the equation:

$$pmf = \Delta\psi - z\Delta pH$$

where $\Delta\psi$ is the electrical potential difference or membrane potential (cytoplasm – matrix),

z is 2.303 RT/F, with values of 59 mV at 25°C.

Thus, pmf (in millivolts) = $\Delta\psi - 59\ \Delta pH$

Measurements on respiring mitochondria have shown that the electrical potential difference across the inner membrane is ~160 mV (negative inside matrix) and that ΔpH is ~1.0 (equivalent to ~60 mV). Thus, the total pmf is ~220 mV, with the transmembrane electric potential responsible for about 70 percent. In mitochondria, the electrical potential difference (~160 mV, inside negative) makes a larger contribution than the pH gradient (~1 pH unit, inside alkaline). As compared to mitochondria, in chloroplasts pH gradient makes a larger contribution than the transmembrane electric potential.

2.3.9 Chemiosmotic theory

What is the actual mechanism by which energy released from respiration is used to drive the synthesis of ATP? In 1961, Peter Mitchell, a British biochemist, proposed a mechanism of *chemiosmotic coupling* to answer this question. This model proposes that energy from electron transport drives an active transport system, which pumps protons out of the mitochondrial matrix into the inter membrane space. This action generates an electrochemical gradient for protons, with a lower pH value outside the inner mitochondrial membrane than inside. The protons on the outside have a thermodynamic tendency of flow back into the matrix so as to equalize pH. When protons do flow back into the matrix, the energy is dissipated, some of it being used to drive the synthesis of ATP.

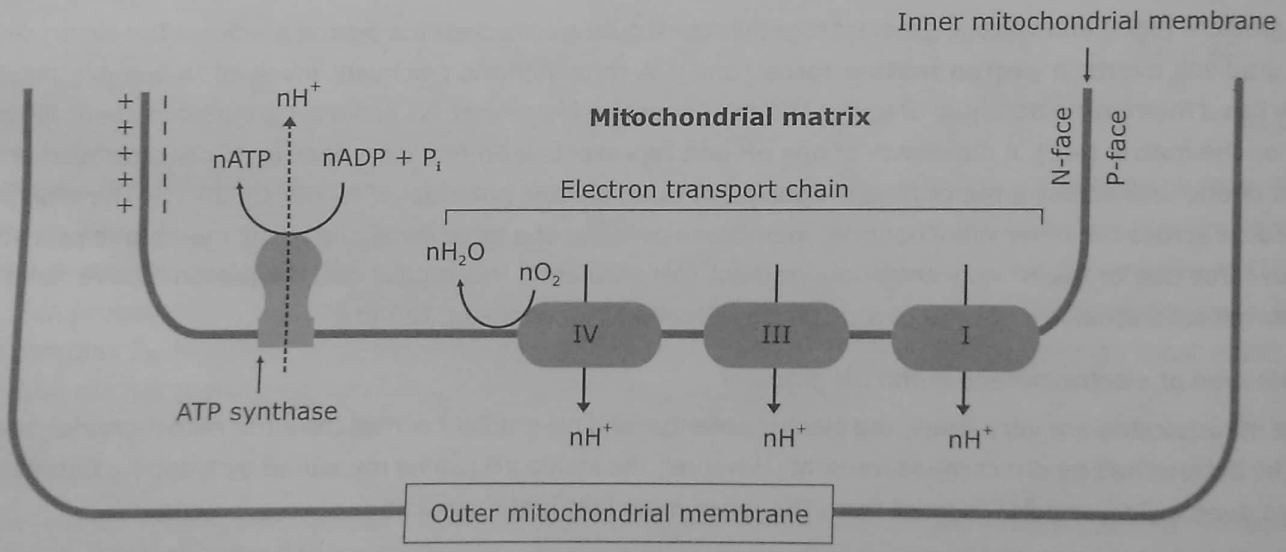


Figure 2.14 The general mechanism of oxidative phosphorylation. As a high-energy electron is passed along the electron-transport chain, some of the energy released is used to drive three respiratory enzyme complexes that pump H^+ out of the matrix space. The resulting electrochemical proton gradient across the inner membrane drives H^+ back through the ATP synthase, a transmembrane protein complex that uses the energy of the H^+ flow to synthesize ATP from ADP and P_i in the matrix.

Experimental proof of chemiosmotic hypothesis

Experimental proof of chemiosmotic hypothesis was provided by Andre Jagendorf and Ernest Uribe in 1966. In an elegant experiment, isolated chloroplast thylakoid vesicles containing F_0F_1 particles were equilibrated in the dark with a buffered solution at pH 4.0. When the pH in the thylakoid lumen became 4.0, the vesicles were rapidly mixed with a solution at pH 8.0 containing ADP and P_i. A burst of ATP synthesis accompanied the transmembrane movement of protons driven by the electrochemical proton gradient. In similar experiments using *inside-out* preparations of submitochondrial vesicles, an artificially generated membrane electric potential also resulted in ATP synthesis.

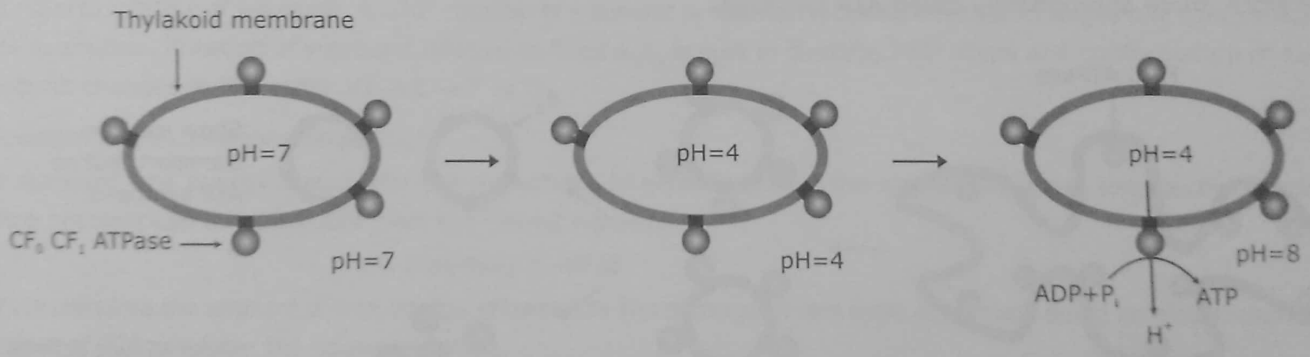


Figure 2.15 Synthesis of ATP by F_0F_1 depends on a pH gradient across the membrane.

2.3.10 ATP synthase

The use of proton motive force for ATP synthesis is catalyzed by ATP synthase. The multiprotein ATP synthase or F_0F_1 complex or complex V catalyzes ATP synthesis as protons flow back through the inner membrane down the electrochemical proton gradient. It consists of two components – F_0 component and F_1 ATPase. The F_0 component is embedded in the inner mitochondrial membrane. F_0 contains one 'a' subunit, two 'b' subunits and 9–12 'c' subunits. The c subunit consists of two α helices that span the membrane. An aspartic acid residue in the second helix lies on the center of the membrane. F_0 is a transmembrane complex that forms a regulated H^+ channel. An antibiotic **oligomycin** completely blocks ATP synthesis by blocking the flow of protons through F_0 of ATP synthase (subscript 'O' denotes its inhibition by antibiotic oligomycin). F_1 ATPase (made up of 3α, 3β, γ, δ and ε) is tightly bound to F_0 and protrudes into the matrix; it contains three β-subunits that are the sites of ATP synthesis. At the center of F_1 ATPase is the γ-subunit. The γ-subunit extends through F_1 and interacts with F_0. The γε and C_{9-12} ring complex is the *rotor* (moving unit) and the a, b_2 and $\alpha_3\beta_3\delta$ complex is the *stator* (stationary unit). Rotational motion is imparted to the rotor by the passage of protons.

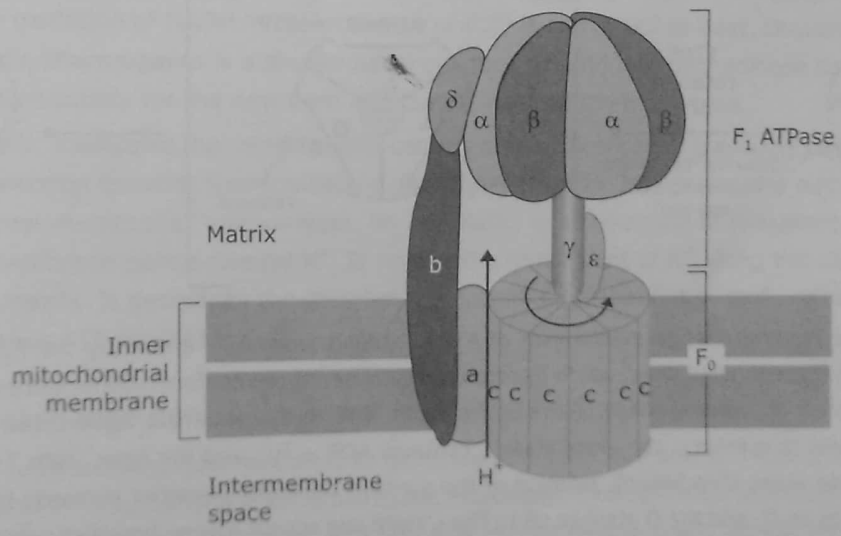


Figure 2.16 The enzyme complex consists of an F_0 component and F_1 ATPase. Proton passing through the disc of 'C' units cause it and the attached γ-subunit to rotate. The γ-subunit fits inside the F_1 ATPase of a three α and three β-subunits, which are fixed to the membrane and do not rotate.

ATP synthase synthesizes ATP by harnessing the proton motive force. ATP synthase can also function in reverse to hydrolyze ATP and pump H^+ across the inner mitochondrial membrane. It thus acts as a *reversible coupling devise*, interconverting electrochemical proton gradient and chemical bond energies, or vice versa.

F_1 ATPase was first extracted from the mitochondrial inner membrane and purified by Efraim Racker and his colleagues. F_1 cannot synthesize ATP from ADP and P_i; because it can catalyze the hydrolysis of ATP. Thus the enzyme was originally called F_1ATPase. The complete F_0F_1 complex, like isolated F_1, can hydrolyze ATP to ADP and P_i, but its biological function is to catalyze the condensation of ADP and P_i to form ATP. The F_0F_1 complex is, therefore, more appropriately called *ATP synthase*.

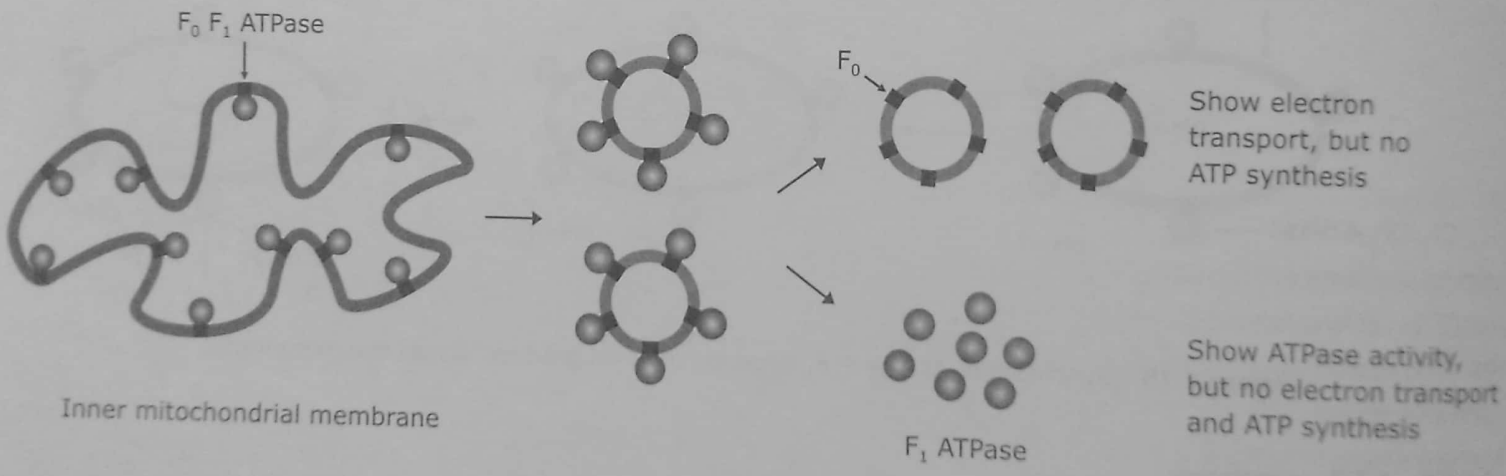


Figure 2.17 F_1 particles are required for ATP synthesis, but not for electron transport. Submitochondrial vesicles from which F_1 is removed by mechanical agitation cannot catalyze ATP synthesis. Because F_1 separated from membranes is capable of catalyzing ATP hydrolysis, it has been called the F_1 ATPase.

2.3.11 Uncoupling agents and ionophores

Uncoupling agents uncouple oxidation from phosphorylation. They allow the oxidation of NADH and $FADH_2$ and reduction of O_2 to continue at high levels but do not permit ATP synthesis. Thus, electron transport continues unabated, but ATP synthesis stops. Most common uncoupling agents are *2,4-dinitrophenol* (DNP), *dicoumarol* and carbonyl cyanide-p-(trifluoromethoxy) phenylhydrazone (FCCP). DNP is a weak acid that is soluble in lipid bilayer both in their protonated neutral forms and in their anionic states. DNP in an anionic state picks up protons in the inter-membrane space and diffuse readily across mitochondrial membranes. After entering the matrix in the protonated form, they can release a proton, thus dissipating the proton gradient and inhibiting ATP synthesis. The energy relased by the oxidation of NADH in the presence of DNP is converted to heat. Dicoumarol and FCCP act in the same way. Similarly, **thermogenin** is a physiological uncoupler found in brown adipose tissue that functions to generate body heat, particularly for the new born and during hibernation in animals.

Ionophores are lipophilic molecules that bind specific cations and facilitate their transport through the membrane. Ionophore uncouple electron transfer from oxidative phosphorylation by dissipating the electrochemical gradient across the mitochondrial membrane. **Valinomycin**, an antibiotic, is an example of ionophore. Its addition makes inner mitochondrial membrane permeable for K^+. It causes the movement of K^+ along the concentration gradient from cytosol into the matrix. It decreases the memberane potential component of pmf (without a direct effect on the pH gradient) and thus ATP synthesis.

2.3.12 ATP–ADP exchange across the inner mitochondrial membrane

Summary of ATP synthesis from the oxidation of one molecule of glucose

Glycolysis (cytoplasm)	NADH	$FADH_2$	ATP
Glucose ⟶ Glucose-6-phosphate			−1
Fructose-6-phosphate ⟶ Fructose-1,6-bisphosphate			−1
2 Glyceraldehyde-3-phosphate ⟶ 2 Glycerate-1,3-bisphosphate	+2		
2 Glycerate-1,3-bisphosphate ⟶ 2 Glycerate-3-phosphate			+2
2 Phosphoenolpyruvate ⟶ 2 Pyruvate			+2
Mitochondrial reactions			
2 Pyruvate ⟶ 2 Acetyl-CoA	+2		
Citric acid cycle			
2 Isocitrate ⟶ 2 α-ketoglutarate	+2		
2 α-ketoglutarate ⟶ 2 Succinyl-CoA	+2		
2 Succinyl-CoA ⟶ 2 Succinate			+2
2 Succinate ⟶ 2 Fumarate		+2	
2 Malate ⟶ 2 Oxaloacetate	+2		
	10 NADH	2 $FADH_2$	4ATP

Total yield of ATP from the complete oxidation of one molecule of glucose (*via* glycolysis, pyruvate dehydrogenase complex reaction, Krebs cycle and oxidative phosphorylation) = 32 (or 30). This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. The total number is either 30 or 32 depending on the mechanisms used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix. In prokaryotic organisms, complete oxidation of one molecule of glucose yields 32 ATP, because no shuttle systems are required for transport of NADH.

2.3.15 Fermentation

Pyruvate, the end product of glycolysis has two fates. In the absence of oxygen, it undergoes *fermentation* and *anaerobic* respiration whereas in the presence of oxygen it enters the *aerobic* respiration. *Anaerobic respiration is different from fermentation.* In **anaerobic respiration**, final electron acceptor in the electron transport chain is an inorganic molecule other than O_2 like nitrate, sulfate and carbonate whereas in *fermentation*, the final electron acceptor is an organic molecule. Fermentation is a self contained process and no outside electron acceptor is involved. It does not involve an electron transport system. In this section we have discussed only fermentation.

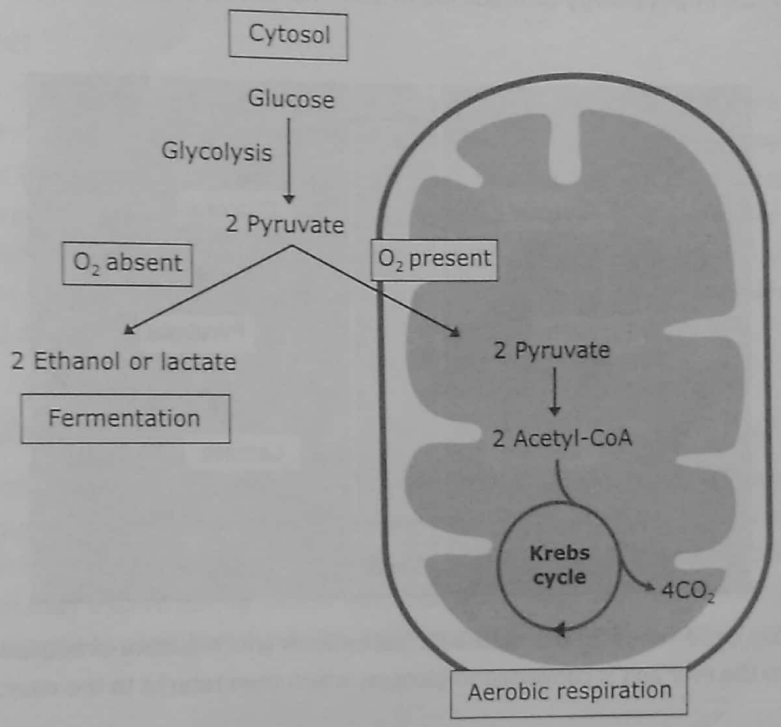


Figure 2.23 Glycolysis is common to fermentation and aerobic respiration. In a facultative anaerobe, which is capable of both aerobic respiration and fermentation, pyruvate is committed to one of those two pathways, usually depending on whether or not oxygen is present.

During fermentation, when O_2 is not present (or the cell cannot use it) NAD^+ is regenerated from NADH by transferring electrons to organic molecules. In this process, pyruvate is converted into organic molecules like lactate, ethanol. Fermentations are classified in terms of either the substrate fermented or the fermentation products formed. Some of the major types of fermentations on the basis of products formed are alcoholic, lactic acid, propionic acid and butyric acid fermentation.

Lactic acid fermentation

Muscle cells and certain bacterial species (e.g. *Lactobacillus*) oxidize NADH by transforming pyruvate into lactate and the process is known as *lactic acid fermentation*. Lactic acid fermentation is catalyzed by an enzyme, *lactate dehydrogenase* (LDH).

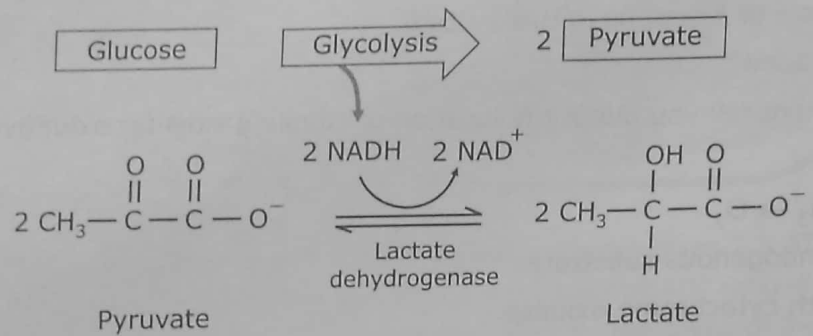


Figure 2.24 In lactic acid fermentation, the NADH formed in the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of pyruvate. The regeneration of NAD^+ in the reduction of pyruvate to lactate sustains the continued operation of glycolysis under anaerobic conditions.

In animals, lactate formed in the muscles is recycled to glucose in the liver. Lactate produced in muscle is transported from the muscle to the liver, where it is reoxidized by liver LDH to pyruvate. By the process of gluconeogenesis, pyruvate is finally converted into glucose in the liver. Liver again exports glucose to muscle for glycolysis. This cycle is referred to as the **Cori cycle** (also known as *Lactic acid cycle*). The Cori cycle is named for Carl and Gerty Cori, who received the Nobel Prize in physiology or medicine in 1947 for their studies of glycogen metabolism and blood glucose regulation.

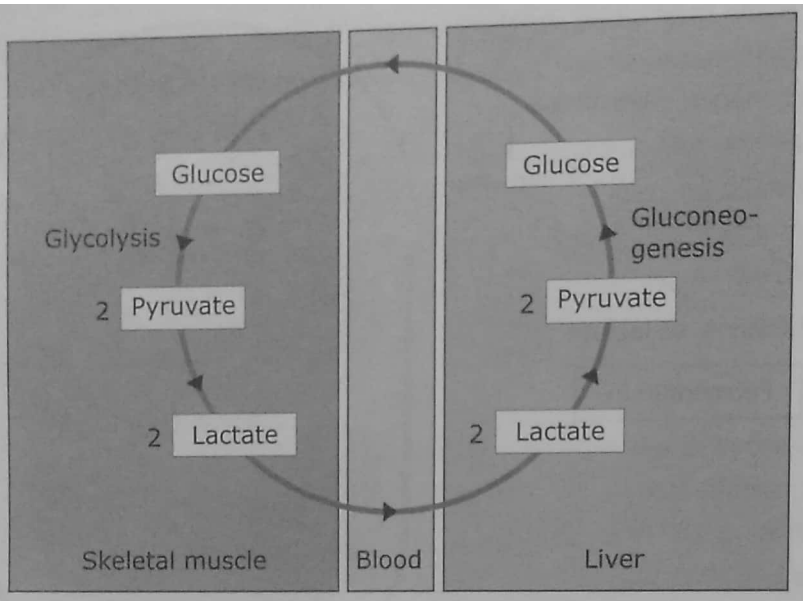


Figure 2.25 The Cori cycle refers to the metabolic pathway in which lactate produced by fermentation in the muscles moves to the liver and is converted to glucose, which then returns to the muscles and is converted back to lactate.

Alcoholic fermentation

In *alcoholic fermentation* that occurs in yeast and several bacterial species, pyruvate is converted into ethanol in a two-step pathway. In yeast, pyruvate is decarboxylated to form acetaldehyde, which is then reduced by NADH to form ethanol. The non-oxidative decarboxylation of pyruvate to acetaldehyde is catalyzed by *pyruvate decarboxylase* and NADH-dependent reduction of acetaldehyde to ethanol is catalyzed by *alcohol dehydrogenase*. Pyruvate decarboxylase requires thiamine pyrophosphate as a coenzyme. This coenzyme, derived from vitamin B_1, participates in a number of group transfer reactions involving an activated aldehyde moiety.

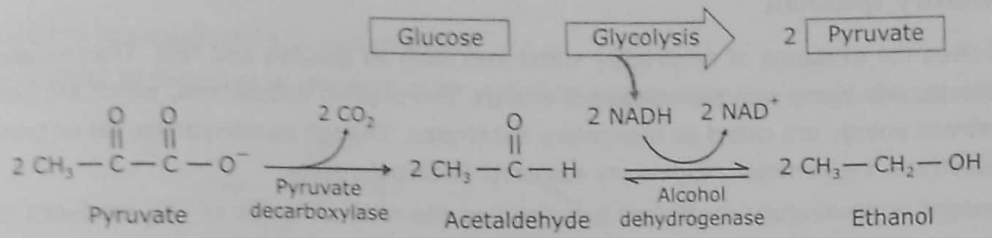


Figure 2.26 In alcoholic fermentation, conversion of glucose into ethanol occurs. The NADH generated by the oxidation of glyceraldehyde 3-phosphate is consumed in the reduction of acetaldehyde to ethanol. Thus, there is no net oxidation-reduction in the conversion of glucose into ethanol.

Table 2.6 Comparison of aerobic respiration and fermentation

Aerobic respiration	Growth condition : Aerobic
	Final electron acceptor : Molecular oxygen
	Type of phosphorylation used to generate ATP : Substrate-level and oxidative
Fermentation	Growth condition : Aerobic/anaerobic
	Final electron acceptor : An organic substance
	Type of phosphorylation used to generate ATP : Substrate-level
Anaerobic respiration	Growth condition : Anaerobic
	Final electron acceptor : An inorganic substance such as nitrate, sulfate but not oxygen
	Type of phosphorylation used to generate ATP : Substrate-level and oxidative

2.3.16 Pasteur effect

Louis Pasteur observed that when yeast is exposed to aerobic conditions (in the presence of oxygen), their glucose consumption and ethanol production drop. Whereas in the absence of oxygen glucose consumption increases several fold. Reason for the decrease in consumption of glucose is that fermentation results in the production of 2 ATPs per glucose whereas aerobic respiration yields 32 ATPs per glucose. Hence, for the generation of same amount of ATP to perform essential metabolic activities, more consumption of glucose is needed in anaerobic condition. This accounts for Pasteur's observation that yeast consumes more glucose when growing anaerobically than aerobically (called *Pasteur effect*).

2.3.17 Warburg effect

Most cancer cells exhibit increased glycolysis and use this metabolic pathway for generation of ATP as a main source of their energy supply. This phenomenon is known as the *Warburg effect*. However, the advantage it confers to cancer cells has been unclear. Biochemical and molecular studies suggest several possible mechanisms by which this metabolic alteration may evolve during cancer development. These mechanisms include mitochondrial defects and malfunction, adaptation to hypoxic tumor microenvironment and abnormal expression of enzyme, pyruvate kinase.

2.3.18 Respiratory quotient

Respiration involves the oxidation of respiratory substrates such as glucose and fats. The oxidation involves the release of carbon dioxide along with the release of energy. The organic substances, which are catabolised in the living cells to release energy are called as *respiratory substrates*. Though carbohydrate, fat or protein may act as a respiratory substrate, the common respiratory substrate is carbohydrate.

Respiratory quotient or *respiratory coefficient* is defined as the ratio of moles of CO_2 produced to the moles of oxygen consumed during complete oxidation of a metabolic fuel to CO_2 and H_2O. The moles of oxygen required as well as the moles of carbon dioxide released depend on the nature of respiratory substrate that is being broken down. For carbohydrates, such as glucose, the respiratory quotient (RQ) is 1.

$$RQ = \frac{6 \text{ mol of } CO_2 \text{ produced}}{6 \text{ mol of } O_2 \text{ consumed}} = 1 \quad (C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O)$$

Here, 6 moles of CO_2 produced to the 6 moles of oxygen consumed during complete oxidation of glucose. Thus RQ is 1. RQ is less than one when respiration is aerobic, but the respiratory substrate is either fat or protein. *RQ is about 0.7 for most of common fats and about 0.9 in case of proteins.*

RQ for palmitate, $C_{16}H_{32}O_2 + 23O_2 \longrightarrow 16CO_2 + 16H_2O$

$$RQ = \frac{16 \text{ mol of } CO_2 \text{ produced}}{23 \text{ mol of } O_2 \text{ consumed}} = 0.7$$

RQ is more than unity when organic acids such as malic acid, oxalic acid are broken down as a respiratory substrate under aerobic condition. For malic acid RQ value is 1.33.

$$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$$

$$RQ = \frac{4 \text{ mol of } CO_2 \text{ produced}}{3 \text{ mol of } O_2 \text{ consumed}} = 1.3$$

Table 2.7 Name of the substance and their RQ:

Carbohydrates	–	1
Proteins	–	0.8 – 0.9
Oleic acid (Lipid)	–	0.71
Malic acid	–	1.33
Oxalic acid	–	4.0