

Block

5

BIOENERGETICS AND METABOLISM

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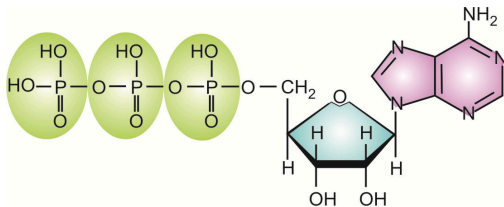
BLOCK 5: BIOENERGETICS AND METABOLISM

In the preceding blocks of this course, you studied about the cell and the various biomolecules present in it. Lipids as the important biomolecules and the process of protein biosynthesis was discussed in Block 4. In the present block viz., Block 5 we will discuss the biogenergetics and intermediary metabolism which includes both catabolism and anabolism. This block consists of four units. In Unit 14, '**Bioenergetics**' we have discussed the generation and consumption of energy taking place in biochemical reactions. The unit describes the laws of thermodynamics as applied to the biological systems. It gives an overview of the energy utilisation and generation during different metabolic processes. Unit 15, '**Carbohydrate Metabolism**' deals with metabolism of carbohydrates, mainly of glucose. The catabolism i.e., the breakdown of glucose and its synthesis via gluconeogenesis is described in the unit. It also elaborates the energetics of the glycolytic process. Unit 16, '**Kreb's Cycle and its Metabolic Role**' deals mainly with the Kreb's cycle which is central to all the types of metabolisms. The electron transport chain responsible for ATP generation via a chemiosmotic mechanism is explained in detail. The last unit of the block as well as of the course, Unit 17 is '**Metabolism of Lipids and Proteins**'. It includes stepwise process of β -oxidation and synthesis of fats. The stepwise description of protein metabolism is given in the unit. Lastly an overview of the interrelationship among the metabolic pathways is given.

Expected Learning Outcomes

After studying this block, you should be able to:

- explain the thermodynamic principles as applied to the biochemical reactions,
- describe the metabolic breakdown and synthesis of carbohydrates,
- explain the energetics of the carbohydrate metabolism,
- describe the Kreb's cycle and its central role in metabolism,
- describe the stepwise process of fat metabolism,
- explain the energetics involved in the fat metabolism,
- explain the stepwise process of protein catabolism, and
- appreciate the interrelationship among the carbohydrate, lipid and protein metabolic pathways.



UNIT 14

BIOENERGETICS

Structure

14.1	Introduction	Structural Basis for the Role of ATP
	Expected Learning Outcomes	
14.2	Thermodynamics of Biochemical Reactions	14.4 Formation of ATP
	The First Law of Thermodynamics	Substrate Level Phosphorylation
	The Second Law of Thermodynamics	Oxidative Phosphorylation
	Conventions in Biochemical Energetics	Photosynthesis
	Additivity of ΔG Values-Coupling Reactions	14.5 Utilisation of ATP or Energy Requiring Functions
14.3	ATP: The Energy Carrier in Biological Energy Transformations	Synthesis of Organic Molecules
	Hydrolysis of ATP	Photosynthesis
	Role of ATP in Biological Energy Transformations	14.6 Thermodynamics and the Living Cells
		14.7 Summary
		14.8 Terminal Questions
		14.9 Answers

14.1 INTRODUCTION

In the previous blocks you have studied about the cell structure, function and the biomolecules present in it. You know that cell has a well defined structure built from complex organic molecules. This is made possible by a continuous flux of energy through the living system and its transformation for various processes. These energy transformations in living systems are governed by the laws of thermodynamics. The study of such transformations comes under the purview of bioenergetics which includes the generation of energy by the breakdown of nutrients, its storage and utilisation in performing various functions of the cell. Further, bioenergetics attempts to unravel the complex network of biochemical pathways involving a large number of interwoven biochemical reactions. Here one tries to apply the principles of

thermodynamics to rationalise the biochemical reactions in terms of free energies of various species.

In this unit we would first recapitulate the fundamentals of thermodynamics and then would evolve a criterion for the spontaneity of biochemical reactions in terms of thermodynamic quantities. Living organisms differ markedly in their appearance yet they have a striking similarity in that they all use the 'wonder molecule', ATP, as the carrier of energy. We would study its formation during phosphorylation and photosynthesis and its utilisation in various energy requiring functions like synthesis of organic molecules, etc. In this unit would also discuss the validity of application of thermodynamic principles to the living system. In the coming units you would study the biosynthesis and breakdown of some biomolecules, which were described in the previous blocks.

Expected Learning Outcomes

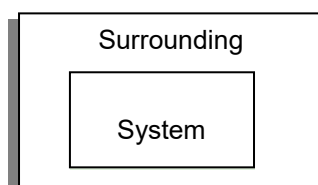
After studying this unit, you should be able to:

- ❖ predict the directions in which the various biochemicals reactions should proceed under the physiological conditions;
- ❖ calculate the energy input or output accompanying these reactions;
- ❖ describe the significance, formation and utilisation of ATP in living systems; and
- ❖ justify the applicability of the thermodynamic principles to living systems.

14.2 THERMODYNAMICS OF BIOCHEMICAL REACTIONS

Energetics in context of biological system is called **bioenergetics**.

Thermodynamics, also known as 'energetics', is better known in terms of some laws which have evolved on the basis of human experience. Before describing the laws and their applications to the biochemical reactions it would be worthwhile to get acquainted with the jargon of the terms used in thermodynamics. These are as follows:



Universe

System: In thermodynamics any portion of the universe which we intend to study constitutes a system and everything else is its surroundings. A system is separated from its surroundings by a boundary (real or imaginary). A thermodynamic system and its surroundings can mutually exchange matter and energy. A system which permits the exchange of matter as well as energy is called an **open** system. On the other hand a system which permits the flow of energy but not of matter is called a **closed** system, whereas the one which does not permit the exchange of either energy or matter, is called an **isolated** system. The three systems are depicted in Fig. 14.1.

State of a System: In thermodynamics the state of a system is defined by assigning the value of thermodynamic variables namely, pressure, temperature and composition for example. 100 cm³ of water at 298K and 1 bar defines a state. Change in any of the parameters produces a new state.

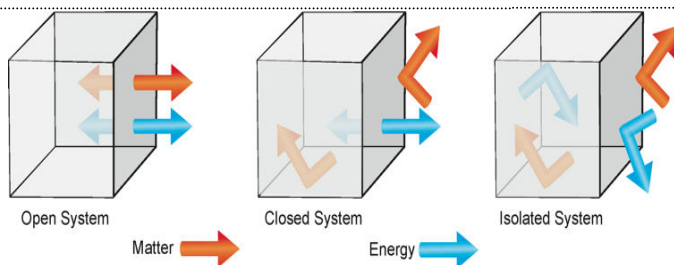


Fig. 14.1: The types of thermodynamic systems.

Spontaneous Process: In thermodynamics, spontaneity refers to the tendency, of the change or, a chemical reaction to take place on its own accord. It has no relationship to the rate of the change. For example, when solid ammonium chloride is added to water, it goes into solution. Similarly, when an acid and an alkali are mixed, they neutralise each other. These are examples of spontaneous processes. The reverse processes in these cases are not spontaneous, e.g., the formation of an acid and an alkali from aqueous solution of a salt. An ordinarily nonspontaneous process may be brought about under special conditions involving input of energy, e.g., electrolysis of salts. Some spontaneous processes may proceed exceedingly slow and the rate of reaction may be close to zero unless the reaction mixture is heated or a suitable catalyst is added. For example, the oxidation of glucose to carbon dioxide and water is a spontaneous process but does not proceed at a measurable rate unless glucose is heated or is acted upon by biological catalysts.

Now we shall state the laws of thermodynamics and see the thermodynamic quantities which emerge out of these. We would attempt to understand their significance and assess them as a criterion of spontaneity.

You know that a catalyst can only alter the rate of a spontaneous reaction. It cannot bring about a reaction which will not proceed spontaneously.

14.2.1 The First Law of Thermodynamics

The first law of thermodynamics states that the total energy of a system and its surroundings, i.e., of the universe, is constant. If a system undergoes a change from state A to state B and in doing so it exchanges with the surroundings and performs some work, then according to the first law of thermodynamics,

$$E_B - E_A = \Delta E = Q - W \quad \dots (14.1)$$

A function depending on only the initial and final states is called the state function.

Where E_A and E_B represent the energy of the system, called internal energy of the system, in state A and B, respectively, Q stands for the heat absorbed by the system from its surroundings and W is the work done by the system on its surroundings. Note that work is also a form of energy.

Internal energy, E , includes the translational, rotational, vibrational and electronic energy of a molecule.

You should note that although transformation of energy may take place, e.g., heat into work or vice versa, but the total energy of the universe remains constant. Therefore, the first law of thermodynamics is also referred to as the law of conservation of energy. This is schematically shown in Fig. 14.2. Note further that the change of the system, ΔE , depends only on the initial and final states of the system and is independent of the pathway of the change, or the number of steps involved. This is an important corollary of the first law.

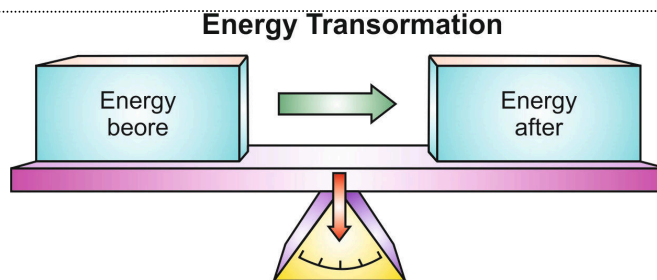


Fig. 14.2: Representation of first law of thermodynamics.

Each system and indeed each of its constituents, is endowed with certain energy. In Eq. 14.1, E_A and E_B refer to the energy of the system, i.e., sum of the energies of all its constituents in state A and B, respectively. In most cases, we do not need to know these absolute values but only the changes in the energy (ΔE) as a result of any process or reaction, e.g., dissolution of ammonium chloride in water or neutralisation of an acid with alkali. This quantity can be experimentally determined by measuring the values of Q and W .

Most of the chemical reactions normally are carried out at a constant pressure. If we assume that the work involved in these is of expansion type then we may rewrite Eq. 14.1 as,

$$\Delta E = Q_p - P\Delta V$$

Where the subscript p denotes the constancy of pressure. Rearranging the above equation we may write,

$$Q_p = (E_B + PV_B) - (E_A + PV_A)$$

Under these conditions a new function called enthalpy, H , defined below; is more pertinent for describing the chemical and biochemical reactions,

$$H = E + PV; \quad \Delta H = \Delta E + P\Delta V$$

Like internal energy, enthalpy is also a state function. We can only determine the change in enthalpy during a reaction, and not the absolute enthalpies of reactants or products. Normally the volume change is not large and the internal energy (E) and enthalpy (H) are only slightly different, more so for biochemical systems where the $P\Delta V$ terms is negligibly small.

So far we have defined two thermodynamic properties name E and H . Let us see if these can provide a criterion for spontaneity. There are examples of spontaneous reactions in which for some the ΔE is negative, while for others it is positive. Clearly ΔE cannot be a criterion for spontaneity.

For quite some time it was believed that ΔH could be used as a criterion for spontaneity, since most of the spontaneous reactions at constant pressure are accompanied by evolution of heat i.e., are exothermic. But in course of time it became evident that there are many reactions that proceed spontaneously though accompanied by +ve ΔH . Both LiCl and $(\text{NH}_4)_2\text{SO}_4$ dissolve readily i.e., spontaneously, in water. The former releases heat, while the latter absorbs heat. Thus, enthalpy change also fails to be a criterion for spontaneity.

We see that the two thermodynamic properties obtained from the first law do not provide a sufficient for the spontaneity of a reaction. We need to have an additional law that may provide the suitable criterion we are looking for. Let us see if the second law does the job.

14.2.2 The Second Law of Thermodynamics

This law can be stated in number of equivalent ways. According to the statement most relevant to the biochemical processes, a change will take place spontaneously if it leads to an increase in randomness (or disorder) in the universe, i.e., of the system and its surroundings. The extent of randomness is represented by the thermodynamic function called entropy and denoted by S . Thus, the entropy of the universe must increase in any spontaneous process. In other words, S (system) + S (surroundings) should be greater than zero for a spontaneous change. We have got entropy change as a criterion for spontaneity but application of this statement to predict the spontaneity of a chemical reaction is not always possible. It is so because entropy changes are not easily measurable. Further, the law requires measurement of entropy changes not only of the systems but of its surroundings also. A convenient for predicting the direction of a reaction was discovered. A convenient function for predicting the direction of a reaction was discovered by J. Gibbs. It is called **free energy** (G) and defined as.

$$G = H - TS; \Delta G = \Delta H - T\Delta S$$

Where ΔG is the change in the free energy of the system undergoing the chemical reaction, or any other process, at constant temperature T and pressure. ΔH and ΔS stand for the changes in the enthalpy and entropy, respectively of the system alone, and excludes the surroundings. Of the total enthalpy changes in a process or a chemical reaction, a certain part (equal to $T\Delta S$) goes to increase the extent of randomness in the system and only the remained amount i.e., $\Delta H - T\Delta S = \Delta G$ is available for performing useful work, which may be mechanical work, work of chemical synthesis or work, work of chemical synthesis or work due to transport of substances against the concentration gradient, etc. The concept of free energy and its applications are best illustrated with biochemical reactions, as will be seen with examples described later in this unit.

According to **Gibbs**, a reaction (or a process) will take place spontaneously if it is accompanied by a decrease in the free energy of the system. In other words, **ΔG (system) must be negative for a spontaneous reaction.** Note that application of this criterion for spontaneity does not require any measurement with the surroundings. Only the reaction system is to be considered.

The value of ΔG depends only on the free energy of the system before and after the chemical reaction, i.e.,

$$\Delta G = G(\text{Products}) - G(\text{Reactants})$$

Therefore, ΔG is independent of the mechanism or the pathway of the reaction. This property of ΔG is useful in many calculations as will be discussed later. Further, it must be kept in mind that ΔG provides no

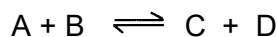
On microscopic level entropy is an index of the number of different ways that a system could be arranged without changing its energy. If there are Q different ways then the absolute entropy (S) per mole of substance is, $S = R \ln Q$, Where R is the gas constant.

Chemical reactions with negative free energy changes are termed **exergonic**. Reactions with positive standard free energy changes are termed **endergonic**.

information about the rate at which the chemical reaction will proceed. Thus, we have got a working criterion for spontaneity. Since it is an important characteristic of different types of reactions.

Free Energy Changes in Reversible Reactions

In a chemical reaction,



the free energy change, ΔG for the conversion of A and B into C and D is given by the following relationship:

$$\Delta G = \Delta G^0 + RT \ln \frac{[C][D]}{[A][B]} \quad \dots (14.3)$$

Where ΔG^0 is a constant which is characteristics of the reaction, R is the molar gas constant, T the absolute temperature in Kelvin and the quantities within square brackets represent the concentration, more precisely the activities, of the respective products and reactants. Significance of the constant ΔG^0 becomes clear if we presume that the reaction is carried out under standard conditions, i.e., where concentrations of all reaction partners (reactants and products) are maintained at unity (one mole per dm³ for solutions and one bar pressure for gases). Under these conditions, $\Delta G = \Delta G^0$, because the second term on the right-hand side of Eq. 14.3 becomes zero. Therefore, ΔG^0 is referred to as the **standard free energy change** (or simply the standard free energy) of the reaction. Note that the actual free energy change will depend on this value and the prevailing concentrations of reactants and products as in Eq. 14.3. The distinction between ΔG and ΔG^0 is very important. The values of ΔG^0 have been tabulated for many reactions. However, it is necessary to calculate the actual ΔG value of a chemical reaction under the prevailing concentration conditions with the help of Eq. 14.3. The criterion for spontaneity and the direction in which a reversible reaction will proceed is ΔG and not ΔG^0 . As stated earlier also the reaction will proceed in the direction of negative ΔG .

The standard free energy of a reversible reaction, ΔG^0 , is related to its equilibrium constant, K_{eq} . Under equilibrium conditions, the free energy of the system is minimum and ΔG of the reaction is equal to zero. Therefore,

$$\Delta G = 0 = \Delta G^0 + RT \ln \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}} \quad \dots (14.4)$$

where the terms within square brackets present concentrations under equilibrium conditions. Since,

$$K_{eq} = \frac{[C]_{eq}[D]_{eq}}{[A]_{eq}[B]_{eq}}$$

$$\Delta G^0 = -RT \ln K_{eq}$$

$$\Delta G^0 = -2.303 \times R \times T \times \log K_{eq} \quad \dots (14.5)$$

As a matter of convention, temperature is fixed at 298 K. Values of K_{eq} of various reactions at this temperature are used to compute their standard free energies. For example, if K_{eq} is equal to 0.1 at 298 K,

$$\Delta G^0 = -2.303 \times 8.314 \times 298 \times (\log 0.1)$$

$$\Delta G^0 = -2.303 \times 8.314 \times 298 \times (-1)$$

$$= 5705.8 \text{ J mol}^{-1}$$

$$= 5.706 \text{ kJ mol}^{-1}$$

Free Energy Change for Redox Reactions

For redox reactions, i.e., the ones involving simultaneous oxidation and reduction, the value of standard free energy and hence that of the equilibrium constant can be obtained from the difference in the standard reduction potentials of the oxidant/reductant pair involved.

The difference in standard reduction potential ΔE^0 is related to the standard free energy change by the following relation:

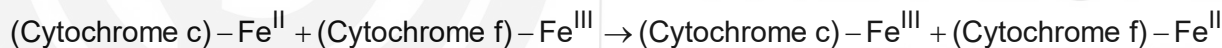
$$\Delta G^0 = -nF\Delta E^0 \quad \dots (14.6)$$

Where, n = number of electrons transfers

F = Faraday, it is the free energy change when one mole of electrons drops through a potential of one volt (96.485 kJ volt⁻¹ mol⁻¹)

ΔG^0 (in volts) = the difference in the standard reduction potentials of the two reaction pairs

For example, for the reaction given below, involved in electron transfer chain from glucose metabolites to molecular oxygen in aerobic organisms.



the value of ΔE^0 is 0.11 V which gives

$$\Delta G^0 = -1 \times 96.485 \times 0.11 = -10.61 \text{ kJ mol}^{-1}$$

Oxidation is the loss of electrons while reduction is the gain of electrons.

standard reduction potential of an oxidant/reductant pair is the measure of the tendency of the reductant to get reduced, i.e., to accept electrons. It is related to the observed potential and the concentration ratio of the electron – donor and electron-acceptor species by Nernst equation which is:

$$E = E^0 + \frac{2.303RT}{nF} \log$$

$$\frac{2[\text{electron – acceptor}]}{[\text{electron – donor}]}$$

SAQ 1

Tick ✓ mark for the correct answer.

The free energy change, ΔG ,

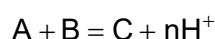
- is equal to zero at equilibrium.
- is directly proportional to the standard free energy change.
- is equal to $-RT$ in K_{eq} .
- can be calculated only when the reactants and products are present at 1 M concentrations.

14.2.3 Conventions in Biochemical Energetics

For biochemical reactions two conventions are very important. The first one concerns the standard state and hence the standard free energy change. You

would recall that the standard state refers to a situation where all the reactants and products are at unit molar concentrations which means that in a reaction involving H^+ ions the standard state would have $[H^+] = 1M$ or $pH = 0$. However, biochemical reactions take place at a pH of about 7, i.e., $[H^+] = 10^{-7} M$ and the above mentioned standard state of $pH = 0$ is not of much relevance. Consequently, a different standard state is adopted for biochemical reactions in which the concentration of H^+ ions is $10^{-7} M$ (and $pH = 7.0$); while that of all other species is 1 M, as with non-biochemical reactions. Further, the corresponding standard free energy is also given a new symbol i.e., $\Delta G^{0'}$ as against ΔG^0 corresponding to the physical chemistry standard state.

For a reaction where H^+ ions are released,



the two standard free energies are related as

$$\Delta G^0 = \Delta G^{0'} + nRT \ln 1/10^{-7}$$

For $n = 1$ and $T = 298 K$,

$$\Delta G^0 = \Delta G^{0'} + 40.0 kJ mol^{-1}$$

i.e., for the reaction producing H^+ , ΔG^0 is greater than $\Delta G^{0'}$ by about $40 kJ mol^{-1}$. On the other hand, for reactions involving H^+ as reactant,



For $n = 1$ and $T = 298 K$,

$$\Delta G^{0'} \text{ is greater than } \Delta G^0 \text{ by about } 40 kJ mol^{-1}$$

For the reactions which do not involve H^+ ions,

$$\Delta G^0 = \Delta G^{0'}$$

You may recall that the criterion for the spontaneity of a reaction is its ΔG value and not ΔG^0 or ΔG . The value of ΔG remains the same irrespective of the standard state chosen.

Hence, proper care should be taken while using the ΔG values (given in the standard tables) for biochemical reactions.

Another convention used in biochemical energetics is that the activity of water molecules in aqueous solutions is taken to be unity although their concentration is about 55 M.

The values of standard free energy changes obtained for different steps of a reaction can be added. The sum value of the ΔG s for individual reaction steps will determine whether the reaction will proceed in forward direction or not. You will study the significance of this additive property of ΔG in the following subsection.

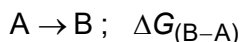
14.2.4 Additivity of ΔG Values - Coupling Reactions

The values of ΔE , ΔH and ΔG depend on the initial and final states of the system only. When a system is transformed from state A to the state B, then

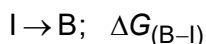
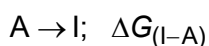
$$\Delta G \text{ (for } A \rightarrow B) = G_B - G_A$$

The values of ΔG is independent of the pathway of transformation, or the number of steps involved in going from state A to state B.

This change may take place in a single step,



Alternatively, the reaction may take place through an intermediate (I) as given below:

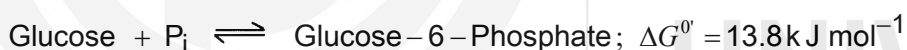


Since ΔG depends on the initial and final states only, it follows that

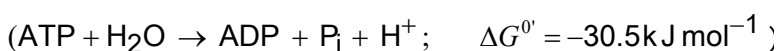
$$\Delta G_{(B-A)} = \Delta G_{(I-A)} + \Delta G_{(B-I)}$$

In other words, the thermodynamic equations can be added or subtracted like algebraic equations. This is a very useful relationship. It helps us in calculating ΔG (or ΔG^0 or $\Delta G^{0'}$) values for reactions where direct determination may not be conveniently carried out.

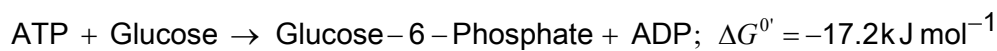
An important corollary of the additivity for free energy values is that certain unfavourable reactions (ΔG +ve) can be made to proceed by suitably **coupling** these with highly favourable reactions. Coupling of the reactions can take place in different ways. One of these is with the aid of suitable enzymes. For example, in the glycolysis (one of the metabolic pathways to be studied in the next unit), first step is the conversion of glucose to glucose-6-phosphate.



As you can see, it is an endergonic process and would not be spontaneous. However, this reaction can be driven by coupling it with a highly exergonic reaction i.e., the hydrolysis of ATP.



The overall reaction is,



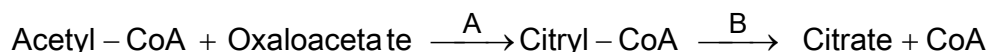
It may however be noted that above two reactions would not couple if they are occurring independently. This reaction is catalysed by an enzyme called hexokinase which facilitates the transfer of phosphate group from ATP to glucose.

The favourable reaction either precedes or follows the unfavourable reaction. If it precedes, it provides the reactant for the unfavourable reaction in large amount to push it ahead. If it allows the unfavourable reaction, it continuously removes the product of first reaction thereby effectively pulling the reaction through in large amount to push it ahead.

ΔG^0 for the oxidation of glucose to carbon dioxide and water will be the same whether it is brought about by combustion in a bomb calorimeter (one step) or via a number of steps as in a living cell.

This mode of coupling is essentially a manifestation of law of mass action.

For an unfavourable reaction $B \rightarrow C$, highly favourable reactions like, $A \rightarrow B$ or $C \rightarrow D$ can act as suitable coupling reactions where the former would have to precede and latter would have to follow the reaction $B \rightarrow C$. For example, in the following sequence of reaction,



The $\Delta G^{0'}$ for step A is close to zero (but -ve) while the step B is highly exergonic and the overall reaction quite favourable and proceeds in the right direction to a good extent. The energy carrier in living systems for various energy requiring processes is adenosine triphosphate (ATP) about which you will study in the next section. Before that try to answer the following SAQ.

SAQ 2

- You have learnt about the similar nature of ΔG^0 and $\Delta G^{0'}$. State when will ΔG^0 and $\Delta G^{0'}$ be actually different?
- For a reaction; $A \rightarrow B$, will $\Delta G^{0'}$ value be able to indicate its feasibility?

A human being with a sedentary occupation needs about 8000 kJ per day, while somebody carrying out manual work requires up to 16000 kJ per day.

ATP is not a 'store' of energy but rather the link between exergonic and endergonic reactions.

The triphosphate component of ATP is responsible for its role as an energy carrier.

14.3 ATP: THE ENERGY CARRIER IN BIOLOGICAL ENERGY TRANSFORMATIONS

You would recall that free energy change under isothermal conditions is the energy available for useful work. Since living cells function isothermally, these must utilise free energy for performing various energy requiring functions. A key function of cellular metabolism lies in transferring of free energy between the systems producing it and those consuming it. It was pointed out before that virtually all organisms make use of adenosine triphosphate, ATP, for this purpose. We would now make an attempt to understand how does ATP act as a carrier of free energy and what makes it the molecule of choice for the purpose.

14.3.1 Hydrolysis of ATP

You have studied in Unit 12 that adenosine triphosphate is a nucleotide consisting of a purine base, adenine, a ribose sugar and a triphosphate unit. Fig. 14.3 (a) gives the structure of ATP and Fig. 14.3 (b) gives its hydrolysis products namely, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and the inorganic phosphate. A large amount of free energy is generated on the hydrolysis of ATP as detailed in the figure.

The actual free energy change (ΔG) for the hydrolysis of ATP to ADP and phosphate ion (P_i) in a living cell is considerably different from $\Delta G^{0'}$ given in Fig. 14.3 and depends on the intracellular concentrations of the reaction partners, which are far from unity and are unequal. For example, the concentrations of ATP, ADP and P_i in human erythrocytes (red cells of the blood) are 2.25, 0.25 and 1.65 mM, respectively.

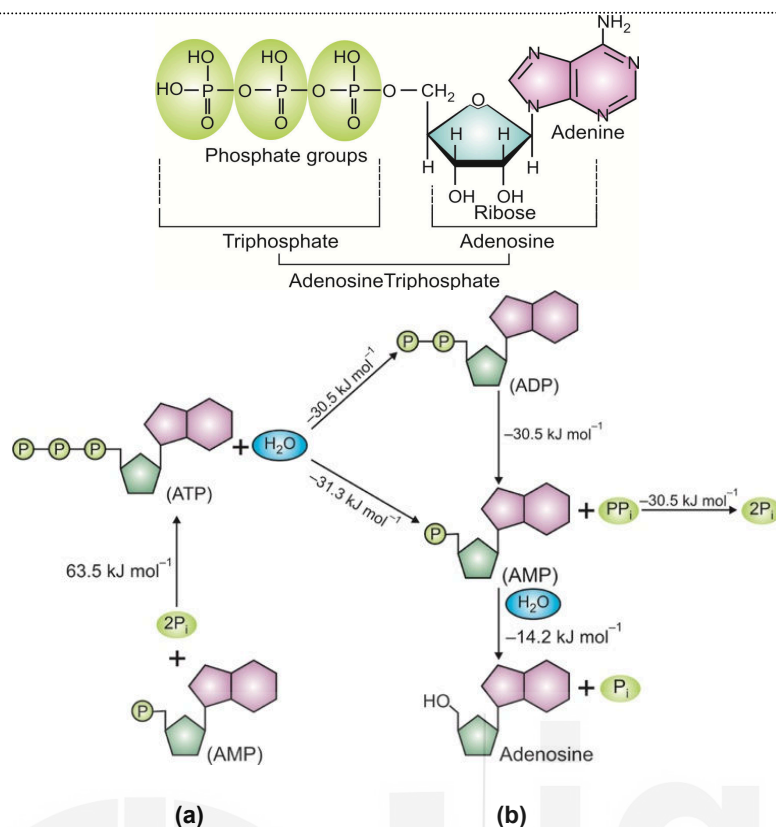


Fig. 14.3: (a) Structure of ATP (b) Hydrolysis of ATP and its components.

Presuming the pH of blood to be equal to 7 and a temperature of 298 K (standard temperature and pH), the actual ΔG value for the hydrolysis of ATP in these cells can be calculated with the help of Eq. 14.3.

$$\begin{aligned}\Delta G &= \Delta G^0 + (2.303 \times R \times T \times \log \frac{[ADP][P_i]}{[ATP]}) \\ &= -30.5 + (2.303 \times 8.314 \times 298 \times \log \frac{(2.5 \times 10^{-4}) (1.65 \times 10^{-3})}{(2.25 \times 10^{-3})}) \\ &= -30.5 - 21.31 \\ &= -51.8 \text{ kJ mol}^{-1}\end{aligned}$$

The numbers pertaining to ΔG^0 given above will be useful when we try to rationalise the choice of ATP as energy carrier. For just now it should suffice to note that a large amount of energy is available on hydrolysis of ATP. Let us see how this energy is harnessed i.e., how does ATP work?

14.3.2 Role of ATP in Biochemical Energy Transformations

The free energy available from the hydrolysis is used to drive a number of reactions which require an input of free energy. This is done by suitably coupling the appropriate reaction. You would recall the example given in subsection 14.2.4. in this process of driving an energy requiring process, ATP gets hydrolysis to ADP. For the sustenance of life we need to have some mechanism of regenerating ATP, ADP and P_i by taking energy from external source.

Chemotrophs are the organisms using chemical source of energy.

In chemotrophs metabolic breakdown of nutrients takes place in such a way that the release free energy is utilised to drive the endergonic (free energy requiring) synthesis of ATP from ADP and P_i (positive $\Delta G^{0'}$). Similarly, during photosynthesis, the light energy is converted into chemical energy in the form of ATP (besides producing some reducing substances).

Thus, ATP is continuously formed and consumed in the living systems. It acts as the energy coinage or “**energy currency**” of the living cells. An analogy of the role of ATP in living cells is seen in exchange of currency when you visit a foreign country. In that country, you have to first get your money changed into the local currency in order to be able to pay for various services and merchandise. Similarly, all energy received by the living cells (chemical energy of the nutrients or light energy during photosynthesis) is converted into its own energy currency, i.e., ATP, before it can be utilised to drive the various energy requiring functions.

The role of ATP as an energy transducing agent, or vehicle, suggests that it should be rapidly “turned over”, i.e., rapidly and repeatedly broken down to ADP to P_i and resynthesised in the living cells. An idea of the rapid turnover of ATP can be had from the fact that a resting adult human being “turns over” 30-40 kg ATP in one day, i.e., converts it into ADP and P_i and then back to ATP, the relative concentrations of ATP, ADP and P_i remain constant.

Before we go into the chemistry of ATP-ADP interconversion, it is important to compare the free energy change involved in this process with that obtainable from the oxidation of a common nutrient, namely, glucose.



You see that a large amount of free energy becomes available on complete oxidation of glucose into carbon dioxide and water. For an efficient utilisation of this energy for the synthesis of ATP, it is necessary that it should be liberated in several smaller packets, each of which should be adequate to drive the synthesis of ATP. An analogous situation occurs in electricity generation from a large water fall when the only available turbine is of small and limited capacity. Since the turbine placed at the bottom cannot produce power more than its capacity (say 100 MW), the rest of the energy of the fall will go waste (dissipated as heat). On the other hand, if several turbines are placed at appropriate heights, the energy of the waterfall can be more efficiently harnessed. You can compare Fig. 14.4 (a) and (b) for this. Similarly, the large amount of free energy made available on oxidation of a glucose molecule can be better conserved if it is released in several packets representing the steps of metabolism. Many more molecules of ATP can be synthesised.

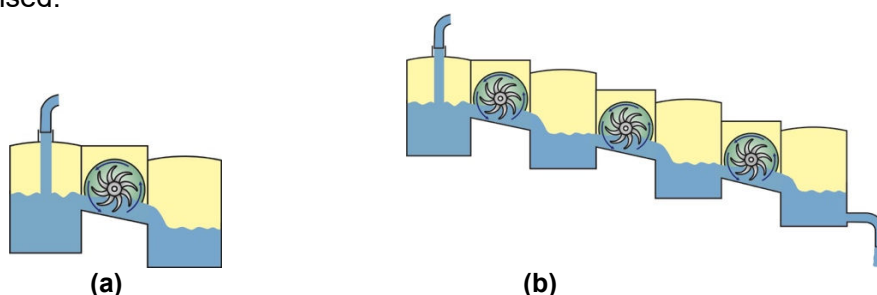
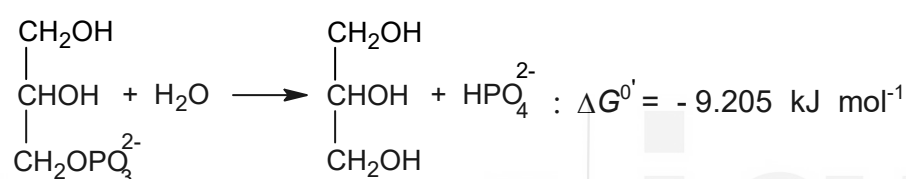


Fig.14.4: Diagrammatic representation of generation of electricity by a large waterfall (a) with one turbine and (b) with several turbines.

Thus, the oxidation of glucose must take place via a large number of steps and, therefore, involve an equal number of intermediates, called **metabolites**. It is indeed found to be so and this is a common feature of all metabolic pathways. Therefore, the metabolism is commonly referred to as “**Intermediary Metabolism**”. Some examples of metabolic pathways will be discussed in Units 15, 16 and 17.

14.3.3 Structural Basis for the Role of ATP

We have seen before that the ΔG^0 value for the hydrolysis of ATP, is much larger than that for AMP. It is, in fact, found to be larger than that for the hydrolysis of all simple phosphate esters, e.g., glycerol-1-phosphate, glucose-6-phosphate, etc. The reaction of hydrolysis of glycerol-1-phosphate and the corresponding value of ΔG^0 is given below.



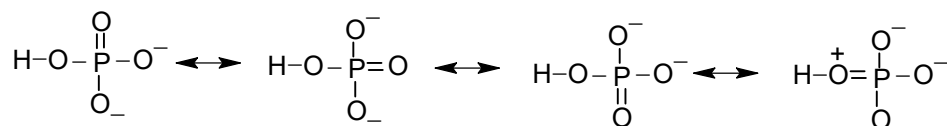
The standard free energy of hydrolysis of some compounds of biological interest is given in Table 14.1.

Table 14.1: Standard Free Energy of Hydrolysis of Some Compounds of Biological Interest at pH 7

S.N.	Compound (Reaction)	ΔG^0 (kcal mol ⁻¹)	ΔG^0 (kJ mol ⁻¹)
1.	Phosphoenol pyruvate	-14.8	-61.92
2.	Carbamoyl phosphate	-12.3	-51.46
3.	1, 3-Diphosphoglycerate (-phosphoglycerate + P _i)	-11.8	-49.37
4.	Phosphocreatine	-10.3	-43.1
5.	Acetyl phosphate	-10.3	-43.1
6.	Pyrophosphate ion (PP _i + H ₂ O → 2P _i)	-7.9	-33.0
7.	ATP (→ AMP + PP _i)	-7.5	-31.38
8.	ATP (→ ADP + P _i)	-7.3	-30.5
9.	ADP (→ AMP + P _i)	-7.3	-30.5
10.	AMP (→ Adenosine + P _i)	-3.4	-14.23
11.	Glucose -1- phosphate	-5.0	-20.92
12.	Fructose-6-phosphate	-3.8	-15.90
13.	Glucose-6-phosphate	-3.3	-13.80
14.	Glycerol-1-phosphate	-2.2	-9.20
15.	Acetyl-AMP (→ Acetate + AMP)	-13.3	-55.65
16.	Acetyl-SCoA (→ Acetate + CoA-SH)	-7.5	-31.38
17.	Carboxylic esters (→ Carboxylate ion + Alcohol)	-4.0	-16.73

Since $\Delta G^{0'}$ values are related to the respective equilibrium constants of these reactions, it follows that ATP has a higher tendency to transfer its terminal phosphate group to water as compared to glycerol-1-phosphate esters. Therefore, it is said to have a higher **phosphate group transfer potential** than the phosphate esters.

The higher phosphate group transfer potential of ATP can be readily explained on the basis of its structure and that of ADP and phosphate ion at pH 7. ATP has four negative charges situated close to one another at this pH. There is strong force of repulsion between them. This repulsion is lesser in ADP which has three negative charges. Furthermore, at pH 7, phosphate ion is resonance stabilised due to delocalisation of its electrons as shown below.



This leads to a lower value of free energy of the products and, therefore, to a numerically larger $\Delta G^{0'}$ value for the reaction, which is equal to the difference between the free energy of the products and the reactants. No such resonance stabilisation is possible for ATP^{4-} .

A compound is referred to as high energy compound if its standard free energy of hydrolysis is negative and numerically larger than 29.2 kJ mol^{-1} i.e., it must be more negative than 29.2 kJ mol^{-1} .

Similar arguments are also applicable for explaining the large $\Delta G^{0'}$ for the hydrolysis of ADP to AMP and P_i , but not for the hydrolysis of AMP, glycerol-1-phosphate or other phosphate esters.

ATP and ADP have been referred to as **high energy phosphate compounds** and the phosphoanhydride or pyrophosphate bond between phosphate groups of these compounds has often been referred to as a **high energy bond**. The latter term must be understood to merely indicate the site of hydrolytic cleavage and not as suggesting that the free energy is somehow localised in that bond. It is always necessary to consider the complete structures of reactants and products.

There are several other “high energy phosphate compounds”, which participate in the metabolic processes, Table 14.1. The phosphate group transfer potentials of some of these are higher than that of ATP. This intermediate position of ATP with respect to phosphate group transfer potential is very significant.

Some compounds, other than phosphate derivatives, also exhibit large $\Delta G^{0'}$ values for hydrolysis. These are also referred to as “high energy compounds”. As we will see, some of these function as intermediates in the utilisation of ATP for biosynthetic work. It may be noted that classification as high energy compounds is based entirely on the $\Delta G^{0'}$ value for the hydrolytic cleavage.

Let us now take up the question why ATP is the molecule of choice for its role as energy carrier for different life processes. In this context the exergonic nature of its hydrolysis is of prime importance. We have already seen that the hydrolysis of ATP provides a large amount of free energy, obvious question is that if the hydrolysis of ATP to ADP or AMP is exergonic to similar extent

(ΔG^0 for both is $\sim -30.5 \text{ kJ mol}^{-1}$) then why we have ATP/ADP pair as the vehicle of free energy and not ATP/AMP.

The answer to the above lies in the fact that the hydrolysis of ATP to AMP gives a molecule of pyrophosphate (PP_i). This molecule gets further hydrolysed giving an additional large amount of free energy i.e.,

$\Delta G^0 = -33.0 \text{ kJ mol}^{-1}$. As a consequence the effective ΔG^0 for the hydrolysis to AMP and inorganic phosphate becomes large which means that the reaction would be almost irreversible. This would make the regeneration of ATP (from AMP) very difficult. On the other hand, in case of ADP the regeneration of ATP would require moderate amount of free energy.

Further, the intermediate position of ATP with respect to phosphate group transfer potential enables it function as an efficient carrier of phosphate group in various metabolic reactions. This is because ATP is able to transfer its phosphate group to a number of metabolites and also a number of molecules are available (with higher phosphate group transfer potential) which can help regenerate ATP.

Another aspect in which ATP scores a point is that it itself and its hydrolysis products are reactants or products for a number of biochemical reactions which makes it an efficient choice for coupling. You would recall that the available free energy can be utilised only if it is possible to suitably couple the reaction.

The purine base and ribose sugar (i.e., the nucleoside component) of AMP, ADP and ATP contribute towards interaction of these with enzymes. It is of importance in regulating enzyme activity. The above mentioned qualities besides the stability of ATP are responsible for its role.

In the process of study of role and mode of action of ATP we have realised that ATP has to be rapidly turned over. Let us now see how this job is accomplished. Before that try the following SAQ.

SAQ 3

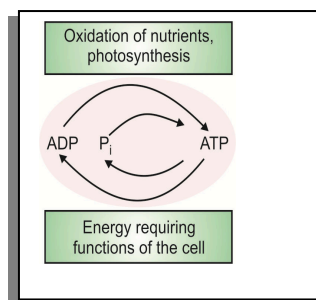
Choose the correct answer.

Each of the following contains a high energy phosphate group except,

- a) ATP
 - b) ADP
 - c) AMP
 - d) pyrophosphate ion
-

14.4 FORMATION OF ATP

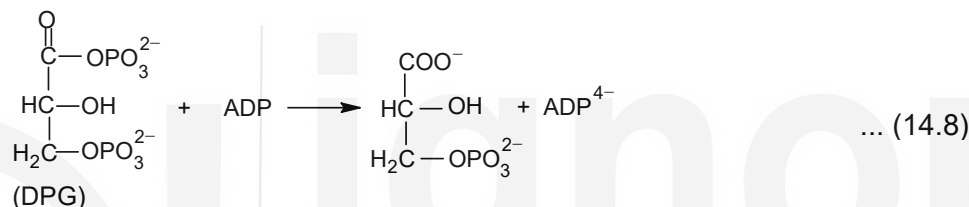
The replenishment of ATP takes place in a number of ways. In this section we would take up two major routes for the synthesis of ATP. These routes include its synthesis during breakdown of nutrients and photosynthesis. In the breakdown of nutrients, some ATP is formed by the reaction of ADP with other



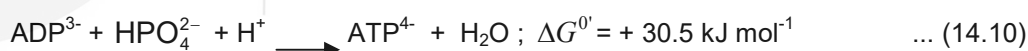
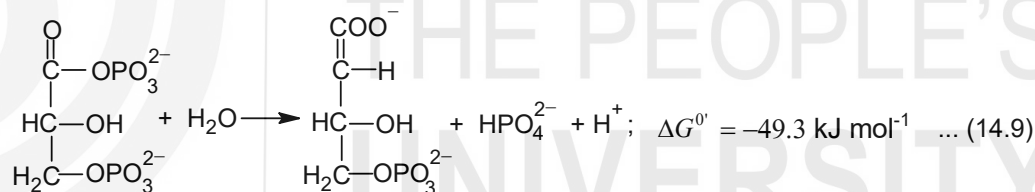
phosphorylated metabolites having a higher phosphate group transfer potential. This method is called **substrate level phosphorylation**. In aerobic organisms, substrates are oxidised by transferring their electrons to coenzymes. The reduced coenzymes so obtained, (NADH, FADH₂, etc.) transfer their electrons eventually to oxygen. The latter reaction is accompanied by the synthesis of several molecules of ATP. This is the major source of cellular ATP in aerobic organisms and is referred to as **oxidative phosphorylation** or **electron transport chain**. Let us study these ATP forming processes.

14.4.1 Substrate Level Phosphorylation

In the conversion of glucose and other sugars to pyruvate (glycolysis) in muscles, two reactions bring about the formation of ATP. You will study about these in Unit 15 also. These are the reactions of ADP with 1,3-diphosphoglycerate (DPG), Eq. 14.8 and with phosphoenolpyruvate (PEP), Eq. 14.11.



The $\Delta G^{0'}$ value for this reaction can be calculated from the standard free energies of hydrolysis of DPG and ATP, Table 14.1.



Adding Eq. 14.9 and 14.10, we get the $\Delta G^{0'}$ value for the reaction of Eq. 14.8, which is equal to $-18.8 \text{ kJ mol}^{-1}$. Thus, this reaction is exergonic. It should, therefore, proceed spontaneously from left to right. The equilibrium constant for this reaction can be calculated as follows.

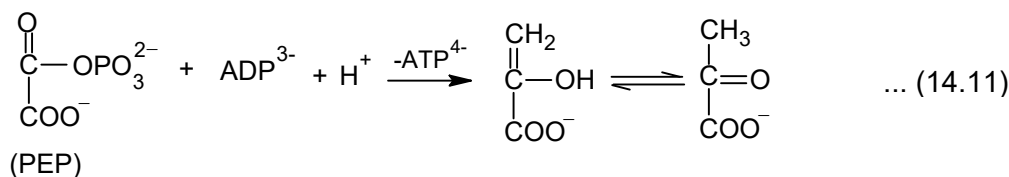
$$\Delta G^{0'} = -RT \ln K'_{\text{eq}} = -2.303 \times R \times T \log K'_{\text{eq}}$$

$$\text{Thus, } \log K'_{\text{eq}} = \frac{-18800}{-(2.303 \times 8.314 \times 298)} = 3.3$$

$$\text{and } \log K'_{\text{eq}} = 1.99 \times 10^3 \text{ at pH 7 and 298 K}$$

Note that the K'_{eq} value of the reaction heavily favours the formation of ATP. This is achieved by “spending” a much larger amount of free energy than is just adequate for the synthesis of ATP.

The second substrate level phosphorylation reaction is that between ADP and phosphoenolpyruvate (PEP), Eq. 14.11.



The $\Delta G^{0'}$ value of the reaction can be computed from the data given in Table 14.1 in a similar manner as explained for the reaction of Eq. 14.8.

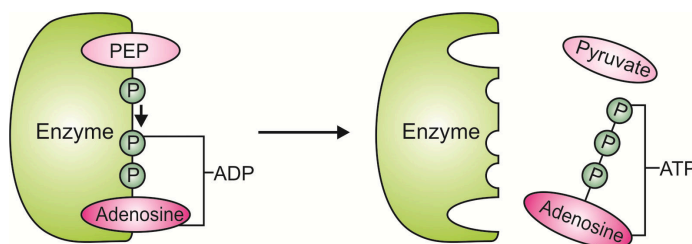


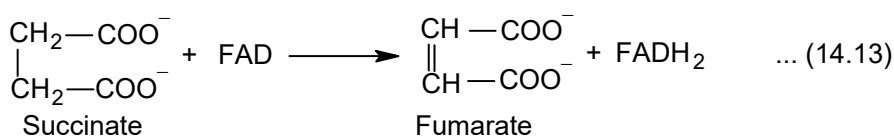
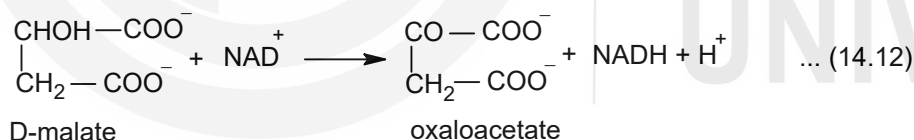
Fig.14.5: Substrate level phosphorylation between ADP and PEP.

SAQ 4

Calculate the $\Delta G^{0'}$ value for the reaction of Eq. 14.11 using the data given in Table 14.1.

14.4.2 Oxidative Phosphorylation

It was mentioned earlier that oxidative phosphorylation involves reactions with reduced coenzyme. Two typical reactions producing the reduced coenzymes are given below:



Coenzyme is an accessory substance, not a protein, necessary to the protein part of an enzyme to work and is noncovalently bound to a protein. You have studied the structures of nicotinamide adenine dinucleotide, NAD and flavin adenine dinucleotide FAD in unit 7 of this course

The reduced coenzymes, NADH and FADH₂, are subsequently reoxidised by a series of reactions called **electron transport chain**, in which molecular oxygen is the **ultimate** electron acceptor. The pathway is also called **oxidative phosphorylation**, because under normal physiological conditions the electron transport is tightly coupled to the synthesis of several molecules of ATP from ADP and P_i. The steps involved in the electron transport chain leading to the synthesis of ATP are explained in Unit 16.

14.4.3 Photosynthesis

You might have studied that photosynthesis can be shown to proceed in a “light reaction” and a “dark reaction”. In the light reaction, photoenergy is

Photosynthesis is the process in which plants use the energy of light to synthesis. Carbohydrates from the simple molecules of carbon dioxide and water.

utilised to produce a reduced coenzyme, NADPH, and ATP, which are required in the dark reaction for fixation and conversion of carbon dioxide into carbohydrates. We will discuss the synthesis of ATP only.

Photosynthesis takes place in plants and some photosynthetic bacteria. In plants the process is brought about in chloroplasts. The latter contain flattened membrane-enclosed vesicles called **thylakoids**. There are the loci of photosynthesis. Chemiosmotic hypothesis applies also to ATP synthesis during photosynthesis. The inner compartment of the thylakoids becomes more acidic on irradiation. The resultant proton gradient then drives the synthesis of ATP in a similar manner as discussed above for the corresponding process in the mitochondria. Here also the results of some simple and straightforward experiments support the chemiosmotic hypothesis which are:

- Isolated chloroplasts were soaked in a buffer of pH 4 to permit a slow equilibration and lowering of pH in the inner compartment. ADP and P_i are added and the pH of the outer medium is suddenly raised to 8, thus setting up an artificial pH gradient where the interior of chloroplasts is more acidic than outside. ATP synthesis is found to take place in dark concomitant with the dissipation of the pH gradient. This shows that a pH gradient represents an energy rich state which can drive ATP synthesis without input of any other kind of energy.
- According to the chemiosmotic hypothesis, the proton pumping across the membrane and ATP synthesis are two separate reactions. These are catalysed by different enzymes and are coupled by virtue of their catalysts being located in a vectorial manner on the same membrane. This has been tested with a simple experiment by taking the proton pumping part from one source and ATP-synthase from a source. The former is the purified bacteriorhodopsin isolated from photosynthetic halobacteria and the latter is ATP synthase isolated from beef heart mitochondria (a non-photosynthetic system). The two are incorporated into membranes which form closed vesicles shown in Fig. 14.6. Such "artificial" or "reconstituted" vesicles are found to bring about the synthesis of ATP on being irradiated with light. This observation supports the hypothesis and shows that the mechanism of ATP synthesis by photophosphorylation is essentially the same as in oxidative phosphorylation.

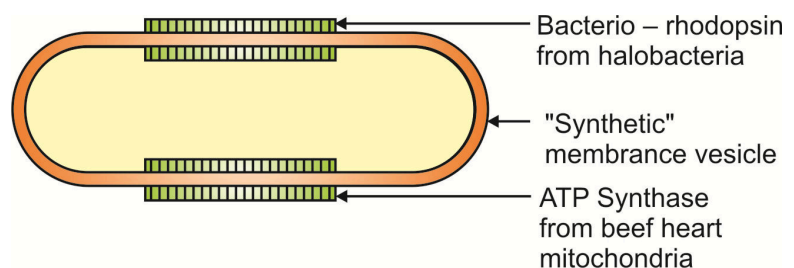


Fig. 14.6: Synthesis of ATP during photosynthesis.

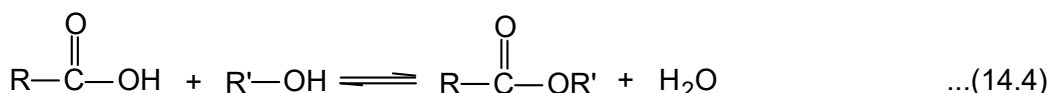
Like ATP, a proton gradient is also a readily accessible form of stored free energy which is put to various use by different organisms. These include active transport of ions, rotation of flagella, ATP synthesis and also heat production during hibernation.

14.5 UTILISATION OF ATP FOR ENERGY REQUIRING FUNCTIONS

In the preceding section, we have discussed some examples of the transmutation of various forms of energy input, chemical nutrients or light into ATP which represents the chemical form of the currency of the living cells. We will now study, in brief, a few examples of the utilisation of the chemical energy of ATP for some energy requiring functions of the cells.

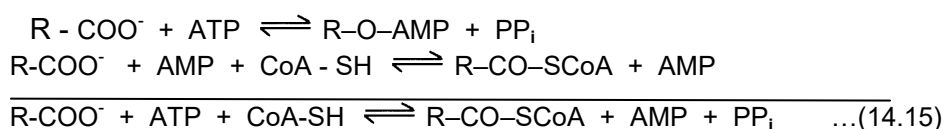
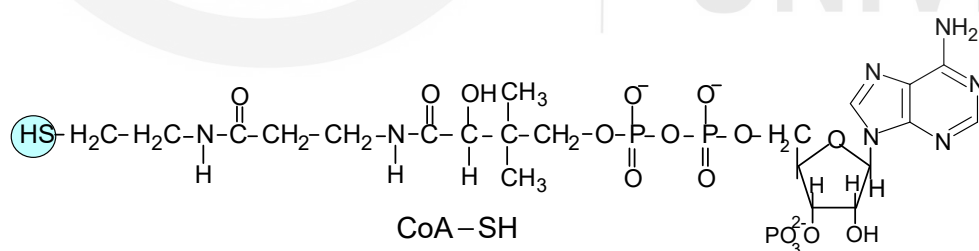
14.5.1 Synthesis of Organic Molecules

Let us consider the reversible reaction of acids and alcohols.



The equilibrium for this reaction lies predominantly towards the left-hand side of the equation, i.e., hydrolysis of esters is favoured. This is especially so in aqueous solutions where the concentration of water molecules is exceedingly large (approx. 55 M). Thus, aqueous suspensions of esters, e.g., oils and fats, tend to yield solutions of acids and alcohols. A familiar example is the rancidification of butter. Formation of esters, therefore, requires special conditions in order to overcome the unfavourable equilibrium constant. In the laboratory, this is achieved by carrying out the reaction of an acid and alcohol under anhydrous conditions and by continuous removal of water produced, e.g., in the presence of concentrated sulphuric acid. The living systems utilised a different approach. They “spend” free energy in order to “drive” this otherwise unfavourable endergonic process in aqueous environments. The free energy is provided by net hydrolysis of a molecule of ATP. This is explained as follows.

In the biological systems, the acid molecule is first “activated” by reaction with ATP and coenzyme-A (CoA-SH). The structure of coenzyme-A is given below:



The equilibrium constant for the above reaction is close to unity (ΔG^0 is close to zero). One “high energy bond” of ATP is broken and another bond of almost equal energy is established in acyl-SCoA (compare the ΔG^0 values for the hydrolysis of ATP and acetyl-CoA in Table 14.1). In the physiological systems, the reaction of formation of esters is driven to completion by removal of pyrophosphate ion (PP_i) product which is hydrolysed with the help of another enzyme, pyrophosphatase.

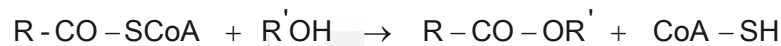
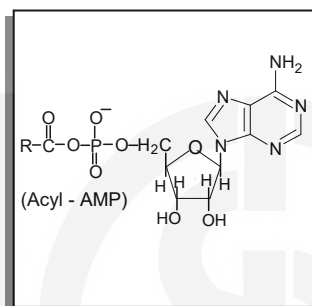


The overall reaction becomes practically irreversible due to large drop in the free energy.



Note the “expenditure” of two high energy pyrophosphate bonds for establishing one high energy thiol-ester linkage. This principle of driving an “uphill”. i.e., an endergonic reaction, to completion by spending a much larger amount of free energy is observed in many biosynthetic pathways. Many of these are rendered irreversible by hydrolysis of pyrophosphate ion.

Acyl-CoA is an “activated” form of acyl moiety in the sense that it has a high acyl group transfer potential. The acyl group can be spontaneously transferred to various acceptors, e.g., alcohols, amines, water etc. Such acyl group transfer reactions proceed with a large free energy drop, i.e., ΔG^0 is large and negative.



A slightly different but recurring motive observed in biosynthetic reactions requiring “activated” acyl groups utilises acyl-adenylate, also called acyl-AMP without the participation of coenzyme-A. For example, in the protein biosynthesis the carboxyl group of each amino acid is activated by the formation of aminoacyl-adenylate and the reaction is rendered irreversible by the hydrolysis of the resultant pyrophosphate ion.

14.5.2 Muscle Contraction

Let us now study how the free energy released on hydrolytic cleavage of ATP is used for mechanical work, namely muscle contraction.

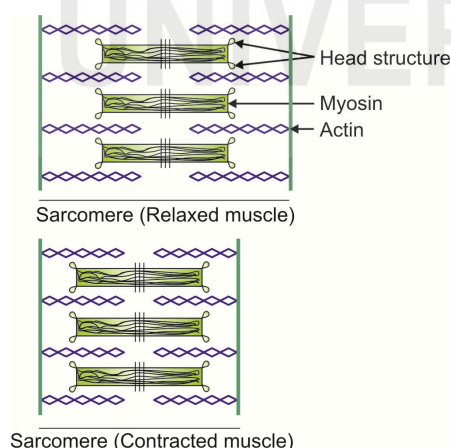


Fig. 14.7: Thick(myosin) and thin (actin) filaments of a muscle ‘sarcomere’ in different stages of contraction.

The contractile muscle fibres consist of overlapping arrays of thick and thin filaments. The thick filaments are made up of bundles of parallel, rod shaped molecules of myosin consist of two strands of fibrous actin (F-actin) twisted around each other. In the muscle fibre, the thick and thin filaments are regularly arranged parallel to each other (inter-digitated) in repeating units, called sarcomeres (Fig. 14.7). During contraction, the thick filaments slide into

the spaces between the thin filaments in each sarcomere, thereby causing a shortening of the entire muscle fibre. This is accompanied by hydrolysis of ATP, which provides the necessary free energy. It was observed by **A. Szent-Gyorgyi** (1940) that the actomyosin fibres contracted on the addition of ATP, but myosin or actin fibres taken separately did not.

Myosin of the thick filaments possesses ATPase activity, i.e. it catalyses the hydrolysis of ATP into ADP and P_i . The actomyosin complex hydrolyses ATP faster than myosin alone. The binding of ATP of actomyosin dissociates the complex, i.e., the contact between actin and myosin is broken. ATP is hydrolysed in the myosin-ATP complex, but the products are not released into the medium. The resulting myosin-ADP- P_i complex binds actin again, which accelerates the dissociation of the products (ADP and P_i) from myosin. The actomyosin so formed is now ready to bind another molecule of ATP (Fig. 14.7). When actin binds to the myosin-ADP- P_i complex, the linkage is not at the same site as in the initial **actomyosin complex**, but to a neighbouring site as depicted in Fig. 14.8. This is due to a conformational change in the “head” part of the myosin molecule on binding ATP. This results in a rowing type action, with the myosin head structure acting as an oar, which is responsible for the sliding motion of the thick filaments.

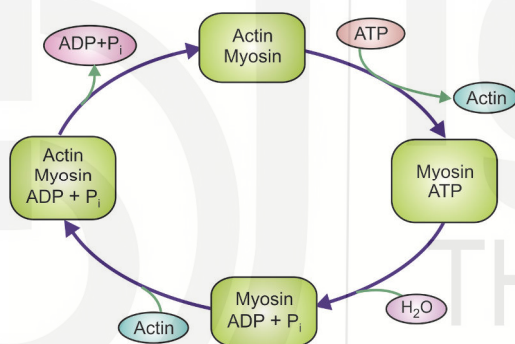


Fig. 14.8: Hydrolysis of ATP by actomyosin complex

14.6 THERMODYNAMICS AND THE LIVING CELLS

In this unit, we have considered some isolated biochemical reactions and processes and the accompanying energy transductions in light of the principles of classical thermodynamics or equilibrium thermodynamics. These principles were earlier arrived at from consideration of closed systems and at equilibrium in which all changes took place slowly and reversibly. The intact living cells, on the other hand, are open systems. Further, the concentrations of various cellular constituents are far from equilibrium values and the metabolic reactions proceed quite fast. It may, therefore, be asked as to whether the laws of equilibrium thermodynamics are strictly applicable to the living cells. In this unit, as also in most textbooks of biochemistry, laws of thermodynamics have been applied for two purposes, namely, to predict the direction of spontaneous reactions and to calculate the free energy changes accompanying such reactions under the concentration conditions prevailing in the living cells. For these purposes, the laws of the thermodynamics apply equally well to the living and non-living systems.

At any given time, a living cell is in steady state, in which the rate of input of matter and energy is equal to the rate of matter and energy. In a steady state, the concentration of cellular constituents, e.g., ATP, remain constant over a period of time because the rate of their formation (and input, if any) exactly equals the rate of their utilisation. For such a system, it will be more appropriate to apply the concepts of nonequilibrium (or irreversible) thermodynamics which are much more complicated and beyond the scope of this course. However, two properties of an open system are relevant here and need to be emphasised:

- (1) An open system in steady state is capable of doing useful work only when its constituents are not at equilibrium with one another. A system at equilibrium is incapable of doing any useful work at constant temperature, because $\Delta G^{0'} = 0$ for such a system.
- (2) A steady state represents the most orderly state of an open system. Rate of entropy of production in an open system is minimum in steady state and the system operates with maximum efficiency. Note that $\Delta G^{0'} = (\Delta H - T\Delta S)$ is maximised when ΔS is minimised. This has been aptly summed up by A. Katchalsky, a pioneer in the application of nonequilibrium thermodynamics to the living systems. He wrote, "But since there is no possibility of escaping the entropic doom imposed on all nature phenomena under the second law of thermodynamics living organisms choose the least evil - they produce entropy at a minimum rate by maintaining a steady state."

SAQ 5

Tick (✓) on the correct statements and (X) on the wrong statements given below.

- a) Living cells are always in equilibrium.
- b) The rate of formation of each component in a cell is exactly equal to its rate of utilisation.
- c) Thermodynamically speaking the living cells constitute a closed system.
- d) The myosin-ATP complex is responsible for muscle contraction.
- e) Generally hydrolysis of ATP provides the energy required for the synthesis of organic molecules in biological systems.

14.7 SUMMARY

Living organisms are highly organised systems, constituted by cells with well defined structure built from complex organic molecules. The living cells depend on randomly distributed and unorganised raw materials. This apparently spontaneous creation of an organised system from unorganised surroundings, i.e., creating order out of chaos, is made possible by a continuous flux of energy through the living system. Exchange of energy, or more precisely of enthalpy (i.e., heat content), between a system and its

surroundings is governed by the law of conservation of energy or the first law of thermodynamics. Out of the total enthalpy of change in a system, a certain part goes to increase the extent of randomness or entropy and the remainder amount (change in free energy) is available for performing useful work at constant temperature. The living cells depend on the latter for capture and utilisation of energy. The changes in enthalpy, ΔH , entropy, ΔS and free energy, ΔG of system are related by the equation, $\Delta S = \Delta H - T\Delta S$.

A physical transformation or a chemical reaction will take place spontaneously, i.e. of its own accord, if the accompanying ΔG of the system is negative. An exergonic process, where ΔG is positive, can be made to proceed spontaneously if it can be coupled with a more strongly exergonic process, so that the net ΔG of the total system is negative. ΔG is related to standard free energy change, ΔG^0 , constant, by the relationship, $\Delta G = \Delta G^0 - RT \ln K_{eq}$. In biochemical systems, all measurements are made at pH 7 which is held constant. The standard free energy change at pH is denoted by $\Delta G^{0'}$.

All biochemical systems depend on the availability of free energy in one form or the other, e.g., chemical and light energy. The immediate source of free energy in living systems is adenosine triphosphate (ATP). The standard free energy of the hydrolytic cleavage of ATP into adenosine diphosphate (ADP) and phosphate ion (P_i) at pH 7 and 298 K, $\Delta G^{0'}$, is found to be equal to $-30.5 \text{ kJ mol}^{-1}$. The externally supplied energy from nutrients or from light in photosynthesis is first converted into ATP, i.e., its net synthesis from ADP and P_i . This is subsequently released by a net hydrolytic breakdown of ATP into ADP and P_i and utilised to drive the various energy requiring (endergonic) processes of the living cell, e.g., chemical synthesis, mechanical work, transport of ions and molecules against osmotic gradient, etc. ATP has been referred to as a "high energy" compound. Due to its unique role, ATP is continuously and rapidly synthesised and broken down, i.e., "turned over" in all living systems.

Thermodynamically, the living cells are said to constitute an open system which exchanges matter and energy continuously with its surrounding. At any given time, a living cell is in a steady state, i.e., the rate of input of matter and energy is equal to the rate of output of matter and energy. The entropy increase is minimum when an open system is in steady state.

14.8 TERMINAL QUESTIONS

Use the data from Table 14.1 for answering the following questions.

1. Predict the direction in which the following reversible reactions will proceed spontaneously at pH 7 and 298 K, if the concentration of each reaction partner is one mole per litre.
 - a) $\text{ATP} + \text{creatine} \rightleftharpoons \text{Phosphocreatine} + \text{ADP}$
 - b) $\text{ATP} + \text{glucose} \rightleftharpoons \text{Glucose-6-phosphate} + \text{ADP}$
 - c) $\text{ATP} + \text{acetate} \rightleftharpoons \text{Acetyl phosphate} + \text{ADP}$
 - d) $\text{ATP} + \text{pyruvate} \rightleftharpoons \text{Phosphate pyruvate} + \text{ADP}$

2. Calculate the ΔG^0 and equilibrium constant K'_{eq} values for the isomerisation of glucose-6-phosphate to glucose-1-phosphate.
3. For the reaction:

$$\text{ATP} + 3\text{-phosphoglycerate} \rightleftharpoons 1,3\text{-Diphosphoglycerate} + \text{ADP}$$
 - a) Calculate the ΔG^0 and K'_{eq} values.
 - b) If ATP/ADP ratio is 10, what will be ratio of 1-diphosphoglycerate to 3-phosphoglycerate?
4. ATP is considered the energy currency of a cell. Justify this statement.

14.9 ANSWERS

Self Assessment Questions

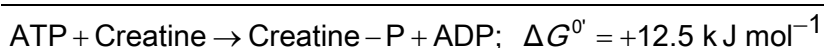
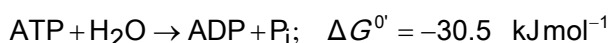
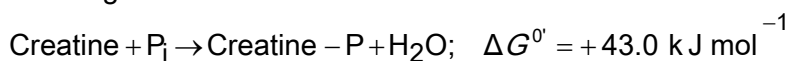
1. i)
2. a) When H^+ is a reactant or product the two will be very different.
 b) ΔG^0 value will be able to indicate the feasibility only under the conditions that this value is similar to the actual free energy change. This conditions may not apply under different conditions of temp., pH or concentration from those of standard one.
3. c)
4. The reaction can be considered to be the sum of the following two reactions, for which the ΔG^0 value are known from Table 14.1.

$$\begin{array}{l} \text{Phosphoenolpyruvate} + \text{H}_2\text{O} \rightleftharpoons \text{Pyruvate} + \text{P}_i; \Delta G^0 = -61.9 \text{ kJ mol}^{-1} \\ \text{ADP} + \text{P}_i \rightleftharpoons \text{ATP} + \text{H}_2\text{O}; \Delta G^0 = 30.5 \text{ kJ mol}^{-1} \\ \hline \text{Sum : Phosphoenolpyruvate} + \text{ADP} \rightarrow \text{Pyruvate}; \Delta G^0 = -31.4 \text{ kJ mol}^{-1} \end{array}$$
5. a) x b) $\checkmark$ c) x d) $\checkmark$ e) $\checkmark$

Terminal Questions

1. Each reaction will proceed spontaneously in the direction in which ΔG value is negative. Since the initial concentration of each reaction partner is unity and pH and temperature are 7 and 298 K, respectively $\Delta G = \Delta G^0$. The value of ΔG^0 for each reaction can be calculated from the data of Table 14.1 by breaking the reaction into appropriate steps and applying the principle of additivity of ΔG values.

For example, the reaction (a) can be considered to be the sum of the following two reactions:



Note that $\Delta G^{0'}$, value has a plus sign if the reaction proceeds from left hand side (LHS) to the to the right hand side (RHS). The value of $\Delta G^{0'}$, value for the same reaction going from RHS to LHS will be $-12.5 \text{ kJ mol}^{-1}$. Answers for the reactions b, c and d can be similarly obtained. It is found that the direction in which these reactions will proceed spontaneously are as follows:

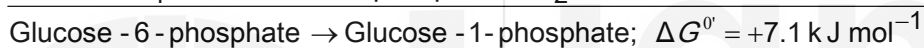
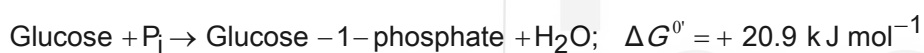
a) $\text{RHS} \longrightarrow \text{LHS}$

b) $\text{LHS} \longrightarrow \text{RHS}$

c) $\text{RHS} \longrightarrow \text{LHS}$

d) $\text{RHS} \longrightarrow \text{LHS}$

2. The reaction; Glucose-6-phosphate $\longrightarrow$ Glucose-1-phosphate, can be considered to be sum of the following reactions:



$$\Delta G^{0'} = -RT \ln K'_{\text{eq}}$$

$$+7100 = -2.303 \times 8.314 \times 298 \times \log K'_{\text{eq}}$$

$$\log K'_{\text{eq}} = +1.244; \quad \text{Therefore, } K'_{\text{eq}} = +5.69 \times 10^{-2}$$

3. a) $\Delta G^{0'}$, and K'_{eq} can be calculated in a similar manner as explained for question No. 2 above.

$$\Delta G^{0'} = +18.8 \text{ kJ mol}^{-1}$$

$$K'_{\text{eq}} = 5.01 \times 10^{-4}$$

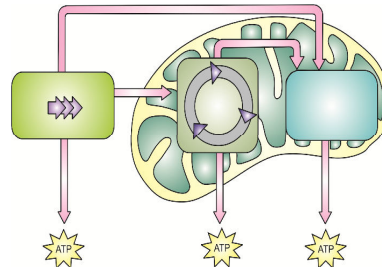
$$\text{b) } K'_{\text{eq}} = \frac{[\text{Disphosphoglycerate}] \times [\text{ADP}]}{[\text{3-phosphoglycerate}] \times [\text{ATP}]} = 5.01 \times 10^{-4}$$

$$\frac{[\text{Disphosphoglycerate}]}{[\text{3-phosphoglycerate}]} = 5.01 \times 10^{-4} \times \frac{[\text{ATP}]}{[\text{ADP}]}$$

$$= 5.01 \times 10^{-4} \times 10$$

$$= 5.01 \times 10^{-3}$$

4. Hint: You can answer this question from Sec. 14.3.



CARBOHYDRATE METABOLISM

Structure

15.1 Introduction

Expected Learning Outcomes

15.2 General Scheme of Metabolic Pathways

15.3 Glycolysis and Alcoholic Fermentation

Conversion of Glucose into Triose Phosphate

Conversion of G-3-P into Pyruvate

Metabolic Fate of Pyruvate

15.4 Energetics of Glycolysis

15.5 Entry of Other Sugars into Glycolytic Pathway

15.6 Some Glycolysis Related Disorders

15.7 Gluconeogenesis

15.8 Summary

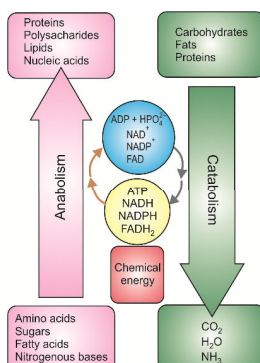
15.9 Terminal Questions

15.10 Answers

15.1 INTRODUCTION

In the previous unit we recapitulated the two laws of thermodynamics and discussed the generation of energy rich source in living cells, the ATP. This complex molecule is produced and utilised in the important metabolic pathways which will be discussed in the present unit and the forthcoming units.

You by now know that the term "metabolism" refers to the totality of chemical reactions brought about by the living cells. These series of reactions, i.e., the metabolic pathways, evolved mainly to bring about transmutation of energy and formation of precursors or building blocks and their assembly into macromolecules like polysaccharides, proteins, nucleic acids, etc. In addition, there are minor, but very important, pathways for the synthesis and breakdown of chemical messengers like hormones, neurotransmitters, etc. Broadly



speaking, the metabolic pathways can be considered under two distinct headings, namely, **catabolism** which refers to the breakdown of complex organic compounds into simpler organic or inorganic molecules with the release and transmutation of energy and **anabolism** which refers to the synthesis of complex organic compounds from simpler molecules with the input of chemical energy. All the metabolic pathways involve several chemical steps and intermediates called metabolites. Therefore, the metabolism is frequently referred to as "**Intermediary Metabolism**". In this unit, you will study the general scheme of metabolism along with the catabolism and anabolism of carbohydrates (glucose to be more specific) viz., **glycolysis** and **gluconeogenesis** respectively. These deal with the biochemical reactions and the energetics involved. In the next unit you will study the further break down of the product of glycolysis via the Krebs's cycle.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ define metabolism and differentiate between catabolism and anabolism;
- ❖ describe the chemical steps through which glucose is degraded into simpler compounds, namely pyruvate, lactate and ethanol;
- ❖ identify the steps where the released metabolic energy is conserved or captured by the cell for its use, i.e., energy transmutation;
- ❖ describe the pathway for the conversion of pyruvate back into glucose i.e., gluconeogenesis; and
- ❖ compare the energetics of breakdown and synthesis of glucose.

15.2 GENERAL SCHEME OF METABOLIC PATHWAYS

The metabolic breakdown of carbohydrates, fats and proteins shows that these may be considered to proceed in three stages as depicted in Fig. 15.1. In the **first stage**, large nutrient molecules are hydrolysed to smaller molecules which are their building blocks. No metabolic energy is released in this stage. In the **second stage** the smaller molecules so produced are further broken down to some common metabolites, e.g., pyruvate. A limited amount of energy is released in this stage, part of which is utilised to drive the net synthesis of a small number of ATP molecules. These two stages can proceed anaerobically. In the **third stage**, the common products obtained in the previous stage are oxidised to carbon dioxide and water via a cyclic process, called the **tricarboxylic acid (TCA) or citric acid cycle**. The metabolites transfer their electrons to coenzymes (NAD^+ and FAD). The reduced coenzymes so formed (NADH and FADH_2) are reoxidised by oxygen in the electron transport chain and concomitant oxidative phosphorylation. Most of the metabolic energy is released in this stage, which also accounts of most of the ATP synthesis. This stage requires oxygen and, therefore, represents the aerobic part of the metabolism.

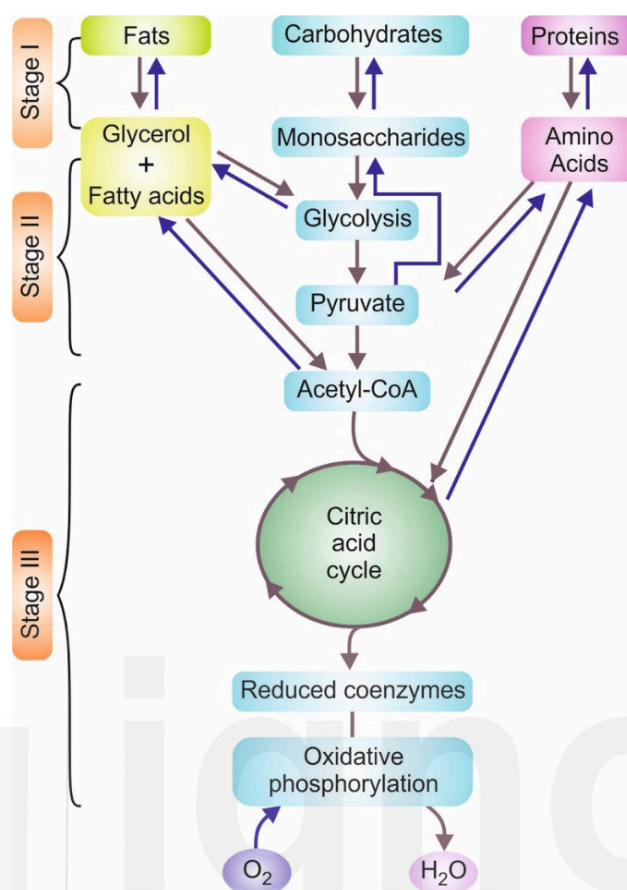


Fig. 15.1: Stages in metabolic breakdown of fats, carbohydrates and proteins. The brown arrows pointing downward represent the breakdown (catabolism) and purple arrows pointing upward show the biosynthetic routes (anabolism).

Fig. 15.1 shows the **convergent** nature of catabolism of fats, carbohydrates and proteins which give rise to some common metabolites. Biosynthesis of these classes of compounds proceeds mostly but not entirely, by a reversal of the steps involved in the breakdown. There are small, but important, differences in a couple of reactions between breakdown and synthesis. Thus, anabolism is **divergent** in nature in the sense that a diverse set of compounds, namely carbohydrates, fats and proteins are synthesised from a common set of precursors. We will describe the energetics of the metabolic pathways only in terms of the total energy drop/used in the breakdown, say of glucose, and its comparison with the amount of energy captured in the form of ATP without going into the mechanism of energy transmutation. In this course we would discuss the metabolic chains/pathways, namely, glycolysis in this unit, tricarboxylic acid cycle in Unit 16, fatty acid biosynthesis and degradation and protein degradation in Unit 17 would be discussed to illustrate the principles of catabolism and anabolism.

A variety of experimental approaches have been employed to elucidate the metabolic pathways. Thin slices of animal and plant tissues were incubated in buffered media containing different metabolites and the accumulated products were identified. Addition of specific inhibitors resulted in larger accumulation of

some compounds whereas others were not formed at all. Genetic defects can be produced in microorganisms, e.g., on exposure to X-rays. Studies with such mutants shows that they could not carry out one or the other metabolic step. Consequently, the metabolites preceding the blocked step accumulated in the medium but those occurring later in the pathway were not formed. Results obtained with a number of such mutants were then combined. Similar information about metabolic pathways was also obtained from studies involving human patients having genetic defects in elucidating the catabolic as well as anabolic pathways. As said before, some of the pathways so elucidated are described in this and the next units. Let us start with glycolysis.

15.3 GLYCOLYSIS AND ALCOHOLIC FERMENTATION

Glycolysis is the anaerobic degradation of six carbon glucose molecules to two three -carbon lactate molecules in the absence of molecular oxygen. It is also called **homolactic fermentation** and is common in many microorganisms and in the cells of most higher animals and plants. Another closely related pathway is **alcoholic fermentation** which is characteristic of many yeasts. In alcoholic fermentation glucose molecule is broken down into two molecules of two carbon compound, ethanol and two molecules of CO₂. The sequence of reactions involved on the two above pathways was discovered by two different parallel studies done by **Buchner and Young** in the early part of the 20th century and later by **Embden, Meyerhoff, Neuberg, Warburg** and **Cori**.

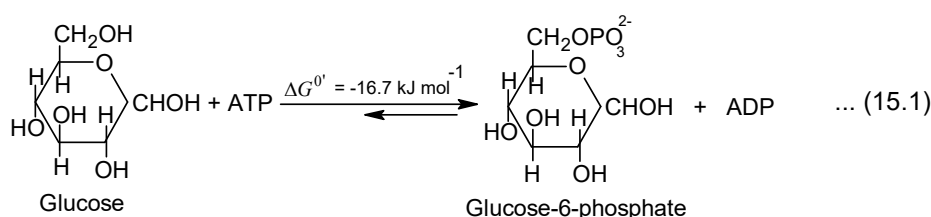
Scheme of glycolysis and alcoholic fermentation is shown in Fig. 15.2. Two distinct stages can be noticed in this. In the first stage different hexoses enter into glycolysis by getting converted to the common product, i.e., glyceraldehyde-3-phosphate. These are energy requiring steps where ATP molecules are used by phosphorylate hexoses. In the second stage all the hexoses follow the same path i.e., glyceraldehyde is converted into lactate. The steps here are energy releasing and produce ATP. Let us study all the steps one by one in the following subsection.

Glycos meaning sugar + lysis meaning dissolution

In liver cells an additional enzyme, glucokinase, is present which phosphorylates only glucose.

15.3.1 Conversion of Glucose into Triose Phosphate

Glucose or any other monosaccharides like fructose, galactose cannot be metabolised until it is activated to a phosphate form. In the **first step** glucose reacts with ATP (donor of phosphate) to give rise to glucose-6-phosphate (Gl-6-P) and ADP. The reaction is catalysed by the enzyme, hexokinase and glucokinase (Eq. 15.1).



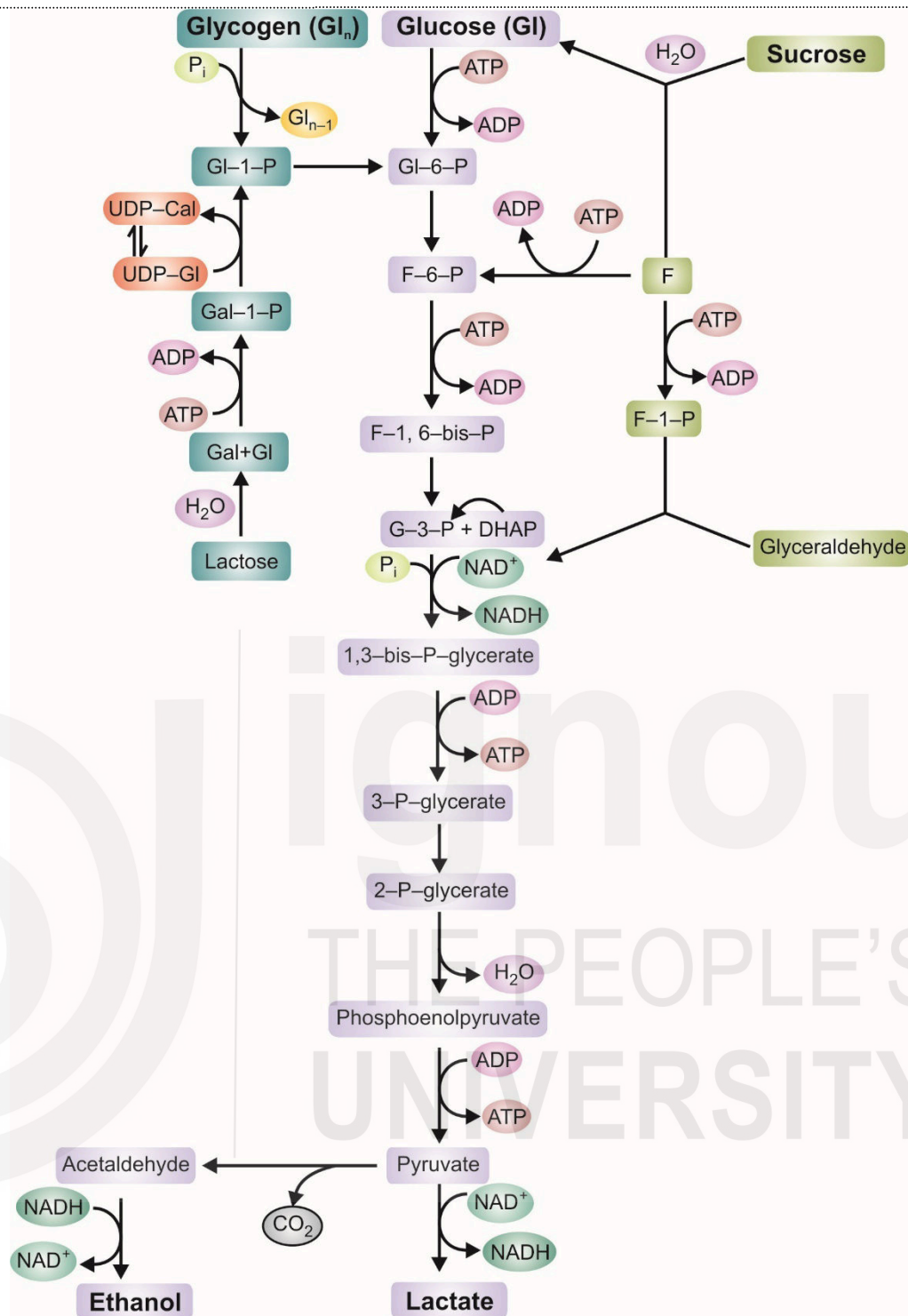
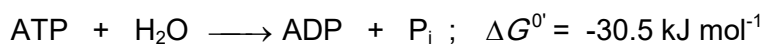


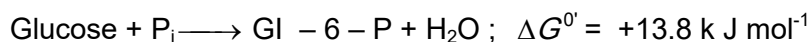
Fig. 15.2: Scheme of glycolysis and alcoholic fermentation showing entry points for various sugars.

Abbreviation: Glucose, GI; Fructose, F; Phosphate group, P; Phosphate ion, P_i ; Glyceraldehyde-3-phosphate, G-3-P; Dihydroxyacetonephosphate, DHAP; Galactose, Gal.

This reaction is practically irreversible because of the large energy drop. The ΔG^0 value of this reaction can be calculated by making use of ΔG^0 values for the hydrolysis of ATP and GI-6-P given in Table 14.1 of Unit 14.



The second reaction can also be written as:



Adding the above equations, we get Eq. 15.1. Thus, the $\Delta G^{0'}$ value for the reaction is found to be $-16.7 \text{ kJ mol}^{-1}$. Using the relationship between $\Delta G^{0'}$ and K'_{eq} we can calculate the equilibrium constant for this reaction at pH 7.0.

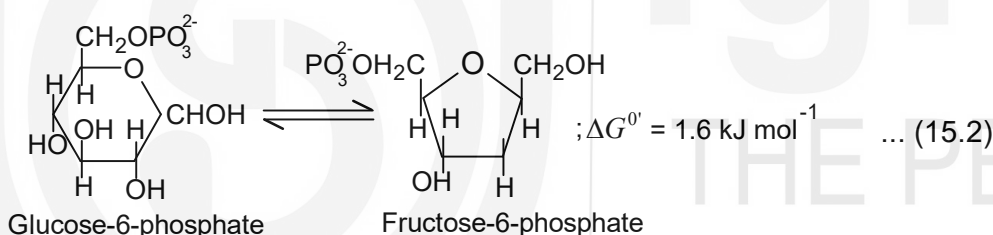
$$\Delta G^{0'} = -R.T. \ln K'_{\text{eq}} = -2.303 \times RT \times \log K'_{\text{eq}}$$

Substituting the values of $\Delta G^{0'}$, R and T , equilibrium constant is found to be equal to 8.45×10^2 at pH 7.0 and 298 K.

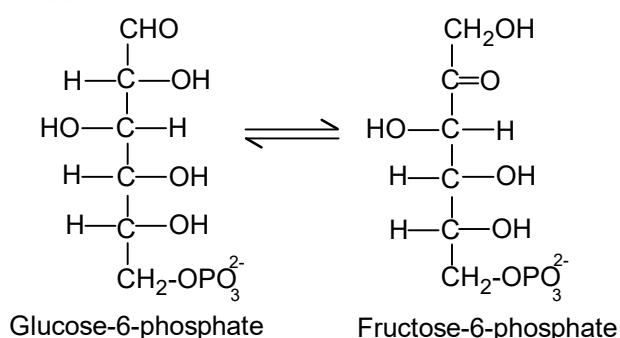
$$K'_{\text{eq}} = \frac{[\text{ATP}] [\text{Gl-6-P}]}{[\text{ATP}] [\text{glucose}]} = 8.45 \times 10^2$$

Thus, the equilibrium constant highly favours the formation of Gl-6-P from glucose and ATP.

The **second step** is an isomerisation reaction catalysed by **phosphoglucose isomerase**, Eq. 15.2.

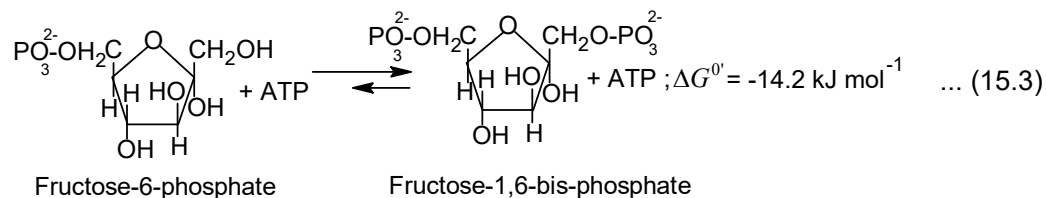


You can understand the simple aldose-ketose isomerisation more clearly by considering the open chain structure of the two sugars.



From Unit 2 you know that in fructose and its derivative, furanose or 5-membered ring structure predominates as against the pyranose or the 6-membered ring structure which predominates in glucose and its derivatives.

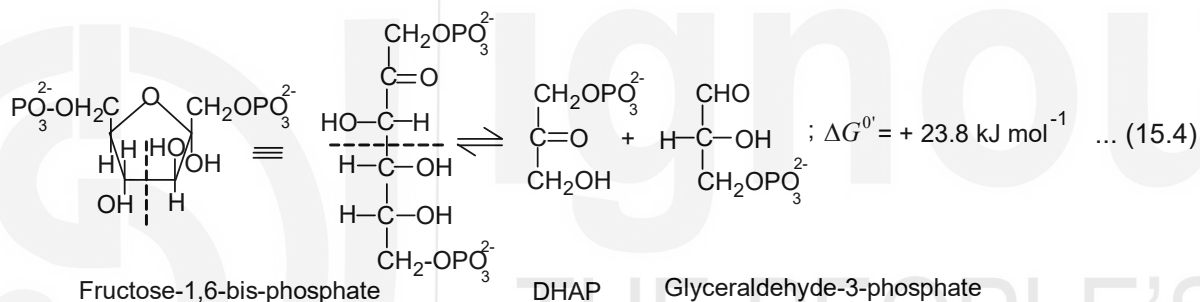
In the next step, F-6-P gets phosphorylated with another molecule of ATP to give rise to fructose-1, 6-bisphosphate (F-1, 6 - bis -P) and the reaction is catalysed by phosphofructokinase (PFK), Eq. 15.3.



You would recall (unit 6) that in allosteric regulation, the first enzyme of a metabolic pathway is strongly inhibited by its end product.

This reaction is also practically irreversible in the direction of formation of F-1, 6-bis-P because of the large energy drop (large negative $\Delta G^{0'}$ value). This reaction is of critical importance in the regulation of the rate of glycolysis, because the enzyme PFK is an allosteric enzyme which is highly sensitive to changes in the cellular concentrations of ADP and AMP both of which are activators, and ATP, citrate and fatty acids all of which are inhibitors of its activity. This aspect will be discussed in Unit 16 (will have to check).

Fructose-1, 6-bisphosphate gets cleaved into one molecule each of glyceraldehydes-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP) as per Eq. 15.4. The enzyme catalysing this reaction is called fructose-1, 6-bisphosphate aldolase or simply aldolase.



If we consider the reverse reaction, it is similar to an aldol condensation, namely reaction of the aldehydic group of G-3-P with a reactive methylene group of DHAP. This explains how the enzyme got its name, aldolase.

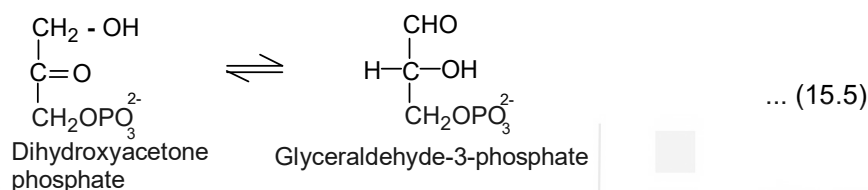
You can see a large positive standard free energy change accompanying this reaction, which corresponds to an equilibrium constant of approximately 10^{-4} M as can be seen from the following calculation,

$$\begin{aligned}
 \Delta G^{0'} &= -RT \ln K'_{\text{eq}} = -2.303 \times RT \times \log K'_{\text{eq}} \\
 \text{Therefore, } \log K'_{\text{eq}} &= \frac{\Delta G^{0'}}{-2.303 \times R \times T} \\
 &= \frac{23.8}{-2.303 \times 8.314 \times 298} = -4.18 \\
 K'_{\text{eq}} &= \frac{[\text{G-3-P}] \times [\text{DHAP}]}{[\text{F-1,6-bis-P}]} = 6.61 \times 10^{-5}
 \end{aligned}$$

Physiological concentration of F-1, 6-bis-P is approximately equal to 10^{-4} M. Substituting this value in the equation it is found that the product of concentrations of G-3-P and DHAP should be equal to 6.6×10^{-9} or approximately 10^{-8} M. Since these are formed in equimolar concentrations from F-1, 6-bis-P, their concentrations should be approximately 10^{-4} M each. This

corresponds to the conversion of approximately 50% of the F-1, 6-Bis-P into G-3-P and DHAP. Since the latter are continuously drawn away for further reactions of glycolysis, their concentrations are depleted. Therefore, more and more of F-1, 6-bis-P is cleaved to form more of G-3-P and DHAP. This example illustrates the points frequently made in this and the preceding units that although ΔG^0 values are adequate in most cases, the real picture about the direction of a chemical change and the accompanying free energy change emerges only when the physiological concentrations of the reactants and products are considered.

Glyceraldehyde and dihydroxyacetone are frequently referred to as 3-carbon sugars or trioses. Their phosphorylated derivatives, G-3-P and DHAP are interconvertible, just like the other aldose-ketose interconversions. This reaction is catalysed by triosephosphate isomerase.



For the next stage of glycolysis, the substrate is G-3-P. However, DHAP is also metabolised because of its conversion into G-3-P. Thus, effectively two molecules of G-3-P are obtained from one molecule of glucose. Consequently, from the next step onward in the second stage each reaction must be multiplied by two in order to arrive at the correct stoichiometry in relation to glucose. This stage includes oxidoreduction and phosphorylation steps.

Before studying the further steps, try answering the following SAQ.

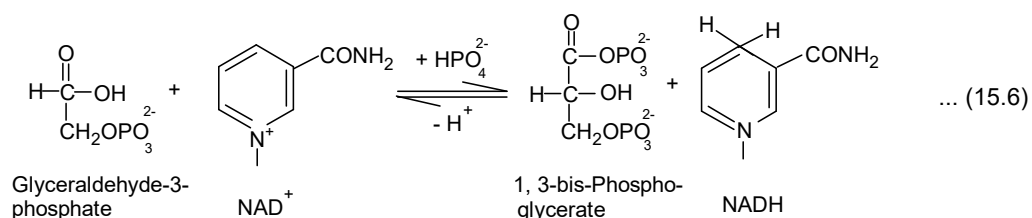
SAQ 1

Which of the following statements are correct and which are wrong. Also give the correct statements for wrong ones.

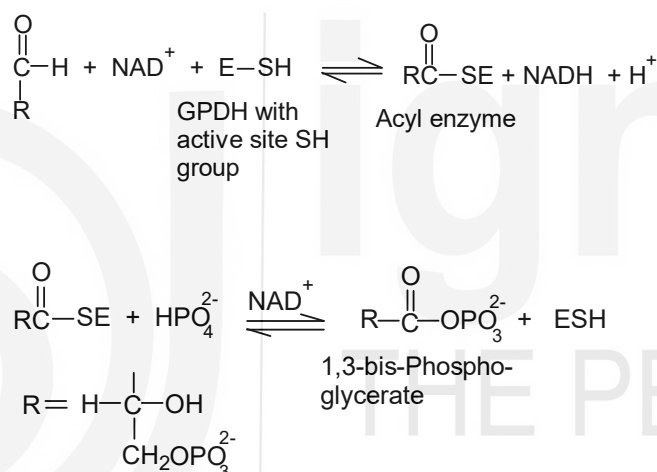
- Glucokinase is specific for the phosphorylation of glucose.
- Phosphofructokinase catalyses the reaction in which glucose-1-phosphate is the substrate.
- The reaction catalysed by PFK is inhibited by high concentration of ATP and citrate.
- Like G-3-P, dihydroxyacetone phosphate can get into glycolytic pathway for its further metabolism.

15.3.2 Conversion of G-3-P into Pyruvate

G-3-P formed by cleavage of F-1, 6-bis-P and isomerisation of DHAP reacts with NAD^+ and phosphate ion to give rise to 1,3-bisphosphoglycerate, Eq. 15.6 the reaction is catalysed by glyceraldehydes-3-phosphate dehydrogenase (GPDH).

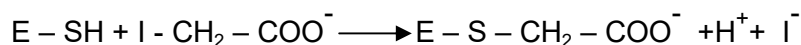


GGPDH has a highly reactive cysteine-SH group at its active site. It has been shown that the reaction proceeds in two readily distinguishable steps. In an oxidation/reduction step the aldehyde group of G-3-P is oxidised to an acyl moiety which remains covalently attached to the enzyme active site SH group in the form of a thiol-ester, with a concomitant reduction of NAD^+ to NADH. In the next, group transfer, step the acyl moiety is transferred to phosphate ion to give rise to the final product, 1, 3-bisphosphoglycerate. This reaction step requires the presence of NAD^+ as an obligatory effector in the absence of which the reaction does not proceed.



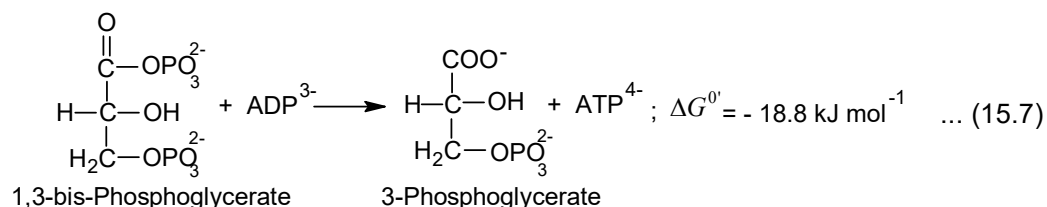
From energy considerations this reaction is very important because a part of the free energy drop in the oxidation of aldehydic group to acyl moiety is conserved in the form of a thiol-ester and next as acyl-phosphate linkage of the final product. Note the role of a covalent enzyme-substrate intermediate in the catalysed reaction, especially for coupling of oxidation and the energy conserving phosphorylation reaction. This is an example of substrate-level phosphorylation.

The highly reactive active site SH group of GPDH reacts with several reagents, e.g., iodoacetate or iodoacetamide.



Blocking of the active site SH group leads to loss of catalytic activity of GPDH. It is well known that iodoacetate and iodoacetamide inhibit glycolytic breakdown of glucose. The reaction of these reagents with GPDH is one of the major loci for attack.

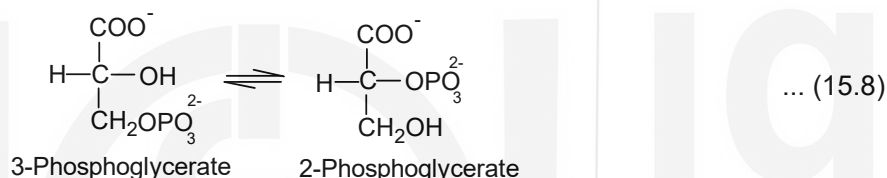
1, 3-Bisphosphoglycerate reacts with ADP in the next step of glycolysis. The reaction is catalysed by phosphoglycerate kinase and yields 3-phosphoglycerate and ATP, Eq. 15.7.



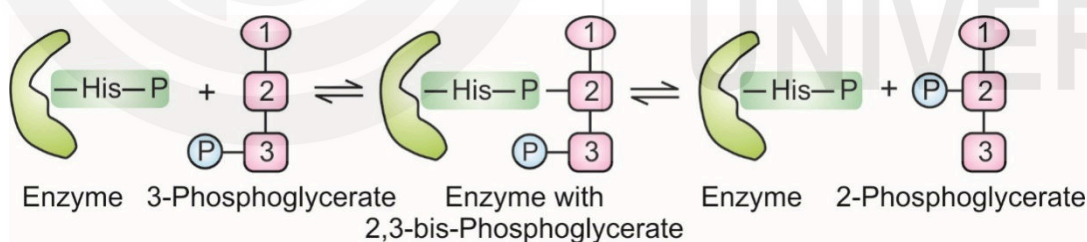
In this reaction, the energy conserved in the preceding step is “harvested” and utilised for a “net” synthesis of ATP. **This is the first ATP-generating step of glycolysis.**

Note that there is no direct reaction between ADP and phosphate ion as happens in the oxidative phosphorylation (Unit 8). Instead, phosphate group is first attached to the acyl moiety (in the preceding step) and the high phosphate transfer potential of acyl-phosphate is utilised in this step to transfer the phosphate moiety to ADP giving rise to ATP.

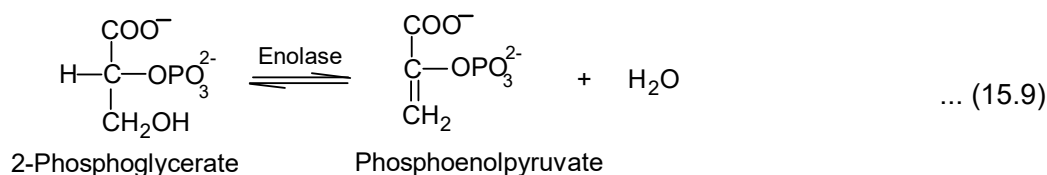
In the next reaction, catalysed by phosphoglyceromutase, 3-phosphoglycerate is isomerised to 2-phosphoglycerate, Eq. 15.8.



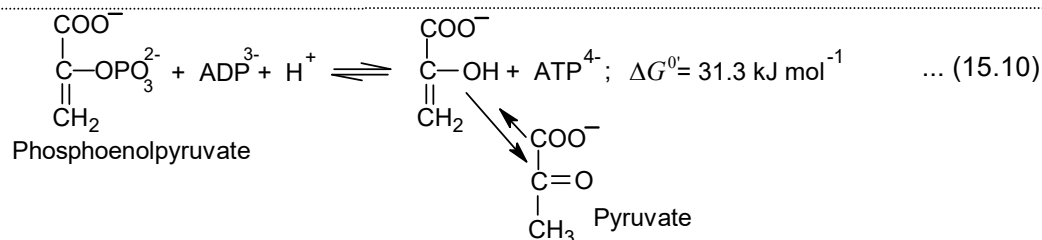
This reaction requires the presence of enzyme-bound 2,3-bisphosphoglycerate. A histidine residue of the enzyme active site functions successively as an acceptor and donor of phosphate group. The isomerisation is believed to proceed as follows:



2-Phosphoglycerate undergoes dehydration to give rise to phosphoenolpyruvate (PEP), Eq. 15.9. The enzyme catalysing this reaction is called enolase.



Phosphate esters of enolic compounds are also energy rich compounds (Unit 14, Table 14.1). They have a high phosphate group transfer potential. This is made use of in the next step to transfer the phosphate group to ADP giving rise to ATP and pyruvate. The reaction is catalysed by pyruvate kinase (PK), Eq. 15.10.



The genetic deficiency of pyruvate kinase(PK) in the erythrocytes leads to hemolytic anemia (excessive erythrocyte destruction). It may be the consequence of the reduced rate of glycolysis. The rate of ATP synthesis thought to be inadequate to meet the energy needs of the cell and to maintain the structural integrity of the erythrocytes.

There is a very large energy drop which renders this reaction practically irreversible. Much of this energy drop comes from the enol-ketone-conversion. The latter is much more stable.

Pyruvate kinase, after phosphofructokinase, is the second important regulatory point to control the rate of glycolysis. It is activated by glucose-6-phosphate, fructose-1, 6-bisphosphate and glyceraldehyde-3-phosphate and is inhibited by alanine, ATP and fatty acids.

A summary of the energetic of the reactions from glucose of pyruvate is given in Table 15.1.

Table 15.1: Energetics of the Reaction of Glycolysis

Eq. Number	Reaction Type	$\Delta G^{0'}$	ΔG
15.1	Phosphate transfer	-16.74	-33.47
15.2	Isomerisation	+ 1.67	-2.51
15.3	Phosphate transfer	-14.22	-22.17
15.4	Aldol cleavage	+23.85	-1.25
15.5	isomerisation	+7.53	+2.57
15.6	Oxidation and phosphorylation	+6.28	-1.64
15.7	Phosphate transfer	-18.83	+1.20
15.8	Phosphate group shifting	+4.60	+0.83
15.9	Dehydration	+1.67	-3.35
15.10	Phosphate transfer	-31.4	16.74

In this table, the actual free energy changes (ΔG) are based on the known $\Delta G^{0'}$ values and the prevailing intracellular concentrations of various metabolites. For determining the latter, it is necessary to stop all reaction before the cells are ruptured. This is achieved by first cooling and freezing the intact tissue to liquid nitrogen temperature which stops all chemical reaction. This tissue is ground in the frozen condition and then extracted in the presence of perchloric acid, which inactivates all the enzymes. The extract is then assayed for the various metabolic reactions/pathways. The data so obtained can then be related to the intracellular concentrations of the metabolites at the time of freezing. If these precautions are not taken, the concentrations of the metabolites in the extract will rapidly approach their equilibrium ratios because of the presence of the enzymes. The metabolite concentrations vary from one tissue to another. The ΔG values given in Table 15.1 have been computed from the human erythrocytes, which derive all their energy requirements from glycolysis.

Further metabolism of pyruvate depends on the nature of the living organism and its physiological state, e.g., on the rate of oxygen supply, etc. These are described below. Before that try to answer the following SAQ.

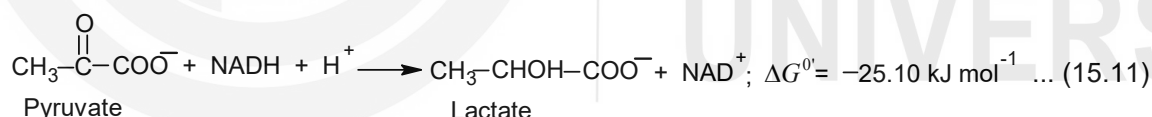
SAQ 2

In what respect is generation of ATP by glycolysis unique?

15.3.3 Metabolic Fate of Pyruvate

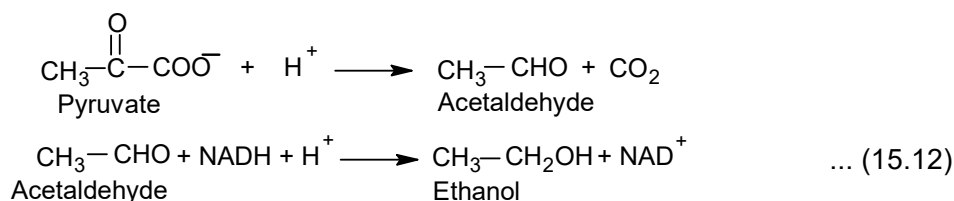
Under aerobic conditions, pyruvate is converted into acetyl-CoA and then oxidised to carbon dioxide via Kreb's tricarboxylic acid cycle also called citric acid cycle, Fig. 16.1. These reactions will be discussed in Unit 16. The reduced coenzymes formed during glycolysis and these reactions are reoxidised via the oxidative phosphorylation, in which the metabolic energy is utilised to drive the synthesis of ATP. This accounts for the major part of conservation and capture of the metabolic energy of the nutrients. The reoxidised coenzymes become available for reaction with more substrate molecule.

The rate of oxidative phosphorylation and, therefore, of the regeneration of reduced coenzymes is limited by the rate of oxygen supply. If the demand of energy, i.e. of ATP is large as happens with intense muscular activity, the rate of delivery of oxygen to the tissues is not adequate to reoxidise the rapidly forming reduced coenzymes. Under these conditions, alternative pathways must operate to reoxidise the reduced coenzymes and thus ensure continuous supply of ATP. In animal muscles, pyruvate is used for this purpose. NADH formed in the oxidation-phosphorylation of glyceraldehyde-3-phosphate (Eq. 15.6) reacts with pyruvate in the presence of lactate dehydrogenase to form lactate and NAD^+ , Eq. 15.11.



The resulting NAD^+ can be used again for the reaction of Eq. 15.6. Note that none of the reactions of glycolysis and the reaction given before require oxygen. This permits continuous generation of ATP even under conditions of limited oxygen supply. Glycolysis is the major source of ATP during strenuous muscular activity. The disposal of accumulated lactate is discussed later.

In yeast cells, pyruvate is not directly used for reoxidation of NADH. It is first decarboxylated in the presence of a thiamine pyrophosphate-dependent enzyme, pyruvate decarboxylase to form acetaldehyde. The latter then reacts with NADH in the presence of alcohol dehydrogenase to give rise to ethanol and NAD^+ , Eq. 15.12.



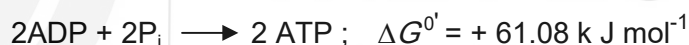
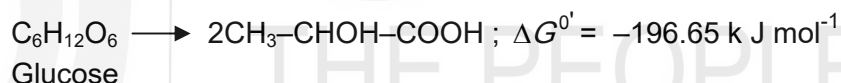
The oxidised coenzyme so produced (NAD^+) can be used again for the reaction given in Eq. 15.6. Thus, this process, called alcoholic fermentation, can proceed in the absence of air or oxygen and supply energy (ATP) to the yeast cells. If the supply of oxygen is plentiful, pyruvate may be oxidised completely as described above.

Another metabolic reaction of pyruvate is its carboxylation to oxaloacetate by pyruvate carboxylase, a biotin-dependent enzyme. This reaction is important for replenishing the TCA cycle intermediates and for providing substrates for gluconeogenesis, as discussed latter.

Having studied the whole pathway let us look into the overall stoichiometry and the energetic of the reactions.

15.3.4 Stoichiometry of ATP Formation and Energetics of Glycolysis

The scheme of glycolysis (Fig. 15.2) shows that two molecules of ATP are consumed in the conversion of glucose into fructose-1, 6-bisphosphate (Eq. 15.1 and 15.3) which then gives rise to two molecules of triose-phosphate (Eq. 15.4). During the conversion of each molecule of triose-phosphate into lactate, two molecules of ATP are produced in these steps for each molecule of glucose. Subtracting the two ATP molecules “spent” in the beginning, there is a net synthesis of two ATP molecules per molecule of glucose converted into two lactate molecules. We can thus write down two partial reactions for the total chemical change.



Considering the $\Delta G^{0'}$ values only, it may be concluded that approximately 31% of the free energy released on the breakdown of glucose into lactate is “captured” in the form of ATP, which represents the readily useable energy currency of the living systems. The actual “conversation” of energy is different because the concentration of various metabolites in the cell are far from standard (1.0M). As explained in Unit 14, the actual free energy of hydrolysis of ATP in human erythrocytes is found to be $-51.8 \text{ k J mol}^{-1}$. The present energy capture comes closer to 50%. The rest of the energy cannot be said to have been wasted. It serves two major purposes. Firstly, the large negative $\Delta G^{0'}$ values ensures that the series of reactions proceed in the desired direction, i.e., in the direction of glycolysis, without significant reversal. This and the presence of necessary enzymes ensures a steady supply of ATP when desired. Secondly, the energy conserved in the form of ATP appears as heat and thus helps maintain the desired body temperature in the event of a fall in ambient temperature. This also explains the necessity of a system for the heat loss from the body during hot weather. In terms of efficiency of energy transduction, no man-made machine compares with the the living cell.

The stoichiometry of reactions and the generation of ATP during glycolysis is given in the following table.

Reaction	Change in ATP per glucose consumed
GI $\rightarrow$ GI-6-P	-1
F-6-P $\rightarrow$ F-1,6-bis-P	-1
2 x 1,3-bis-P-Glycerate $\rightarrow$ 2 x 3-P-Glycerate	+2
2 x PEP $\rightarrow$ 2 x Pyruvate	+2
Net	+ 2 ATP

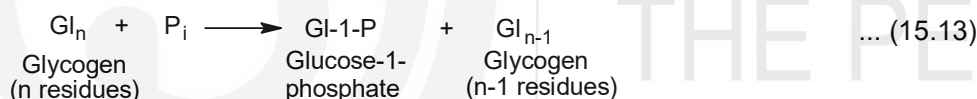
SAQ 3

Give a scheme showing different metabolic fate of pyruvate.

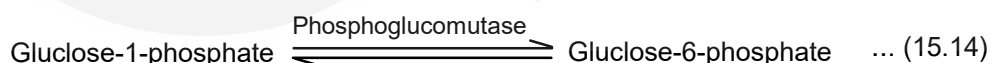
15.3.5 Entry of Glycogen and other Sugars into Glycolytic Pathway

Carbohydrates other than glucose are also metabolised via glycolysis. These enter the pathway at various points (Fig. 15.2). Let us consider the entry of some major nutrient carbohydrates.

- i) **Entry of glycogen:** Glycogen is a branched chain reserve polysaccharide of animal origin, which is structurally similar to amylopectin fraction of starch (see Unit 2). At the time of necessity, glucose residues are removed one-by-one from the nonreducing ends of glycogen by the action of glycogen phosphorylase (which is the main enzyme of glycogenolysis), Eq. 15.13.

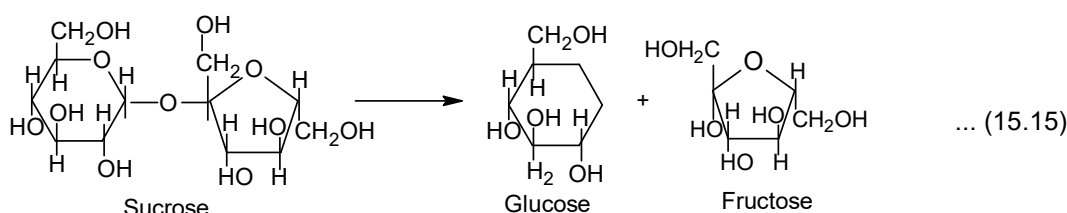


Glucose-1-phosphate is isomerised to glucose-6-phosphate in the presence of the enzyme phosphoglucomutase, Eq. 15.14.

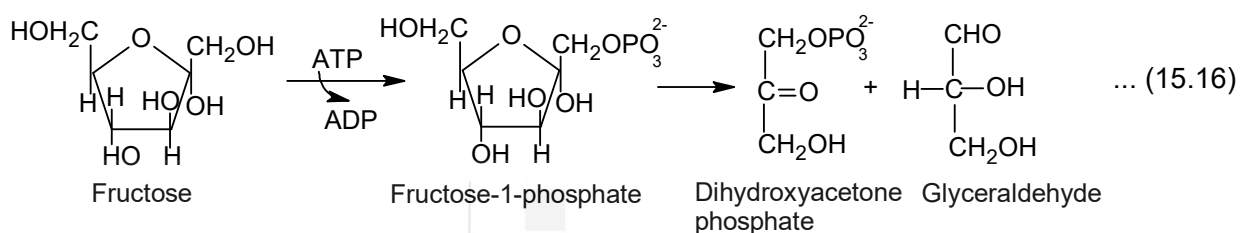


The rest of the reactions from G1-6-P onward are identical to those described before. Note that no ATP is required in this case for the formation of GI-6-P. Consequently, glycolysis of each glucose residue of glycogen leads to a net generation of three molecules of ATP.

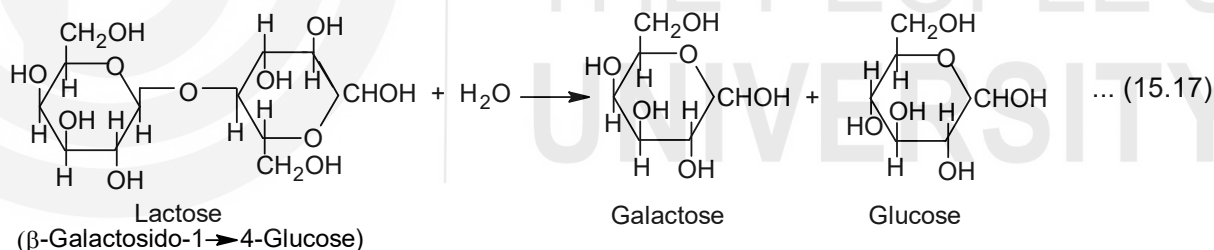
- ii) **Entry of sucrose and fructose:** Disaccharides, like sucrose, are not absorbed through the intestines. Only monosaccharides are absorbed. Sucrose is hydrolysed in the presence of intestinal mucosa giving rise to glucose and fructose. Eq. 15.15.



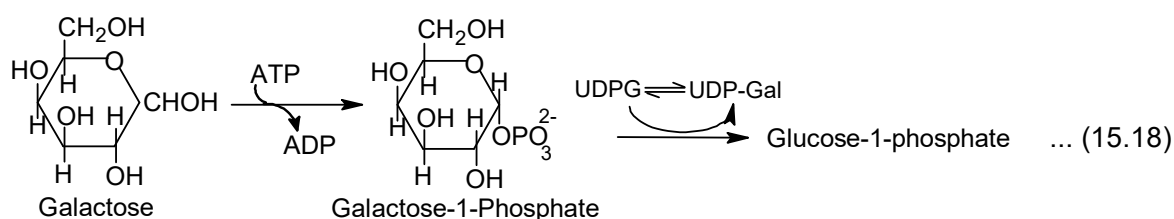
Fructose enters the glycolytic pathway to two routes. In the minor route, fructose is converted into fructose-6-phosphate on reaction with AP catalysed by hexokinase, the same enzyme which converts glucose into glucose-6-phosphate. Further, metabolism is as per the scheme of Fig. 15.2. Most of fructose, however, reacts with ATP in the presence of fructokinase forming fructose-1-phosphate (F-1-P). The latter is cleaved to give to rise to glyceraldehyde and dihydroxyacetone phosphate in the presence of F-1-P-aldolase, Eq. 15.16, the action of which is similar to reaction of Eq. 15.4 discussed before. Further, metabolism of the products is clear from Fig. 15.2.



iii) **Entry of lactose and galactose:** You have read that lactose, the milk sugar, is a disaccharide made up of one residue each of galactose and glucose. It gets hydrolysed to the monosaccharides in the intestine with the help of lactase (or B-galactosidase) present in the intestinal mucosa, Eq. 15.17.



Galactose is phosphorylated at position-1 with ATP in the presence of galactokinase. The resulting galactose-1-phosphate (Gal-1-P) reacts with uridinediphospho glucose (UDPG) giving rise to uridine-diphospho-galactose (UDP-Gal) and glucose-1-phosphate (GI-1-P) in the presence of galactose-1-phosphate uridyl transferase. The resulting UDP-Gal is epimerised to UDPG in the presence of UDPG epimerase, Eq. 15.18.



15.3.6 Some Glycolysis Related Disorders

You have studied in Unit 6 that metabolism is largely controlled by increasing or decreasing the activity of key enzymes. Also, the lack or deficiency of certain enzymes causes some or the other disease in human beings. Let us look into some such deficiency diseases related to the glycolytic pathway. In some cases where the enzyme is lacking or deficient, the respective substrates accumulate in the body. This may lead to some disease, which are referred to as the “inborn errors of metabolism”. Some metabolic disorders are related to genetic deficiencies, many of which are transmitted as recessive traits. Some metabolic disorders are related to the entry of the sugars, other than glucose, into the glycolytic pathway.

Some infants suffer from galactosemia, in which the galactose metabolism is blocked at the galactose-1-phosphate stage because of the lack of galactose-1-phosphate uridyl transferase. Initial symptoms of galactose accumulation are vomiting or diarrhoea, enlargement of liver and jaundice. In later stages, the patients suffer from mental retardation, clouding of the eye lens. This condition is frequently irreversible because of mental retardation. It may be noted that galactosemia is caused by accumulation of toxic substances and not by lack of any essential nutrient. Further, the patients eventually outgrow this problem because other pathways of galactose utilisation are developed at a higher age.

Fructose intolerance is a metabolic disorder caused by lack of fructose-1-phosphate aldolase.

Many individuals, especially in Asia and Africa, develop lactose intolerance in adulthood because of inadequate levels of lactase in their intestinal mucosa. Lactose, being a disaccharide, is not absorbed by the intestines and consequently accumulates there. This increases the osmotic pressure of the intestinal contents which leads to diffusion of water into the intestines causing flatulence. Further, lactose is readily taken up and metabolised by the intestinal flora leading to their increased multiplication and gas formation. This is not a serious condition, because lactose is not an essential nutrient and can be easily avoided. Such individuals find it difficult to digest milk but can easily consume curd (also called yoghurt). In the latter, most of, if not all, lactose has been converted into lactate during curd formation.

SAQ 4

Fill in the blank space with suitable words.

- The conversion of glycogen to lactate in glycolytic pathway is accompanied by a net gain of of ATP.
- An intense muscular activity leads to an increased..... formation.
- Galactosemia is observed as a result of accumulation in human body.

15.4 GLUCONEOGENESIS

Gluconeogenesis occurs primarily in the liver

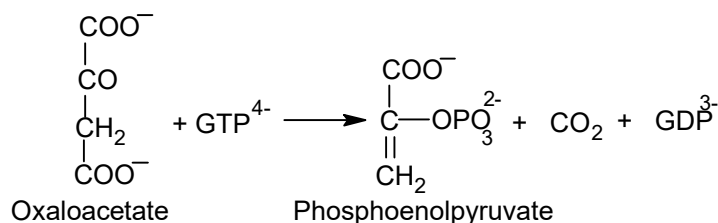
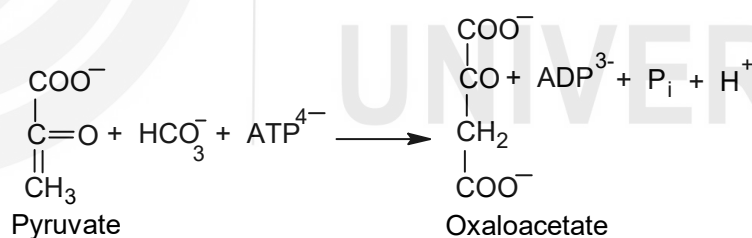
As already mentioned, glycolysis is the major source of ATP for the skeletal muscles during strenuous activity. As a result, lactate accumulates in the muscles. In the resting condition, excess lactate is transported to liver where it is reconverted into glucose. Conversion of a noncarbohydrate precursor, like lactate, into glucose is called **gluconeogenesis**.

Glycolysis and gluconeogenesis make up a pseudocycle or pair of oppositely directed reaction sequences.

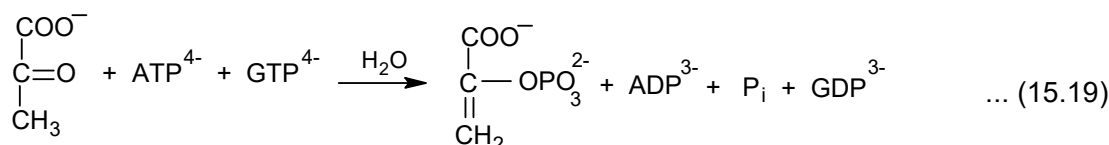
Glycerol and some amino acids are other starting materials for gluconeogenesis, which become important under conditions of starvation. Under these conditions, glucose levels in the blood have to be maintained at about 80-140 mg per 100 ml by converting noncarbohydrate material into glucose because brain is highly dependent on glucose as the primary fuel.

The pathways of gluconeogenesis and entry points for lactate, amino acids and glycerol are shown in Fig. 15.3. It may be noted that many reactions are common to glycolysis but proceeding in opposition direction. The differences lie in three steps, namely (i) the conversion of pyruvate into phosphoenolpyruvate, (ii) the conversion of fructose-1,6-bisphosphate into fructose-6-phosphate and (iii) the conversion of glucose-6-phosphate into glucose. It may be recalled that these are points that represent the steps of large energy drops and practically irreversible in the direction of glycolysis (Table 15.1).

Pyruvate is carboxylated in presence of ATP and biotin-dependent pyruvate carboxylase to form oxaloacetate. The latter is then decarboxylated and phosphorylated by its reaction with another high energy phosphate compound namely guanosine triphosphate (GTP). This reaction is catalysed by phosphoenolpyruvate carboxykinase.



The sum of these reactions is:



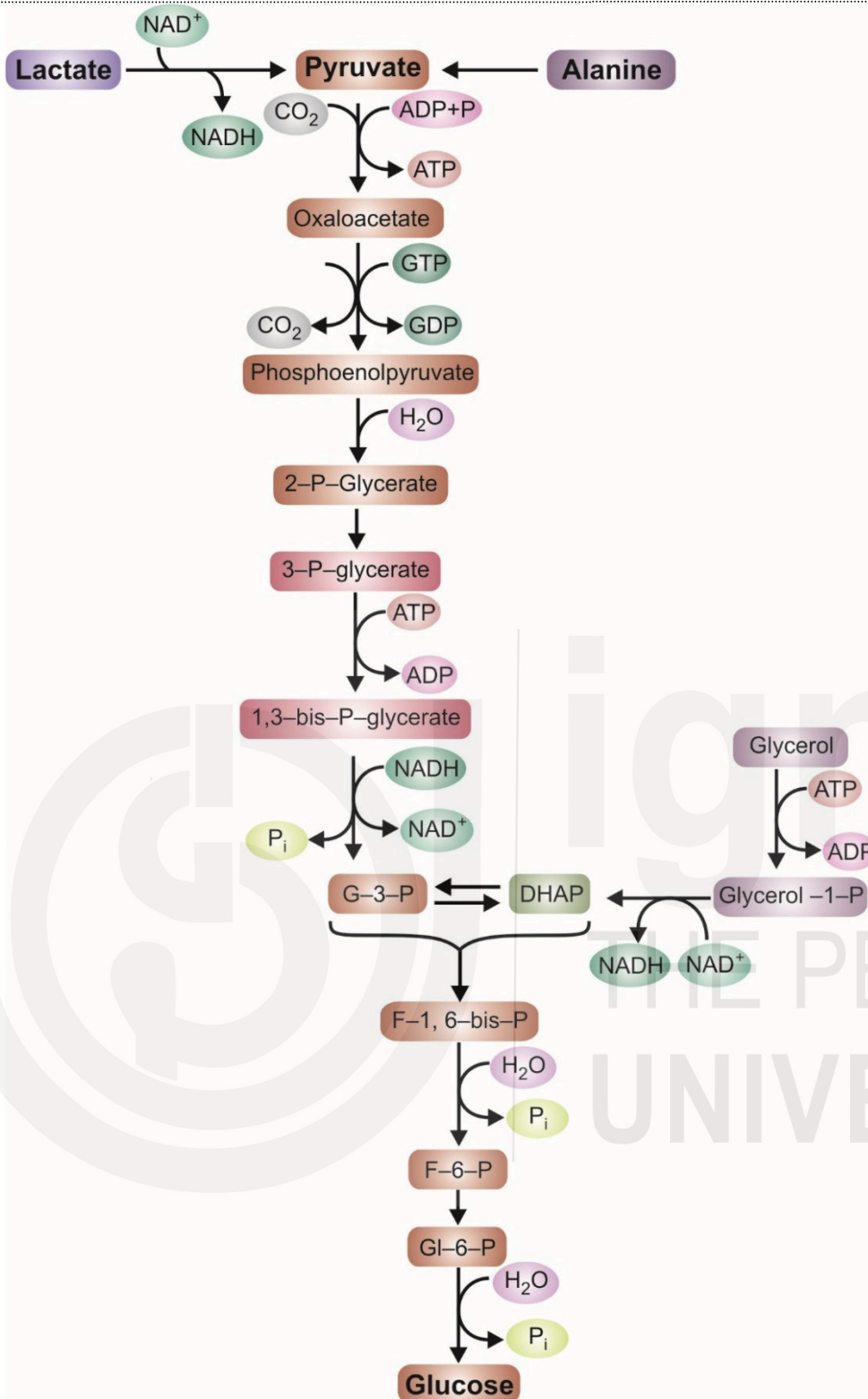
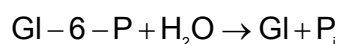


Fig. 15.3: Scheme of gluconeogenesis showing entry points for lactate, amino acids and glycerol

Note the “expenditure” of two high-energy phosphate bonds, ATP and GTP, in the conversion of pyruvate into phosphoenolpyruvate whereas only one such bond was established as ATP in the direction of glycolysis (Eq. 15.10). This extra energy input makes the otherwise energetically unfavourable conversion of pyruvate into phosphoenolpyruvate, ($\Delta G^0 = + 61.8 \text{ k J mol}^{-1}$) feasible.

Fructose-1,6-bisphosphate and glucose-1-phosphate are hydrolysed in the presence of respective phosphatases.



In the direction of glycolysis, each of these reactions required the input of one molecule of ATP.

A summary of differences in the reactions and enzymes involved in glycolysis and gluconeogenesis is given in Table 15.2.

Table 15.2: Differences in the reactions and enzymes of glycolysis and gluconeogenesis

Glycolysis		Gluconeogenesis	
Reaction	Enzyme	Reaction	Enzyme
$GI + ATP \rightarrow GI-6-P + ADP$	Hexokinase	$GI-6-P + H_2O \rightarrow GI + P_i$	GI-6-Phosphatase
$F-6-P + ATP \rightarrow F-1,6\text{-bis-P} + ADP$	Phosphofructokinase	$F-1,6\text{-bis-P} + H_2O \rightarrow F-6-P + P_i$	F-1,6-bis-Phosphatase
$\text{Phosphoenolpyruvate} + ADP \rightarrow \text{Pyruvate} + ATP$	Pyruvatekinase	$\text{Pyruvate} + ATP + GTP + CO_2 \rightarrow \text{PEP} + ADP + P_i + GDP + CO_2$	Pyruvate-carboxylase + Phosphoenolpyruvate carboxykinase

Abbreviations: Glucose, GI; Fructose, F; Phosphate group, P; Phosphate ion, P_i ; Phosphoenolpyruvate, PEP.

Let us consider the energy input required to convert two molecules of pyruvate into one molecule of glucose. For each molecule of pyruvate, one molecule of ATP and one of GTP are required for its conversion into phosphoenolpyruvate. One more ATP is required in the formation of 1,3-bisphosphoglycerate from 3-phosphoglycerate. Thus, in all six high energy bonds are “consumed” for the synthesis of one molecule of glucose by the gluconeogenesis pathway. Contrast this with the net generation of only two ATP molecules in glycolysis. The “expenditure” of four extra high energy bonds is required to drive the otherwise thermodynamically “uphill” process of the formation of glucose from pyruvate. For the complete chain of gluconeogenesis there is large negative free energy change.

SAQ 5

What are the physiological functions of gluconeogenesis?

15.5 SUMMARY

Let us summarise what all we studied about the two metabolic pathways in this unit.

- The sets of reactions brought about by the living cells are collectively referred to as metabolism, which may be broadly considered to be of two

types namely degradation of complex organic compounds into simpler ones (catabolism) and biosynthesis of more complex molecules from simpler starting materials (anabolism).

- The catabolic chains of reactions, or pathways, help to release the metabolic energy and convert it into a form which is suitable for use by the living cells, i.e., as ATP. These pathways also provide precursors for synthesis of complex molecules.
- The catabolism of glucose to pyruvate proceeds in ten steps. In the first three steps glucose is successively converted into glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-bisphosphate. Two molecules of ATP are used for each molecule of glucose in these steps. Fructose-1,6-bisphosphate is cleaved to give two molecules of triosephosphate. These triosephosphate molecules get finally converted to two molecules of pyruvate. This sequence of reactions is called glycolysis. It helps in the net synthesis of two molecules of ATP from ADP per molecule of glucose.
- In anaerobic alcoholic fermentation of glucose, pyruvate is decarboxylated to acetaldehyde which is reduced to ethanol with the concomitant oxidant of NADH to NAD⁺.
- Under aerobic conditions pyruvate is converted into acetyl-coenzyme A and the oxidised completely to carbon dioxide.
- Glycogen, sucrose, fructose, lactose and galactose are some other sugars which enter the glycolytic pathway and get metabolised.
- Deficiency of certain enzymes leads to metabolic disorders and therefore some diseases in human beings. Some glycolysis related metabolic disorders have been discussed.
- Lactate which accumulates in muscles during strenuous activity is transported to liver where it is reoxidised to pyruvate and then converted into glucose (gluconeogenesis).
- Gluconeogenesis differs from glycolysis in three steps. For the conversion of two molecules of pyruvate into one molecule of glucose by the gluconeogenesis pathway, six high energy bonds are utilised as compared to the net synthesis of only two high energy bonds in the glycolytic process.

15.6 TERMINAL QUESTIONS

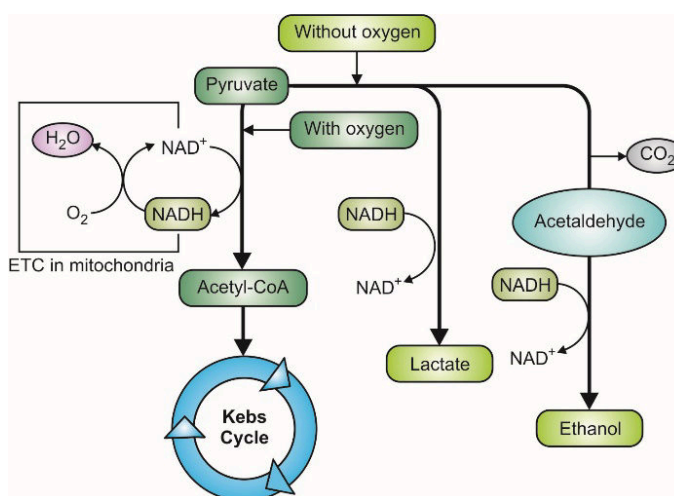
1. a) Write a balanced equation for the conversion of glucose into lactate via the glycolytic pathway.
b) Calculate the ΔG° value for this reaction from the data of Table 8.1.
c) Calculate the ΔG° value for this reaction if the concentrations of various reaction partners are: glucose, 5.0 mM; lactate, 0.05 mM; ATP, 2.0 mM; ADP, 0.2 mM; and P_i, 1.0 mM. (Hint: Consult Unit 14).

2. Is gluconeogenesis just the reverse of glycolysis. If not, how?
3. In a solution, all the species participating in the reaction of Eq. 15.10 are at equilibrium with one another at pH 7.0 and 298 K. If $[ATP]/[ADP]$ ratio is equal to 10, what must be ratio of the concentrations of pyruvate and phosphoenolpyruvate?
4. Alcoholic fermentation was carried out using glucose labelled with ^{14}C in various positions and the isolated products, CO_2 and ethanol (methyl carbon and alcoholic group carbon), were analysed for the ^{14}C label.
 - a) If glucose is labelled in C-1 position, where will the label appear in the products?
 - b) Which position of glucose should have ^{14}C in order that it is entirely isolated in CO_2 ?
5. Describe the significance of the enzyme PFK and PK in glycolytic pathway.
6. How is alcoholic fermentation in yeast similar to lactate production in skeletal muscles?

15.7 ANSWERS

Self Assessment Questions

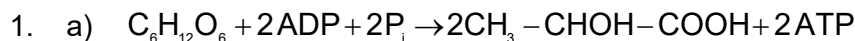
1.
 - a) correct
 - b) Wrong, PFK catalyses the reaction of glucose-6-phosphate.
 - c) Correct
 - d) Wrong, DHAP has to get converted to G-3-P to get into glycolysis.
2. The generation of ATP by glycolysis is unique in that it occurs by substrate level phosphorylation.
- 3.



4.
 - a) 3 moles
 - b) lactate
 - c) galactose

5. To produce glucose from noncarbohydrate precursors, specially from lactate which will otherwise be a waste material. It helps to replenish glycogen of liver and muscle and to maintain blood sugar levels.

Terminal Questions



b) $\Delta G^{0'}$ value is $-123.4 \text{ kJ mol}^{-1}$ glucose

c)
$$\Delta G^{0'} = \Delta G^{0'} + 2.303 \times R \times T \times \log \frac{[\text{lactate}]^2 \times [ATP]^2}{[\text{glucose}] \times [ATP]^2 \times [P_i]^2}$$

$$= (-123,500) + 2.303 \times 8.3014 \times 298 \times \log \frac{(0.050 \times 10^{-3})^2 \times (2.0 \times 10^{-3})^2}{5 \times 10^{-3} \times (0.2 \times 10^{-3})^2 \times (1 \times 10^{-3})^2}$$

$$= -113.70 \text{ kJ mol}^{-1} \text{ glucose}$$

2. It is not just the reverse of glycolysis and differs in three steps viz., conversion of pyruvate into PEP, conversion of F-1, 6-bis-P into F-6-P and conversion of Gl-6-P into glucose.



$$\Delta G^{0'} = -2.303 \times R \times T \times \log K'$$

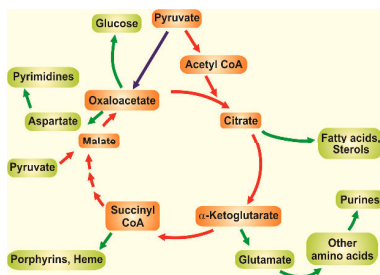
$$\log K' = \frac{-31,300}{-2.303 \times 8.314 \times 298} = 5.486$$

$$K' = 3.06 \times 10^5 = [\text{Pyruv}][ATP] / [\text{PEP}][ADP]$$

$$\text{If } [ATP]/[ADP] \approx 10$$

$$[\text{Pyruv}][\text{PEP}] = 3.06 \times 10^5 / 10 \approx 3.06 \times 10^4$$

4. a) Methyl carbon atom of ethanol
b) In C-3 or C-4 position.
5. They are the important regulatory points controlling the rate of glycolysis.
6. Both are anaerobic in nature and reoxidise the NADH produced in glycolysis by donating electrons to pyruvate or to a product produced from pyruvate.



KREB'S CYCLE AND ITS METABOLIC ROLE

Structure

16.1	Introduction	Stereochemistry of the TCA Cycle
	Expected Learning Outcomes	
16.2	Conversion of Pyruvate into Acetyl-CoA	Stoichiometry and Energetics of the TCA Cycle
16.3	Tricarboxylic Acid Cycle	Central Role of the TCA Cycle
	Entry of Acetyl-CoA in the Cycle	16.4 Electron Transport Chain
	Other Reactions of the TCA Cycle	16.5 Summary
		16.6 Terminal Questions
		16.7 Answers

16.1 INTRODUCTION

In the previous unit, you studied the metabolic breakdown of glucose and other sugars to pyruvate. Under anaerobic conditions pyruvate is subsequently converted into lactate in animal muscle or to ethanol in the yeast cells. These last reactions serve the purpose of regenerating NAD^+ , i.e., reoxidising NADH , so that glycolytic process can be continued. In aerobic conditions however, NADH is reoxidised by transferring its electrons to oxygen through electron transport chain. Under these conditions, pyruvate is metabolised further to CO_2 and H_2O by getting first converted into acetyl-coenzyme A. The acetate moiety of acetyl-CoA is oxidised via a cyclic metabolic pathway, called **tricarboxylic acid cycle** or **Kreb's cycle** or **citric acid cycle**. We would discuss this cycle in the present unit. Since pyruvate is also produced from an amino acid, alanine, and acetyl-CoA itself is a product of fatty acid degradation, the tricarboxylic acid cycle plays a central role in metabolism where the metabolism of carbohydrates, fats and proteins converge (Unit 15). Tricarboxylic acid cycle is directly related to the metabolism of several other acids through some of its metabolites. Further, it provides precursors for biosynthesis of some biomolecules. In this unit, we will study the conversion of pyruvate into acetyl-CoA, its oxidation via the tricarboxylic acid cycle to carbon

dioxide and water along with formation of NADH, FADH₂ and ATP molecules. The reduced coenzymes, NADH and FADH₂ are the electron carriers that enter into the last stage of cellular respiration, i.e., the electron transport chain to drive synthesis of ATP molecules through oxidative phosphorylation. You will study the steps involved in the electron transport chain and the mechanism involved therein.

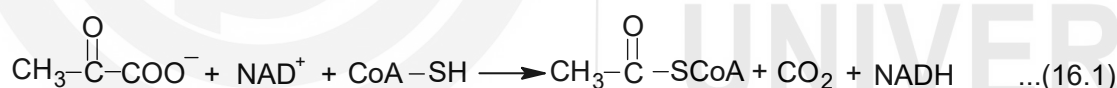
Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ describe reactions involved in the conversion of pyruvate to acetyl-CoA;
- ❖ describe the steps involved in the tricarboxylic acid cycle;
- ❖ explain the central role of tricarboxylic acid cycle in metabolism including making precursors available for a variety of biomolecules;
- ❖ explain the steps involved in the oxidative phosphorylation of the electron transport chain.

16.2 CONVERSION OF PYRUVATE INTO ACETYL-CoA

As mentioned in the introduction, under aerobic conditions pyruvate gets further metabolised through tricarboxylic acid (TCA) cycle. To enter the TCA cycle, pyruvate must first be converted into acetyl-CoA. For this, pyruvate is transported into mitochondria where it undergoes oxidative decarboxylation and condensation with coenzyme A (CoA-SH; see Unit 14 for structure.)



This reaction is catalysed by an assembly of enzymes or a “multienzyme complex” called **pyruvate dehydrogenase complex**.

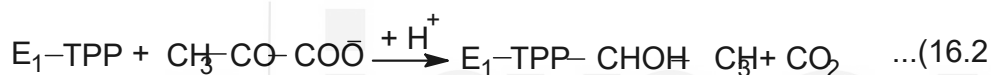
This complex consists of three distinct enzymes held together by strong noncovalent interactions. Consequently, they are isolated in the form of the complex rather than as three individual enzymes in contrast to many other enzymes which are isolated individually. Under some special condition, the complex can be dissociated and the constituent enzymes isolated. On bringing the isolated enzymes together, they combine with a definite stoichiometry to reconstitute the initial complex thus suggesting that the forces holding them together must be very strong and that under the physiological conditions the complex is the more stable species than the individual enzymes existing independently. The three constituent enzymes and their respective prosthetic groups are listed in Table 16.1. In the complex the enzyme E₂ is present in the centre flanked by E₁ and E₃. In addition to the prosthetic groups of the constituent enzymes, the complex as a whole requires NAD⁺ as the coenzyme.

Table 16.1: Constituent Enzymes of Pyruvate Dehydrogenase Complex

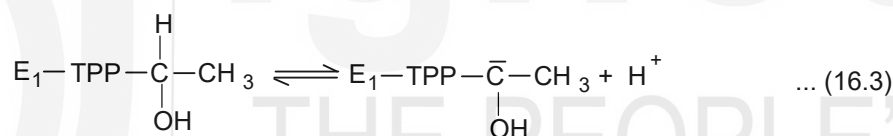
	Constituent enzyme	Abbreviation	Prosthetic group
1	Pyruvate decarboxylase	E ₁	Thiamine pyrophosphate (TPP)
2	Dihydrolipoyl transacetylase	E ₂	Lipoic acid
3	Dihydrolipoyl dehydrogenase	E ₃	Flavin adenine dinucleotide (FAD)

In the complex the enzymes E₁, E₂ and E₃ act upon the pyruvate one by one. Individual reaction steps are outlined below.

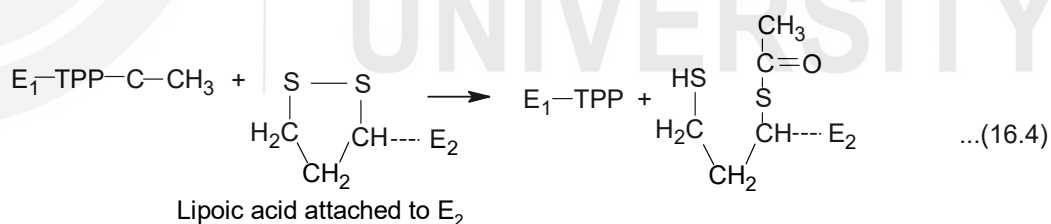
First of all, pyruvate undergoes decarboxylation catalysed by E₁. You may recall from Unit 9 that decarboxylation of pyruvate yields acetaldehyde. In the present case, the aldehyde is not released but remains covalently attached to thiamine pyrophosphate (TPP), the prosthetic group of E₁, Eq. 16.2.



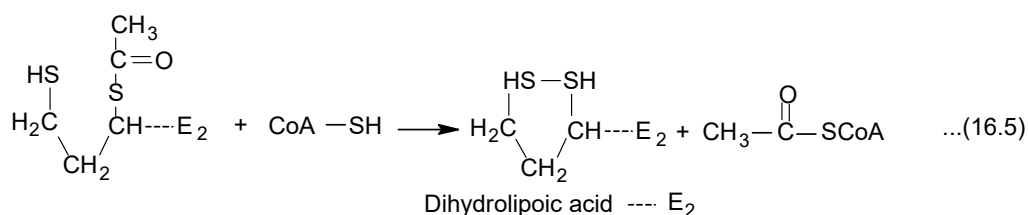
The "hydroxyl ethyl" group attached to TPP is not an alcoholic group but a "potential aldehyde", because it can readily lose a proton giving rise to a carbanion which is the "active aldehyde" form.



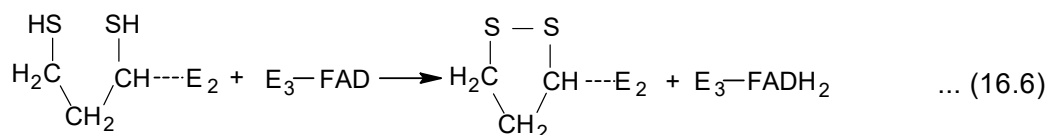
The potential aldehyde, or its active form, reacts with lipoic acid, the prosthetic group of E₂, Eq. 16.4.



You can see that in this reaction, the potential aldehyde has been oxidised to the carboxylic acid state and lipoic acid moiety has also been reduced to dihydrolipoic acid. Further, the acid has been retained in the enzyme complex in the form of a thiolester. In the next step, the acetyl group is transferred from the dihydrolipoic acid moiety to CoA-SH, Eq. 16.5.



Dihydrolipoic acid is reoxidised to lipoic acid moiety by reaction with E₃-FAD, Eq. 16.6.



Finally, the reduced prosthetic group of E_3 , i.e., FADH_2 is reoxidised by NAD^+ , Eq. 16.7.



Summation of Eq. 16.2 and 16.4 to 16.7 gives rise to Eq. 16.1. It may be pointed out that pyruvate has undergone several reactions, namely, decarboxylation, oxidation and condensation with the thiol group of CoA-SH . None of the intermediates is released into the medium. They remain attached to the enzyme complex and are transferred directly from one enzyme to another. This phenomenon is called **substrate channelling**.

A summary of these reactions is given in Fig. 16.1, in which the pyruvate dehydrogenase complex is shown by a box. Pyruvate, CoA-SH and NAD^+ enter the box as shown and CO_2 , acetyl-CoA and NADH come out of it.

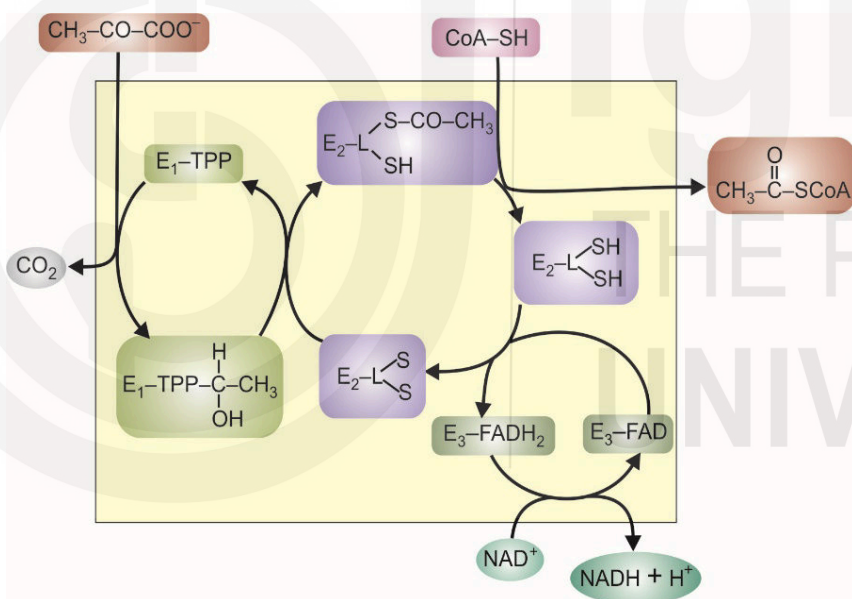


Fig. 16.1: Summary of the reactions involved in the conversion of pyruvate into acetyl-CoA catalysed by pyruvate dehydrogenase complex (box). L stands for lipoic acid moiety given above.

It was mentioned in Unit 14 (Table 14.1) that a thiolester linkage is an energy rich linkage, the requisite energy coming from the oxidation of the aldehydic group (or of the potential aldehyde). It is partially conserved in the thiolester linkage, the rest of it being conserved in the form of reduced coenzyme, i.e., NADH . You would recall that this part of the energy can subsequently be recovered in the form of three ATP molecules when NADH is oxidised by the electron transport chain. Thus, this multienzyme complex is very efficient in terms of conservation. After studying the conversion of pyruvate to acetyl-CoA let us describe the fate of the acetyl-CoA which enters into the earlier mentioned TCA cycle. Before we proceed further try the following SAQ.

SAQ 1

Tick $\checkmark$ on the right and **x** in front of wrong statements given below.

The conversion of pyruvate to acetyl-coenzyme A and CO_2 :

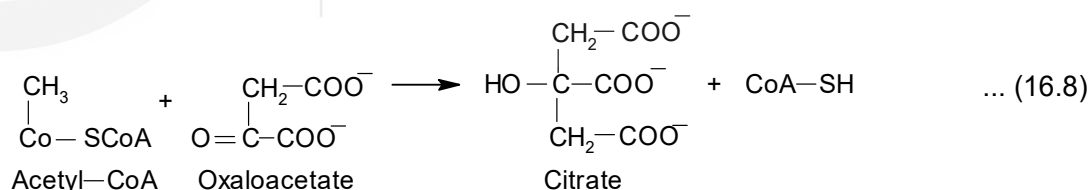
- a) is catalysed mainly by lipoic acid.
- b) involves decarboxylation, oxidation and condensation reactions.
- c) is essentially irreversible.
- d) forms a part of the tricarboxylic acid cycle.

16.3 TRICARBOXYLIC ACID CYCLE

Acetyl moiety of acetyl-CoA generated from pyruvate and by the degradation of fatty acids (to be taken up later) is oxidised to carbon dioxide and water via a cyclic set of reactions, known as **citric acid cycle** or **Kreb's tricarboxylic acid (TCA) cycle**, after the name of the person who discovered it. As mentioned earlier, this cycle occupies a central position in the metabolic pathways, because its precursors, pyruvate and acetyl-CoA, may be derived from carbohydrates, fatty acids and some amino acids. A few other amino acids are degraded into metabolites which are intermediates in this cycle. In addition, some intermediates of the cycle serve as precursors in the biosynthesis of several biomolecules. Acetyl-CoA enters the cycle by reacting with oxaloacetate giving rise to citrate. This and the subsequent reactions of the cycle are described below.

16.3.1 Entry of Acetyl-CoA in the Cycle

Acetyl-CoA reacts with oxaloacetate to give rise to citrate and coenzyme A is set free, Eq. 16.8. The enzyme catalysing this reaction is called **citrate synthase**. In earlier literature, it was referred to as the condensing enzyme.



You can see the similarity of this reaction with aldol condensation, i.e., the condensation of an α -CH group (next to CO) of acetyl-CoA with a carbonyl compound. This is the most commonly employed reaction in the physiological systems for establishing a new C-C linkage. You can compare the formation of fructose-1, 6-bis-phosphate from glyceraldehydes-3-phosphate and dihydroxyacetone phosphate during gluconeogenesis (Unit 15).

16.3.2 Other Reactions of TCA Cycle

In the above reaction, a 4-carbon compound condenses with a 2-carbon acetate unit to form a 6-carbon compound, namely, citrate. The latter

undergoes a series of reactions, summarised in Fig. 16.2 during which two molecules of CO₂ are lost and the original 4-carbon compound is regenerated.

To start with, citrate is isomerised to isocitrate in the presence of the enzyme aconitase. The latter is iron-sulphur protein in which iron is not complexed to any heme moiety. Therefore, is also referred to as a nonheme-iron protein. It has been suggested that citrate undergoes a dehydration followed by a rehydration step with *cis* aconitate as an intermediate as shown in Eq. 16.9. This mechanism has, however, not been confirmed.

Aconitase, the dehydration product of citrate and isocitrate gives the enzyme its name, aconitase.

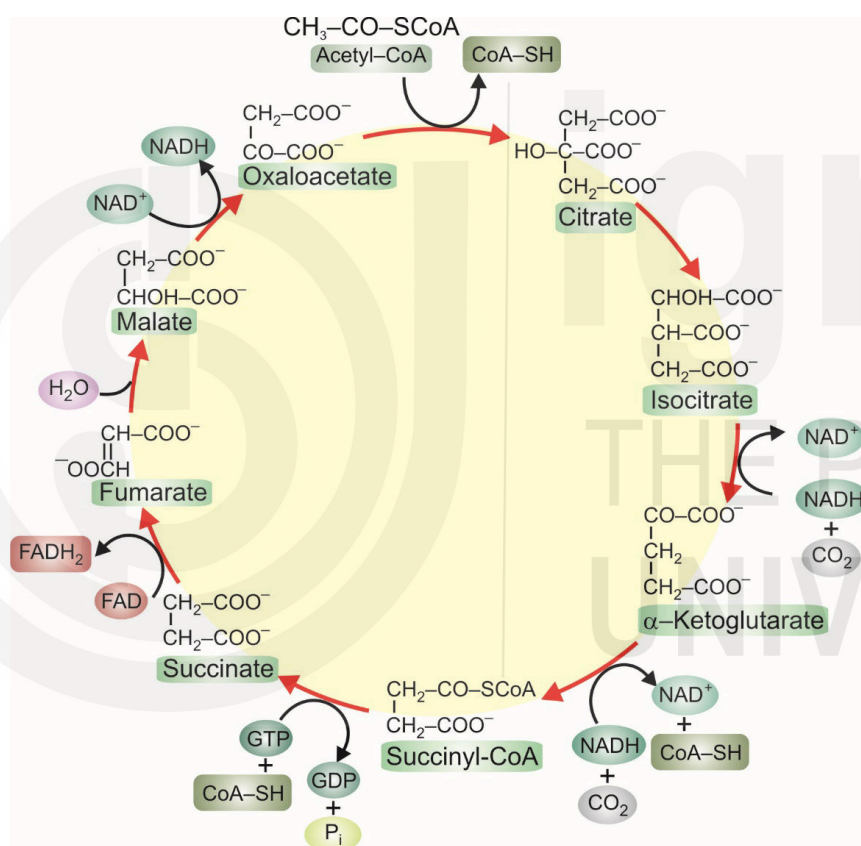
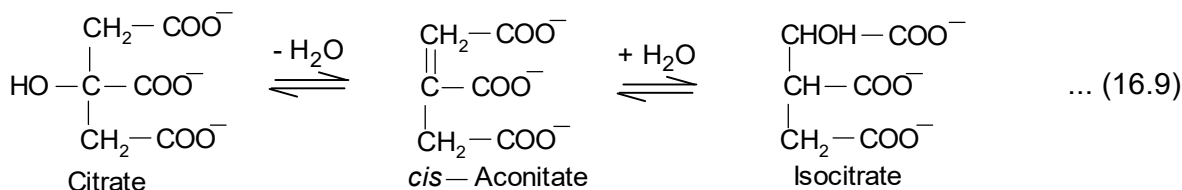
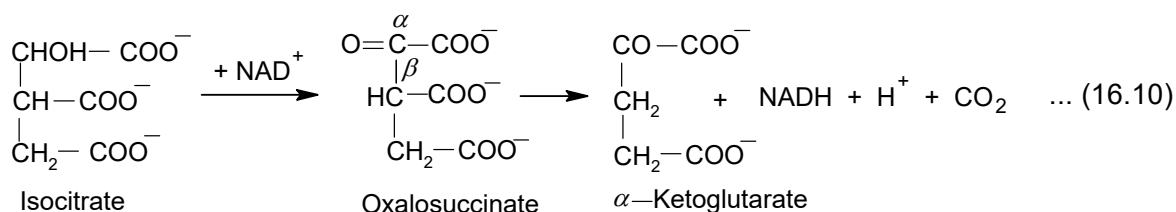


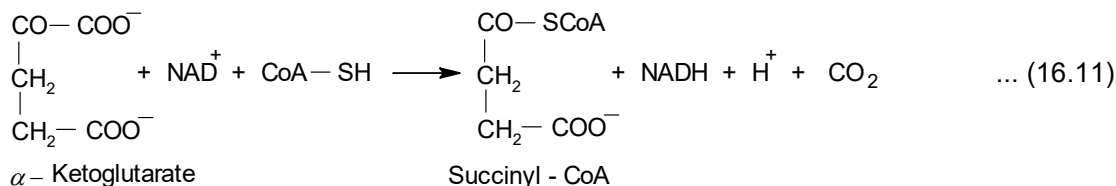
Fig. 16.2: Summary of the reactions of tricarboxylic acid cycle.

Isocitrate reacts with NAD⁺ in the presence of **isocitrate dehydrogenase** giving rise to α-ketoglutarate, a 5-carbon compound. This is achieved in two steps. In the first step the secondary alcohol is oxidised to ketone while the second step involves β-decarboxylation.

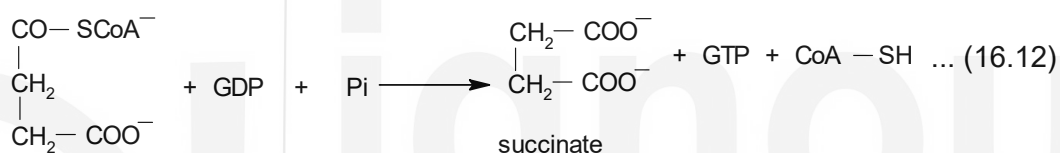


The loss of CO_2 helps push this reaction to completion towards right hand side.

The next reaction takes place in the presence of another multienzyme complex, namely **α -ketoglutarate dehydrogenase complex**. This complex is very similar to pyruvate dehydrogenase complex and so is the nature of the catalysed reaction as can be seen by comparing Eq. 16.11 with Eq. 16.1.

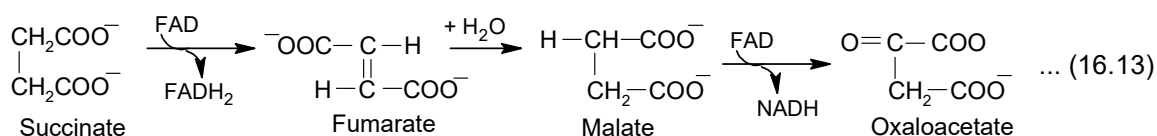


You may recall that the thiol-ester bond, which is present in succinyl-CoA, is an energy-rich linkage. This energy is recovered in the form of a pyrophosphate linkage of a nucleosidetriphosphate, GTP, in the next reaction which is catalysed by succinyl-CoA synthetase (name derived from the reverse reaction of Eq. 16.12).



You see that two molecules of CO_2 have been lost, one each in reactions of Eq. 16.10 and 16.11. consequently, a 4-carbon acid has been formed. The next three reactions help to convert succinate into oxaloacetate, the original 4-carbon compound, which will complete the cycle and enable another molecule of acetyl-CoA to be taken up.

Succinate undergoes successively on oxidation, hydration and another oxidation reaction. In the first reaction, catalysed by **succinate dehydrogenase**, two hydrogen atoms are transferred from succinate to flavin adenine dinucleotide (FAD) to form reduced coenzyme (FADH_2) and an unsaturated acid, fumarate. It may be pointed out here that nature employs flavin nucleotide coenzymes wherever a saturated organic compound, generally an acid or its derivatives, is to be converted into an unsaturated compound by removal of two hydrogen atoms and introduction of a $\text{C}=\text{C}$ double bond. On the other hand, pyridine nucleotide coenzymes, NAD^+ or NADP^+ , are utilised for the oxidation of alcoholic or aldehyde groups. Fumarate adds a molecule of water in the presence of fumarase to form malate and the latter is oxidised by NAD^+ to oxaloacetate in the presence of malate dehydrogenase. These three reactions are represented below:



Some of the intermediates of this cycle are involved in the metabolism of other biomolecules. The implications of these links will be discussed later in this unit. First try to answer the following SAQ.

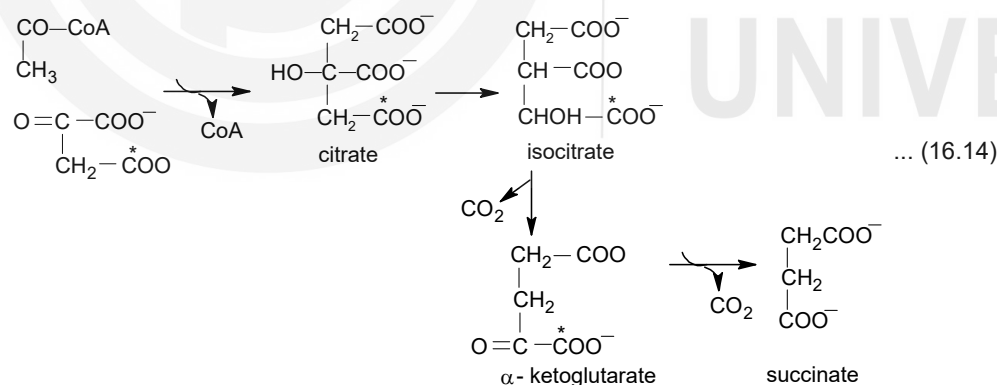
SAQ 2

Tick ✓ the correct answer.

- a) For continuity, the TCA cycle requires the regeneration of:
- pyruvic acid
 - oxaloacetate
 - citrate
 - malate
- b) Before entering the TCA cycle, pyruvate gets converted to:
- Acetyl-CoA
 - Citrate
 - Ethanol
 - Oxaloacetate

16.3.3 Stereochemistry of the TCA Cycle

The stereochemistry of interaction between enzymes and a certain type of substances was generalised by the application of isotopic tracer technique to the TCA cycle. For example, if the carboxyl carbon farthest from the keto group of oxaloacetate is labelled with ^{14}C , the entire radioactivity is found to be present in α -ketoglutarate and is recovered as CO_2 in the next step, i.e, in the reaction of Eq. 16.11. The resulting succinate (Eq. 16.12) is completely devoid of any radioactivity. These results can be explained only if the sequence of reactions from citrate to succinate proceeds as depicted below:



You would see that in the symmetrical citrate molecule, the two $\text{CH}_2\text{-COO}^-$ groups are chemically identical. There should have been equal changes of either one of them being involved in the conversion of citrate into isocitrate. If that were so, only half of the radioactivity should have been recovered as CO_2 and the remaining half should have been present in succinate. The experimental observation that the entire amount of radioactivity is recovered as CO_2 suggests that the enzyme aconitase somehow distinguishes between the two chemically identical $\text{CH}_2\text{-COO}^-$ groups. Ogston pointed out that this was possible if three groups of the substrate were to be bound to three specific sites, or groups, of the enzyme as explained in Fig. 16.3.

Compounds, like citrate, having the general formula $CXYH_2$ are referred to as prochiral which can be transformed into chiral molecules, e.g., $CXYZH$, in a single step. Two isomeric chiral molecules can be obtained depending on the specific H atom which is replaced by Z. In the enzyme-substrate complex of the type depicted in Fig. 16.3, the two CH_2-COO^- groups are no longer chemically identical because one of them is bound to the enzyme and the other is exposed. They cannot interchange their positions because of the specific binding of the other groups, namely, OH and COO^- , to their respective sites. Many other cases of distinction between chemically identical in prochiral molecules encountered in biochemical reactions.

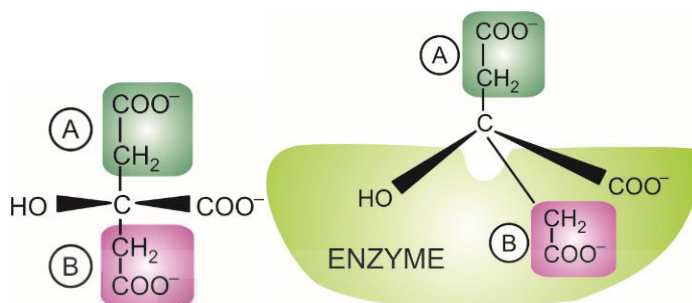
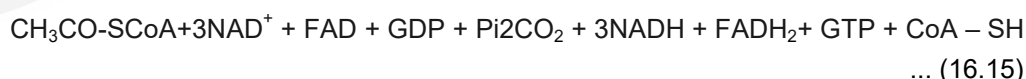


Fig.16.3: Postulated structure of citrate-aconitase complex.

As you can observe in Fig. 16.3, the enzyme active site has specific complementary sites for binding OH, COO^- and CH_2-COO^- groups. The OH and COO^- groups cannot interchange their positions. Consequently, only the CH_2-COO^- marked B in the structure of citrate can bind to the active site. The CH_2-COO^- group marked A cannot be bound in the given geometry of the active site, because it will displace the OH and COO^- groups from their respective specific sites.

16.3.4 Stoichiometry and Energetics of TCA Cycle

From Fig. 16.2 or by summing up the reactions of TCA cycle, we can arrive at its stoichiometry which is given below:



In this equation, water molecules and protons have been left out. It was shown in Unit 14, that oxidation of each NADH molecule via the electron transport chain is coupled to the synthesis of three molecules of ATP. Similarly, oxidation of each $FADH_2$ molecule causes the synthesis of two ATP molecules. Further, GTP is equivalent to and can be converted into ATP by the following reaction.



Thus, a total of $(3 \times 3) + (2 \times 1) + 1 = 12$ ATP molecules are synthesised for the oxidation of the 2-carbon acetyl moiety of acetyl-CoA via the TCA cycle. If we consider the complete oxidation of one molecule of glucose via glycolysis. Conversion of the resultant two pyruvate molecules into two molecules of acetyl-CoA and their oxidation through the TCA cycle, the energy balance sheet works out as follows:

Reaction	ATP	Reduced coenzymes
Glucose $\rightarrow$ 2Pyruvate (glycolysis)	2	2NADH
2Pyruvate + 2CoA $\rightarrow$ 2Acetyl – CoA + 2CO ₂ (pyruvate dehydrogenase complex)	-	2NADH
2Acetyl – CoA $\rightarrow$ 4CO ₂ + 2CoA (TCA cycle)	2	6 NADH + 2 FADH ₂
Total: Glucose $\rightarrow$ 6CO ₂	4	10NADH + 2FADH ₂

Considering that each NADH is equivalent to 3 ATP and each FADH₂ is equivalent to two ATP, the total generation of ATP works out to be 38 molecules of ATP for each molecule of glucose oxidised. It may be noted that only two ATP molecules are formed during glycolysis and the remaining thirty six are produced on the oxidation of two molecules of pyruvate. Further, major part of ATP synthesis (about 90%), takes place on the reoxidation of reduced coenzymes by the electron transport chain.

The overall efficiency of energy conservation, i.e., utilisation for ATP synthesis, is very high. Complete oxidation of glucose under standard conditions liberates 3085 kJ of free energy per mole. Free energy utilised for the synthesis of ATP from ADP and P_i under standard conditions works out to be equal to $38 \times 30.5 = 1159$ kJ. This corresponds to $1159 \times 100/3085 \sim 37.5\%$ efficiency. The actual efficiency is much higher, because the conditions prevailing in the cell are different from standard conditions (see Unit 8).

You may recall that if glucose unit for oxidation is derived from glycogen, the number of ATP molecules synthesised during glycolysis will rise to three. Consequently, complete oxidation of glucose unit of glycogen will give rise to the synthesis of thirty nine ATP molecules.

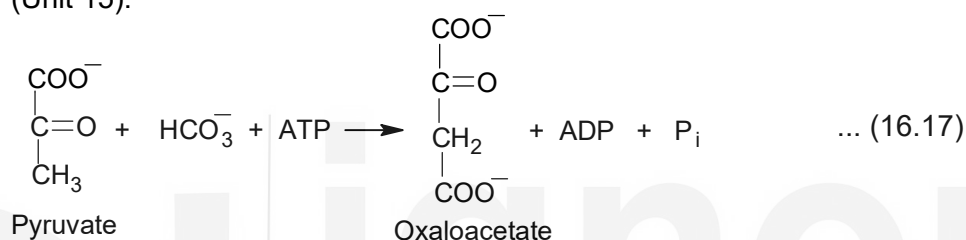
Having studied the steps, reaction, the stoichiometry and energetic of TCA cycle, let us try to understand its significance in metabolism.

16.3.5 Central Role of TCA Cycle

Besides bringing about the oxidation of acetyl moiety of acetyl-CoA to carbon dioxide, a catabolic function, TCA cycle also provides precursors for the biosynthesis of several other compounds, i.e., it performs as anabolic function. Therefore, it is often referred to as an **amphibolic** pathway. Let us study how this cycle is able to accomplish this.

It was mentioned in Unit 15 that catabolic, i.e., biodegradative, pathways are convergent. Some common metabolites are formed from a variety of starting materials, or nutrients, and these metabolites are further degraded by a common pathway. You saw the formation of pyruvate from carbohydrates and amino acids and that of acetyl-CoA from pyruvate and fatty acid. The acetyl-CoA is oxidised via the TCA cycle. Several amino acids, related to glutamate, and aspartate, are metabolised by their conversion into α -ketoglutarate and oxaloacetate, respectively, both of which are intermediates of this cycle. Some other intermediates of the cycle, e.g., succinate, are precursors of other biomolecules. These conversions illustrate the convergent nature of catabolism and the central role played by the TCA cycle.

Oxaloacetate and other metabolites of the TCA cycle have a “catalytic” role in overall reaction (Eq. 16.15) in the sense that they are regenerated on the completion of the cycle. Their concentrations must be maintained at optimum levels to ensure an efficient running of the cycle and, therefore, of the metabolic breakdown of carbohydrates, fats and proteins. When some metabolites of this cycle are drawn as precursors for the synthesis of other biomolecules, their concentrations decrease. As a consequence, the concentrations of all the metabolites of the cycle are decreased because of the cyclic nature of the sequence of reactions. This leads to slowing down of the cycle and accumulation of acetyl-CoA and a decrease in the rate of ATP generation. It becomes necessary, therefore, to replenish some of the intermediates. One such replenishment reaction, significant in mammals, is the formation of oxaloacetate from pyruvate, CO_2 and ATP catalysed by pyruvate carboxylase (Eq. 16.17). Recall that this reaction is also involved in the conversion of pyruvate into phosphoenolpyruvate during gluconeogenesis (Unit 15).



Replenishment of any one intermediate of the cycle will increase the concentrations of all the metabolites of the cycle as explained above. The process of replenishment is called **anaplerosis** (meaning to “fill up”).

Not that the major source of pyruvate in animal body is the glycolytic breakdown of carbohydrates. Availability of pyruvate is critical for anaplerosis and, therefore, for the efficient running of TCA cycle. As we will study later in Unit 17, fats are degraded to acetyl-CoA and the latter is normally metabolised via the TCA cycle. Thus, carbohydrates indirectly help in the catabolism or “burning” of fats in the body cells. This explains the necessity of including a certain amount of carbohydrates in the diets of persons who are trying to lose weight by “burning” some of the body fats. It has been estimated that about one-fifth of the calorie, or energy, requirement of such persons must be provided as dietary carbohydrates. This ensures adequate supply of pyruvate which helps maintain optimum levels of TCA cycle intermediates. The metabolic role of TCA cycle can be depicted as given in Fig. 16.4.

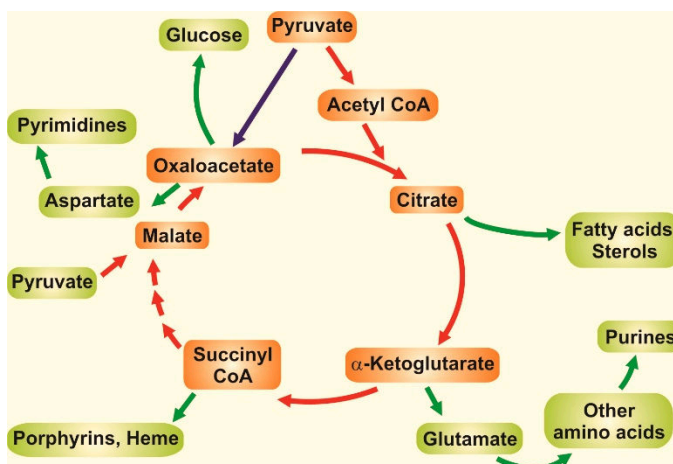


Fig. 16.4: Diagram depicting the central metabolic role of TCA cycle.

SAQ 3

Complete the statement by choosing a correct answer:

- a) The number of ATP molecules produced by the oxidation of one molecule of pyruvate via TCA cycle is
- 2
 - 4
 - 10
 - 15
- b) The TCA cycle is said to have a central role in metabolism because:
- It has a central place in the metabolism.
 - It has a cyclic nature.
 - It helps both in synthesising biomolecules (anabolism) and in catabolic pathways.
 - Several amino acids are metabolised through this cycle.

16.4 ELECTRON TRANSPORT CHAIN

The steps of electron transport chain are shown in Fig. 16.5 in which the sites of ATP synthesis are indicated. The reactions of Fig. 16.5 and the coupled ATP synthesis are brought about by the enzymes located on the inner membrane of mitochondria (see Unit 1 for the structure of mitochondria). With the exception of coenzyme Q, all the other membranes of this chain are associated with protein.

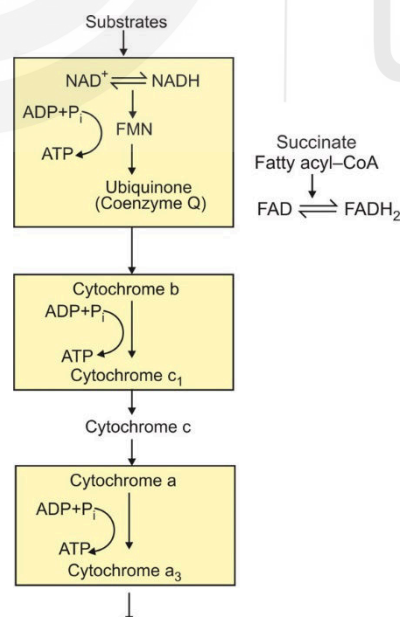


Fig. 16.5: Electron transport chain showing the direction of flow of electrons and the sites of coupling with ATP synthesis (in boxes).

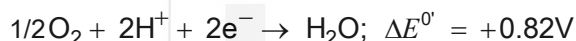
In the first step of the electron transport chain NAD^+ is reduced to NADH by a dehydrogenase which removes two hydrogen atoms from its substrate. NADH

The requirement for oxygen in electron transport process results in its another name, the **respiratory chain**.

carries a proton and a hydride ion and transfers them to an enzyme complex, NADH dehydrogenase. This complex has a cofactor, i.e., flavin mononucleotide (FMN) which gets converted to FMNH₂ by accepting the two hydrogen atoms. The hydrogen atoms are next transferred to ubiquinone known as coenzyme Q. The remaining members of the chain are cytochromes. Electrons are passed down the chain to cytochromes b, c₁, c, a and a₃. Cytochrome a₃ is the only cytochrome that can react directly with molecular oxygen. It is at this site that the transported electrons, molecular oxygen and free protons are brought together to produce water.

You studied earlier that in oxidation-reducing reactions, the standard free energy change and, therefore, the equilibrium constant of the reaction can be calculated from the difference in the standard reduction potentials of the oxidant-reductant pairs involved. The overall standard free energy change in the oxidation of NADH by molecular oxygen (O₂) can be calculated from the standard reduction potentials of O₂/H₂O and NAD⁺/NADH pairs as per the following reactions.

E° indicates standard potential at pH 7, i.e., at biochemical standard state.



Subtracting and rearranging the above reactions, we get,



Substituting the values of n (=2) and ΔE° (1.14 volts) in Eq. 14.6 we can calculate the value of ΔG° for the oxidation of NADH by O₂.

$$\Delta G^{\circ} = -2 \times 96.48 \times 1.14 = -219.97 \text{ kJ mol}^{-1}$$

Note a large drop in the standard free energy of the system. If harnessed properly, this free energy can be utilised to drive the synthesis of several molecules of ATP ($\Delta G^{\circ} = +30.5 \text{ kJ mol}^{-1}$) as indeed it happens in various steps of electron transport in the living cell, Fig. 16.4.

The above discussion shows that thermodynamically the synthesis of ATP may be coupled to specific steps of electron chain where, ΔE° may be adequate to provide the necessary free energy input. However, it does not explain as to how the coupling is brought about (as is the case with all thermodynamic calculations).

16.4.1 Mechanism of Oxidative Phosphorylation–Chemiosmotic Hypothesis

Mechanism of coupling of electron transport and ATP synthesis has been a subject of research for many years. A suggestion was made that an intermediate with a high phosphate group transfer potential might be formed during the electron transport and that it might then transfer its phosphate group to ADP. The reaction between ADP and the postulated intermediate would be similar to the reactions of ADP with DPG and PEP (Eq. 15.7 and 15.10). However, all experiment designed to detect such an intermediate ended in failures. No such intermediate has been detected so far.

It has been found that a complex enzyme assembly located in the inner mitochondrial membrane is responsible for the synthesis of ATP. This enzyme was first discovered through its catalytic action in bringing about the hydrolysis of ATP, i.e., the reverse of synthesis. Accordingly, it was named as an **ATPase**. Specifically, it is called H^+ -ATPase or F_1F_0 -ATPase. These names were assigned, respectively, on the basis of the role of protons in its activity and the subunit structure of the enzyme. The physiological role of this enzyme is to catalyse the synthesis of ATP from ADP and P_i . Therefore, it is also referred to as **ATP-synthase**.

A novel suggestion was made by Peter Mitchell in 1961 to explain the role of ATP-synthase and the coupling of electron transport to ATP synthesis. According to him, the free energy released in specific steps of electron transport is utilised to “pump protons from the mitochondrial matrix, i.e., from inside the inner membrane, to the inner membrane space, i.e., to outside the inner membrane, as represented in Fig. 16.6.

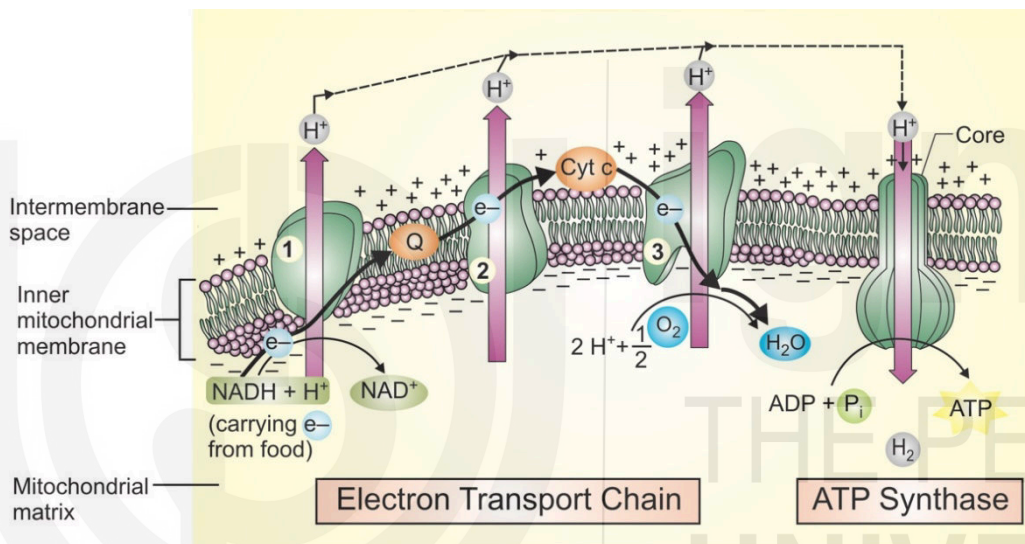


Fig. 16.6: Oxidative phosphorylation on the mitochondrial membrane. Electron transport brings about “pumping” of protons from the matrix into the inner membrane space. As the protons “flow” down the gradient through ATP synthase, these drive the endergonic synthesis of ATP.

The unidirectional proton pumping is a consequence of the unique vectorial, i.e., directional, location of the electron transport proteins across the membrane. Since the inner mitochondrial membrane is not freely permeable to protons, this results in storage of some free energy in the form of a pH gradient with the outside becoming more acidic than the inside and a membrane potential where the outside has more positive charges than the inside. Together, the two gradients constitute a **proton motive force**. The excess protons “flow” back into the matrix through the ATP-synthase. This enzyme is located in the membrane and provides a channel for the flow of protons through an otherwise impermeable membrane. The free energy stored in the proton motive force drives the synthesis of ATP from ADP and P_i .

Mitchell’s hypothesis has been referred to as the **chemiosmotic hypothesis**. This simple and elegant hypothesis is supported by a variety of experimental results which are as follows:

- It has been demonstrated that a proton gradient is established across the inner mitochondrial membrane during electron transport.
- ATP synthesis is found to take place when a pH gradient is imposed on the inner mitochondrial membrane even when no electron is taking place.
- According to this hypothesis, a closed compartment (or a vesicle) is necessary for the coupling of electron transport and ATP synthesis because otherwise the protons can move freely on both sides and no proton gradient can be established. Consistent with this prediction, no ATP synthesis is observed when electron transport is catalysed by fragments of the inner membrane. The liberated free energy is dissipated as heat.

Some substances help transport protons by providing an alternative proton channel through the membrane. Such substances do not allow any proton gradient to be established. In their presence, electron transport proceeds unhindered but ATP is not synthesised. Presumably the proton flow through the channels provided by these compounds is more facile than that through ATP-synthase. Such compounds are referred to as **uncouplers**. In their presence also, the free energy released on electron transport is dissipated as heat. 2, 4-Dinitrophenol is a commonly employed uncoupler.

SAQ 4

Complete the following sentence with the correct answer.

In oxidative phosphorylation, dinitrophenol,

- a) acts to inhibit the electron transport.
 - b) allows electron transport to proceed without ATP synthesis.
 - c) inhibits the cytochrome action.
 - d) helps in the synthesis of ATP during photosynthesis.
-

16.5 SUMMARY

Let us summarise what we have discussed in this unit about the TCA cycle and metabolism of fatty acids.

Pyruvate formed on glycolysis or from alanine is converted into acetyl-CoA. This oxidative decarboxylation of pyruvate and condensation of the resultant acetyl moiety pyruvate dehydrogenase complex. Acetyl-CoA is also formed by the breakdown of fatty acids the acetyl moiety of acetyl-CoA is oxidised completely to carbon dioxide via a cyclic sequence of reactions, called Kreb's tricarboxylic acid (TCA) cycle or citric acid cycle. Acetyl moiety of acetyl-CoA is transferred to oxaloacetate to give rise to citrate. The latter undergoes a series of reactions in which two molecules of carbon dioxide are lost and oxaloacetate is regenerated. It then combines with another molecule of acetyl-CoA and the cycle goes on. TCA cycle occupies a central place in metabolism because it helps oxidise the metabolites formed from carbohydrates, amino acids and fats. In addition, some metabolites of the cycle serve as precursors in the synthesis of porphyrins and some amino

acids. The concentrations of the metabolites of TCA cycle are not allowed to be depleted and are maintained at optimum levels by formation of oxaloacetate by carboxylation of pyruvate (anaplerosis).

The reduced coenzymes formed in the tricarboxylic acid cycle and earlier steps are reoxidised, and thereby regenerated via the electron transport chain with the net synthesis of ATP from ADP and phosphate ion. Calculations show that about 90% of the free energy transduction in carbohydrate catabolism (for the formation of ATP) takes place in the TCA cycle and electron transport chain.

Oils and fats are hydrolysed by various lipases to give glycerol and free fatty acids. Glycerol is metabolised via glycolysis. Each fatty acid is converted into the corresponding acyl-CoA which is transported from the cytoplasm to the mitochondrial matrix. Here through a sequence of reactions called β -oxidation, the entire aliphatic chain of acyl-CoA is broken down to several acetyl-CoA molecules. The acetyl group of the latter is then oxidised via TCA cycle. Biosynthesis of fatty acids utilises acetyl-CoA as the precursor and takes place in cytoplasm. It is catalysed by an assembly of several enzymes called fatty acid synthase. The latter contains a centrally located acyl carrier protein (ACP) surrounded by the enzymes which catalyse the various steps. Synthesis of palmitate via a sequence of reactions of fatty acid biosynthesis has been described in the unit. The unsaturated fatty acids and higher saturated fatty acid will derive from palmitate. Much more energy is "spent" during biosynthesis of palmitate from eight molecule of acetyl-CoA than that which is "recovered" on breakdown of palmitate to eight acetyl-CoA molecules. This is generally true for any metabolism.

16.6 TERMINAL QUESTIONS

- The following radioactively labelled compounds (position of label shown by an asterisk) are catabolised with a cell extract containing all the enzymes and cofactors of pyruvate dehydrogenase complex and TCA cycle. Predict the fate of the label (^{14}C), assuming only one turn of the TCA cycle.
(a) $\overset{*}{\text{C}}\text{H}_3\text{-CO-COO}^-$ (b) $\text{CH}_3\text{-}\overset{*}{\text{C}}\text{O-COO}^-$ (c) $\text{CH}_3\text{-CO-}\overset{*}{\text{C}}\text{OO}^-$
(d) $\overset{*}{\text{C}}\text{H}_3\text{-CO-SCoA}$ (e) $\text{CH}_3\text{-}\overset{*}{\text{C}}\text{O-SCoA}$
- Glucose was catabolised to carbon dioxide and water by minced pigeon breast muscle via glycolysis, TCA cycle and electron transport chain. The overall rate of metabolism was measured in terms of the rate of oxygen consumption. It was found that (i) addition of small amounts of oxaloacetate stimulated the rate of oxygen consumption and (ii) the increase in oxygen consumption was several times larger than that required to oxidise oxaloacetate to carbon dioxide and water. Explain (i) and (ii).
- α -Ketoglutarate is required for the biosynthesis of several amino acids. From your study of this unit, write a sequence of known enzymatic reactions for a net synthesis for α -ketoglutarate without depleting the concentration of any metabolite of tricarboxylic acid cycle.

4. Given below are two statements. Explain whether they are true or false giving reasons.
- The methyl group of each acetyl-CoA molecule entering the TCA cycle is derived from the methyl group of pyruvate.
 - Malate cannot be converted to fumarate because the TCA cycle is unidirectional.

16.7 ANSWERS

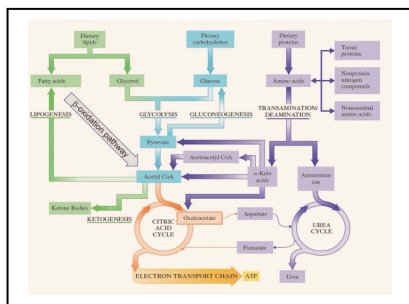
Self Assessment Questions

- x
 - ✓
 - ✓
 - x
- ii)
 - i)
- iv)
 - iii)
- b)

Terminal Questions

- Label is distributed in C-2 and C-3 of oxaloacetate.
 - Label is distributed in C-1 and C-4 of oxaloacetate.
 - Label is lost as CO_2 in the conversion of pyruvate to acetyl-CoA.
 - Same as in (a)
 - Same as in (b)
- Oxaloacetate has a "catalytic" role in the TCA cycle, because it is regenerated at the end of the cycle and is used over and over again. Increasing the concentration of oxaloacetate increases the rate of formation of citrate, as observed with all enzymes at higher substrate concentrations. This increases the rate of reactions of tricarboxylic acid cycle, which is reflected in the enhanced rate of oxygen consumption.
 - Since oxaloacetate is regenerated, it is used over and over again for catabolism of more and more of glucose. Consequently, much larger amount of oxygen is consumed than that of required for the oxidation of the added oxaloacetate only.
- Pyruvate $\xrightarrow[\text{ATP} \rightarrow \text{ADP} + \text{P}_i]{\text{CO}_2}$ Oxaloacetate $\xrightarrow[\text{CoA}]{\text{Acetyl-CoA}}$ Citrate $\rightarrow$ Isocitrate $\xrightarrow[\text{NADH} + \text{CO}_2]{\text{NAD}^+}$ α -Ketoglutarate
- False. The acetyl-CoA is also produced from β -oxidation of long chain fatty acids or from amino acid metabolism.
 - False. Malate can easily be dehydrated to fumarate by reversal of fumarase reaction.

UNIT 17



METABOLISM OF LIPIDS AND PROTEINS

Structure

17.1	Introduction	Comparison of Biosynthesis and Degradation of Fatty Acids
	Expected Learning Outcomes	
17.2	Degradation of Fatty Acids	17.4 Catabolism of Amino Acids
	Conversion of Fatty acids into Acyl-CoA	Removal of Amino Group from Amino Acids
	Energetics of Oxidation of Acyl-CoA	Oxidation of the Carbon Skeleton
	Energetics of Oxidation of Fatty Acids	17.5 Interrelationships Among Metabolic Pathways
17.3	Biosynthesis of Fatty Acids	17.6 Summary
		17.7 Terminal Questions
		17.8 Answers

17.1 INTRODUCTION

In the previous units, you studied the metabolic breakdown of glucose and other sugars to pyruvate and the central role of the tricarboxylic acid cycle. Our body needs various lipids and proteins apart from carbohydrates for biological functions. You have studied that to ensure proper body functions, most of the lipids and proteins are synthesized in our body, mostly from carbohydrates and amino acids. The body also derives energy from dietary lipids, 90% of which are triacylglycerols. Essential fatty acids are the only lipids that are not synthesised in the body. The body maintains a reservoir of lipids as the source of energy for emergency situations such as starvation when dietary fuels are not available. During the normal growth and physiological changes in our body, lipids and proteins also undergo changes like any other substances.

In this unit you will study the metabolism of fats and proteins. The fatty acids produced from fat molecules degrade on oxidation to form acyl-CoA, finally entering the Krebs cycle. The energetics involved in these reactions justify fats as rich sources of energy. The biosynthesis of fatty acids and its

comparison with the degradation is also described here. You have learnt the process of biosynthesis of proteins in Unit 13 of the previous block. The catabolism of amino acids produced by the breakdown of proteins and urea cycle is described in this unit. An interrelationship amongst all the metabolisms is also discussed in brief. 1

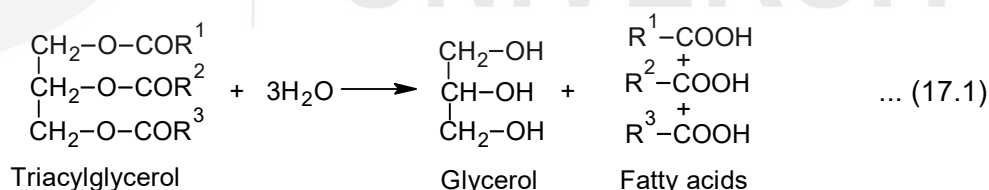
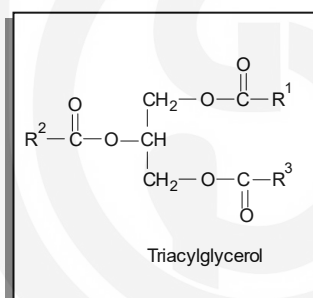
Expected Learning Outcomes

After studying this unit and having the experiments performed, you should be able to:

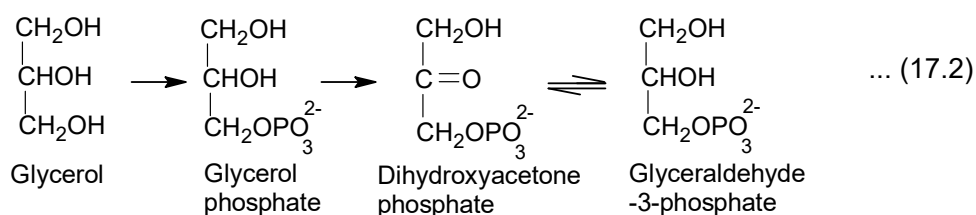
- ❖ describe the reactions involved in conversion of fatty acids to acyl-CoA as part of fatty acid degradation;
- ❖ explain the energetics of degradation of fatty acids;
- ❖ describe the process of biosynthesis of fatty acids;
- ❖ compare the degradation and biosynthesis of fatty acids;
- ❖ describe the urea cycle as part of the catabolism of proteins; and
- ❖ justify the interrelationship amongst various metabolic pathways.

17.2 DEGRADATION OF FATTY ACIDS

You know that oils and fats are major energy reserves of living beings including humans. You have studied their structure in Unit 10 of Block 4. They are esters of glycerol with fatty acids, i.e., they are triacylglycerols. The three acids, namely, R^1 -COOH, R^2 -COOH and R^3 -COOH, may be identical or different. Major differences between the fatty acids lie in (i) the chain length of the alkyl groups. R^1 , R^2 and R^3 and (ii) the presence and position of double bonds in these groups. The triacylglycerols are hydrolysed in the cytoplasm, Eq. 17.1. The reaction, which takes place in more than one steps, is catalysed by various lipases, secreted into the intestinal lumen.



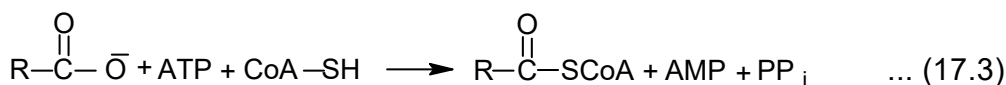
Glycerol is phosphorylated and oxidised to dihydroxyacetone phosphate which is isomerised to glyceraldehyde-3-phosphate, Eq. 17.2 and then degraded via the glycolytic pathway (Unit 15), TCA cycle and finally the electron transport chain (Unit 16).



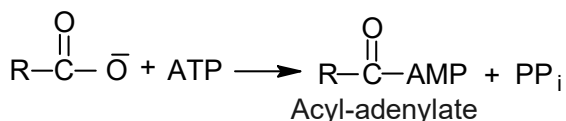
Each fatty acid is converted into the corresponding acyl-CoA and transported into the mitochondrial matrix where it is degraded. In this section, we will discuss the metabolic breakdown of saturated fatty acids only.

17.2.1 Conversion of Fatty acids into Acyl-CoA

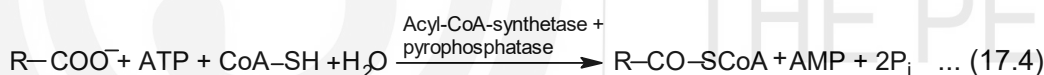
Free energy stored in pyrophosphate linkage of ATP is utilised to drive the formation of thiol-ester linkage between the carboxyl group of the fatty acid and SH group of coenzyme-A (CoA-SH; Eq. 17.3). The reaction is catalysed by **acyl-CoA synthetase**, also called fatty acid **thiokinase**.



An identical reaction was discussed in Unit 14 as part of esterification of acids. It may be recalled that the above reaction proceeds in two steps. In the first step the fatty acid and ATP react at the enzyme active site with the formation of enzyme-bound acyl-adenylate (also called acyl-AMP). In the next step, acyl-adenylate reacts with CoA-SH with the formation of acyl-CoA and AMP. The structure of acyl-adenylate is given in Unit 14, Section 14.5.1.



As mentioned in Unit 14, the equilibrium constant of the above reaction is close to unity because the ΔG^0 values for the hydrolysis of ATP ($\rightarrow \text{AMP} + \text{PP}_i$) and of acyl-CoA ($\rightarrow \text{acylate} + \text{CoA-SH}$) are approximately equal (Unit 14, Table 14.1). The reaction is driven to completion by the removal of pyrophosphate ion (PP_i) which is hydrolysed to two phosphate ions in the presence of **pyrophosphatase**. Thus, the total reaction can be represented as:

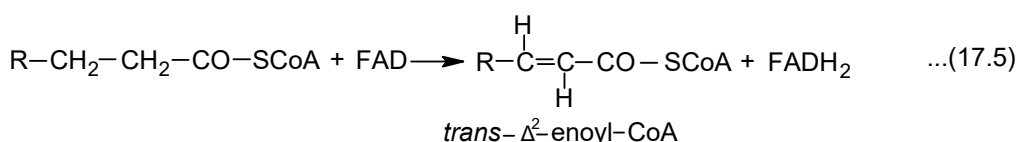


In this reaction, two high energy pyrophosphate linkage have been broken and only one high energy thiol-ester linkage established. The extra energy is utilised to drive the reaction to completion in the desired direction. As mentioned earlier, this is a recurring endergonic reaction to completion (also see gluconeogenesis, Unit 15).

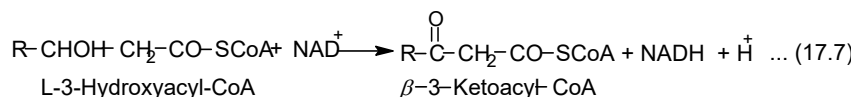
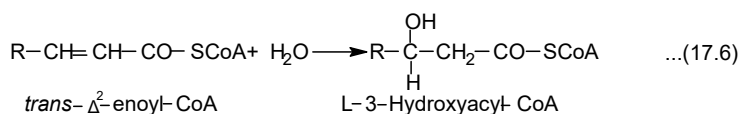
17.2.2 Oxidative Degradation of Acyl-CoA

Acyl-CoA undergoes a sequence of four reactions, namely an **oxidation**, **hydration** (i.e., addition of a water molecule), another oxidation and a **thiolytic cleavage**. As a result of these reactions, it loses a two-carbon fragment in the form of acetyl-CoA. The residual acyl-CoA, which is now shorter by two carbon atoms, undergoes the same sequence of reactions and this is repeated till the entire long chain fatty acyl-CoA has been broken down to several molecules of acetyl-CoA.

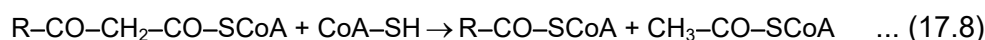
The first oxidation reaction is catalysed by an **acyl-CoA dehydrogenase** with FAD as the hydrogen acceptor.



The resulting *trans*-2, 3-unsaturated enoyl-CoA adds a molecule of water in the presence of **enoyl-CoA hydratase**. The addition of water is stereospecific giving rise to L-3-hydroxyacyl-CoA, which is oxidised in the presence of **L-3-hydroxy-acyl-CoA dehydrogenase** with NAD^+ as the coenzyme.



In the final reaction of this series, β -ketoacyl-CoA reacts with CoA-SH in the presence of **β -ketothiolase** (also called thiolase) giving rise to acetyl-CoA and another acyl-CoA which is two carbons shorter than the starting one.



The latter can again undergo the reactions like those of Eq. 17.5 to 17.8 and the process is repeated till the entire aliphatic chain is broken down to several molecule of acetyl-CoA. Note that because of reactions of Eq. 17.5 to 17.7 the β -methylene group of acyl-CoA is oxidised to a keto group therefore, the above pathway is referred to as the **β -oxidation pathway**. A summary of the reactions of this pathway is given in Fig. 17.1.

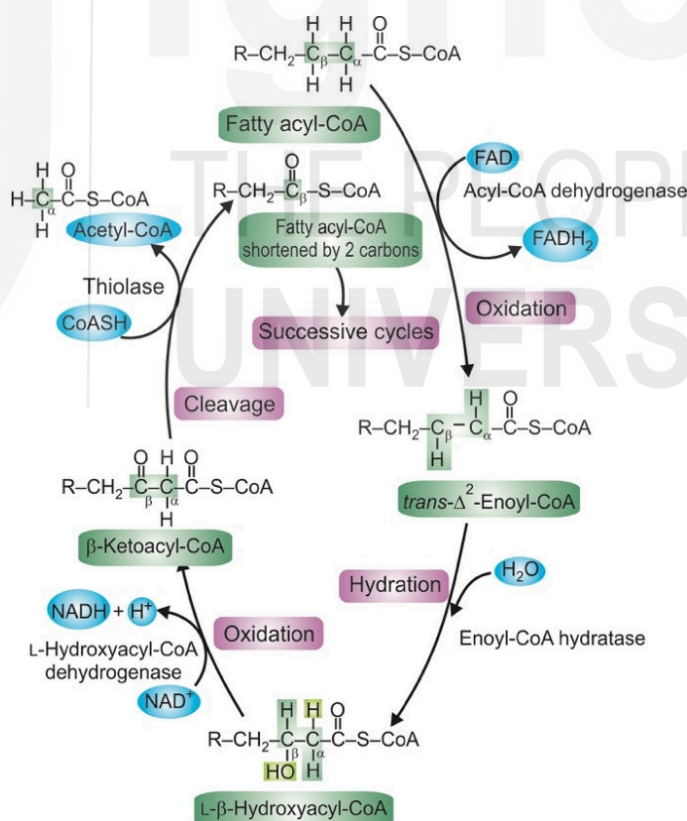


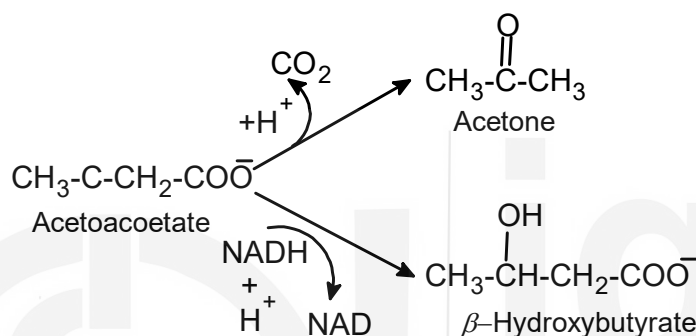
Fig. 17.1: Summary of the reactions of β -oxidation pathway of fatty acids.

Similarity of the reactions of Eq. 17.5 to 17.7 to last three reactions of TCA cycle, namely succinate $\longrightarrow$ fumarate $\longrightarrow$ malate $\longrightarrow$ oxaloacetate, is remarkable. It appears that similar reactions are utilised by nature in different metabolic pathways when the overall required chemical transformations are identical.

As a consequence of the reactions given in Eq. 17.5 to 17.8, the acyl-CoA molecule loses a two-carbon fragment at a time. It is noteworthy that most of the fatty acids present in oils and fats have an even number of carbon atoms. Thus, the entire carbon chain is broken down to acetyl-CoA molecule. Fatty acids with odd number of carbon atoms are less commonly found. In those cases, a three-carbon fragment is left behind in the form of propionyl-CoA. The latter is carboxylated to succinyl-CoA which then enters the TCA cycle.

Ketone Bodies

Under certain metabolic conditions such as starvation, associated with a high rate of fatty acid oxidation, the liver produces considerable quantities of acetoacetate and β -hydroxybutyrate. Acetoacetate undergoes spontaneous decarboxylation to yield acetone. These substances are collectively called **ketone bodies**. The structures are as given below.



Ketone body production is characteristic of starvation and diabetes mellitus. Presence of higher-than-normal quantities of ketone bodies in blood and urine constitute **ketonemia** and **ketonuria**, respectively and the overall condition is called **ketosis**.

Before you study about the energetics of the degradation of fatty acids, attempt the following SAQ.

SAQ 1

State whether true or false.

- The first oxidation reaction of acyl-CoA is catalysed by an acyl-CoA dehydrogenase.
- Dihydroxyacetone phosphate is an isomer of glyceraldehyde-3-phosphate.
- Presence of higher than normal quantities of ketone bodies in blood is ketonuria.
- Fatty acid degradation is also called the β -oxidation.

17.2.3 Energetics of the Degradation of Fatty Acids

The oxidation of fatty acids yields large amount of ATP. Let us understand this by taking the example of a common fatty acid found in many oils and fats,

namely **palmitic acid**. Its molecular formula is $C_{16}H_{32}O_2$ (i.e., $C_{15}H_{31}COOH$). Two high-energy pyrophosphate linkages are utilised to convert it into its active form, namely, palmitoyl-CoA or $C_{15}H_{31}-CO-SCoA$. Since a two-carbon atom fragment is removed in each cycle of β -oxidation pathway, seven such cycles will be required for the complete breakdown of palmitoyl-CoA giving rise to eight molecules of acetyl-CoA. The reactions of the degradation of palmitic acid are given in Fig. 17.2 (a) and (b). The stoichiometry of the β -oxidation breakdown of palmitoyl-CoA can be written as given below leaving out H^+ and water molecules.

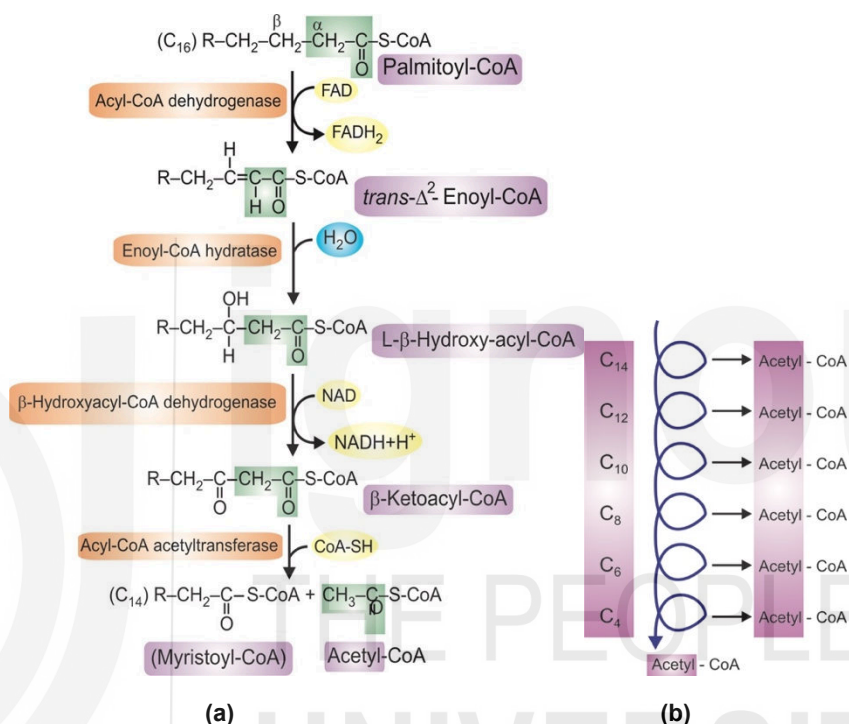
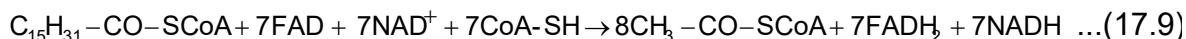


Fig. 17.2: Summary of the reactions of β -oxidation pathway of palmitic acid
 (a) One sequence of reactions with formation of one acetyl-CoA
 (b) Six more sequences with formation of seven acetyl-CoA

No useful energy is captured directly during the reactions of β -oxidation pathway. The energy transduction takes place only on the oxidation of acetyl-CoA and of the reduced coenzymes. It may be recalled from the previous unit that complete oxidation of the acetyl moiety of one acetyl-CoA molecule results in the net synthesis of twelve molecules of ATP. In addition, two molecules of ATP are synthesised on the oxidation of each NADH molecule. Thus, the total ATP synthesis from the products of the reaction is found to be equal to $(12 \times 8) + (7 \times 2) + (7 \times 3) = 131$ molecules for each molecule of palmitoyl-CoA. Since two energy-rich linkages were "spent" for the formation of palmitoyl-CoA from palmitate, net ATP synthesis will be 129 molecules per molecule of palmitate.

Standard free energy of oxidation of palmitic acid is reported to be equal to $-9780 \text{ kJ mol}^{-1}$. The standard free energy for the synthesis of 129 ATP molecules is found to be equal to $129 \times (+30.5) = 3935 \text{ kJ mol}^{-1}$. Thus, the efficiency of energy transduction based on the **standard free energies** is $3935/9780 = 0.402$ of approximately 40%, which is like that of glycolysis, TCA cycle and oxidative phosphorylation.

It is interesting to compare the energetics of metabolic oxidation of glucose and palmitic acid on mass basis. One mole glucose (molar mass 180), on complete oxidation leads to the synthesis of 38 moles ATP, i.e., 38/180 or 0.211 mole ATP per gram glucose. On the other hand, one mole palmitic acid, molar mass 288, on complete oxidation causes the synthesis of 129 moles ATP corresponding to $129/288 = 0.448$ mole ATP per gram palmitic acid. Note that on the mass basis fatty acid oxidation yields more than twice as many ATP molecules as are formed on the oxidation of carbohydrates. This is also true of the standard free energy of oxidation (or combustion) of fats and carbohydrates (approximately 33.9 and 15.9 kJ g⁻¹, respectively). In terms of the number of ATPs produced per carbon atom, it comes to 38/6 or ~ 6 ATPs per carbon atom from glucose and 129/16 or ~ 8 ATPs per carbon atom for palmitate. The chemical reason for this difference is that most of the carbons in palmitate are in the completely reduced state whereas glucose is partially oxidised, with six oxygens in the molecule.

Having studied in fatty acid catabolism, let us see how fatty acids are synthesised, but before that try to answer the following SAQ.

SAQ 2

Fill in the blank spaces in the following.

- One mole of glucose (molar mass 180), on complete oxidation synthesises _____ moles of ATP.
- One mole of palmitic acid (molar mass 288), on complete oxidation synthesises _____ moles of ATP.

17.3 BIOSYNTHESIS OF FATTY ACIDS

Fatty acid biosynthesis takes place in most living systems. In each case, the starting material is acetyl-CoA. The biosynthesis is not merely a reversal of the β -oxidation pathway, but some new reactions are involved.

The biosynthesis of fatty acids is catalysed by **fatty acid synthase**, which may be multienzyme complex or may carry all the activities on different parts of a single protein. In each case, the reaction steps and the constituent catalytic activities are identical. Also, the growing acyl moiety remains covalently attached to SH group of the acyl carrier protein (ACP). This SH group is part of a phosphopantetheine moiety also forms part of the structure of coenzyme-A, as shown in Fig. 17.3.

Acyl carrier protein carries all the intermediates during fatty acid synthesis.

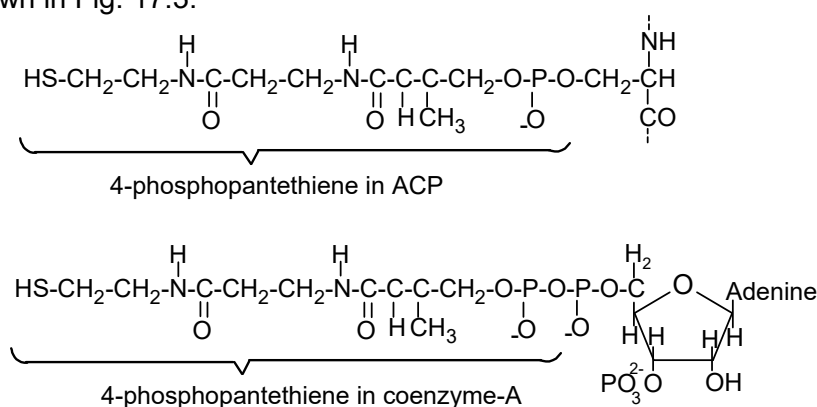
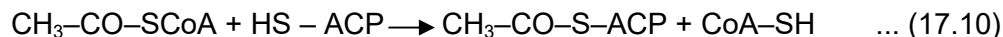
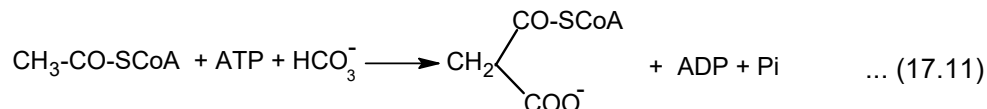


Fig. 17.3: Phosphopantetheinyl moiety in ACP and coenzyme-A

Acetyl group of acetyl-CoA is first transferred to ACP. This reaction is catalysed by **acetyl transacylase**.

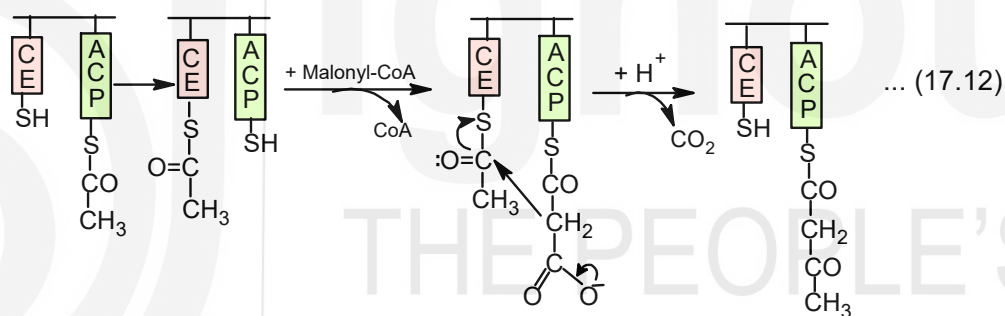


Another molecule of acetyl-CoA is 'activated' by carboxylation. The reaction is catalysed by a biotin-enzyme, **acetyl-CoA carboxylase**, and requires the participation of ATP which is hydrolysed to ADP and P_i as shown below:



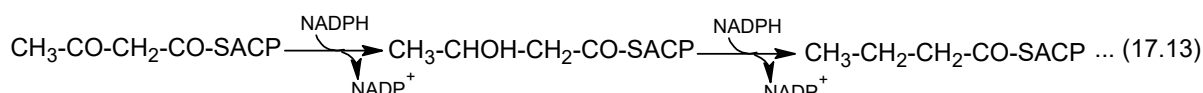
Note the similarity of this reaction to that of formation of oxaloacetate from pyruvate (Unit 16). This is another example where chemically similar reactions are brought about by identical mechanisms even though they require different enzymes which are specific for different substrates.

The acetyl group of acetyl-ACP is transferred to the active site of condensing enzyme and thus liberated SH group of ACP reacts with malonyl-CoA to give rise to malonyl-ACP (Eq. 17.12).



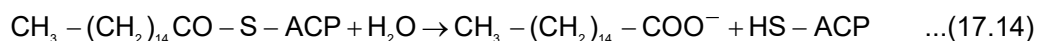
The condensation reaction is rendered irreversible by the loss of CO_2 . Note the significant difference between this reaction and the corresponding reaction in the degradative pathway catalysed by β -**keto-thiolase** (Eq. 17.8).

Acetoacetyl-ACP undergoes successively a reduction, a dehydration and another reduction reaction catalysed by **β -ketoacyl-ACP reductase**, **3-hydroxyacyl-ACP dehydratase** and **enoyl-ACP reductase**, respectively (Eq. 17.13). All these enzyme activities are constituents of fatty acid synthase.



As a result of above reactions, the acyl chain of acetyl-ACP has grown by two carbon atoms to butyryl-ACP. The butyryl group is transferred to the condensing enzyme (as in the first step of Eq. 17.12) and another malonyl group is attached to ATP (as in the second step of Eq. 17.12). This is followed by the condensation (third step of Eq. 17.12) and the reactions of Eq. 17.13. The acyl chain has by now grown to six carbon atoms. This process goes on till palmitoyl-ACP ($\text{C}_{15}\text{H}_{31}\text{-CO-S-ACP}$) is formed. Palmitoyl moiety

does not fulfil the specificity requirements of the condensing enzyme and is, therefore, not accepted by it. Instead, palmitoyl-ACP is a substrate for a hydrolytic enzyme, **thioesterase**, which is also one of the constituent enzymes of fatty acid synthase. Accordingly, it is hydrolysed releasing palmitate and making ACP available for starting synthesis of another fatty acyl chain.



Further elongation of the aliphatic chain or the introduction of double bonds, where required, is brought about by other enzymes. These are not discussed here. Let us now compare the degradation and biosynthesis of fatty acids.

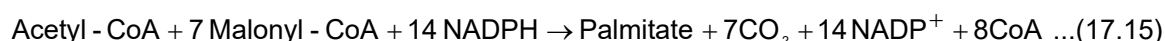
17.3.1 Comparison of Degradation and Biosynthesis of Fatty Acids

As is clear from the above, fatty acid degradation and biosynthesis differ from each other in many respects. The major differences between the two pathways are listed below.

- The site of biosynthesis and organisation of the participating enzymes are different. Degradation of fatty acids takes place in the mitochondrial matrix and peroxisomes while their synthesis take place in the cytosol.
- The enzymes of fatty acid degradation seem to exist independently, but those involved in fatty acid biosynthesis exist either as a strong multienzyme complex, e.g., in bacteria, or are joined together covalently in a single protein, e.g., in higher organisms. In each case it is referred to as **fatty acid synthase**.
- In the breakdown of fatty acids, the acyl group is always attached to SH group of coenzyme-A. In biosynthesis the acyl group remains attached to SH group of the prosthetic group of a specific protein, called **acyl carrier protein** (ACP), which is a constituent of the fatty acid synthase.
- In fatty acid synthesis chain elongation takes place by a sequential addition of two-carbon atom fragment derived from acetyl-CoA, corresponding to the removal of two-carbon fragments in the degradative pathway. However, in the synthetic pathway acetyl-CoA is first "**activated**" by carboxylation to malonyl-CoA at the expense of a high energy linkage of ATP. The condensation reaction between malonyl-CoA and acyl moiety of acyl-ACP is driven to completion by the loss of CO₂.
- In contrast to the use of FAD and NAD⁺ as oxidants in fatty acids degradation, the only reductant employed in the biosynthetic pathway is NADPH.

Let us now compare the energetics of biosynthesis and degradation of fatty acids.

Starting with acetyl-CoA and going through the reactions of Eq. 17.12 and 17.13 seven times each and finally hydrolysis, Eq.17.14, we get



Combining this with Eq. 17.11 on the formation of malonyl-CoA, we get the following reaction for the synthesis of palmitate (a C₁₆ fatty acid) from eight molecules of acetyl-CoA.



For the β -oxidative breakdown of palmitate, you would recall that two high-energy pyrophosphate linkages are required to convert it into palmitoyl-CoA. Subsequently seven cycles of β -oxidation reactions produce eight molecules of acetyl-CoA with concomitant reduction of seven molecules each of NAD⁺. Thus, the total reaction may be written as,



For comparing the energetic of reactions of Eq. 17.16 and 17.17, it is necessary to consider the **equivalents** of high energy linkages involved in the two cases. As explained earlier, each NADH or NADPH molecule is energetically equivalent to three ATP molecules or three high-energy linkages and each FADH₂ molecule is equivalent to two such linkages. Accordingly, the number of high-energy linkages “spent” in converting eight molecules of acetyl-CoA into one of palmitate is equal to $7 + (14 \times 3) = 49$ and the number of such linkage “gained” in the reaction of Eq. 17.17 is equal to $(7 \times 3) + (7 \times 2) - 2 = 33$. This number is different from that given in subsection 17.2.3 because here we are considering the breakdown of palmitate to acetyl-CoA only and not its complete oxidation. Note that much more energy must be “spent” or “pumped in” for performing biosynthetic work than can be “obtained” or “recovered” from the degradation of the synthesised molecule.

The expenditure of extra energy helps to take the biosynthetic reaction to completion. It may be recalled that much more energy is spent in the synthesis of glucose from pyruvate than that recovered on the breakdown of glucose to pyruvate. These two examples help to illustrate a general principle. **In all biosynthetic routes more energy is spent on the synthesis of a compound than what can be recovered by its metabolic breakdown.** The extra energy helps to take the biosynthetic reaction to completion. This may partly explain the necessity of having different pathways for breakdown and biosynthesis.

In the next section you will study the metabolism of amino acids that are obtained by the breakdown of proteins. However, before that answer the following simple question to assess your understanding.

SAQ 3

Fill in the blanks in the following:

- Fatty acid biosynthesis takes place in
 - The cofactor of enzyme acetyl-CoA carboxylase is
 - The synthesis of palmitate via fatty acid synthesis cycle requires.....
 - The formation of fatty acids from malonyl-CoA uses as reductant.
-

17.4 CATABOLISM OF AMINO ACIDS

You know that proteins are constituted of amino acids as the monomeric units and during metabolism amino acids are formed as the degradation products of proteins. Amino acids are not stored in the body. Only 5-10% of the total body energy requirement is met by amino acids. Hence unlike carbohydrates and lipids their role as energy fuels is very limited. It is increased only in prolonged fasting, starvation etc. The amino acids obtained from the degradation of endogenous proteins (from within the body) or from the diet can be used for the biosynthesis of new proteins. The excess of free amino acid which is not immediately required by the body are catabolized i.e., broken down into smaller fragments. The protein metabolism is essentially the catabolism of amino acids. During catabolism, the fate of α -amino group nitrogen is different from the remaining carbon skeleton. Nitrogen is ultimately converted into urea which is a waste product excreted by kidneys. Carbon skeleton is either used for the synthesis of important compounds or for gluconeogenesis and lipogenesis (formation of fats).

The catabolism of amino acids takes place in three stages as given below and depicted schematically in Fig. 17.4.

The three stages are as under:

1. **Removal of the amino group**, leaving the carbon skeleton of the amino acid
2. **Breakdown of the carbon skeleton** to an intermediate of the glycolytic pathway or citric acid cycle, or to acetyl-S-CoA
3. **Oxidation** of these intermediates to carbon dioxide and water with the production of ATP

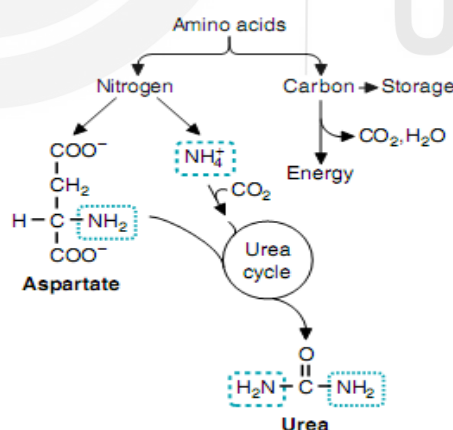


Fig. 17.4: Schematic representation of catabolism of amino acids.

First let us study the process of removal of amino group in the following subsection.

17.4.1 Removal of Amino Group from Amino Acids

The amino groups extracted from amino acids involve the following pathways in the liver:

- the conversion of amino groups from all amino acids into a single product, glutamate, by **transamination**
- the conversion of glutamate into α -ketoglutarate by **oxidative deamination**, releasing NH_4^+
- the conversion of NH_4^+ into the nontoxic compound urea, which the blood carries to the kidneys for excretion.

The removal of α amino group nitrogen usually occurs by two mechanisms viz., **Transamination and Deamination** as mentioned above too. These processes are explained below.

Transamination: It is a chemical reaction in which the amino group from an amino acid is transferred to a keto acid. It is catalysed by an enzyme called **transaminase** or **aminotransferase**. In Fig 17.5 you can see that an amino acid (Amino Acid 1) to be catabolised is converted to **ketoacid**. Another keto acid takes up the amino group and forms an amino acid. This reaction is catabolised by **transaminase**. Pyridoxine (vitamin B6) is used as a co-enzyme and carrier of amino group. It participates in the reaction as **pyridoxal phosphate (PLP)**.

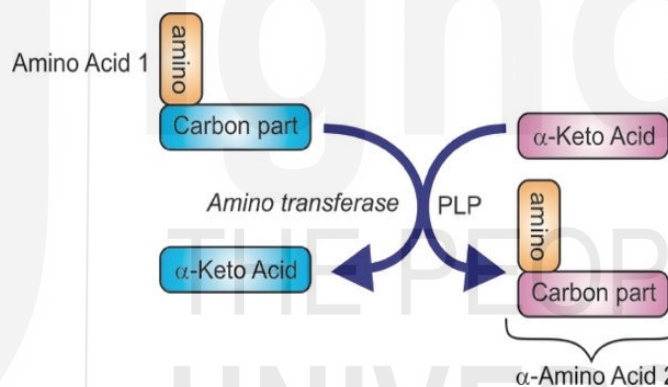


Fig. 17.5: Transfer of amino group by enzyme aminotransferase.

Deamination: In deamination the amino group is directly removed from the amino acid. It occurs in two ways viz., (i) Oxidative deamination and (ii) Non-oxidative deamination

Oxidative deamination is catalysed by two enzymes **glutamate dehydrogenase** and **L-amino acid oxidase**. Glutamate dehydrogenase is the more actively used enzyme. In the breakdown of amino acids for energy, the final acceptor of the α -amino group is α -ketoglutarate, forming glutamate. Glutamate can then undergo oxidative deamination in which it loses its amino group as an ammonium (NH_4^+) ion and is oxidized back to α -ketoglutarate. The latter is ready to accept another amino group.

Nonoxidative deamination is the mechanism of direct removal of amino group by simple hydrolytic cleavage without any cleavage.

The oxidative deamination reaction occurs primarily in liver mitochondria. Most of the NH_4^+ ion formed by oxidative deamination of glutamate is converted to urea and excreted in the urine in a series of reactions known as the **urea cycle**. Let us understand the steps involved in this cycle.

Urea Cycle

The ammonia formed during amino acid catabolism is toxic and cannot be excreted by the body. It gets converted to urea which is less toxic and easily excreted. The process of conversion of ammonia to urea is called the **urea cycle** or the **Krebs Henseleit cycle** which is carried out in the liver. This pathway takes place in both mitochondria and cytoplasm. The reactions of urea cycle are depicted diagrammatically in Fig. 17.6.

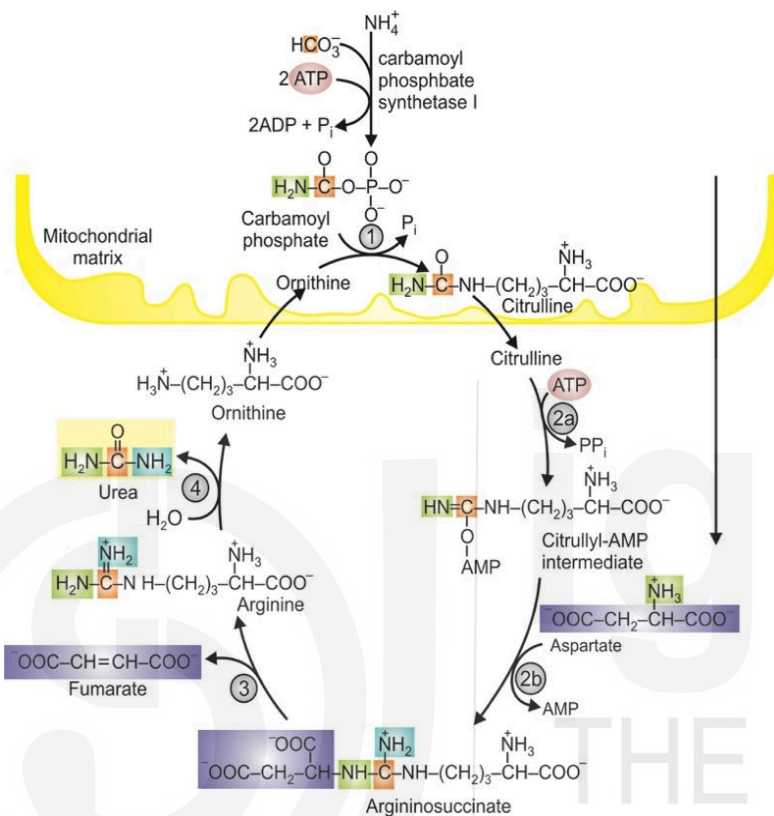


Fig. 17.6: Steps involved in Urea cycle.

The cycle starts in the mitochondria. The step-wise details of the reactions are described as follows.

Step 1: Formation of carbamoyl phosphate: Condensation of ammonium ion with bicarbonate ion results in the formation of carbamoyl phosphate with the help of the enzyme **carbamoyl phosphate synthetase-I** which is present in the liver mitochondria. The reaction requires two ATP molecules.

Step 2a: Formation of citrulline

Carbamoyl phosphate formed in the first step combines with the amino acid, ornithine resulting in the synthesis of citrulline aided by the enzyme **citrulline synthase** or **ornithine transcarbamoylase**. Citrulline is easily permeable to the mitochondrial membrane and hence it diffuses into the cytosol.

Step 2b: Synthesis of Argininosuccinate

Citrulline after diffusing into cytoplasm binds with the amino acid, aspartate to form argininosuccinate. This reaction is catalysed by the enzyme **argininosuccinate synthase**. This marks the entry of the second amino group for ammonia from aspartate.

Step 3: Formation of arginine

Argininosuccinate is cleaved reversibly into arginine and fumarate with help of the enzyme **argininosuccinase**. Fumarate enters the TCA cycle and becomes the linkage between TCA and urea cycle. It is known as **Krebs bicycle**. The linkage between the urea and the Krebs cycle is represented in Fig. 17.7.

Step4: Formation of urea

Enzyme arginase lyses arginine into ornithine and urea. You may note here that though arginine is synthesized in the body it is not available for protein synthesis. Therefore, arginine is known as **semi-essential amino acid**. In other words it is required to be present in the food we consume. Ornithine generated in this step is a potent inhibitor of the enzyme arginase. Ornithine is transported into the mitochondrion to initiate another round of the urea cycle.

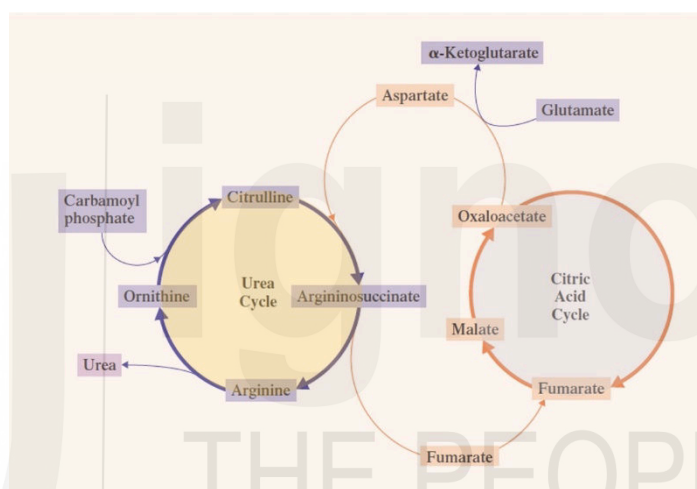


Fig. 17.7: Linkage between the urea cycle and the citric acid cycle.

SAQ 4

Fill in the blanks in the following:

- Oxidative deamination is catalysed by mainly the enzyme _____.
- Transamination is catalysed by an enzyme called _____.
- _____ is the mechanism of direct removal of amino group by simple hydrolytic cleavage without any cleavage.

17.4.2 Oxidation of the Carbon Skeleton

As stated above, in the second stage of amino acid catabolism, the breakdown or oxidation of the carbon skeleton of the deaminated α -keto acid takes place. The α -keto acids of many amino acids are the same as those found in the glycolytic pathway and the citric acid cycle. These keto acids thus replenish the citric acid cycle intermediates. The carbon skeletons end up as either pyruvate or a citric acid cycle intermediate (carboxylation to oxaloacetate) and may be used for energy production or they may be used to synthesise glucose by the gluconeogenesis pathway. Other α -keto acids produced from

amino acids are broken down to acetyl-S-CoA or acetoacetyl-S-CoA or both and thus can enter the citric acid cycle or be converted into ketone bodies. Just like fatty acids that produce acetyl-S-CoA, these keto acids also do not replenish the citric acid cycle intermediates.

The amino acids whose carbon skeletons yield pyruvate or a citric acid cycle intermediate are potentially a source of glucose, are referred to as **glucogenic** amino acids. The amino acids that are converted into ketone bodies through ketogenesis are called **ketogenic** amino acids. Every amino acid has a different degradative pathway. All the pathways lead to intermediates that find their way into the citric acid cycle for complete oxidation to CO_2 and H_2O . Six amino acids are degraded to pyruvate, seven to acetyl-S-CoA, four to α -ketoglutarate, three to succinyl-S-CoA, two to fumarate, and two to oxaloacetate. The fate of the carbon cycle is given in Fig. 17.8.

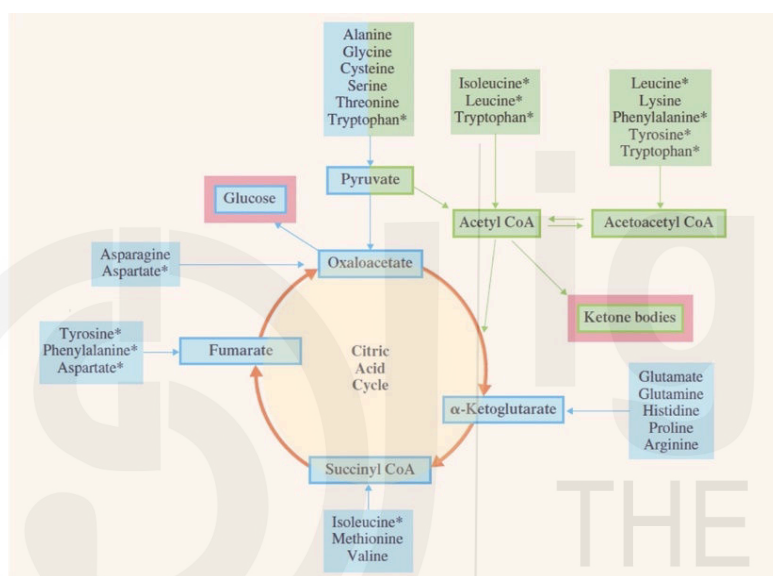


Fig. 17.8: The fate of carbon skeleton.

SAQ 5

Fill in the blanks in the following.

- Fumarate enters the TCA cycle and becomes the linkage between TCA and urea cycle. It is known as _____.
- Arginase is inhibited by _____.
- The amino acids that are converted into ketone bodies through ketogenesis are called _____ amino acids.

17.5 INTERRELATIONSHIP AMONG METABOLIC PATHWAYS

You must have observed that we do not take food continuously and eat only after a gap of few hours, still are able to maintain energy throughout the day. How does it happen? In fact, this is possible by an integration of the metabolic pathways associated with the major types of food taken by us. In other words, it is due to the existence of an interrelationship of pathways of carbohydrate, protein, and fat metabolism. Thus, these pathways are not independent of each

other but, rather, are integrally linked. The numerous connections among pathways mean that a change in one pathway can affect the other pathways.

The interrelationship among the metabolic pathways is essential to:

- interconvert biochemical metabolites as per the cellular need.
- meet the bodies fuel demand continuously.
- regulate the levels of intermediary metabolites and maintain their equilibrium.
- coordinate with the various cells, tissues, and organs for existence.
- impart normal biochemical environment and maintain health.

A summary of the interrelationship among all the three metabolisms is given in Fig. 17.9. As is clear from the diagram and your familiarity with the metabolic pathways studied so far, it is learnt that all the organisms utilise the three major foodstuffs to form acetyl CoA or the metabolites of the Krebs cycle. These, in turn, supply energy upon subsequent oxidation by the cycle.

Acetyl CoA is the primary link and is the degradation product of glucose, glycerol, fatty acids, and amino acids. It is also the starting material for the biosynthesis of fatty acids, cholesterol, and ketone bodies. You may also observe that one nonreversible reaction is the conversion of pyruvic acid to acetyl CoA. Thus, an acetyl CoA molecule derived from fatty acid degradation cannot be directly transformed into pyruvic acid. There is, therefore, no direct route for the synthesis of carbohydrates from fatty acids. Plants and some microorganisms that can convert fatty acids to carbohydrates and proteins utilize a series of enzymic reactions not found in animal tissues referred to as the glycolate cycle.

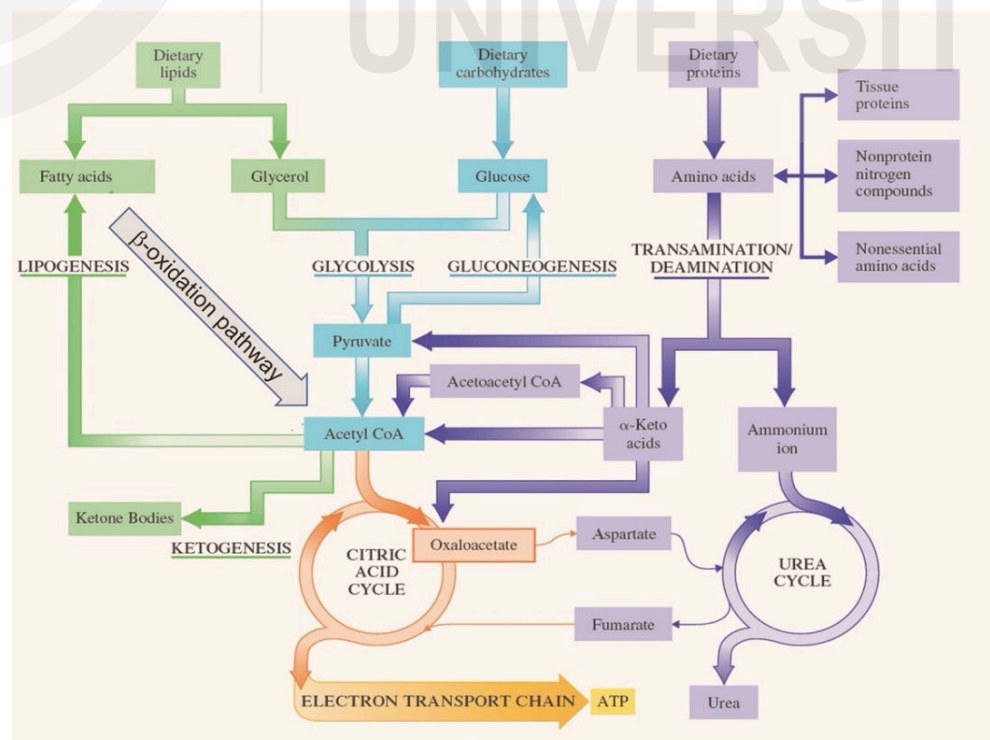


Fig. 17.9: Interrelationship among the three metabolic pathways.

The interrelationships among pathways can very well be understood and appreciated by the comparison of the food intake by an individual in three conditions. These are:

1. **Overfed condition**, when eaten beyond requirement: In this condition a limited amount of the excess food gets stored as glycogen and the remaining gets stored as fat.
2. **Fasting condition** i.e., not eating for a short duration: In this condition the stored glycogen and the fat is utilised for the energy requirements.
3. **Starvation**, not eaten for a long period: When there is a state of no consumption of food, all the stored glycogen is used. In this condition the body protein is broken to amino acids that are used to synthesise glucose. The glucose serves as the source of energy for brain and the nervous system. In the liver, fats are converted to ketone bodies which again are the energy source for brain.

SAQ 6

Match the items of column A with those given in column B.

A	B
a) Starvation	(i) glycogen stored
b) fasting	(ii) glycogen used
c) Overfed state	(iii) ketone bodies

17.6 SUMMARY

Let us summarise what we have discussed in this unit about the metabolism of lipids and proteins.

- Oils and fats are the major energy reserves of living beings including humans. Oils and fats are hydrolysed by various lipases to give glycerol and free fatty acids. Glycerol is metabolised via glycolysis. Each fatty acid is converted into the corresponding acylcoenzyme-A and transported from cytoplasm to the mitochondrial matrix where it is degraded.
- Acyl-CoA undergoes a sequence of four reactions, namely an oxidation, hydration (i.e., addition of a water molecule), another oxidation and a thiolytic cleavage. The oxidation of fatty acids yields large amount of ATP.
- Biosynthesis of fatty acids utilises acetyl-CoA as the precursor and takes place in cytoplasm. It is catalysed by an assembly of several enzymes called fatty acid synthase. Much more energy is “spent” during biosynthesis of palmitate from eight molecule of acetyl-CoA than that which is “recovered: on breakdown of palmitate to eight acetyl-CoA molecules. This is generally true for any metabolism.
- Protein metabolism is basically the metabolism of amino acids because proteins are polymers of amino acids. The catabolism of amino acids

occurs in two stages i.e., the removal of α -amino group nitrogen as NH_3 by two mechanism transamination and deamination and the disposal of the remaining carbon skeletons.

- All the types of metabolic pathways are interrelated to one another by several common intermediates that is helpful in maintaining the fuel requirement of the body.

17.7 TERMINAL QUESTIONS

1. Compare the β -oxidation and biosynthesis of fatty acids with respect to the following aspects:
 - a) Intracellular location of the process
 - b) nature of acyl group carrier
 - c) nature of the oxidants and reductants employed and
 - d) organisation of the participating enzymes
2. Given below are two statements. Explain whether these are true or false giving reasons.
 - a) The methyl group of each acetyl-CoA molecule entering the TCA cycle is derived from the methyl group of pyruvate.
 - b) Malate cannot be converted to fumarate because the TCA cycle is unidirectional.
3. Discuss the transamination and deamination of protein catabolism.
4. With help of diagram discuss the steps of urea cycle.
5. What is the importance of interrelationship of metabolic pathways?

17.8 ANSWERS

Self Assessment Questions

1. (a) True (b) True (c) False (d) True
2. (a) 38 (b) 129
3. (a) Cytosol (b) biotin (c) NADPH (d) Seven
4. a) glutamate dehydrogenase
b) aminotransferase
c) nonoxidative deamination
5. (a) Krebs bicycle (b) ornithine (c) ketogenic
6. (a) Starvation (i) ketone bodies
(b) fasting (ii) glycogen used
(c) Overfed state (iii) glycogen stored

Terminal Questions

1. β -Oxidation

a) Mitochondrion

b) Coenzyme-A

c) FAD and NAD^+ (oxidants)

d) Isolated as individual enzymes
synthase complex

Biosynthesis

Cytoplasm

Acyl carrier protein

NADPH (reductant)

Isolated as fatty acid

2. a) False. The acetyl-CoA is also produced from β -oxidation of longchain fatty acids or from amino acid metabolism.
- b) False. Malate can easily be dehydrated to fumarate by reversal of fumarase reaction.
3. During the catabolism of protein the removal of α -amino group nitrogen as NH_3 by two mechanism transamination and deamination. Transamination is a chemical reaction in which the amino group from an amino acid is transferred to a keto acid. It is catalysed by an enzyme called transaminase or aminotransferase. In deamination amino group is directly removed from amino acid.
4. Refer to Fig. 17.6.
5. The importance of integrated Metabolism is to Interconvert biochemical metabolites as per the cellular need. It helps the body to fulfill its fuel demand. It helps to regulate the levels of intermediary metabolites and maintain their equilibrium and also to coordinate with the various cells, tissues and organs for existence and for normal biochemical environment and maintain health.