UNIT 9 METABOLISM-I

Structure

9.1 INTRODUCTION

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

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 no less 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, we will study the catabolism and anabolism of carbohydrates.

Objectives

After studying this unit you should be able to :

- 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 back conversion of pyruvate into glucose, and
- compare the energetics of breakdown and synthesis of glucose.

9.2 GENERAL SCHEME OF METABOLIC PATHWAYS

A survey of metabolic breakdown of carbohydrates, fats and proteins shows that these may be considered to proceed in three stages, Fig.9.1. 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 **tricarboxylic acid** or **citric acid cycle**. The metabolites transfer their electrons to some 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 for most of the ATP synthesis. This stage requires oxygen and, therefore, represents the aerobic part of the metabolism.

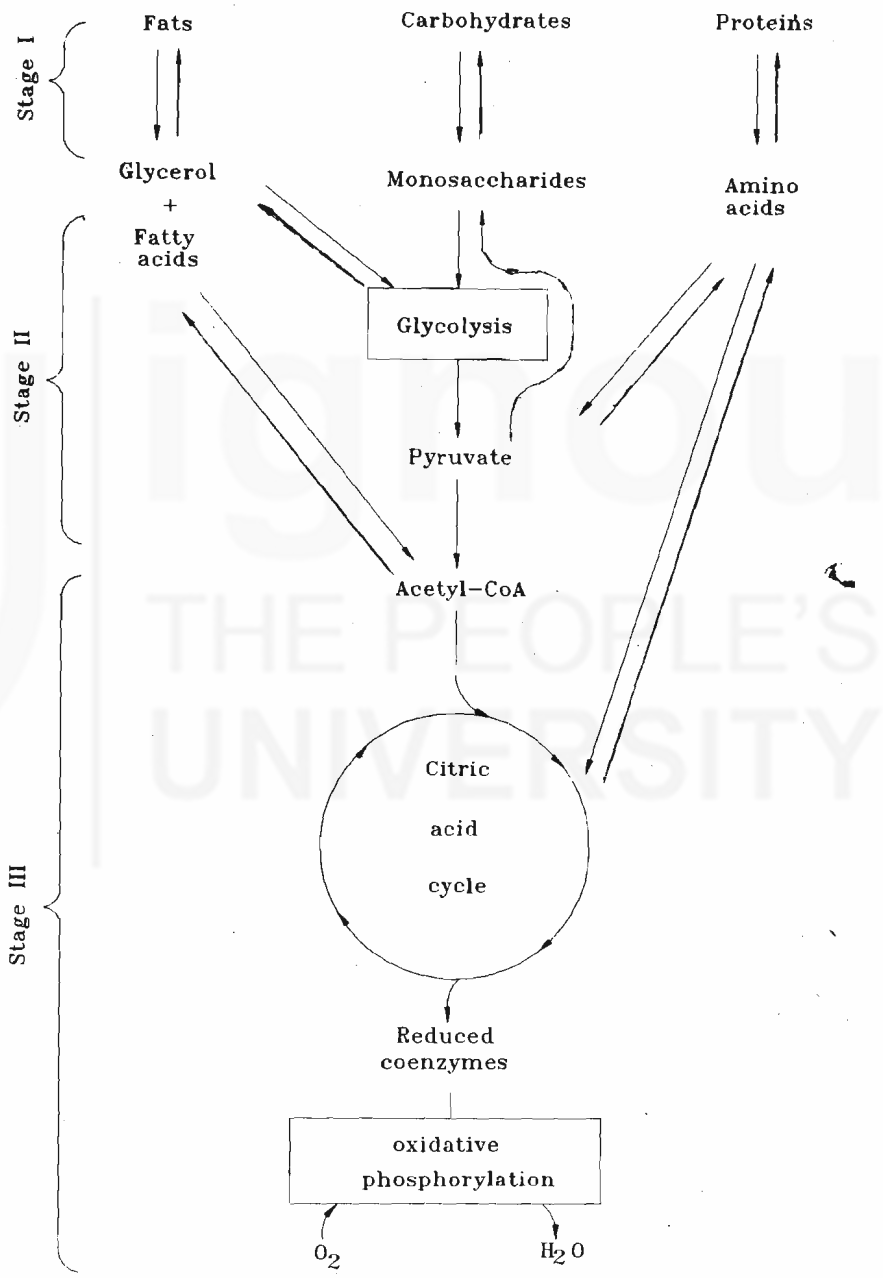


Fig. 9.1 : Stages in the metabolic breakdown of fats, carbohydrates and proteins. The black arrows pointing downward represent the breakdown (catabolism) and brown arrows pointing upward show the biosynthetic routes (anabolism).

Fig. 9.1 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 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. We will not discuss detailed metabolism of all the common compounds in this course. Only a few metabolic chains, namely, glycolysis in this unit, tricarboxylic acid cycle and fatty acid degradation in Unit 10 will be discussed to illustrate the principles of catabolism. Similarly, only two anabolic processes, namely, gluconeogenesis in this unit and fatty acid biosynthesis in Unit 10, will be described mainly to point out the significance of differences in the pathways for degradation and biosynthesis.

A variety of experimental approaches were 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 microorganism, e.g., on exposure to X-rays. Studies with such mutants showed 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 could then be combined. Similar information about metabolic pathways was also obtained from studies involving human patients having one or other genetic help in elucidating the catabolic as well as anabolic pathways. In the latter, the origin of various carbon atoms can be traced to specific precursors by this technique. As said before, some of the pathways so elucidated are described in this and the next units. We shall start with glycolysis.

9.3 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. Here glucose molecule is broken down into two molecules of two carbon compound, ethanol and two molecules of CO_2. The sequence of reactions involved was discovered by parallel studies on the two pathways by Buchner and Halden and Young in the early part of this century and later by Embden, Meyerhoff, Neuberg, Warburg and Cori.

Glycos meaning sugar + lysis meaning dissolution.

Scheme of glycolysis and alcoholic fermentation is shown in Fig. 9.2. 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 expended to 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.

9.3.1 Conversion of Glucose into Triose Phosphate

In the first step glucose reacts with ATP to give rise to glucose-6-phosphate (Gl-6-P) and ADP. The reaction is catalysed by the enzyme, hexokinase, Eq. 9.1.

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

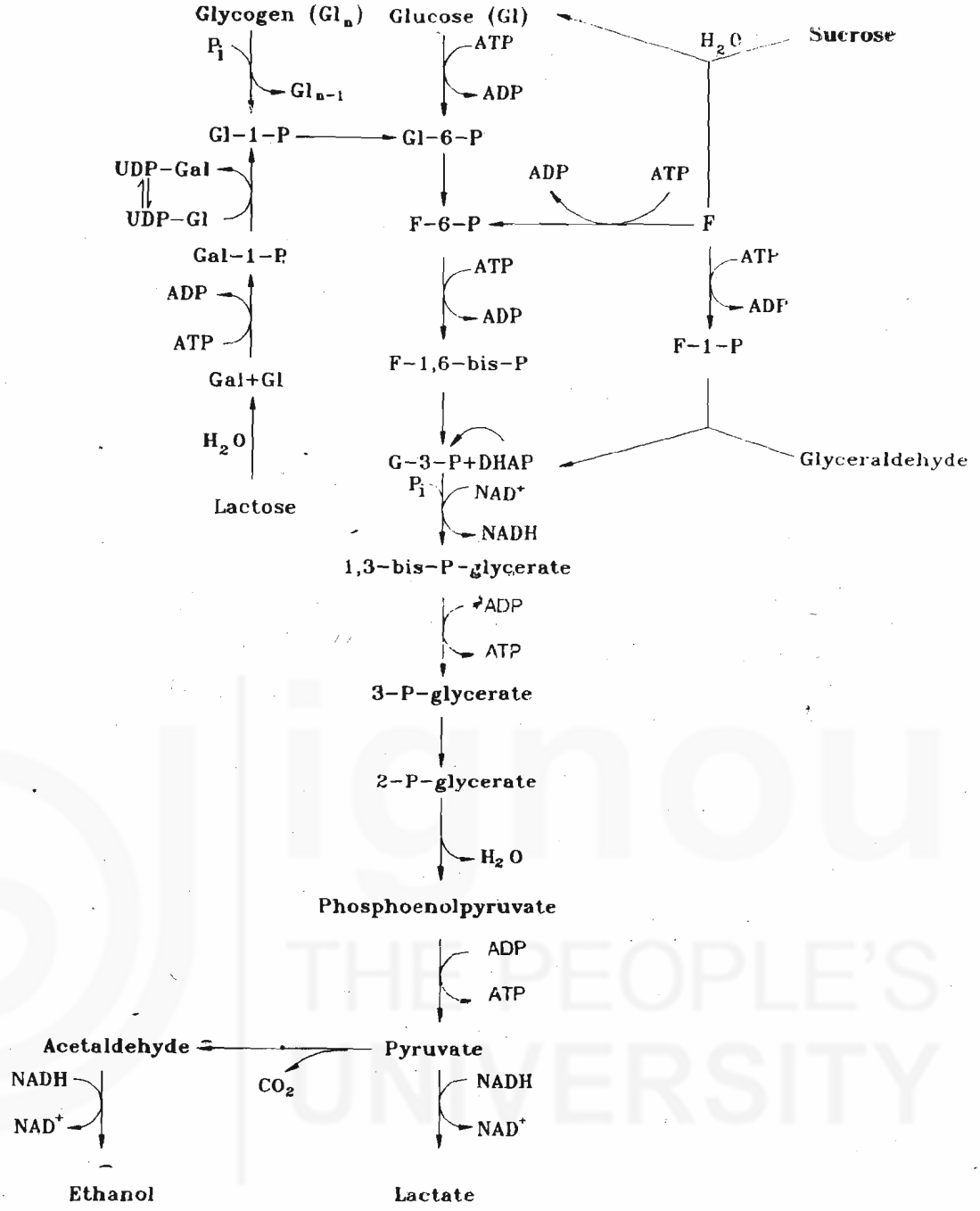
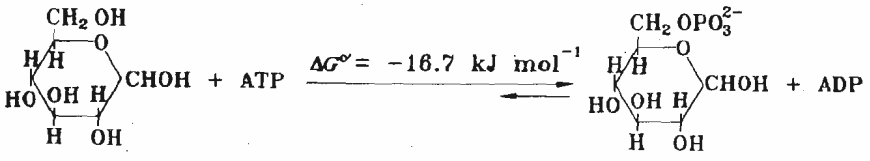


Fig. 9.2 : Scheme of glycolysis and alcoholic fermentation showing entry points for various sugars. The brown arrows are shown for the irreversible reactions.
Abbreviations: Glucose, Gl; Fructose, F; Phosphate group, P; Phosphate ion, P_i; Glyceraldehyde-3-phosphate, G-3-P; Dihydroxyacetonephosphate, DHAP; Galactose Gal.



...(9.1)

This reaction is practically irreversible because of the large energy drop. The $\Delta G^{o\prime}$ value of this reaction can be calculated by making use of $\Delta G^{o\prime}$ values for the hydrolysis of ATP and Gl-6-P given in Table 8.1 of Unit 8.

$$ATP + H_2O \longrightarrow ADP + P_i \; ; \; \Delta G^{o\prime} = -30.5 \text{ kJ mol}^{-1}$$

$$Gl-6-P + H_2O \longrightarrow \text{glucose} + P_i \; ; \; \Delta G^{o\prime} = -13.8 \text{ kJ mol}^{-1}$$

The second reaction can also be written as:

$$\text{Glucose} + P_i \longrightarrow Gl-6-P + H_2O \; ; \; \Delta G^{o\prime} = +13.8 \text{ kJ mol}^{-1}$$

Adding the above equations, we get Eq. 9.1. Thus, the $\Delta G^{o\prime}$ value for the reaction is found to be –16.7 kJ mol^{-1}. Using the relationship between $\Delta G^{o\prime}$ and K'_{eq}, we can calculate the equilibrium constant for this reaction at pH 7.0.

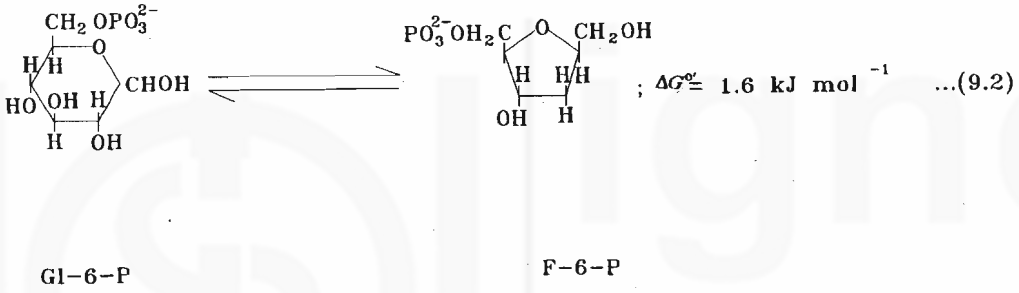
$$\Delta G^{o\prime} = -R.T.\ln K'_{eq} = -2.303 \times RT \times \log K'_{eq}$$

Substituting the values of $\Delta G^{o\prime}$, R and T, equilibrium constant is found to be equal to 8.45×10^2 at pH 7.0 and 298 K.

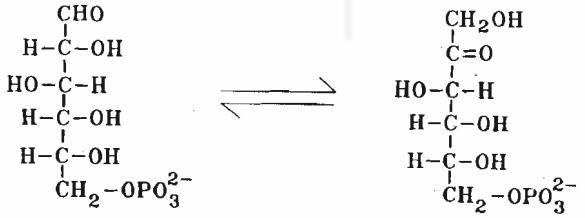
$$K'_{eq} = \frac{[ATP].[Gl-6-P]}{[ATP]\cdot[\text{glucose}]} = 8.45 \times 10^2$$

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. 9.2.



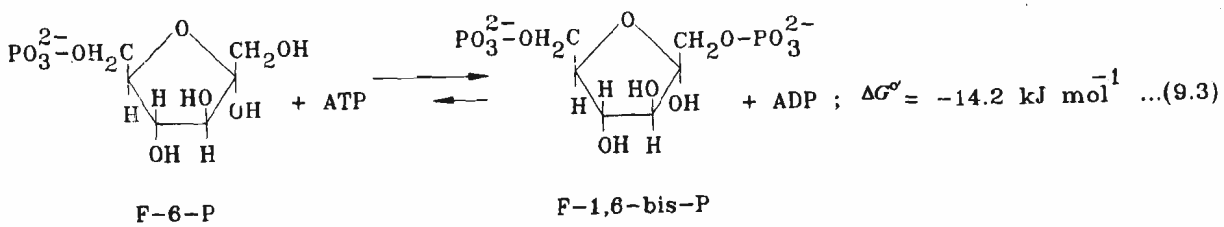
You can understand the facile aldose-ketose isomerisation more clearly by considering the open chain structures of the two sugars,



Gl – 6 – P **F – 6 – P**

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. 9.3.



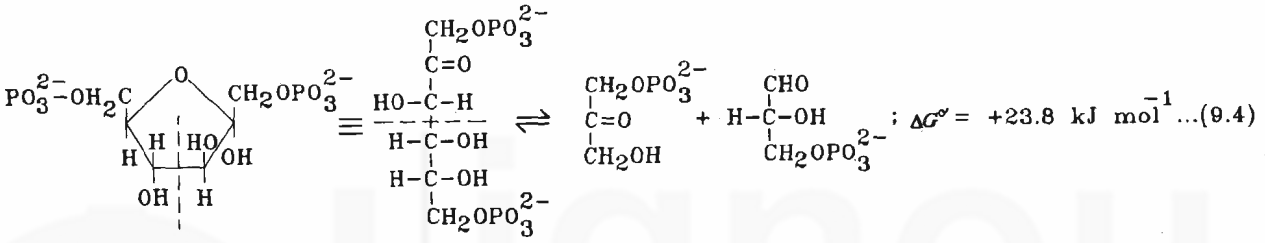
F-6-P F-1,6-bis-P

$$\Delta G^{o\prime} = -14.2 \text{ kJ mol}^{-1} \quad ...(9.3)$$

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 $\Delta G^{o\prime}$ value). This reaction is ot 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 11.

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

Fructose-1,6-bisphosphate gets cleaved into one molecule each of glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP) as per Eq. 9.4. The enzyme catalysing this reaction is called fructose-1,6-bisphosphate aldolase or simply aldolase.



F-1,6-bis-P DHAP G-3-P

$$\Delta G^{o\prime} = +23.8 \text{ kJ mol}^{-1} \quad ...(9.4)$$

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,

$$\Delta G^{o\prime} = -R \cdot T \cdot \ln K'_{eq} = -2.303 \times R \times T \times \log K'_{eq}$$

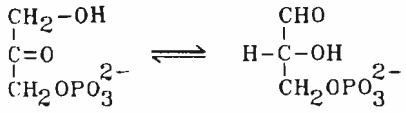
$$\text{Therefore, } \log K' = \frac{\Delta G^{o\prime}}{-2.303 \times R \times T}$$

$$= \frac{23.8}{-2.303 \times 8.314 \times 298} = -4.18$$

$$K'_{eq} = \frac{[\text{G-3-P}] \times [\text{DHAP}]}{[\text{F-1,6-bis-P}]} = 6.61 \times 10^{-5}$$

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 $10^{-8}M^2$. 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 $\Delta G^{o\prime}$ 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.



DHAP G-3-P ... (9.5)

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.

SAQ 1

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

i) Glucokinase is specific for the phosphorylation of glucose.
.....

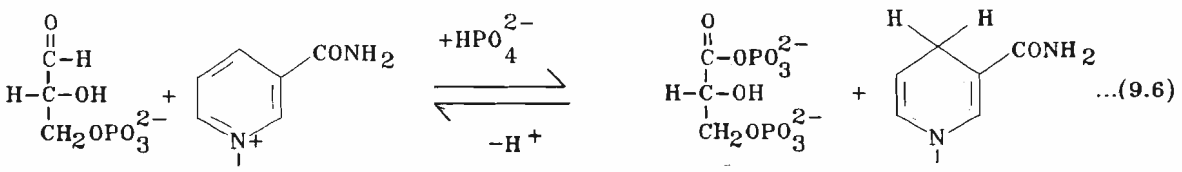
ii) Phosphofructokinase catalyses the reaction in which glucose-1-phosphate is the substrate.
.....

iii) The reaction catalysed by PFK is inhibited by high concentration of ATP and citrate.
.....

iv) Like G-3-P, dihydroxyacetone phosphate can get into glycolytic pathway for its further metabolism.
.....

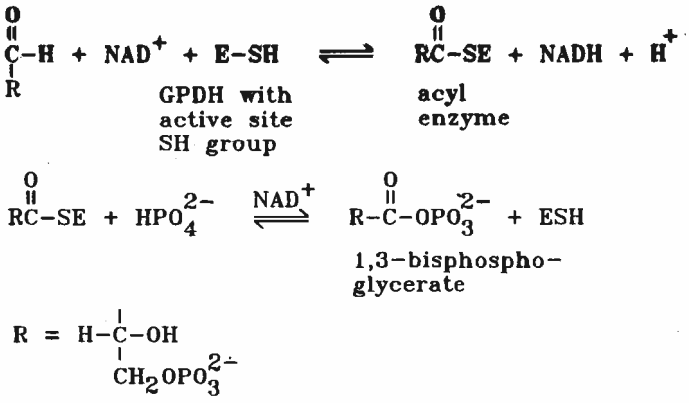
9.3.2 Conversion of G-3-P into Pyruvate

G-3-P formed by the 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. 9.6. The reaction is catalysed by glyceraldehyde-3-phosphate dehydrogenase (GPDH).



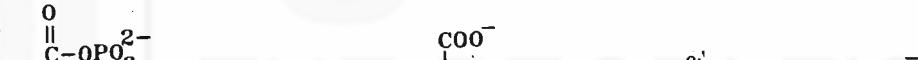
G-3-P NAD^+ 1,3-bisphospho-glycerate NADH ...(9.6)

GPDH has a highly reactive cystein-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, specially 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 readily 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 of attack.

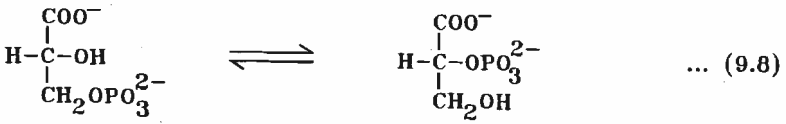
1,3-Bisphosphoglycerate reacts with ADP in the next step of glycolysis. The reaction is catalysed by phosphoglycerate kinase and yields 3-phosphoglycerate and ATP, Eq. 9.7.

3-phosphoglycerate

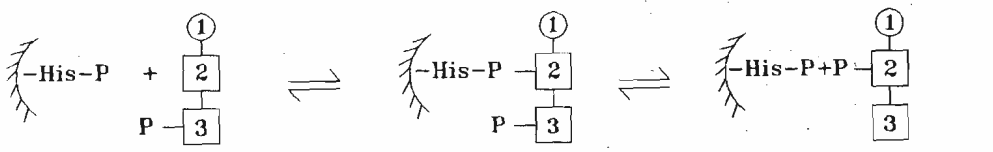
In this reaction, the energy conserved in the preceding step is "harvested" and utilised for a "net" synthesis of ATP. **This is the first ATP-generating step of glycolysis.**

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 group 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. 9.8.

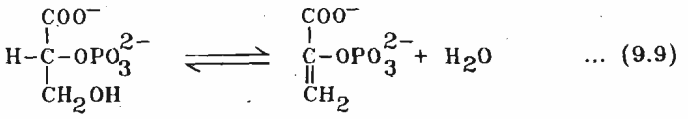


This reaction requires the presence of enzyme-bound 2,3-bisphosphoglycerate. A histidine residue of the enzyme active site functions successively as an acceptor and donor of phosphate group. The isomerisation is believed to proceed as follows :



enzyme 3-phospho-glycerate enzyme with bound 2,3-bisphosphoglycerate enzyme + 2-phospho-glycerate

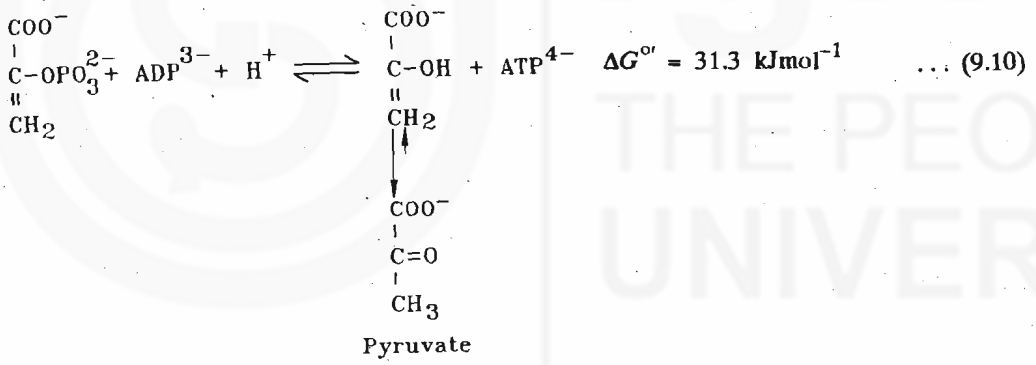
2-Phosphoglycerate undergoes dehydration to give rise to phosphoenolpyruvate (PEP), Eq. 9.9. The enzyme catalysing this reaction is called enolase.



... (9.9)

phosphoenol pyruvate

Phosphate esters of enolic compounds are also energy rich compounds (Unit 8, Table 8.1). They have a high phosphate group transfer potential. This is made use of in the next step to transfer the phosphate ground to ADP giving rise to ATP and pyruvate. The reaction is catalysed by pyruvate kinase (PK), Eq. 9.10.



... (9.10)

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. The significance of these properties will be discussed in Unit 11.

A summary of the energetics of the reactions from glucose to pyruvate is given in Table 9.1.

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

Table 9.1 : Energetics of the Reactions of Glycolysis

Eq. Number	Reaction Type	ΔG^{o} (kJ mol^{-1})	ΔG (kJ mol^{-1})
9.1	Phosphate transfer	–16.74	–33.47
9.2	Isomerisation	+ 1.67	–2.51
9.3	Phosphate transfer	–14.22	–22.17
9.4	Aldol cleavage	+ 23.85	–1.25
9.5	Isomerisation	+ 7.53	+ 2.57
9.6	Oxidation and phosphorylation	+ 6.28	–1.64
9.7	Phosphate transfer	–18.83	+ 1.20
9.8	Phosphate group shifting	+ 4.60	+ 0.83
9.9	Dehydration	+ 1.67	–3.35
9.10	Phosphate transfer	–31.4	–16,74

In this table, the actual free energy changes (ΔG) are based on the known $\Delta G^{o\prime}$ values and the prevailing intracellular concentrations of various metabolites. For determining the latter, it is necessary to stop all reactions before the cells are ruptured. This is achieved by first cooling and freezing the intact tissue to liquid nitrogen temperature which stops all chemical reactions. The 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 metabolites. 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 9.1 have been computed for 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?

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9.3.3 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. 9.1. These reactions will be discussed in Unit 10. 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 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. 9.6) reacts with pyruvate in the presence of lactate dehydrogenase to form lactate and NAD^+, Eq. 9.11

$$CH_3-\overset{O}{\overset{\|}{C}}-COO^- + NADH + H^+ \longrightarrow CH_3-CHOH-COO^- + NAD^+ \quad ...(9.11)$$

$$\Delta G^{\circ} = -25.10 \text{ kJ mol}^{-1}$$

The resulting NAD^+ can be used again for the reaction of Eq. 9.6. Note that none of the reactions of glycolysis and the reaction given above 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. 9.12.

With lactobacilli, lactate is the end product.

$$CH_3-\overset{O}{\overset{\|}{C}}-COO^- + H^+ \longrightarrow \underset{\text{acetaldehyde}}{CH_3-CHO} + CO_2$$

$$CH_3-CHO + NADH + H^+ \longrightarrow \underset{\text{ethanol}}{CH_3-CH_2OH} + NAD^+ \quad ...(9.12)$$

The oxidised coenzyme so produced (NAD^+) can be used again for the reaction given in Eq. 9.6. 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 glycolytic pathway let us look into the overall stoichiometry and the energetics of the reactions.

9.3.4 Stoichiometry of ATP Formation and Energetics of Glycolysis

The scheme of glycolysis (Fig. 9.2) shows that two molecules of ATP are consumed in the conversion of glucose into fructose-1,6-bisphosphate (Eqs. 9.1 and 9.3) which then gives rise to two molecules of triose-phosphate (Eq. 9.4). During the conversion of each molecule of triose-phosphate into lactate, two molecules of ATP are generated in the reactions catalysed by phosphoglycerate kinase (Eq. 9.7) and pyruvate kinase (Eq. 9.10). Thus, in all four molecules of ATP are produced in these steps for each molecule of glucose. Substracting 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.

Reaction	Change in ATP per glucose consumed
Gl → Gl - 6 - P	-1
F - 6 - P → F - 1,6- bis- P	-1
2 × 1,3 - bis- P- glycerate → 2 × 3 - P - glycerate	+ 2
2 × PEP → 2 × pyruvate	+ 2
Net	+ 2ATP

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow 2\,CH_3 - CHOH - COOH\,;\ \Delta G^{o\prime} = -196.65\ kJ\ mol^{-1}$$

$$2\,ADP + 2\,P_i \rightarrow 2ATP\,;\ \Delta G^{o\prime} = +61.08\ kJ\ mol^{-1}$$

Considering the $\Delta G^{o\prime}$ 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 "conservation" of energy is different because the concentration of various metabolites in the cell are far from standard (1.0M). As explained in Unit 8, the actual free energy of hydrolysis of ATP in human erythrocytes is found to be –51.8 k J mol^{-1}. The percent 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 $\Delta G^{o\prime}$ value 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 the necessary enzymes ensures a steady supply of ATP when desired. Secondly, the difference between the energy released on breakdown of nutrients and that captured or 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 efficient heat loss from the body during hot weather. In terms of efficiency of energy transduction no man made machine matches the living cell.

SAQ 3

Give a scheme showing different metabolic fates of pyruvate.

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SAQ 4

How is alcoholic fermentation in yeast similar to lactate production in skeletal muscle?

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9.3.5 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. 9.2). 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, Section 2.5 for structure). Surplus glucose of the body is stored in this form. At the time of necessity, glucose residues are removed one-by-one from the nonreducing ends by the action of glycogen phosphorylase, Eq. 9.13.

$$Gl_n + P_i \longrightarrow Gl - 1 - P + Gl_{n-1} \qquad ...(9.13)$$

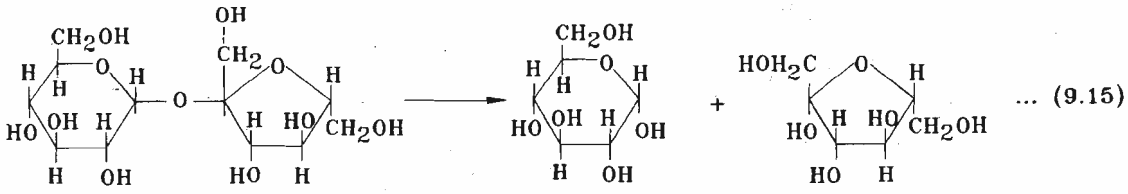
Glycogen having n glucose residues	phosphate ion	glucose-1-phosphate	glycogen having n-1 glucose residues

Glucose-1-phosphate is isomerised to glucose-6-phosphate in the presence of the enzyme phosphoglucomutase, Eq. 9.14.

$$Gl - 1 - P \rightleftharpoons Gl - 6 - P \qquad ...(9.14)$$

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

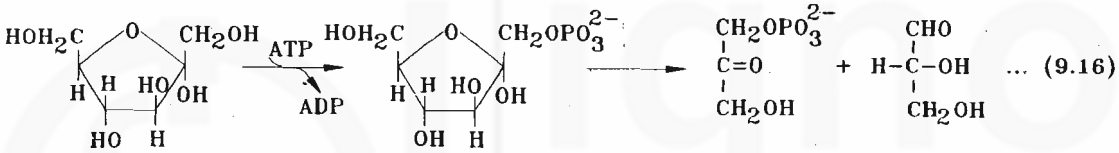
ii) **Entry of sucrose and fructose:** Disaccharides, like sucrose, are not absorbed through the intestines. Only monosaccharides are. Sucrose is hydrolysed in the presence of invertase of intestinal mucosa giving rise to glucose and fructose, Eq. 9.15.



... (9.15)

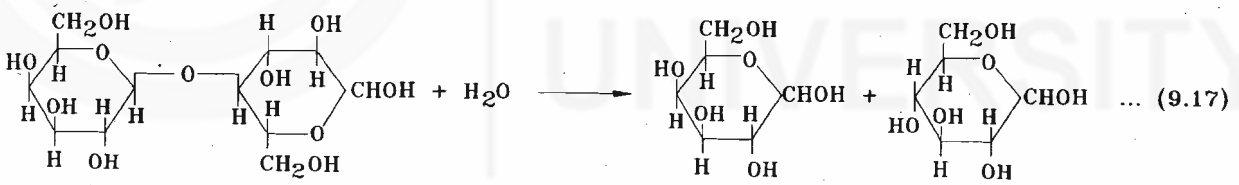
sucrose glucose fructose

Fructose enters the glycolytic pathway by two routes. In the minor route, fructose is converted into fructose-6-phosphate on reaction with ATP catalysed by hexokinase, the same enzyme which converts glucose into glucose-6-phosphate. Further, metabolism is as per the scheme of Fig.9.2. 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 rise to glyceraldehyde and dihydroxyacetone phosphate in the presence of F-1-P-aldolase, Eq. 9.16, the action of which is similar to the reaction of Eq. 9.4 discussed before. Further, metabolism of the products is clear from Fig. 9.2.



... (9.16)

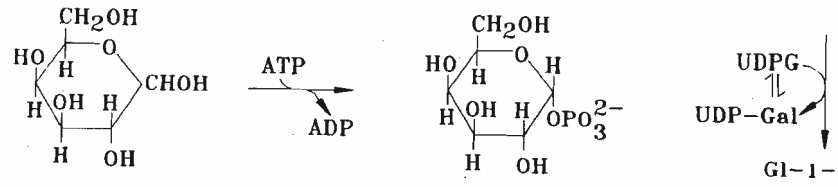
iii) **Entry of lactose and galactose:** You have read that lactose, the milk sugar, is a disaccharide made up of one residue each of galactose and glucose, It gets hydrolysed to the monosaccharides in the intestines with the help of lactase (or β-galactosidase) present in the intestinal mucosa, Eq. 9.17.



... (9.17)

lactose (β-galactosido-1→4-glucose) galactose glucose

Galactose is phoshorylated at position-1 with ATP in the presence of galactokinase. The resulting galactose-1-phosphate (Gal-1-P) reacts with uridine-diphospho-glucose (UDPG) giving rise to uridine-diphospho-galactose (UDPGal) and glucose-1-phosphate (Gl-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. 9.18.



... (9.18)

galactose Gal-1-P

Thus, the combined action of galactose-1-phosphate uridyl transferase and UDPG-epimerase brings about an isomerisation of Gal-1-P into Gl-1-P, which enters glycolysis as described above for the entry of glycogen.

9.3.6 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 diseases, which are referred to as the "Inborn errors of metabolism". These are related to genetic deficiencies, many of which are transmitted as recessive traits. Some metabolic disorders are related to the entry of 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 vomitting or diarrhoea, enlargement of liver and jaundice. In later stages, the patients suffer from mental retardation, clouding of the eye lense and may even die. It is best treated in the early stages only by excluding galactose from diet because mental retardation is frequently irreversible. 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 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 5

Fill in the blank spaces with suitable words

i) The conversion of glycogen to lactate in glycolytic pathway is accompanied by a net gain of.....of ATP.

ii) An intense muscular activity leads to an increased.....formation.

iii) Galactosemia is observed as a result of ..... accumulation in human body.

9.4 GLUCONEOGENESIS

Gluconeogenesis occurs primarily in the liver

As already mentioned, glycolysis is the major source of ATP for the skeletal muscle during strenuous activity. As a result, lactate accumulates in the muscle. 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.**

Glycerol and some amino acids are other starting materials for gluconeogenesis, which become important under conditions of starvation. Under these conditions, glucose

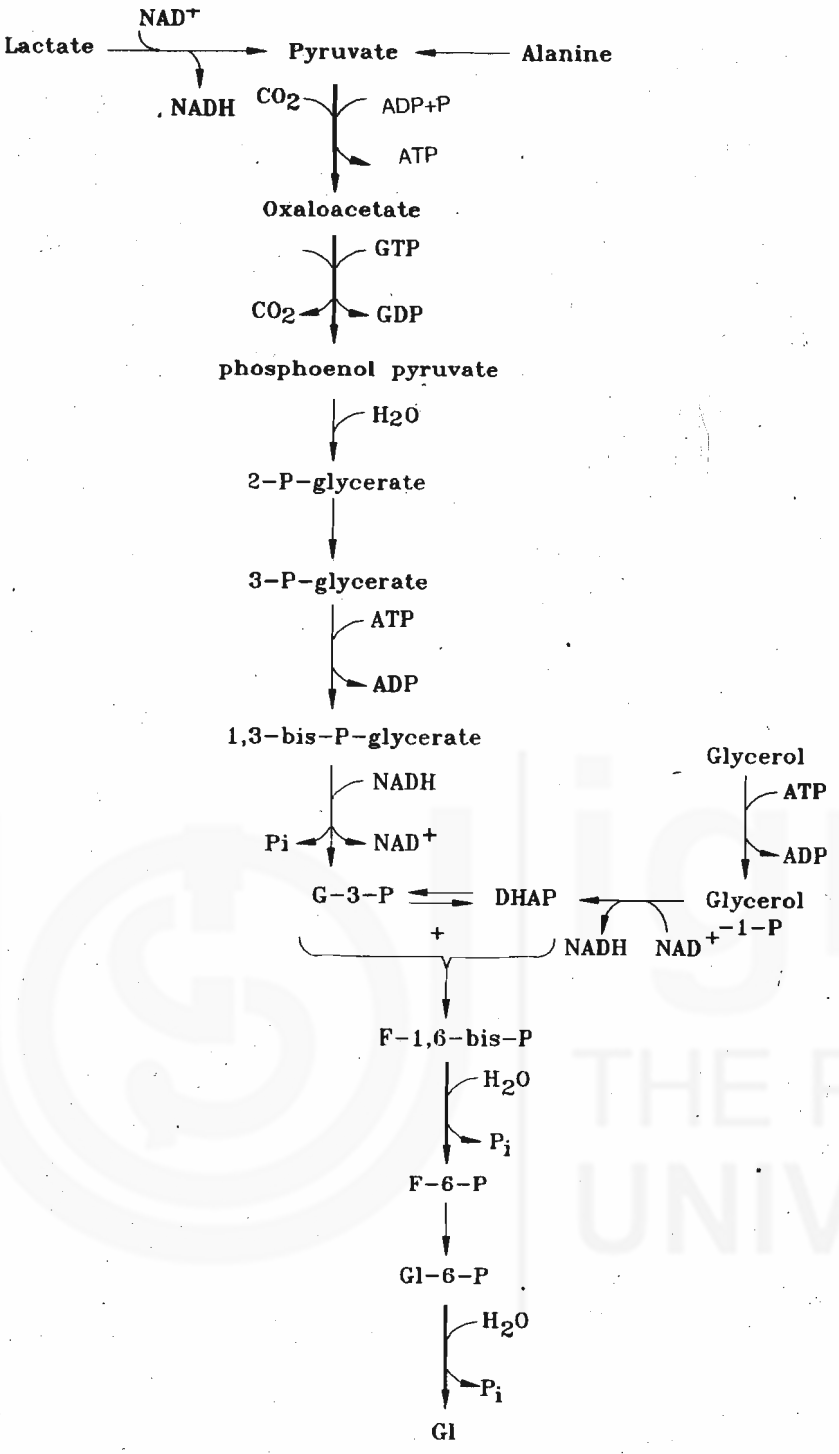


Fig. 9.3 : Scheme of gluconeogenesis showing entry points for lactate, amino acids and glycerol

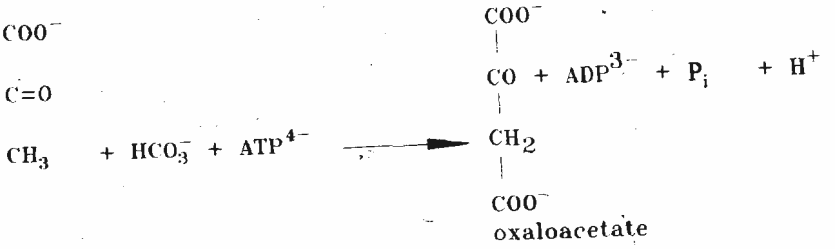
levels in the blood have to be maintained at about 80 mg per 100 ml by converting noncarbohydrate material into glucose because brain is highly dependent on glucose as the primary fuel.

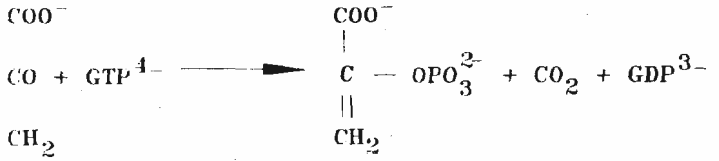
Glycolysis and gluconeogenesis make up a pseudocycle or pair of oppositely directed reaction sequences.

The pathway of gluconeogenesis and entry points for lactate, amino acids and glycerol are shown in Fig. 9.3. It may be noted that many reactions are common to glycolysis but proceeding in opposite 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 three points represent the steps of large energy drops and are practically irreversible in the direction of

glycolysis (Table 9.1). These are also the loci of regulation of this pathway, as will be discussed in Unit 11.

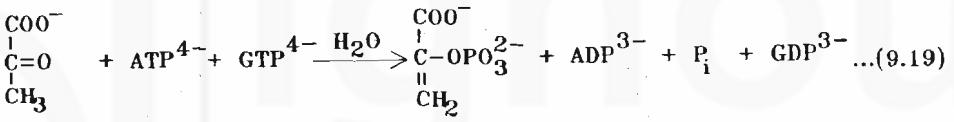
Pyruvate is first carboxylated in the presence of ATP and biotin-dependent pyruvate carboxylase to form oxaloacetate. The latter is then decarboxylated and phosphorylated by its reaction with another high energy phosphate compound, namely guanosine triphosphate (GTP). This reaction is catalysed by phosphoenolypyruvate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





phosphoenolpyruvate

The sum of these reactions is :



...(9.19)

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. 9.10). This extra energy input makes the otherwise energetically unfavourable conversion of pyruvate into phosphoenolpyruvate, ($\Delta G^{o\prime}$ = + 61.8 k J mol^{-1}) feasible.

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

$$F-1,6-\text{bisphosphate} + H_2O \rightarrow F-6-P + P_i$$

$$Gl-6-P + H_2O \rightarrow Gl + P_i$$

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 9.2.

Table 9.2 : Differences in the reactions and enzymes of glycolysis and gluconeogenesis

Glycolysis		Gluconeogenesis	
Reaction	**Enzyme**	**Reaction**	**Enzyme**
Gl+ ATP → Gl-6-P+ ADP	Hexokinase	Gl- 6- P + H_2O → Gl + P_i	Gl- 6- Phosphatase
F-6-P + ATP → F-1,6-bis-P + ADP	Phosphofructokinase	F-1,6-bis-P + H_2O → F-6-P + P_i	F-1, 6-Bis-phosphatase
Phosphoenolpyruvate + ADP → Pyruvate + ATP	Pyruvatekinase	Pyruvate + ATP + GTP + CO_2 → PEP + ADP + P_i + GDP + CO_2	Pyruvate-carboxylase + Phosphoenol - pyruvate carboxykinase

Abbreviations : Glucose, Gl; Fructose, F; Phosphate group, P; Phosphate ion, P_i ; Phosphoenolypyruvate, 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 phosphoenol pyruvate. 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 a large negative free energy change.

SAQ 6

What are the physiological functions of gluconeogenesis?

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9.5 SUMMARY

Let us summarise what all we have 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 oxidation of NADH to NAD^+. Under aerobic conditions pyruvate is converted into acetyl-coenzyme A and then 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). This pathway 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.

9.6 TERMINAL QUESTIONS

1) a) Write a balanced equation for the conversion of glucose into lactate via the glycolytic pathway.

   b) Calculate the $\Delta G^{o\prime}$ value for this reaction from the data of Table 8.1.

   c) Calculate the $\Delta G^{o\prime}$ value for this reaction if the concentrations of various reaction partners are: glucose, 5.0 mm; lactate, 0.05 m mol; ATP, 2.0 m mol; ADP, 0.2 m mol; and P_i, 1.0 m mol. (Hint : Consult Unit 8).

2) Is gluconeogenesis just the reverse of glycolysis. If not, how?

3) In a solution, all the species participating in the reaction of Eq. 9.10 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.

9.7 ANSWERS

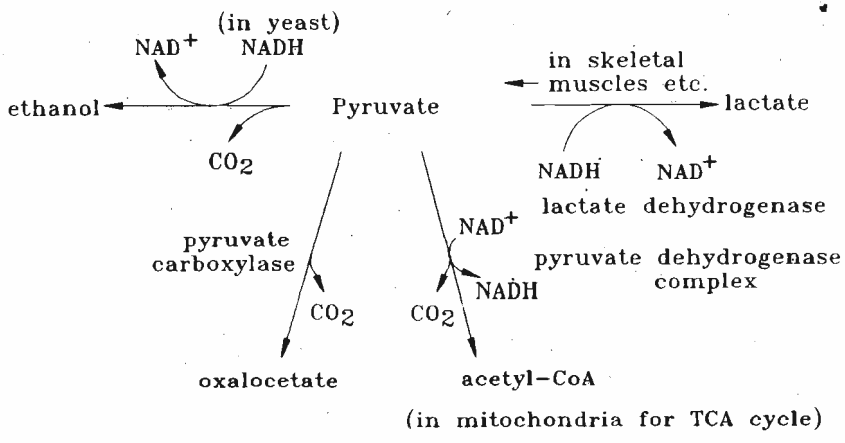
Self Assessment Questions

1) i) Correct (ii) Wrong, PFK catalyses the reaction of glucose-6-phosphate.

   iii) Correct (iv) 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) Both are anaerobic in nature and reoxidise the NADH produced in glycolysis by donating electrons to pyruvate or to a product produced from pyruvate.

5) i) 3 moles (ii) lactate (iii) galactose

6) 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

1) a) $C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2CH_3 - CHOH - COOH + 2ATP$

b) $\Delta G^{o'}$ value is -123.4 k J mol^{-1} glucose

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

$$= (-123{,}500) + 2.303 \times 8.314 \times 298 \times \log \frac{(0.050 \times 10^{-3})^2 \times (2.0 \times 10^{-3})^2}{5 \times 10^{-3} \times (0.2 \times 10^{-3})^2 \times (1 \times 10^{-3})^2}$$

$= -113.70$ kJ mol^{-1} 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.

3) PEP + ADP $\rightarrow$ pyruvate + ATP; $\Delta G^{o'} = -31.3$ k J mol^{-1} glucose

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

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

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

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

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

4) a) Methyl carbon atom of ethanol

b) In C-3 or C-4 position.

5) They are the important regulatory points controling the rate of glycolysis.

UNIT 10 METABOLISM-II

Structure

10.1 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 discussed in Unit 8. Under these conditions, pyruvate is metabolised further to CO_2 and H_2O by getting first converted into acetyl-coenzymeA. 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 9). Tricarboxylic acid cycle is directly related to the metabolism of several other amino 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 as well as the metabolism of fatty acids. The next unit deals with the regulation of metabolism.

Objectives

After studying this unit, you should be able to:

- describe the reactions inolved in the conversion of pyruvate into 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,
- describe the pathways for the breakdown and biosynthesis of fatty acids, and
- explain the energetics of the above processes.