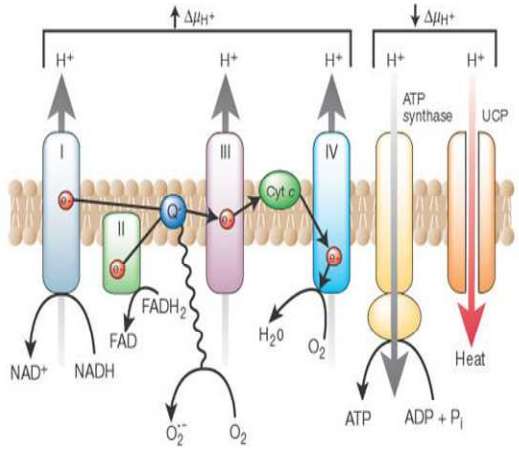
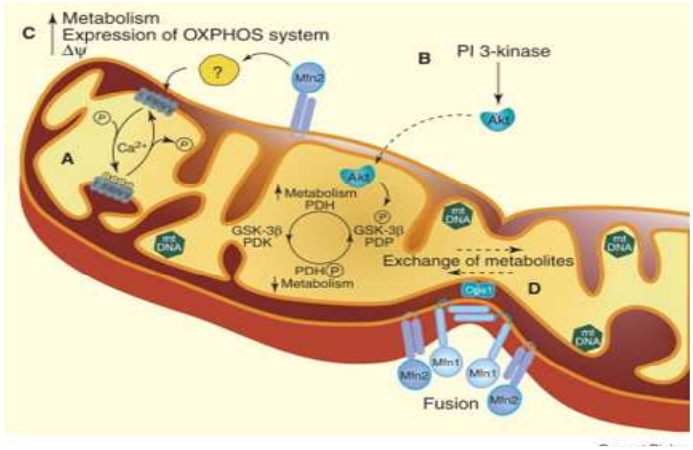
Karnataka State Open University

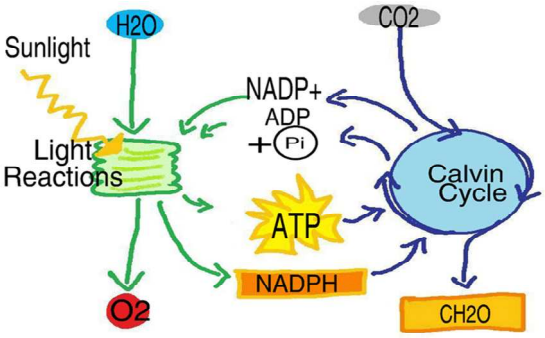
Mukthagangothri, Mysore-507006

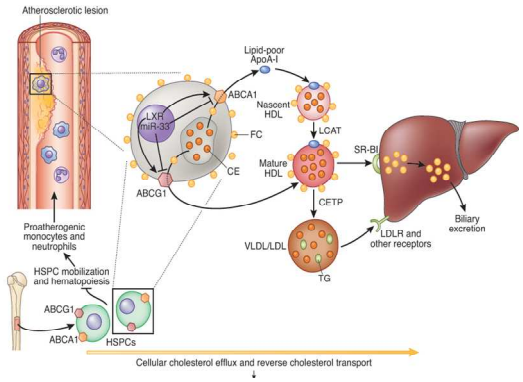
M.Sc. Biotechnology

First Semester









Biochemical Transformation and Clinical Significance

BT- 1.4 **BLOCK- I,II,III and IV** **UNITS - 1**

Karnataka State Open University

M.Sc. in Biotechnology

FIRST SEMESTER

BT 1.4 BIOCHEMICAL TRANSFORMATION AND CLINICAL SIGNIFICANCE

(Blocks -I, II, III and IV; UNITS- 1 TO 16)

1.4 CHEMICAL TRANSFORMATION AND CLINICAL SIGNIFICANCE

COURSE DESIGN

Prof. M. G. Krishnan
Vice Chancellor
Karnataka State Open University
Mukthagangotri
Mysore-570006

Prof. S. N. Vikram Raje Urs
Dean (Academic)
Karnataka State Open University
Mukthagangotri
Mysore-570006

COURSE EDITOR

Dr. N.G.Raju
Asst. Professor & Chairman, Department Biotechnology
Karnataka State Open University, Mukthagangotri, Mysore-570006

COURSE WRITERS

NAME	COURSE	BLOCKS	UNITS
Dr. Pramod Asst. Professor Dept of Biochemistry, Saiyadri College, shimoga.	Course – BT 1.4	Block BT 1.4A	1,2,3 and 4
Dr. Chandra Shekhar G. Joshi Asst. Professor Department of Biochemistry Cauvery Campus, Madikeri.	Course – BT 1.4	Block BT 1.4B	5, 6, 7 and 8
Dr. Bhuvana Rajan Professor and HOD Department of Biochemistry St. George College of Management and Science, Banaswadi, Bangalore	Course – BT 1.4 Course – BT 1.4	Block BT 1.4C Block BT 1.4D	9, 10, 11 and 12 13, 14, 15and 16

PUBLISHER

Registrar
Karnataka State Open University
Mukthagangotri, Mysore-570006

Developed by Academic Section, KSOU, Mysore

Further information on the Karnataka State Open University Programmes may be obtained from the University's Office at Mukthagangotri, Mysore-570006

Printed and Published on behalf of Karnataka State Open University, Mysore-570006 by

Registrar (Administration)

TABLE OF CONTENTS

INTRODUCTION TO BIOCHEMICAL TRANSFORMATION AND CLINICAL SIGNIFICANCE

All actions of living organisms are controlled by four classes of macromolecules namely Proteins, Carbohydrates Lipids and Nucleic acids. Living organisms transform matter and energy into different forms, show Response to changes in the environment, Grow and Reproduce which involves various biochemical reactions such as Oxidation, Reduction, Hydrolysis, Phosporylation, Deamination, Transamination *etc*.

The success of various endeavours in the emerging and expanding field of Biotechnology depend on appropriate design, conduct and analysis of clinical trials for the development of new therapy. No doubt the products from such technologies are potential in treating different previous untreated diseases.

In the text of this course attempt has been made to provide maximum information on different aspects of Biochemical transformation and clinical significance. All the units have been brought up to date by collecting information from different sources and modified in keeping pace with the learning interest and potential of Open University Students.

Each Unit begins with clearly stated learner-oriented objectives followed by terms important for thorough understanding of the text. Every unit at the end includes key words to easily remember the subject and questions to help the readers to self evaluate their grasp of the concepts. The complete format of self learning material of this course should definitely help in creating interest not only towards understanding various biochemical reactions but also for appropriate designing, conducting and analysing new technologies for the development of new therapy.

The content of this book is organized into 4 blocks, each block with 4 units.

The Block I consists of four units (1-4). Units-1 and 2 describe glycolysis, gluconeogenesis, synthesis and breakdown of glycogen, TCA cycle and its regulation, pentose phosphate pathway and glyoxylate cycle.

Unit-3 explains regulation of carbohydrate metabolism and related clinical disorders such as Diabetes mellitus, Lactose intolerance, glycogen storage disorder, Galactose urea, Fructose urea, and pentose urea. Unit-4 throughs light on bioenergetics related topics such as Electron transport chain, oxidative phosphorylation and organization of respiratory chain complexes.

The Block II consists of four units (5-8). Unit-5 explains structure and functions of oxidative phosphorylation, mechanism of ATP synthesis and ATP synthetase complex. Unit-6 describes proton motive force, Mitchell's hypothesis and integration of metabolism to bioenergetics. In Units-7 and 8 topics related to Photosynthesis such as chemistry and structural components of photo systems I and II, light harvesting antennae complex, Calvin cycle, C3 and C4 cycle, photorespiration are explained.

Block III consists of units (9-12). Unit-9 erxplains β-oxidation of saturated, unsaturated and branched chain fatty acids, paroxysomal β -oxidation and omega oxidation. Units-10and 11 deal with biosynthesis of saturated, unsaturated and branched chain fatty acids, biosynthesis of phospholipids by de novo pathway, interconversion; biosynthesis and regulation of cholesterol, atherosclerosis and other disorders of metabolism. Unit-12 explains Amino acid degradation by de-amination, trans-amination, degradation of individual amino acids and inherited diseases of proteins.

Block IV consists of four units (13-16). Units-13 and 14 deal with Urea cycle, biosynthesis of individual amino acids, in-born errors of amino acid metabolism, Pathway for degradation of purines and pyrimidine. The Unit-15 explains de novo synthetic pathways of ribo-nucleotides, salvage pathways, Gout, Lesch-Nyhan Syndrome and the last

Unit-16 explains biosynthesis, regulation of degradation and biosynthesis of deoxy ribonucleotides and its metabolic disorders.

Constructive suggestions, comments and criticism for the improvement of this book are most welcomed.

Dr. N. G. Raju
Chairman, Department of Biotechnology
KSOU, Mysore

BLOCK - I

UNIT- 1:

Carbohydrates: Glycolysis and Gluconeogenesis, Glycogen metabolism-synthesis and breakdown

STRUCTURE

1.0 OBJECTIVES

After studying this Unit you will be able to understand

- The catabolic and anabolic fates of glucose through glycolysis and gluconeogenisis
- Regulation of glucose metabolism
- The consequence of glycogen synthesis and breakdown
- The cellular dependency on glucose for deriving energy.

1.1. INTRODUCTION: CARBOHYDRATES

Carbohydrates are one of the four major classes of biomolecules along with proteins, nucleic acids, and lipids. Carbohydrates are aldehyde or ketone compounds with multiple hydroxyl groups. They make up most of the organic matter on Earth because of their extensive roles in all forms of life. They are precisely defined as polyhydroxy aldehydes and ketones.

- First, carbohydrates serve as energy stores, fuels, and metabolic intermediates.
- Second, ribose and deoxyribose sugars form part of the structural framework of RNA and DNA.
- Third, polysaccharides are structural elements in the cell walls of bacteria and plants. In fact, cellulose, the main constituent of plant cell walls, is one of the most abundant organic compounds in the biosphere.
- Fourth, carbohydrates are *linked to many proteins and lipids*, where they play key roles in mediating interactions among cells and interactions between cells and other elements in the cellular environment.

Animals can synthesize carbohydrate from lipid glycerol and amino acids, but most animal carbohydrate is derived ultimately from plants. **Glucose** is the most important carbohydrate; most dietary carbohydrate is absorbed into the bloodstream as glucose, and other sugars are converted into glucose in the liver. Glucose is the major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus. It is the precursor for synthesis of all the other carbohydrates in the body, including **glycogen** for storage; **ribose** and **deoxyribose** in nucleic acids; and **galactose** in lactose of milk, in glycolipids, and in combination with protein in glycoproteins and proteoglycans. Diseases associated with carbohydrate metabolism include **diabetes mellitus, galactosemia, glycogen storage diseases,** and **lactose intolerance.**

Carbohydrate Metabolism:

A carbohydrate plays a several crucial roles in the metabolic process of living organisms. They serve as energy sources and as structural elements in living cells. This unit deals with the role of carbohydrates in energy production. As glucose is a prominent energy source in almost all living cells, its synthesis, degradation, and storage is described elaborately.

Living cells are in a state of ceaseless activity. To maintain its "life," each cell depends on highly coordinated biochemical reactions. Carbohydrates are an important source of the energy that drives these reactions.

This chapter discusses the energy-generating pathways of carbohydrate metabolism are discussed. During **glycolysis**, an ancient pathway found in almost all organisms, a small amount of energy is captured as a glucose molecule is converted to two molecules of pyruvate. Glycogen, a storage form of glucose in vertebrates, is synthesized by **glycogenesis** when glucose levels are high and degraded by **glycogenolysis** when glucose is in short supply. Glucose can also be synthesized from noncarbohydrate precursors by reactions referred to as **gluconeogenesis**.

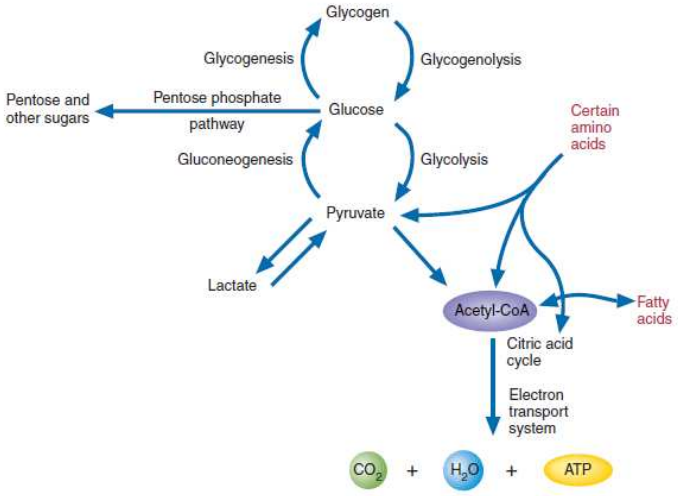


Fig 1.1 Pathways of Carbohydrate metabolism

1.2 GLYCOLYSIS – ITS BIOMEDICAL IMPORTANCE

Most tissues have at least some requirement for glucose. In brain, the requirement is substantial. Glycolysis, the major pathway for glucose metabolism, occurs in the cytosol of all cells. It is unique in that it can function either aerobically or anaerobically. Erythrocytes, which lack mitochondria, are completely reliant on glucose as their metabolic fuel and metabolize it by anaerobic glycolysis. However, to oxidize glucose

beyond pyruvate (the end product of glycolysis) requires both oxygen and mitochondrial enzyme systems such as the pyruvate dehydrogenase complex, the citric acid cycle, and the respiratory chain.

- Glycolysis is both the principal route for glucose metabolism and the main pathway for the metabolism of fructose, galactose, and other carbohydrates derived from the diet.
- The ability of glycolysis to provide ATP in the absence of oxygen is especially important because it allows skeletal muscle to perform at very high levels when oxygen supply is insufficient and because it allows tissues to survive anoxic episodes.
- Glycolysis, occurs, at least in part, in almost every living cell. This series of reactions is believed to be among the oldest of all the biochemical pathways. Both the enzymes and the number and mechanisms of the steps in the pathway are highly conserved in prokaryotes and eukaryotes.
- It is an anaerobic process, which would have been necessary in the oxygen-poor atmosphere of pre-eukaryotic Earth. In glycolysis, also referred to as the *Embden-Meyerhof-Parnas pathway*, each glucose molecule is split and converted to two three-carbon units (pyruvate).
- Glycolysis (Figure 1.2), which consists of 10 reactions, occurs in two stages:

  1. Glucose is phosphorylated twice and cleaved to form two molecules of glyceraldehyde-3-phosphate (G-3-P). The two ATP molecules consumed during this stage are like an investment, because this stage creates the actual substrates for oxidation in a form that is trapped inside the cell.

  2. Glyceraldehyde-3-phosphate is converted to pyruvate. Four ATP and two NADH molecules are produced. Because two ATP were consumed in stage 1, the net production of ATP per glucose molecule is 2.

1.2.1 The Glycolytic Pathway

Glycolysis is summarized in fig 1.2 which involve 10 reactions. The 10 reactions of glycolytic pathway are described below. The glycolytic pathway can be summed up in the following equation:

$$\textbf{D-Glucose + 2 ADP + 2 Pi + 2 NAD}^{+} \rightarrow \textbf{2 pyruvate + 2 ATP + 2 NADH + 2H}^{+} \textbf{ + 2H}_2\textbf{O}$$

During glycolysis, each glucose molecule is converted into two pyruvate molecules in addition to production of two molecules each of ATP and NADH. Reactions with double arrows are reversible reactions, and those with single arrows are irreversible reactions that serve as control points in the pathway.

The Reactions of the Glycolytic Pathway

1. Synthesis of glucose-6-phosphate. Glucose and other sugar molecules soon after entering a cell are first phosphorylated. The enzymes, called the hexokinases, catalyze the phosphorylation of hexoses and ATP acts as Phosphate donor and will be hydrolysed to ADP.

2. Conversion of glucose-6-phosphate to fructose-6-phosphate.In the second reaction of glycolysis, the open chain form of the aldose glucose-6-phosphate is converted to the open chain form of the ketose fructose-6-phosphate by phosphoglucose isomerase (PGI) in a readily reversible reaction:

3. The phosphorylation of fructose-6-phosphate. Phosphofructokinase-1 (PFK-1) irreversibly phosphorylates fructose-6-phosphate to form fructose-1,6-bisphosphate by investing a second molecule of ATP.

4. Cleavage of fructose-1,6-bisphosphate. It involves the cleavage of fructose-1,6-bisphosphate into two three-carbon molecules: glyceraldehyde-3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP). This reaction is catalyzed by aldolase enzyme.

5. The interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Only G-3-P serves as a substrate for the next reaction in glycolysis. The reversible DHAP is converted to G-3-P with the help of triose phosphate isomerase.

6. Oxidation of glyceraldehyde-3-phosphate. G-3-P undergoes oxidation and phosphorylation. The product, glycerate-1,3-bisphosphate, contains a high-energy phosphoanhydride bond, It requires inorganic phosphate group and convert NAD+ to $NADH+H^+$. This complex process is catalyzed by glyceraldehyde-3-phosphate dehydrogenase enzyme

7. Formation 2-phoConversion of Glyceraldehyde-1,3-bis phosphate to 3-phospho glycerate: In this reaction ATP is synthesized as phosphoglycerate kinase catalyzes the transfer of the high-energy phosphoryl group of glycerate-1,3-bisphosphate to ADP: Reaction 7 is an example of a substrate-level phosphorylation.

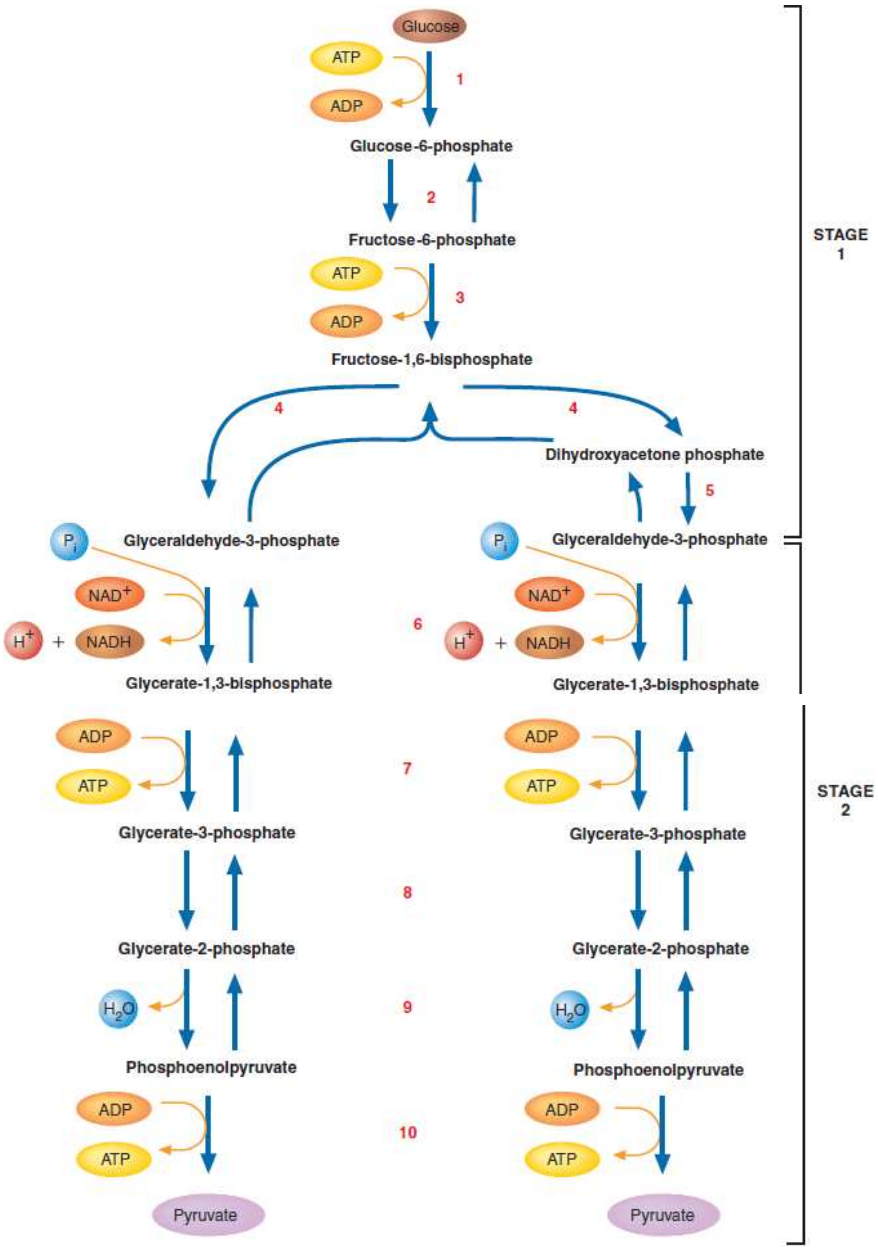


Fig 1.2 Summary of Glycolytic Pathway

8. The interconversion of 3-phosphoglycerate and 2-phosphoglycerate. Glycerate-3-phosphate has a low phosphoryl group transfer potential. Phosphoglycerate mutase catalyzes the conversion of a C-3 phosphorylated compound to a C-2 phosphorylated compound through a two-step addition/elimination cycle.

9. Dehydration of 2-phosphoglycerate tp phosphor enol pyravate: Enolase catalyzes the dehydration of glycerate-2-phosphate to form PEP: The *enol*form contains a carbon-carbon double bond and a hydroxyl group.

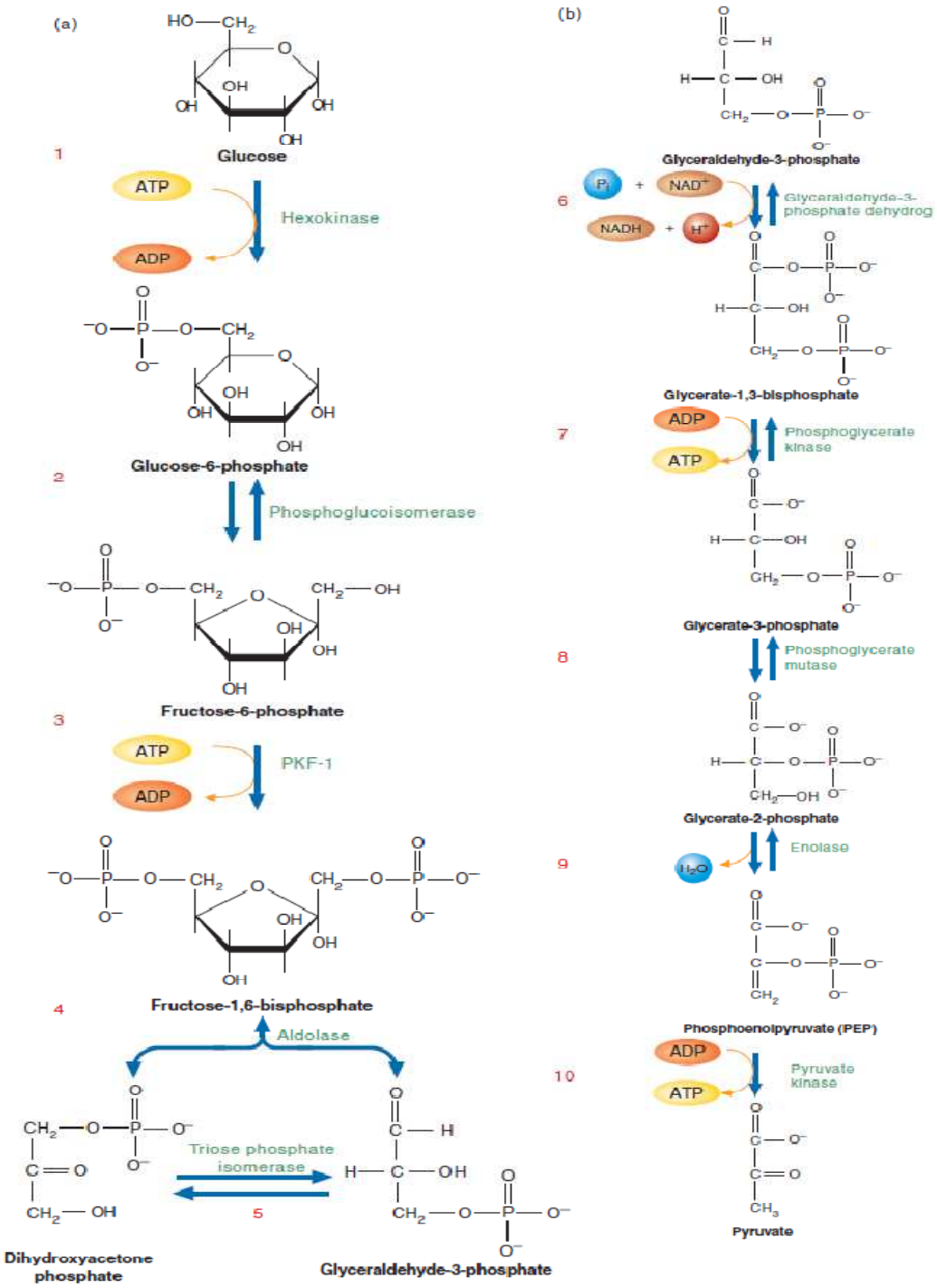


Fig 1.3 Summary of Reactions of Glycolytic Pathway

10. Synthesis of pyruvate. In the final reaction of glycolysis, pyruvate kinase catalyzes the transfer of a phosphoryl group from PEP to ADP. Two molecules of ATP are formed for each molecule of glucose. PEP is irreversibly converted to pyruvate because in this reaction—the transfer of a phosphoryl group from a molecule with a high transfer potential to one with a lower transfer potential. The detail reaction of glycolysis are illustrated in Fig-1.3.

1.2.2 The Fates of Pyruvate

In terms of energy, the result of glycolysis is the production of two ATPs and two NADHs per molecule of glucose. Pyruvate, the other product of glycolysis, is still an energy-rich molecule, which can yield a substantial amount of ATP. Whether or not further energy can be produced, however, depends on the cell type and the availability of oxygen.

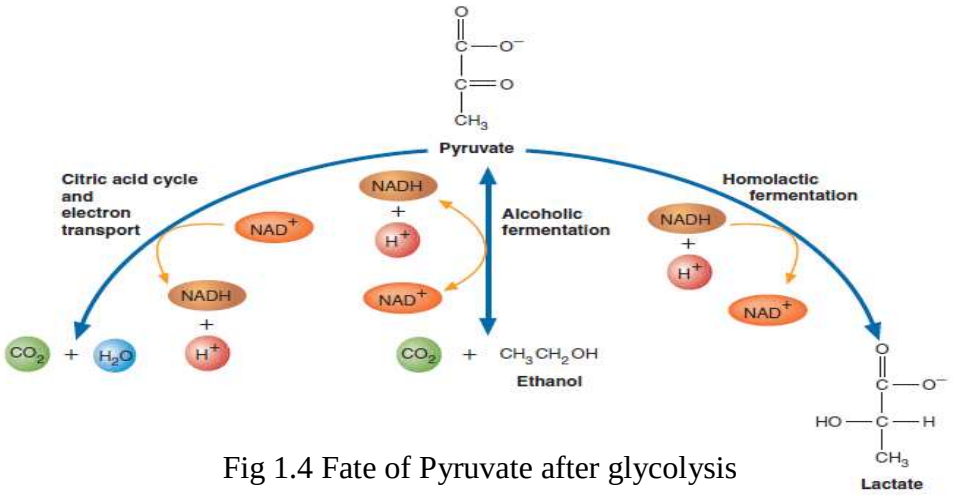


Fig 1.4 Fate of Pyruvate after glycolysis

- Under aerobic conditions, most cells in the body convert pyruvate into acetyl-CoA through pyruvate dehydrogenase enzyme complex in mitochondria, the entry-level substrate for the **citric acid cycle**, an amphibolic pathway that completely oxidizes the two acetyl carbons to form CO2 and the reduced molecules NADH and FADH2 (Fig 1.4).
- Under anaerobic conditions, further oxidation of pyruvate is impeded. A number of cells and organisms compensate by converting this molecule to a more reduced organic compound and regenerating the NAD+ required for glycolysis to continue. This process of NAD+ regeneration is referred to as **fermentation**. Muscle cells, red blood cells, and certain bacterial species (e.g., *Lactobacillus*) produce NAD+ by transforming pyruvate into lactate: In rapidly contracting muscle cells, the demand for energy is high. After the O2supply is depleted.

- Under strict anaerobic condition like in yeast and certain bacterial species, pyruvate is decarboxylated to form acetaldehyde, which is then reduced by NADH to form ethanol. (In a **decarboxylation** reaction, an organic acid loses a carboxyl group as CO2.). This process, called alcoholic fermentation, is used commercially to produce wine, beer, and bread.

1.2.3 The Energetic of Glycolysis:

During glycolysis, the energy released as glucose is broken down to pyruvate is coupled to the phosphorylation of ADP with a net yield of 2 ATP and 2 $NADH+H^+$ (Fig. 1.2 and 1.3). However, evaluation of the standard free energy changes of the individual reactions does not explain the efficiency of this pathway. A more useful method for evaluating free energy changes takes into account the conditions under which cells actually operate. Free energy changes measured in red blood cells indicate that only three reactions have significantly negative δG values. These reactions, catalyzed by hexokinase, PFK-1, and pyruvate kinase, respectively, are for all practical purposes irreversible; that is, each goes to completion as written. The values for the remaining reactions (2, 4–9) are so close to zero that they operate near equilibrium. Consequently, these latter reactions are easily reversible; small changes in substrate or product concentrations can alter the direction of each reaction.

1.2.4 Regulation of Glycolytic Pathway – It is Tightly Controlled

The glycolytic pathway has a dual role: it degrades glucose to generate ATP, and it provides building blocks for the synthesis of cellular components.

- The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs.
- Under physiologic conditions, the reactions of glycolysis are readily reversible except for the ones catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase (Table 1.1).
- Phosphofructokinase, the most important control element in glycolysis, is inhibited by high levels of ATP and citrate, and it is activated by AMP and fructose 2,6-bisphosphate.
- In the liver, this bisphosphate signals that glucose is abundant. Hence, phosphofructokinase is active when either energy or building blocks are needed.

- Hexokinase is inhibited by glucose 6-phosphate, which accumulates when phosphofructokinase is inactive.
- ATP and alanine allosterically inhibit pyruvate kinase, the other control site, and fructose 1,6-bisphosphate activates the enzyme. Consequently, pyruvate kinase is maximally active when the energy charge is low and glycolytic intermediates accumulate.

Table 1.1 Activator and Inhibitor of glycolytic regulatory enzymes

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

1.3 GLUCONEOGENESIS

Glucose Can Be Synthesized from Non-carbohydrate Precursors? We now turn to the *synthesis of glucose from noncarbohydrate precursors*, a process called *gluconeogenesis*. This metabolic pathway is important because the brain depends on glucose as its primary fuel and red blood cells use only glucose as a fuel.

The gluconeogenic pathway converts pyruvate into glucose. Noncarbohydrate precursors of glucose are first converted into pyruvate or enter the pathway at later intermediates such as oxaloacetate and dihydroxyacetone phosphate. The major noncarbohydrate precursors are *lactate*, *amino acids*, and *glycerol*. Lactate is formed by active skeletal muscle when the rate of glycolysis exceeds the rate of oxidative metabolism.

1.3.1. Gluconeogenisis: Is Not a Reversal of Glycolysis

In glycolysis, glucose is converted into pyruvate; in gluconeogenesis, pyruvate is converted into glucose. However, *gluconeogenesis is not a reversal of glycolysis*. Several reactions must differ because the equilibrium of glycolysis lies far on the side of pyruvate formation. Most of the decrease in free energy in glycolysis takes place in the three essentially irreversible steps catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase.

1. **Synthesis of PEP**. PEP synthesis from pyruvate requires two enzymes: pyruvate carboxylase and PEP carboxykinase (fig 1.5). Pyruvate carboxylase, found within

mitochondria, converts pyruvate to oxaloacetate (OAA): The transfer of CO2 to form the product OAA is mediated by the coenzyme *biotin*, which is covalently bound within the enzyme's active site. OAA is then decarboxylated and phosphorylated by PEP car boxykinase in a reaction driven by the hydrolysis of guanosine triphosphate (GTP): PEP carboxykinase is found within the mitochondria of some species and in the cytoplasm of others.

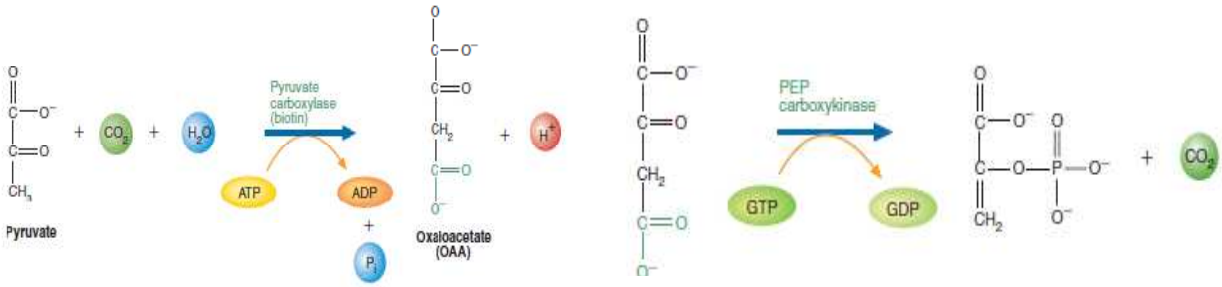


Fig 1.5 Synthesis of PEP from pyruvate through Oxaloacetate

In humans this enzymatic activity is found in both compartments. Because the inner mitochondrial membrane is impermeable to OAA, cells that lack mitochondrial PEP carboxykinase transfer OAA into the cytoplasm by using the **malate shuttle**. In this process, OAA is converted into malate by mitochondrial malate dehydrogenase.

2. **Conversion of fructose-1,6-bisphosphate to fructose-6-phosphate.**The irreversible PFK-1–catalyzed reaction in glycolysis is bypassed byfructose-1,6-bisphosphatase: This exergonic reaction is also irreversible under cellular conditions. ATP is not regenerated, and inorganic phosphate (Pi) is also produced. Fructose-1,6-bisphosphatase is an allosteric enzyme. Its activity is stimulated by citrate and inhibited by AMP and fructose- 2,6-bisphosphate (Fig 1.6).

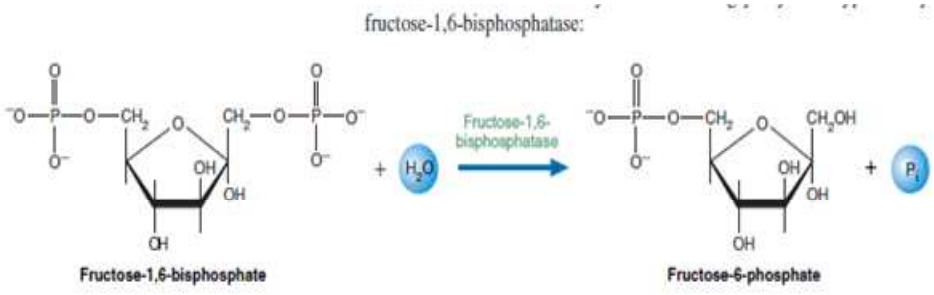


Fig 1.6 Conversion of fructose-1,6-bisphosphate to fructose-6-phosphate

3. **Formation of glucose from glucose-6-phosphate**. Glucose-6-phosphatase, found only in liver and kidney, catalyzes the irreversible hydrolysis of glucose-6-phosphate to form glucose and Pi. Glucose is subsequently released into the blood (Fig 1.7). Each of the foregoing reactions is matched by an opposing irreversible reaction in glycolysis.

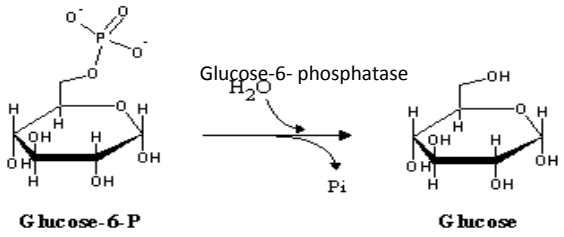


Fig. 1.7. Conversion of Glucose-6-phosphate into Glucose

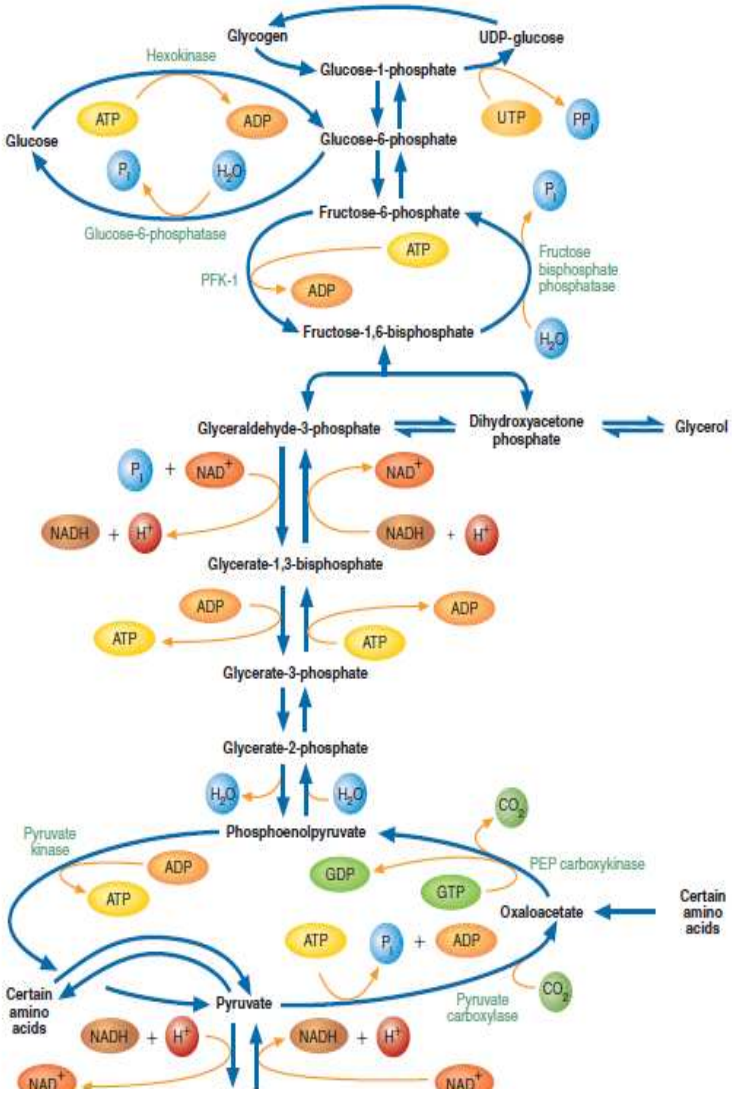


Fig. 1.8 Steps in Gluconeogesis which differs the reversibility of glycolysis

The following illustration in Fig 1.8 depicts the steps that differ in the Gluconeogesis from Glycolysis. The three steps which are not reversible will be the key difference between reversals of glycolysis.

The distinctive reactions and enzymes of this pathway are shown in red in fig 1.11. The other reactions are common to glycolysis. The enzymes for gluconeogenesis are located in the cytosol, except for pyruvate carboxylase (in the mitochondria) and glucose 6-phosphatase (membrane bound in the endoplasmic reticulum).

1.3.2. Reciprocal Regulation of Gluconeogenesis and Glycolysis in the Liver.

The level of fructose 2,6- bisphosphate is high in the fed state and low in starvation. Another important control is the inhibition of pyruvate kinase by phosphorylation during starvation.

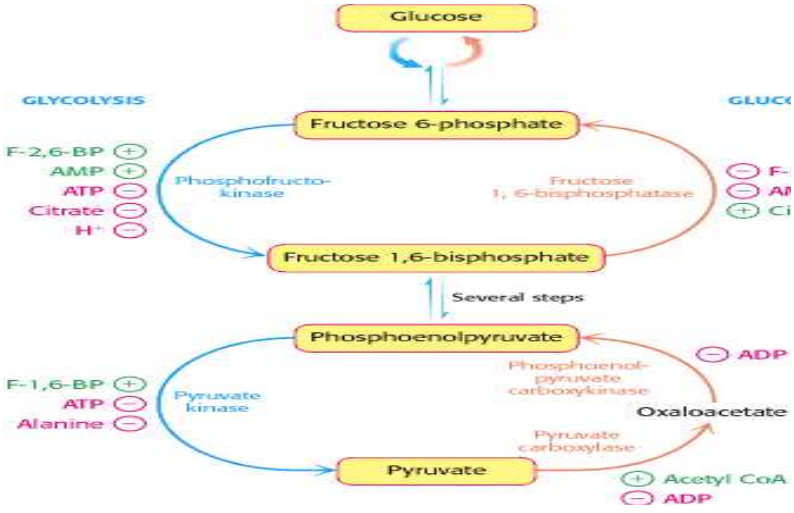


Fig. 1.9. Reciprocal regulation of Gluconeogenesis and Glycolysis

Glycolysis and gluconeogenesis are coordinated, in a tissue-specific fashion, to ensure that the glucose-dependent energy needs of all cells are met. **The Cori Cycle.** Lactate formed by active muscle is converted into glucose by the liver. This cycle shifts part of the metabolic burden of active muscle to the liver.

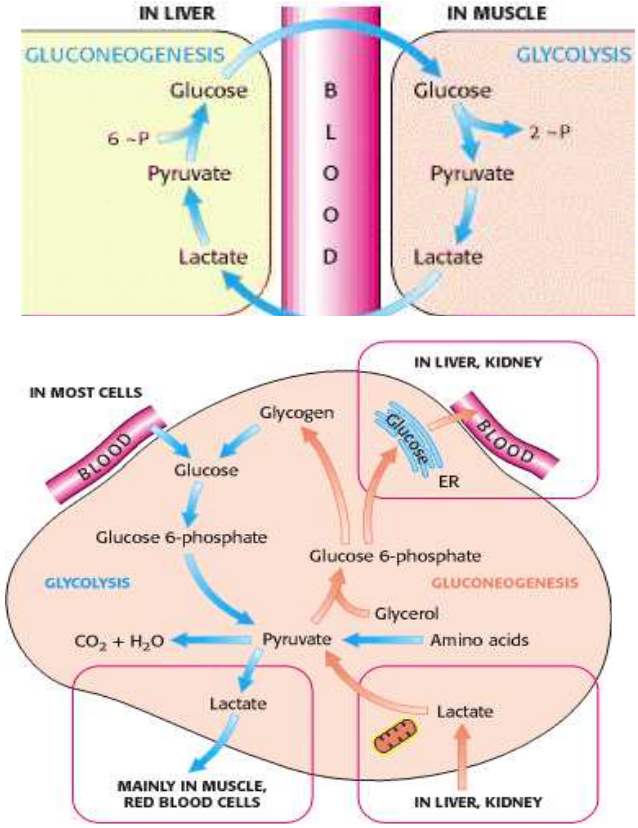


Fig 1.10 Cooperation between Glycolysis and Gluconeogenesis.

1.4 GLYCOGEN METABOLISM

Glycogen is a *readily mobilized storage form of glucose.* It is a very large, branched polymer of glucose residues that can be broken down to yield glucose molecules when energy is needed. Most of the glucose residues in glycogen are linked by alpha-1,4-glycosidic bonds. Branches at about every tenth residue are created by alpha-1,6-glycosidic bonds.

Glycogen Structure. In this structure of two outer branches of a glycogen molecule, the residues at the non-reducing ends are shown in red and residue that starts a branch is shown in green. The rest of the glycogen molecule is represented by R.

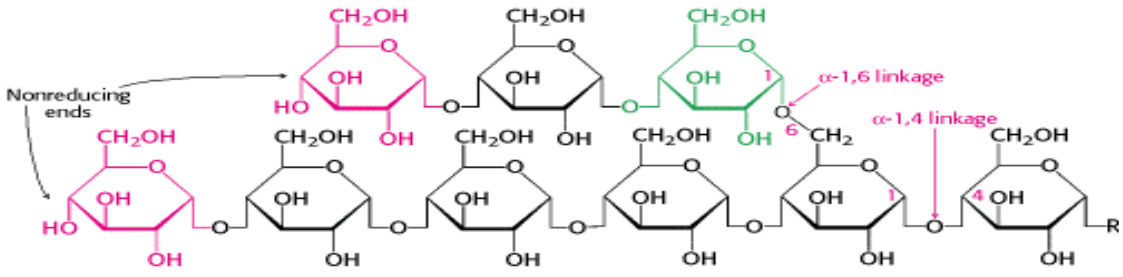


Fig 1.11 Structure of Glycogen

The two major sites of glycogen storage are the liver and skeletal muscle. The concentration of glycogen is higher in the liver than in muscle (10% versus 2% by weight), but more glycogen is stored in skeletal muscle overall because of its much greater mass. Glycogen is present in the cytosol in the form of granules ranging in diameter from 10 to 40 nm. In the liver, glycogen synthesis and degradation are regulated to maintain blood-glucose levels as required to meet the needs of the organism as a whole.

The glycogen in muscle is there to provide a quick source of energy for either aerobic or anaerobic metabolism. Muscle glycogen can be exhausted in less than an hour during vigorous activity. Liver glycogen serves as a reservoir of glucose for other tissues when dietary glucose is not available (between meals or during a fast); this is especially important for the neurons of the brain, which cannot use fatty acids as fuel. Liver glycogen can be depleted in 12 to 24 hours. Glycogen granules are complex aggregates of glycogen and the enzymes that synthesize it and degrade it, as well as the machinery for regulating these enzymes.

The synthesis and degradation of glycogen are carefully regulated so that sufficient glucose is available for the body's energy needs. Both glycogenesis and glycogenolysis are controlled primarily by three hormones: insulin, glucagon, and epinephrine.

1.4.1 Glycogenesis-Glycogen synthesis

Glycogen synthesis occurs after a meal, when blood glucose levels are high. It has long been recognized that the consumption of a carbohydrate meal is followed promptly by liver glycogenesis. The synthesis of glycogen from glucose-6-phosphate involves the following set of reactions.

1. **Synthesis of glucose-1-phosphate**. Glucose-6-phosphate is reversibly converted to glucose-1-phosphate by phosphoglucomutase, an enzyme that contains a phosphoryl group attached to a reactive serine residue: The enzyme's phosphoryl group is transferred to glucose-6-phosphate, forming glucose- 1,6-bisphosphate. As glucose-1-phosphate forms, the phosphoryl group attached to C-6 is transferred to the enzyme's serine residue.

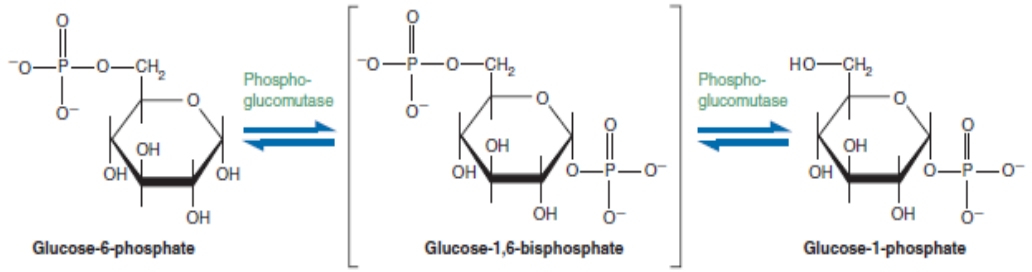


Fig 1.12 Synthesis of glucose-1-phosphate

2. Synthesis of UDP-glucose. Glycosidic bond formation is an endergonic process. Uridine diphosphate glucose (UDP-glucose) is more reactive than glucose and is held more securely in the active site of the enzymes catalyzing transfer reactions. Because UDP-glucose contains two phosphoryl bonds, it is a highly reactive molecule. Formation of UDP-glucose is a reversible reaction catalyzed by UDP-glucose pyrophosphorylase.

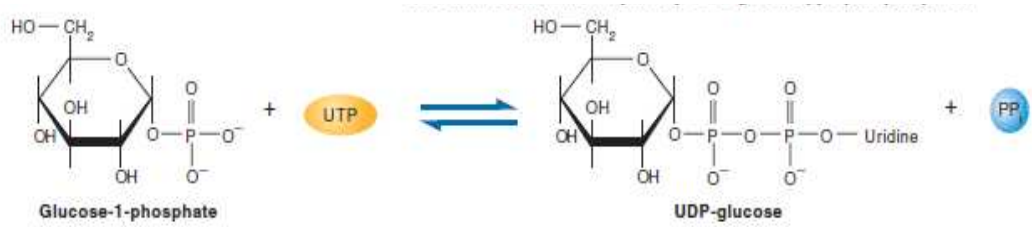


Fig 1.13 Synthesis of UDP-glucose.

2. **Synthesis of glycogen from UDP-glucose**. The formation of glycogen from UDP-glucose requires two enzymes: (a) glycogen synthase, which catalyzes the transfer of the glucosyl group of UDP-glucose to the non-reducing ends of glycogen amylo-alpha (1,4 →1,6)-glucosyl transferase (branching enzyme), which creates the alpha (1,6) linkages for branches in the molecule. Glycogen synthesis requires a preexisting tetrasaccharide composed of four alpha (1,4)-linked glucosyl residues. The first of these residues is linked to a specific tyrosine residue in a "primer" protein called *glycogenin.* The glycogen chain is then extended by glycogen synthase and branching enzyme. Large glycogen granules, each consisting of a single highly branched glycogen molecule, can be observed in the cytoplasm of liver and muscle cells of well-fed animals. The enzymes responsible for glycogen synthesis and degradation coat each granule's surface.

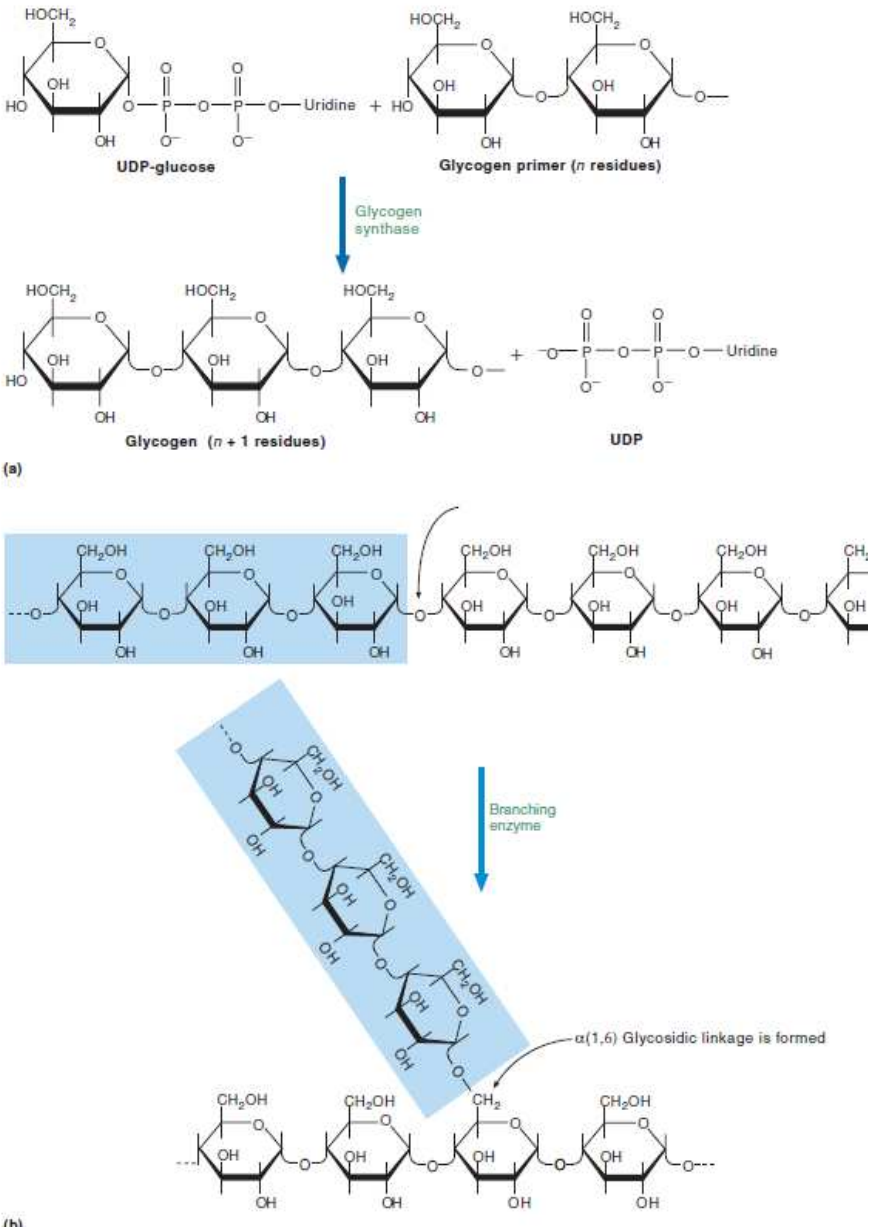


Fig 1.14 Synthesis of glycogen from UDP-

1.4.2 Glycogen Breakdown

The efficient breakdown of glycogen to provide glucose 6-phosphate for further metabolism requires four enzyme activities: one to degrade glycogen, two to remodel glycogen so that it remains a substrate for degradation, and one to convert the product of glycogen breakdown into a form suitable for further metabolism.

1. Glycogen Breakdown Is Catalyzed by Glycogen Phosphorylase: *Glycogen phosphorylase*, the key enzyme in glycogen breakdown, cleaves its substrate by the addition of orthophosphate (Pi) to yield *glucose 1-phosphate*. The cleavage of a bond by the addition of orthophosphate is referred to as *phosphorolysis*.

$$\underset{(n \text{ residues})}{\text{Glycogen}} + P_i \rightleftharpoons \text{glucose 1-phosphate} + \underset{(n-1 \text{ residues})}{\text{glycogen}}$$

- In skeletal muscle and liver, the glucose units of the outer branches of glycogen enter the glycolytic pathway through the action of three enzymes: glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase.
- Glycogen phosphorylase catalyzes the reaction in which an (*alpha*1-4) glycosidic linkage between two glucose residues at a nonreducing end of glycogen undergoes attack by inorganic phosphate (Pi), removing the terminal glucose residue as *alpha*-D-glucose 1-phosphate.
- Glycogen phosphorylase acts repetitively on the nonreducing ends of glycogen branches until it reaches a point four glucose residues away from an (*alpha* 1-6) branch point, where its action stops.
- Further degradation by glycogen phosphorylase can occur only after the **debranching enzyme,** formally known as **oligo (*alpha* 1-6) to (*alpha* 1-4) glucantransferase,** catalyzes two successive reactions that transfer branches.
- Once these branches are transferred and the glucosyl residue at C-6 is hydrolyzed, glycogen phosphorylase activity can continue.

2. A Debranching Enzyme is needed for the Breakdown of Glycogen:

Glycogen phosphorylase, the key enzyme in glycogen breakdown, can carry out this process by itself only to a limited extent before encountering an obstacle.

- The alpha-1,6-glycosidic bonds at the branch points are not susceptible to cleavage by phosphorylase. Indeed, phosphorylase stops cleaving alpha-1-4, linkages when it reaches a terminal residue four residues away from a branch point.
- Because about 1 in 10 residues is branched, glycogen degradation by the phosphorylase alone would come to a halt after the release of six glucose molecules per branch.

- Two additional enzymes, a transferase and alpha 1-6 glucosidase, remodel the glycogen for continued degradation by the phosphorylase. The transferase shifts a block of three glycosyl residues from one outer branch to the other. This transfer exposes a single glucose residue joined by an α-1,6-glycosidic linkage.
- Alpha-1,6-Glucosidase, also known as the debranching enzyme, hydrolyzes the alpha-1, 6-glycosidic bond, resulting in the release of a free glucose molecule. This free glucose molecule is phosphorylated by the glycolytic enzyme hexokinase.
- Thus, the transferase and alpha-1,6- glucosidase convert the branched structure into a linear one, which paves the way for further cleavage by phosphorylase.

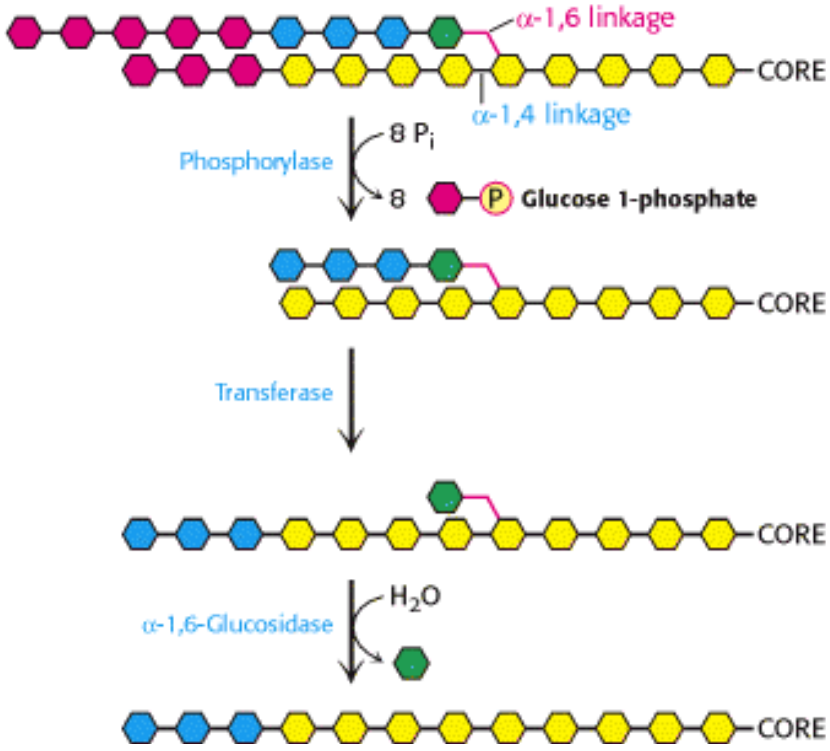


Fig 1.15 Glycogen Remodelling

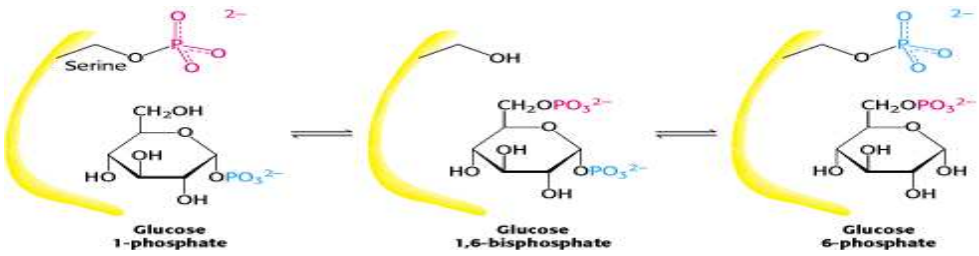


Fig 1.16 Conversion of Glucose 1-phosphate to glucose 6-phosphate

3. Glycogen Remodelling. First, alpha-1,4-glycosidic bonds on each branch are cleaved by phosphorylase, leaving four residues along each branch. The transferase shifts a block of three glycosyl residues from one outer branch to the other. In this reaction, the alpha-1,4-glycosidic link between the blue and the green residues is broken and a new alpha-1,4 link between the blue and the yellow residues is formed. The green residue is then removed by alpha-1,6-glucosidase, leaving a linear chain with all alpha-1,4 linkages, suitable for further cleavage by phosphorylase.

Glucose 1-Phosphate Can Enter Glycolysis or, in Liver, Replenish Blood Glucose: Glucose 1-phosphate, the end product of the glycogen phosphorylase reaction, is converted to glucose 6-phosphate by **phosphoglucomutase** (fig 1.16), which catalyzes the reversible reaction. A phosphoryl group is transferred from the enzyme to the substrate, and a different phosphoryl group is transferred back to restore the enzyme to its initial state. Glycogen phosphorylase cleaves the alpha (1,4) linkages of glycogen to yield glucose-1-phosphate until it comes within our glucose residues of a branch point. Debranching enzyme transfers three of these residues to a nearby nonreducing end and releases the fourth residue as free glucose. The repeated actions of both enzymes can lead to the complete degradation of glycogen.

1.4.3 Regulation of glycogen metabolism:

Glycogen metabolism is carefully regulated to avoid wasting energy. Both synthesis and degradation are controlled through a complex mechanism involving insulin, glucagon, and epinephrine, as well as allosteric regulators. Glucagon is released from the pancreas when blood glucose levels drop in the hours after a meal. It binds to receptors on hepatocytes and initiates a signal transduction process that elevates intracellular cAMP levels. cAMP amplifies the original glucagon signal and initiates a phosphorylation cascade that leads to the activation of glycogen phosphorylase along with a number of other proteins. Within seconds, glycogenolysis leads to the release of glucose into the bloodstream. When occupied, the insulin receptor becomes an active tyrosine kinase enzyme that causes a phosphorylation cascade that ultimately has the opposite effect of the glucagon/cAMP system: the enzymes of glycogenolysis are inhibited and the enzymes of glycogenesis are activated. Insulin also increases the rate of glucose uptake into several types of target cells, but not liver or brain cells.

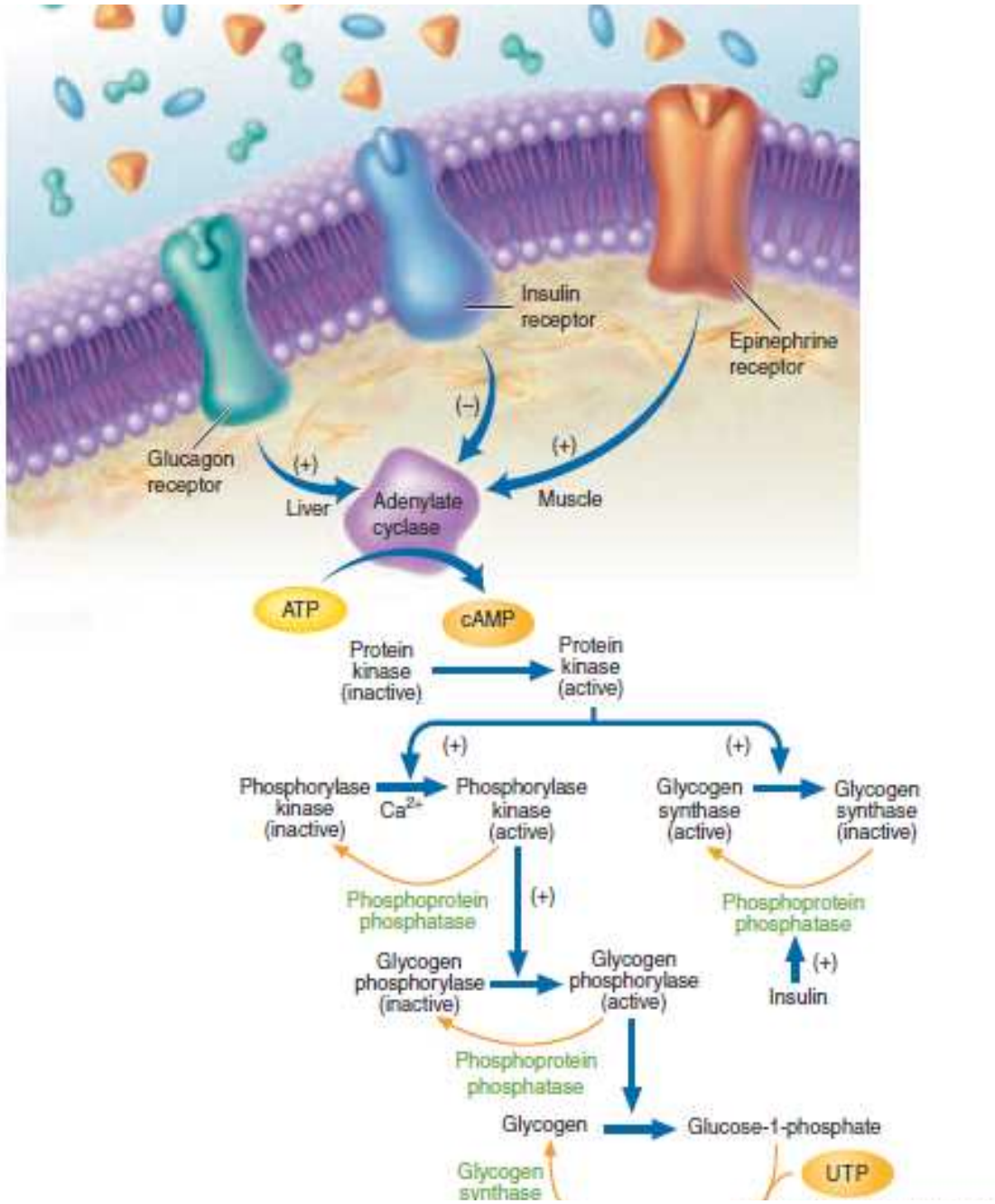


Fig 1.17 Regulation of glycogen metabolism

1.5 SUMMARY

Living cells are in a state of ceaseless activity. To maintain its "life," each cell depends on highly coordinated biochemical reactions. Carbohydrates are an important source of the energy that drives these reactions.

In This unit the energy-generating pathways of carbohydrate metabolism are discussed. During **glycolysis**, an ancient pathway found in almost all organisms, a small amount of energy is captured as a glucose molecule is converted to two molecules of pyruvate. Glycogen, a storage form of glucose in vertebrates, is synthesized by **glycogenesis** when glucose levels are high and degraded by **glycogenolysis** when glucose is in short supply. Glucose can also be synthesized from non-carbohydrate precursors by reactions referred to as **gluconeogenesis**.

1.6 KEYWORDS

- **Glycolysis** : a catabolic pathway for conversion of glucose into pyruvate
- **Gluconeogenesis**: an anabolic pathway for synthesis of glucose from non carbohydrate intermediates derived from lipids and amino acids.
- **Glycogenesis**: Synthesis of Glycogen from cellular glucose when there is excess of cellular or circulating glucose.
- **Glycogenolysis**: Breakdown of Glycogen during depletion of cellular or circulating glucose level.

1.7 QUESTIONS FOR SELF STUDY

1. Briefly explain the importance of glycolysis in glucose utilization by living cells.
2. Outline the glycolytic pathway and give its relevance to energy metabolism
3. Explain the Gluconeogenisis process and give its significance.
4. Detail on the synthesis and breakdown of glycogen in Liver and muscle cells.
5. How glycolysis is regulated give its regulatory mechanisms.

1.8 REFERENCES FOR FURTHER READING

1. Nelson DL and Cox MM. Principles of Biochemistry,5^{h} Editon, W.H. Freeman and Company
2. Berg J,Tymoczko JL and Stryer L. Biochemistry, 5th Editon, W.H. Freeman and Company
3. Satyanarayana U and Chakrapani U. Biochemistry, 3rd Edition, Books and allied (p) Ltd.
4. Janin JL, Jain S and Jain N. Fundamentals of Biochemistry, S.Chand and Company Ltd.

5. Geoffrey L. Zubey, William W. Parson, Dennis E. Vance. Wm. Principles of Biochemistry- C. Brown Publishers.2007

6. Dohald Voet and Judith, G. Voet. Biochemistry 3rd Ed.. Jhon Wiley and Sons Inc.2004