

- Acetyl-CoA is also an important precursor in fatty acid biosynthesis and cholesterol biosynthesis.

### CITRIC ACID CYCLE

Citric acid cycle is also called *Krebs cycle* or *tricarboxylic acid (TCA) cycle*.

- It is called citric acid cycle because citrate was one of the first compounds known to participate.
- It is called Krebs cycle, because its reactions were formulated into a cycle by *Sir Hans Krebs*.
- The most common name for this pathway is, the **tricarboxylic acid** or **TCA cycle**, due to involvement of the tricarboxylates—**citrate** and **isocitrate**.

#### Definition

The citric acid cycle is a series of reactions in mitochondria that brings about the catabolism of acetyl-CoA to CO<sub>2</sub> and H<sub>2</sub>O with generation of ATP.

#### Location of Citric Acid Cycle

The reactions of citric acid cycle are located in the **mitochondrial matrix**.

#### Reactions of Citric Acid Cycle (Figure 12.6)

1. First reaction of the citric acid cycle is the condensation of acetyl-CoA with oxaloacetate to yield citrate, catalyzed by *citrate synthase*.
2. Citrate is converted to isocitrate by an enzyme *aconitase*. This conversion takes place in two steps:
  - Dehydration to cis-aconitate
  - Rehydration to isocitrate.
3. Isocitrate undergoes dehydrogenation in the presence of *isocitrate dehydrogenase* to form oxalosuccinate. There follows a decarboxylation to  $\alpha$ -ketoglutarate, also catalyzed by isocitrate dehydrogenase. *The formation of NADH and liberation of CO<sub>2</sub> occurs at this stage.*
4. Next  $\alpha$ -ketoglutarate undergoes oxidative decarboxylation, catalyzed by a multi-enzyme complex,  *$\alpha$ -ketoglutarate dehydrogenase*, an  $\alpha$ -ketoglutarate dehydrogenase complex requires thiamine pyrophosphate (TPP), Lipoate, NAD, FAD and coenzyme-A and results in the formation of succinyl-CoA, a high energy compound, this reaction is physiologically *irreversible*. At this stage, *second NADH is produced along with liberation of second CO<sub>2</sub> molecule.*
5. Succinyl-CoA is converted to succinate by the enzyme *succinate thiokinase*. This reaction is coupled with the phosphorylation of GDP to GTP. This is a **substrate level phosphorylation**. This GTP is converted to ATP.

6. Succinate is oxidized further by *succinate dehydrogenase* to fumarate with the production of FADH<sub>2</sub>.
7. Next, *fumarase* catalyzes the addition of water to fumarate to give malate.
8. Malate is converted to oxaloacetate by *malate dehydrogenase*, and requires NAD<sup>+</sup>. *The synthesis of third NADH occurs at this stage.* The oxaloacetate is regenerated which can combine with another molecule of acetyl-CoA and continue the cycle.

#### Energetics of Citric Acid Cycle

- As a result of oxidation of acetyl-CoA to H<sub>2</sub>O and CO<sub>2</sub> by citric acid cycle, *three molecules of NADH* and *one FADH<sub>2</sub>* are produced.
- Oxidation of 3 NADH by electron transport chain results in the synthesis of 9 ATP, whereas FADH<sub>2</sub> generates 2 ATP molecules.
- One molecule of ATP is generated at substrate level during the conversion of succinyl-CoA to succinate. Thus, a total of 12 ATP are generated from one molecule of acetyl-CoA. (Table 12.3).

#### Significance of Citric Acid Cycle

- The primary function of the citric acid cycle is to provide energy in the form of ATP.
- Citric acid cycle is the final common pathway for the oxidation of carbohydrates, lipids, and proteins as glucose, fatty acids and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle.
- Citric acid cycle is an **amphibolic process**. Citric acid cycle has a dual function, it functions in both catabolism (of carbohydrates, fatty acids and amino acids) and anabolism. (Figure 12.7). Some metabolic pathways end in the constituent of the citric acid cycle while other pathways originate from the cycle, such as:
  - Gluconeogenesis
  - Transamination
  - Fatty acid synthesis
  - Heme synthesis.
- **Gluconeogenesis:** All major members of the citric acid cycle from citrate to oxaloacetate are glucogenic. They can give rise to glucose by gluconeogenesis.
- **Transamination :** Oxaloacetate and  $\alpha$ -ketoglutarate respectively, serve as precursors for the synthesis of aspartate and glutamate by transamination which in turn are used for the synthesis of other nonessential amino acids, purines and pyrimidines.
- **Fatty acid synthesis :** Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl-CoA for the biosynthesis of fatty acids and steroids.
- **Heme synthesis :** Succinyl-CoA (intermediate of TCA cycle) together with glycine is used for the synthesis of heme.

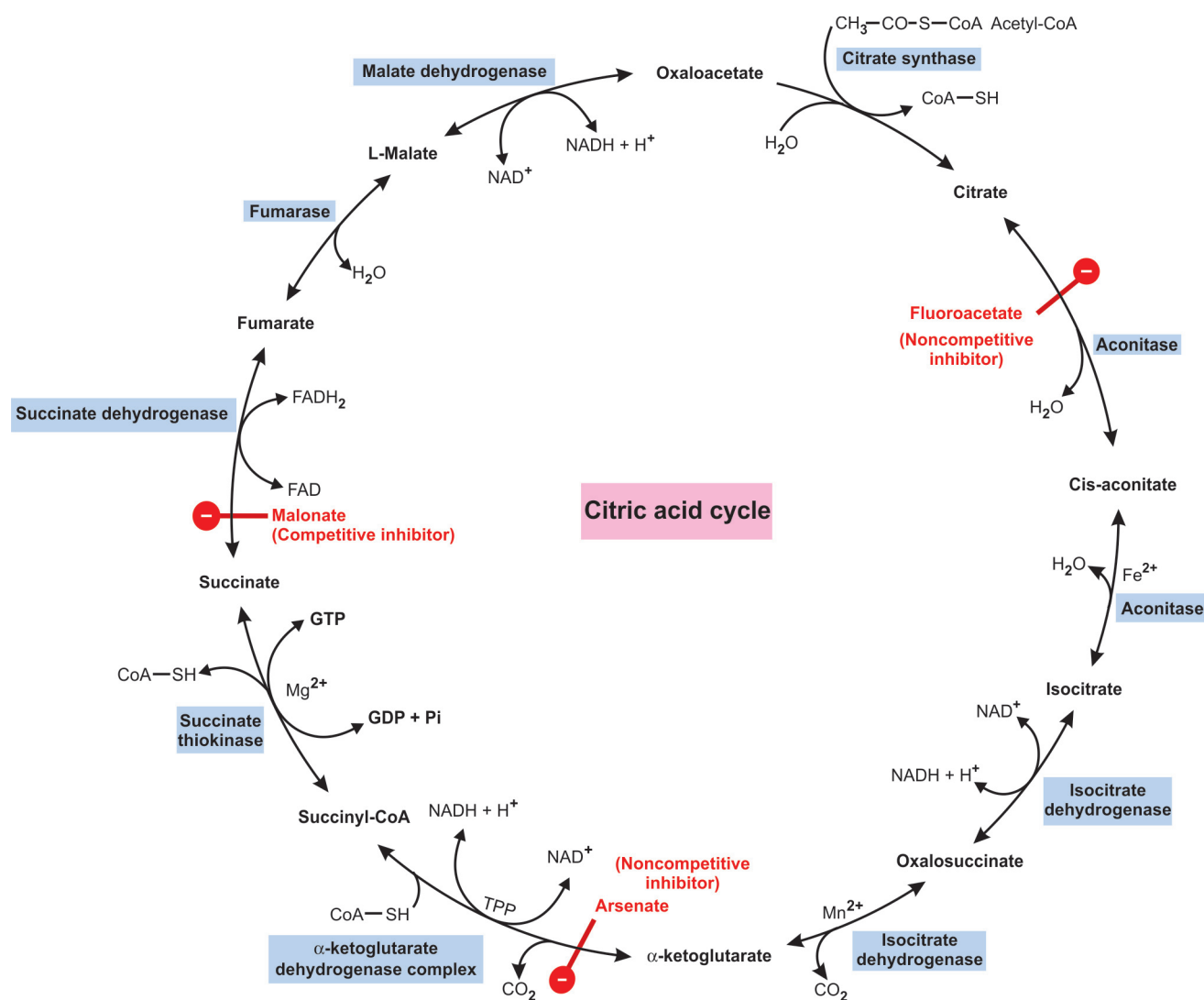
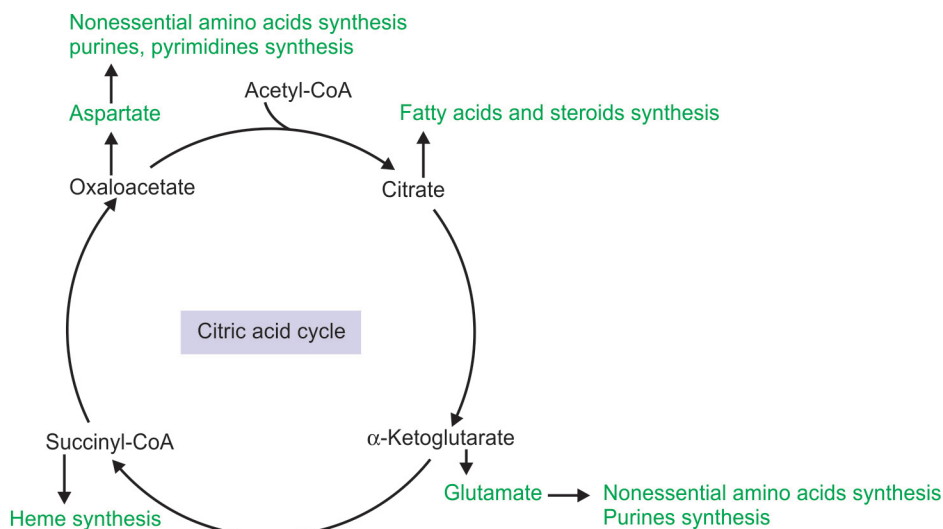


Figure 12.6: Reactions of citric acid cycle

Table 12.3 : Production of ATP in citric acid cycle

Reaction	Reaction catalyzed by	No. of ATP formed per acetyl-CoA molecule
Isocitrate to α-ketoglutarate	Isocitrate dehydrogenase	+3
α-Ketoglutarate to succinyl-CoA	α-Ketoglutarate dehydrogenase	+3
Succinyl-CoA to succinate	Succinyl thiokinase	+1
Succinate to fumarate	Succinate dehydrogenase	+2
Malate to oxaloacetate	Malate dehydrogenase	+3
<b>Number of ATPs formed per acetyl-CoA molecule in citric acid cycle = 12</b>		



**Figure 12.7:** Amphibolic role of the citric acid cycle

### Regulation of Citric Acid Cycle

- Citric acid cycle is regulated at three steps. These are catalyzed by:
  1. Citrate synthase
  2. Isocitrate dehydrogenase
  3.  $\alpha$ -ketoglutarate dehydrogenase.
- Activities of these enzymes are dependent on the energy status of the cycle.
- Excess of ATP, NADH and succinyl-CoA, which signals high energy status of the cell, inhibit these enzymes.
- High level of ADP which signals low energy status of the cell stimulates the operation of the cycle.

## GLUCONEOGENESIS

### Definition

The synthesis of glucose from noncarbohydrate precursors is called *gluconeogenesis* (i.e. synthesis of new glucose).

### Precursors for gluconeogenesis

The major noncarbohydrate substrates for gluconeogenesis are:

- Lactate

- Glycerol
  - Glucogenic amino acids
  - Propionate
  - Intermediates of the citric acid cycle.
1. **Lactate** : Lactate is formed from glucose by anaerobic glycolysis in the muscle. It is transported to the liver by **Cori's cycle** (discussed later) and is converted to glucose by gluconeogenesis.
  2. **Glycerol** : Glycerol is formed in adipose tissue by hydrolysis of triacylglycerol. Glycerol cannot be utilized by adipose tissue due to poor content of enzyme **glycerol kinase**. Therefore, it is delivered to the liver where it is converted to glucose by gluconeogenesis.
  3. **Glucogenic amino acids and intermediates of TCA cycle**: The carbon skeleton of glucogenic amino acids are converted to pyruvate or intermediates of TCA cycle, which are then converted to glucose by gluconeogenesis.
  4. **Propionate**: Fatty acids with an odd number of carbons and carbon skeleton of some amino acids produce propionate. Propionate enters the gluconeogenic pathway via citric acid cycle after conversion of succinyl-CoA (see Figure 13.8).

### Location of Gluconeogenesis

Gluconeogenesis occurs mainly in the cytosol although some precursors are produced in the mitochondria. **Liver** is the major tissue for gluconeogenesis. During starvation, the **kidney** is also capable of making glucose by gluconeogenesis. Certain enzymes required in gluconeogenesis are present only in these organs.

#### Characteristics of Gluconeogenesis

- Gluconeogenesis involves **glycolysis**, the **citric acid cycle** plus some **special reactions**.
- Glycolysis and gluconeogenesis share the same pathway but in opposite direction.
- Seven of the reactions of glycolysis are reversible and are used in the synthesis of glucose by gluconeogenesis.
- However, three of the reactions of glycolysis are irreversible and must be circumvented by four special reactions which are unique to gluconeogenesis and catalyzed by:
  1. Pyruvate carboxylase
  2. Phosphoenol pyruvate carboxykinase
  3. Fructose-1,6-bisphosphatase
  4. Glucose-6-phosphatase.

### Reactions of Gluconeogenesis (Figure 12.8)

1. **Carboxylation of pyruvate to oxaloacetate:** In gluconeogenesis, pyruvate is first carboxylated to oxaloacetate. *Pyruvate carboxylase* which in presence of ATP, vitamin biotin and  $\text{CO}_2$  converts pyruvate to oxaloacetate in mitochondria.  
**Transport of oxaloacetate to cytosol:** Oxaloacetate, formed in mitochondria, must enter the cytosol, where the other enzymes of gluconeogenesis are located. However, as oxaloacetate is unable to cross the inner mitochondrial membrane directly, it must be reduced to malate which can be transported from the mitochondria to the cytosol. In the cytosol, malate is reoxidized to oxaloacetate.
2. **Decarboxylation of cytosolic oxaloacetate to phosphoenol pyruvate (PEP):** Oxaloacetate is decarboxylated and phosphorylated in the cytosol by *phosphoenol pyruvate carboxykinase*. High energy phosphate in the form of GTP is required in this reaction. PEP then enters the reversed reaction of glycolysis until it reaches fructose-1, 6-bisphosphate.
3. **Dephosphorylation of fructose-1,6-bisphosphate to fructose-6-phosphate:** Hydrolysis of fructose-1, 6-bisphosphate to fructose-6-phosphate by *fructose-1,6-bisphosphatase* bypasses the irreversible

phosphofructokinase-I reaction of glycolysis. Fructose-6-phosphate is then converted to glucose-6-phosphate by reversed reaction of glycolysis.

4. **Dephosphorylation of glucose-6-phosphate to glucose:** Hydrolysis of glucose-6-phosphate to glucose by *glucose-6-phosphatase* bypasses the irreversible glucokinase and hexokinase reaction of glycolysis.

### Regulation of Gluconeogenesis

Gluconeogenesis is regulated by four key enzymes.

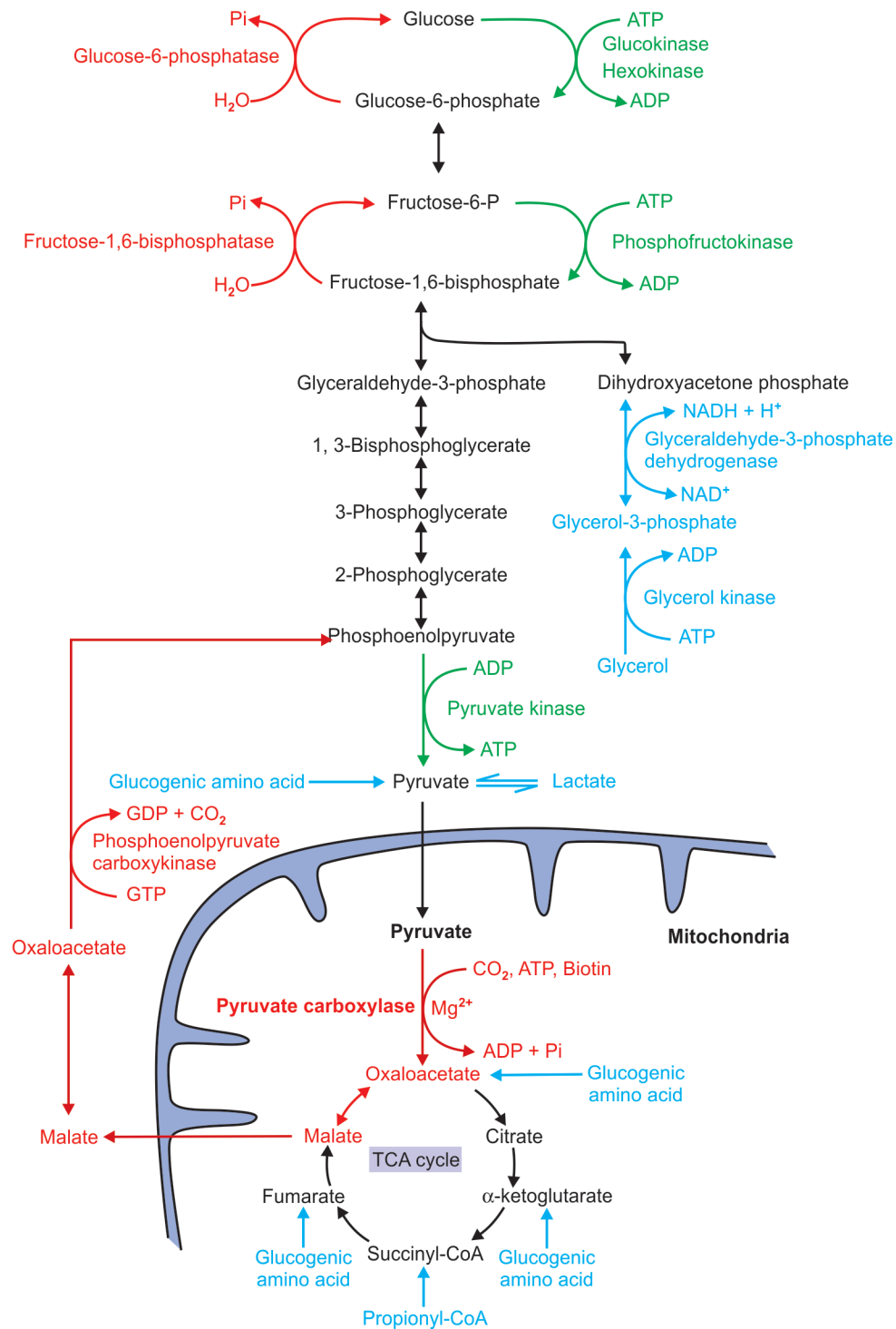
1. Pyruvate carboxylase
  2. Phosphoenolpyruvate carboxykinase
  3. Fructose-1,6-bisphosphatase
  4. Glucose-6-phosphatase.
- The hormones **glucagon** and **epinephrine** stimulate gluconeogenesis by inducing the synthesis of the key enzymes, while **insulin inhibits** the gluconeogenesis by repressing their synthesis.
  - During starvation and in diabetes mellitus, a high level of glucagon stimulates gluconeogenesis. However in well-fed state, insulin suppresses the gluconeogenesis.
  - Pyruvate carboxylase is an allosteric enzyme, which is stimulated by acetyl-CoA and inhibited by ADP.
  - Fructose-1,6-bisphosphatase stimulated allosterically by c-AMP and inhibited by AMP.

#### Significance of Gluconeogenesis

- Gluconeogenesis maintains blood glucose level when carbohydrate is not available in sufficient amounts from the diet.
- During starvation when hepatic glycogen reserve is totally depleted, glucose is provided by gluconeogenesis to the brain and other tissues like erythrocytes, lens, cornea of the eye and kidney medulla. They require a continuous supply of glucose as a source of energy.
- Gluconeogenesis is used to clear the products of the metabolism of other tissues from the blood, for example,
  - Lactate, produced by muscle and erythrocytes
  - Glycerol produced by adipose tissue
  - Propionyl-CoA produced by oxidation of odd carbon number fatty acids and carbon skeleton of some amino acids.

### CORI CYCLE OR LACTIC ACID CYCLE

Lactate is produced in skeletal muscles during anaerobic oxidation of glucose. The lactate thus produced cannot be further metabolized in skeletal muscles. Through blood,



**Figure 12.8:** Pathway of gluconeogenesis

- Special reactions and enzymes of gluconeogenic pathway are shown in red.
- Irreversible reactions of glycolysis are shown in green.
- Remaining reactions which are common to glycolysis and gluconeogenesis are shown in black.
- The entry points of substrate are shown in blue.

lactate is transported to the liver where it is oxidized to pyruvate. Pyruvate so produced, is converted to glucose by gluconeogenesis, which is then transported to the muscle. The glucose thus reformed from lactate again becomes available for energy purpose in skeletal muscle.

This cycling of lactate between muscle and liver is known as the **Cori Cycle** or **Lactic acid cycle** (Figure 12.9).

### GLUCOSE-ALANINE CYCLE

- Because muscle is incapable of synthesizing urea, most of the ammonia formed by protein catabolism is transferred to pyruvate to form **alanine** by transamination reaction.
- Alanine enters the blood and is taken up by the liver.
- In the liver, the amino groups of alanine is removed to form urea, and the resulting pyruvate is converted to glucose by gluconeogenesis which is then transported to the muscle, where it is oxidized to pyruvate.
- The pyruvate acts again as the acceptor for another amino group.
- These reactions transport amino groups from muscle to the liver in the form of **alanine**. This cycle is called

the **glucose-alanine cycle** or the **Cahill cycle** (Figure 12.9).

Alanine is the predominant amino acid released from muscle to liver during fasting.

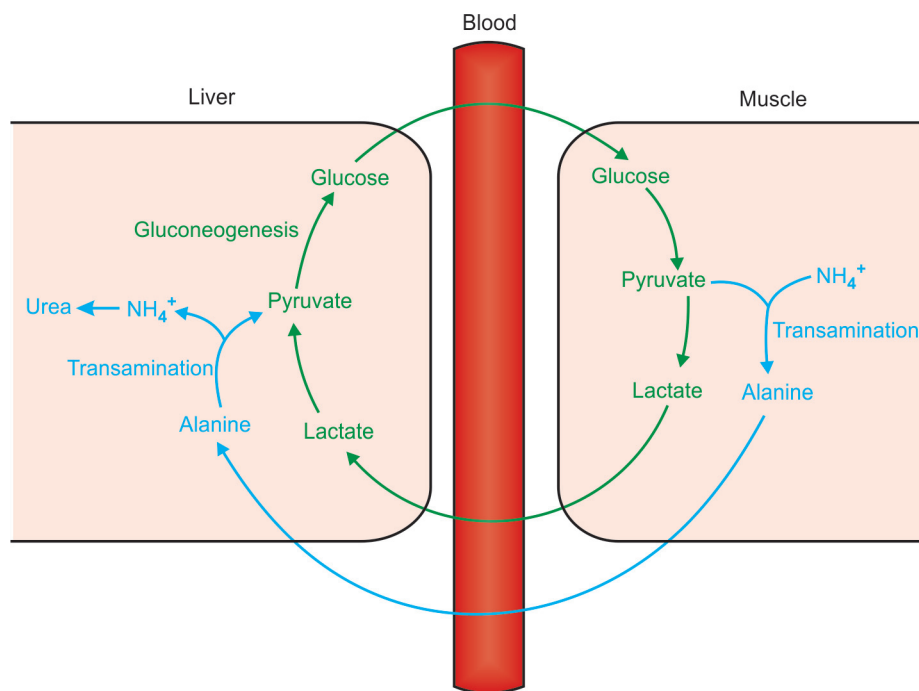
### GLYCOGEN METABOLISM

- Glycogen is the major storage form of glucose mainly in the liver and muscle.
- The concentration of liver glycogen (up to 6%) is greater than in muscle (1%) tissues. However, because muscle tissue comprises a large mass, its total capacity to storage is three to four times that of the liver.
- The synthesis, **glycogenesis** and degradation, **glycogenolysis** occur via different pathways. Glycogenesis and glycogenolysis are both cytosolic processes.

### Glycogenesis

#### Definition

Glycogenesis is the pathway for the formation of glycogen from glucose. This process requires energy, supplied by ATP and uridine triphosphate (UTP). It occurs in muscle and liver.



**Figure 12.9:** Cori cycle or lactic acid cycle and glucose alanine cycle. The pathway of Cori cycle is shown in green and glucose alanine cycle in blue



### Reactions of Glycogenesis (Figure 12.10)

1. Glucose is phosphorylated to glucose-6-phosphate catalyzed by *hexokinase* in muscle and *glucokinase* in liver.
2. Glucose-6-phosphate is converted to glucose-1-phosphate by the enzyme *phosphoglucomutase*.
3. Glucose-1-phosphate reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPGlc). The reaction is catalyzed by the enzyme *UDP-glucose pyrophosphorylase*.
4. By the action of the enzyme *glycogen synthase*, the C<sub>1</sub> of the glucose of UDPGlc forms a glycosidic bond with C<sub>4</sub> of a terminal glucose residue of pre-existing glycogen molecule (glycogen primer), liberating uridine diphosphate (UDP). Thus, pre-existing glycogen molecule must be present to initiate this reaction.
5. In the above reaction, a new  **$\alpha$ -1,4 linkage** is established between carbon atom 1 of incoming glucose and carbon 4 of the terminal glucose of a glycogen primer.

6. When the chain has been lengthened to a minimum of 11 residues, a second enzyme, the **branching enzyme**, transfers a part of the 1,4-chain (minimum length of 6-glucose residues) to a neighboring chain to form  $\alpha$ -1,6-linkage, thus establishing a branching point in the molecule (**Figure 12.11**). The branches grow by further additions of glucose units and further branching.

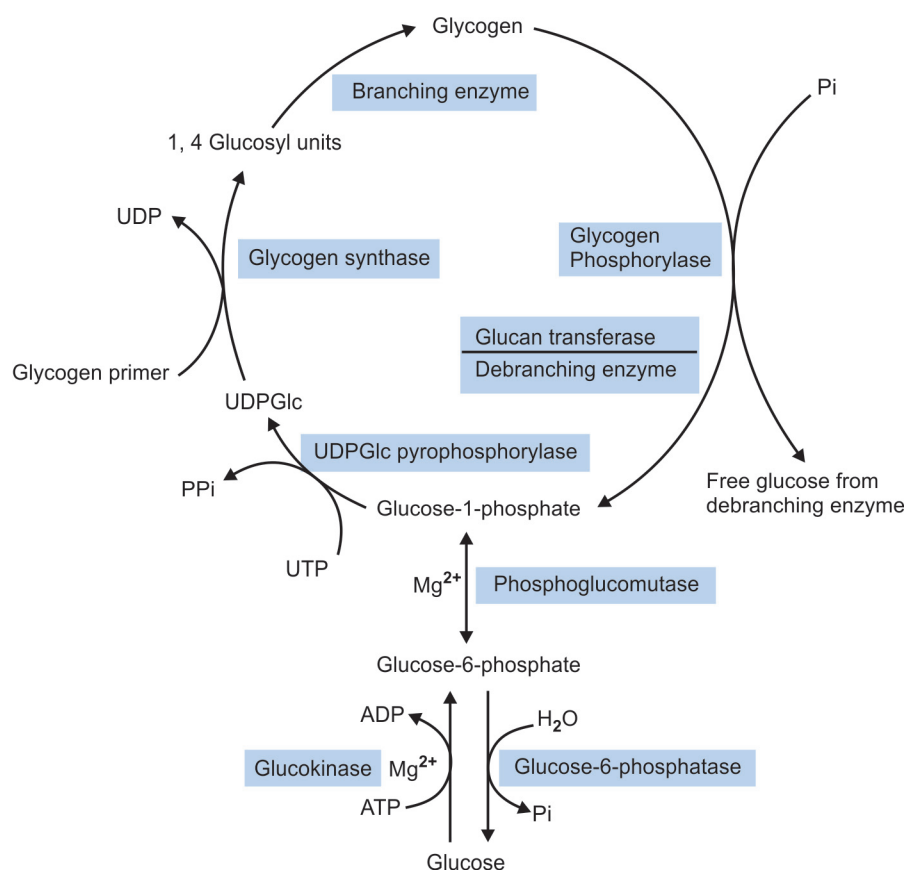
### Glycogenolysis

#### Definition and Location

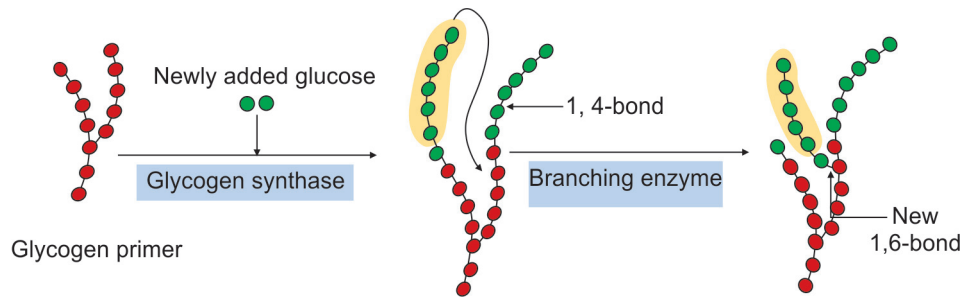
Glycogenolysis is the degradation of glycogen to glucose-6-phosphate and glucose in muscle and liver respectively. Glycogenolysis is not the reverse of glycogenesis but is a separate pathway.

### Reactions of Glycogenolysis (Figure 12.10)

1. Glycogenolysis occurs primarily by phosphorolytic breaking of  $\alpha$ -1,4-glycosidic bonds of glycogen to



**Figure 12.10:** Pathway of glycogenesis and glycogenolysis in liver where, UTP = Uridine triphosphate, UDP = Uridine diphosphate, UDPGlc = Uridine diphosphate glucose



**Figure 12.11:** Schematic representation of glycogenesis (mechanism of branching)

yield glucose-1-phosphate and residual glycogen molecule. This process is catalyzed by the enzyme *glycogen phosphorylase*. The glucose residues from outermost chain of the glycogen molecule are removed sequentially until approximately four glucose residues remain on either side of a branch point having  $\alpha$ -1,6 linkage (**Figure 12.12**).

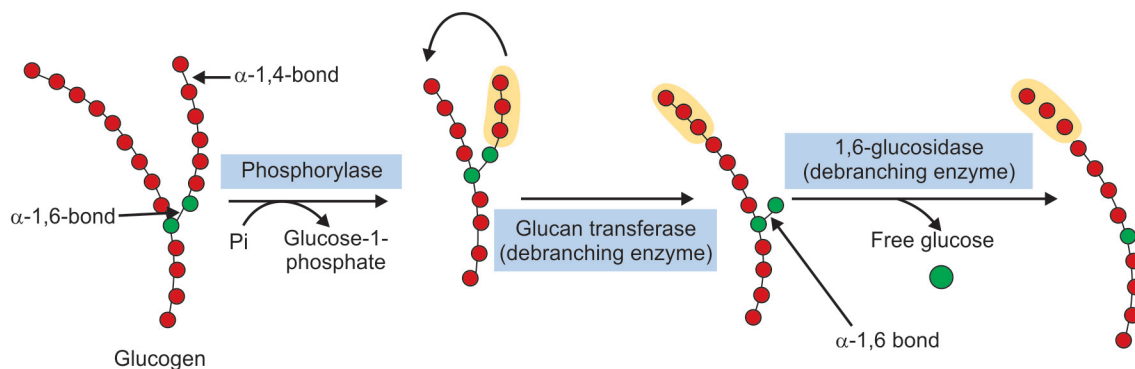
2. Phosphorolysis cannot continue until the branch is removed. This is accomplished by *debranching enzyme*. It has two catalytic activities—*glucan transferase* and *1,6-glucosidase*.
    - First, it acts as a *glucan transferase* and transfers three of the remaining residues from one branch to the other. This exposes the  $\alpha$ -1,6 branch point.
    - In the second step, the hydrolytic splitting of the  $\alpha$ -1,6 linkages occurs by the action of *1,6-glucosidase*. This step releases free glucose. Further splitting of the glycogen can then proceed by the actions of phosphorylase until another branch point is reached. The action of glucan transferase and 1,6-glucosidase are repeated.
- The combined action of *phosphorylase* and *debranching enzyme* leads to the complete break-

down of glycogen with the formation of glucose-1-phosphate and free glucose (from hydrolytic cleavage of the 1,6-glycosidic bond).

3. Next, glucose-1-phosphate is converted to glucose-6-phosphate by *phosphoglucomutase*. This is a reversible reaction.
4. In the liver but *not in the muscle*, there is a specific enzyme, *glucose-6-phosphatase*, that cleaves glucose-6-phosphate to glucose and diffuse from the hepatic cell into the blood. As glucose-6-phosphatase is absent in muscle, free glucose cannot be produced from glucose-6-phosphate in muscle. Moreover, glucose-6-phosphate cannot diffuse out of the muscles. Therefore, muscle cannot provide glucose to maintain blood glucose level.

### Lysosomal Degradation of Glycogen

A small amount of glycogen is continuously degraded by the lysosomal enzyme  *$\alpha$ -1,4-glucosidase (acid maltase)*. The significance of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in the cytosol resulting in *glycogen storage disease type II Pompe's disease*.



**Figure 12.12:** Schematic representation of glycogenolysis (mechanism of debranching)



### Significance of Glycogenolysis and Glycogenesis

The functional role of glycogen differs considerably from tissue to tissue, as we can see in the case of liver and muscle.

#### In liver

Following a meal, excess glucose is removed from the portal circulation and stored as glycogen by glycogenesis. Conversely, between meals, blood glucose levels are maintained within the normal range by release of glucose from liver glycogen by glycogenolysis.

#### In muscle

The function of muscle glycogen is to act as a readily available source of glucose within the muscle itself during muscle contraction. *The muscle cannot release glucose into the blood, because of the absence of glucose-6-phosphatase that hydrolyzes glucose 6-phosphate to glucose.* Therefore, muscle glycogen stores are used exclusively by muscle.

### Regulation of Glycogenesis and Glycogenolysis

The principal enzymes controlling glycogen metabolism are *glycogen phosphorylase* and *glycogen synthase*

which are regulated reciprocally. Regulation of these enzymes involve:

- Hormonal regulation
- Allosteric regulation.

#### Hormonal Regulation

- **Epinephrine** and **glucagon** regulate glycogen breakdown and glycogen synthesis.
- Epinephrine (in liver and muscle) and glucagon (in liver) stimulates glycogen breakdown (glycogenolysis) and inhibits glycogen synthesis (glycogenesis).

#### Regulation of glycogenesis (Figure 12.13)

- **Glycogen synthase** is the regulatory enzyme of glycogenesis. It exists in two forms: **Glycogen synthase-a**, an active or dephosphorylated form and **Glycogen synthase-b**, an inactive or phosphorylated form.
- Glucagon in liver and epinephrine in liver and muscle activates **adenylate cyclase** enzyme that catalyzes the synthesis of **c-AMP**. c-AMP in turn activates **c-AMP dependent protein kinase**.
- c-AMP dependent protein kinase then **phosphorylates glycogen synthase** and thereby inactivates glycogen synthase and synthesis of glycogen is inhibited.

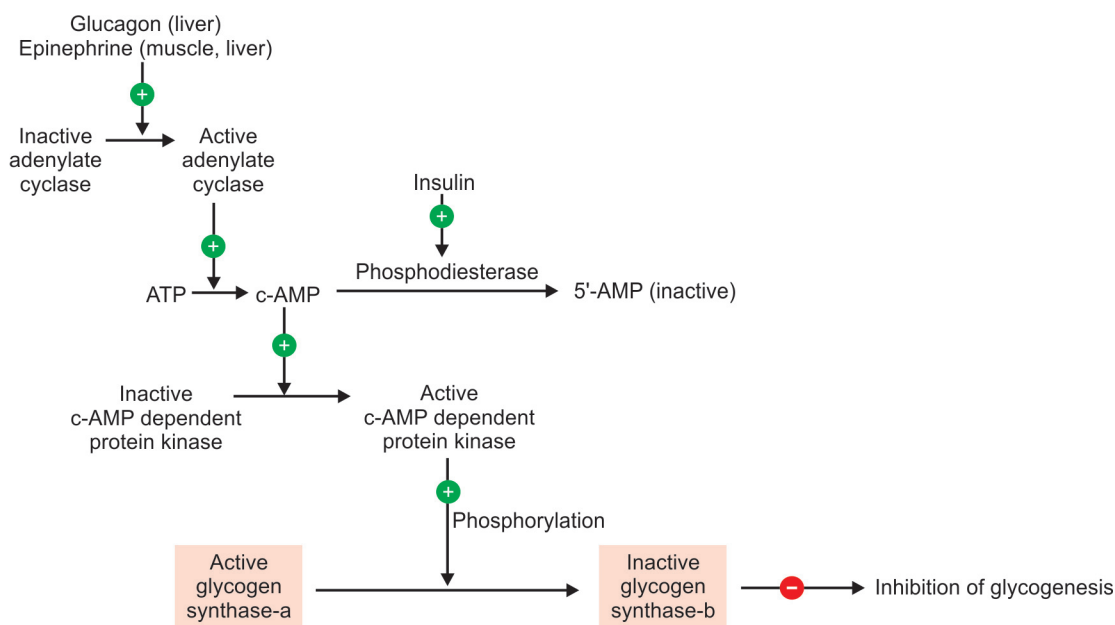


Figure 12.13: Hormonal regulation of glycogenesis

- The hormone **insulin** increases the phosphodiesterase activity in liver and lowers the c-AMP levels and inhibits the action of glucagon and epinephrine.

#### Regulation of glycogenolysis (Figure 12.14)

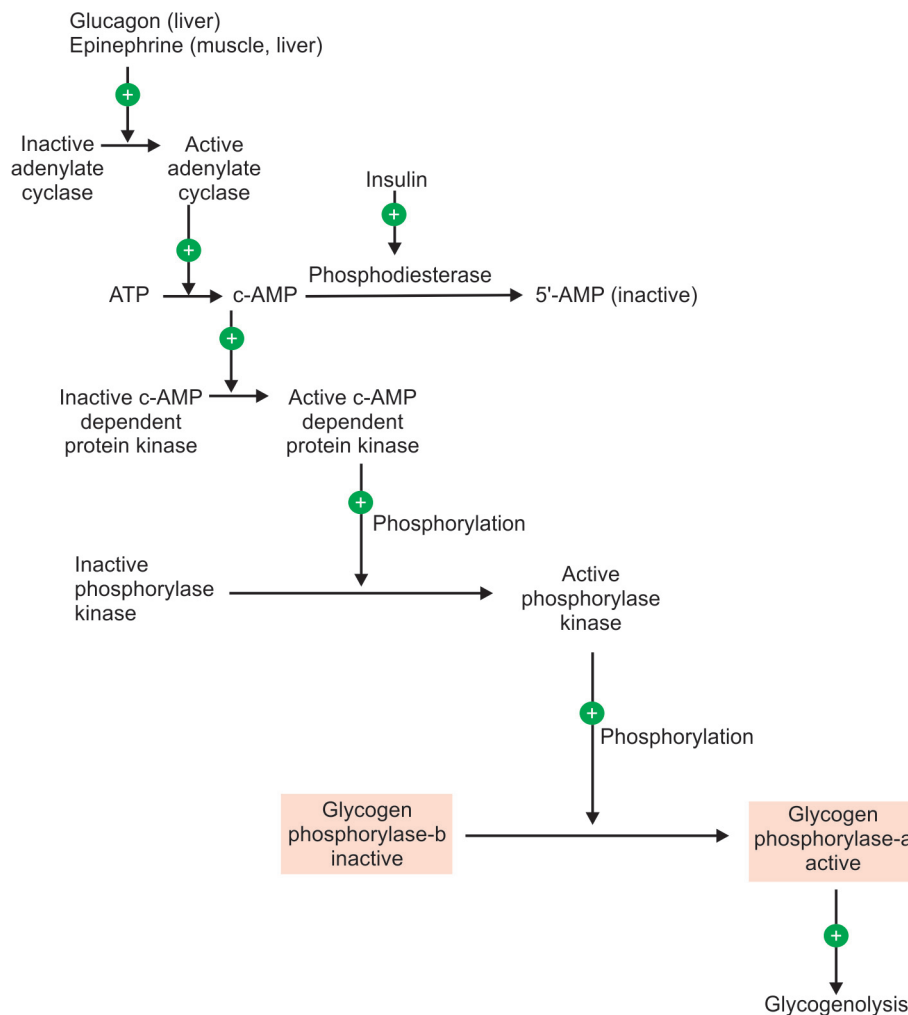
- Glycogen phosphorylase** is the regulatory enzyme of glycogenolysis. It exists in two forms: **Glycogen phosphorylase-a**, an active or phosphorylated form and **Glycogen phosphorylase-b**, an inactive or dephosphorylated form.
- Degradation of glycogen is stimulated by epinephrine in the muscle and by glucagon in the liver via activation of **adenylate cyclase** that catalyzes the synthesis of c-AMP.
- The consequent increase in levels of c-AMP in turn activates **c-AMP dependent protein kinase**. Active

c-AMP dependant protein kinase phosphorylates the inactive form of **phosphorylase kinase** to its active form.

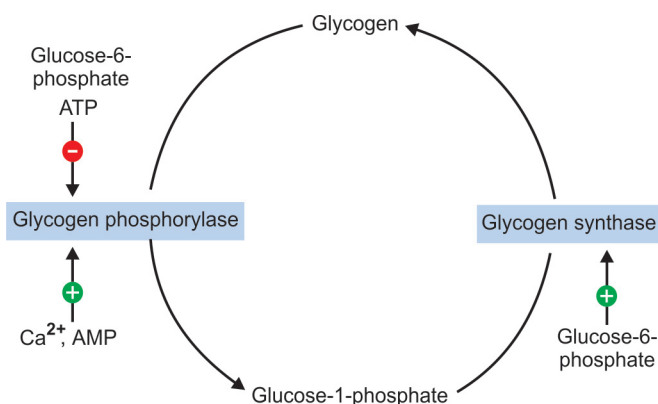
- Active phosphorylase kinase eventually activates inactive form of glycogen phosphorylase to its active form. Active form of **glycogen phosphorylase** stimulates breakdown of glycogen to glucose-1-P (Figure 12.14).

#### Allosteric Regulation (Figure 12.15)

- Glycogen synthase** is allosterically activated by glucose-6-phosphate when it is present in elevated concentrations.
- In contrast, **glycogen phosphorylase** is allosterically inhibited by glucose-6-phosphate.



**Figure 12.14:** Hormonal regulation of glycogenolysis



**Figure 12.15:** Allosteric regulation of glycogenesis and glycogenolysis

- The  $\text{Ca}^{2+}$  ions stimulate the glycogenolysis by activation of glycogen phosphorylase.
- Increased level of AMP in vigorously contracting muscles stimulates glycogen breakdown by stimulating glycogen phosphorylase allosterically. However, in resting muscles ATP inhibits the glycogen breakdown by allosteric inactivation of glycogen phosphorylase.

### Glycogen Storage Disease

This is a group of genetic diseases, that result from a defect in an enzyme required for either glycogen

synthesis or degradation and characterized by deposition of either normal or abnormal glycogen in the specific tissues. Some of the more common forms of these diseases and their characteristics are summarized in **Table 12.4**.

### PENTOSE PHOSPHATE PATHWAY

#### Definition

The pentose phosphate pathway is an alternative route for the oxidation of glucose. It is the pathway for formation of pentose phosphate. It is also called *hexose monophosphate shunt*.

#### Characteristics of Pentose Phosphate Pathway

- It is a multicyclic process in which three molecules of glucose-6-phosphate give rise to three molecules of  $\text{CO}_2$  and three molecules of 5-carbon sugars, (ribulose-5-phosphate).
- The three molecules of ribulose-5-phosphate are arranged to generate two molecules of fructose-6-phosphate and one molecule of glyceraldehyde-3-phosphate.
- It does not generate ATP.

The difference between glycolysis and pentose phosphate pathway is shown in **Table 12.5**.

**Table 12.4:** Glycogen storage diseases

Type	Name	Enzyme affected	Primary organ involved	Manifestations
I	Von Gierke's disease	Deficiency of glucose-6-phosphatase	Liver or kidney	Hypoglycemia, lactic acidemia, hyperlipemia ketosis and hyperuricemia
II	Pompe's disease	Deficiency of lysosomal $\alpha$ -1, 4 glucosidase (acid maltase)	All organs with lysosomes	Infantile form, early death, cardiac failure, accumulation of glycogen in lysosomes
III	Limit dextrinosis, Forbe's or Cori's disease	Absence of debranching enzyme	Liver, skeletal muscle, heart	Accumulation of abnormal glycogen having short outer chains, hypoglycemia
IV	Amylopectinosis, Andersen's disease	Absence of branching enzyme	Liver	Accumulation of abnormal glycogen having few branches, early death due to cardiac or liver failure
V	Mc-Ardle syndrome	Absence of muscle glycogen phosphorylase	Skeletal muscle	Excessive induced muscular pain, cramps, decrease serum lactate after exercise
VI	Her's disease	Deficiency of liver glycogen phosphorylase	Liver	High content of liver glycogen, mild hypoglycemia and ketosis
VII	Tarui's disease	Deficiency of phosphofructokinase in muscle and erythrocytes	Muscle and RBC	As in type V, in addition hemolytic anemia

### Location

The enzymes of pentose phosphate pathway are present in cytosol. The pathway is found in all cells.

### Reactions of the Pentose Phosphate Pathway (Figure 12.16)

The reactions of the pathway are divided into two phases:

1. Phase I : Oxidative irreversible phase
2. Phase II : Nonoxidative reversible phase.

#### Reactions of phase I (oxidative irreversible phase)

In the first phase, glucose-6-phosphate undergoes dehydrogenation and decarboxylation to give pentose, ribulose-5-phosphate with generation of NADPH.

1. Dehydrogenation of glucose-6-phosphate to 6-phosphogluconolactone, catalyzed by *glucose-6-phosphate dehydrogenase* which is an NADP dependent enzyme.
2. 6-phosphogluconolactone is hydrolyzed by *6-phosphogluconolactone hydrolase* to 6-phosphogluconate.
3. The subsequent oxidative decarboxylation of 6-phosphogluconate is catalyzed by *6-phosphogluconate dehydrogenase*, which also requires NADP as hydrogen acceptor. This irreversible reaction produces ribulose-5-phosphate, CO<sub>2</sub> and second molecule of NADPH.

#### Reactions of phase II (nonoxidative, reversible phase)

In the second phase, ribulose-5-phosphate is converted to fructose-6-phosphate by a series of reactions.

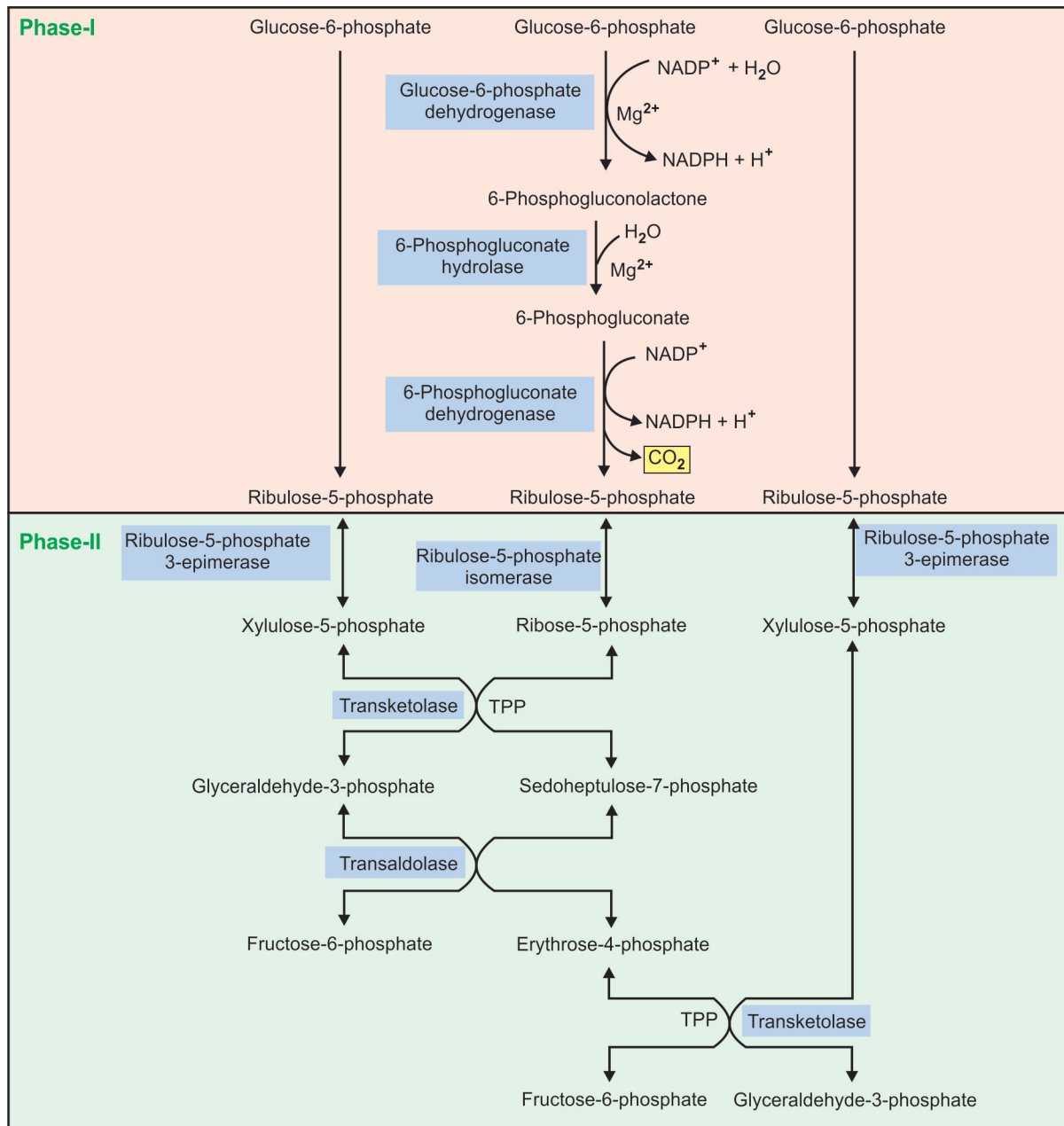
4. Ribulose-5-phosphate formed in the phase I now serves as substrate for two different enzymes:
  - i. **Ribulose-5-phosphate epimerase** catalyzes the epimerization of ribulose-5-phosphate to xylulose-5-phosphate.
  - ii. **Ribulose-5-phosphate isomerase** catalyzes the isomerization of ribulose-5-phosphate to ribose-5-phosphate.
5. **Transketolase** catalyzes the transfer of two carbon units from xylulose-5-phosphate to ribose-5-phosphate, producing a 7-carbon,

sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate. The reaction requires coenzyme *thiamine pyrophosphate (TPP)* and Mg<sup>2+</sup> ions.

6. **Transaldolase** catalyzes the transfer of a three carbon dihydroxyacetone group from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and the 4-carbon, erythrose-4-phosphate.
7. Further reaction again involves *transketolase*, which catalyzes the transfer of the two carbon units from xylulose-5-phosphate to erythrose-4-phosphate producing fructose-6-phosphate and glyceraldehyde-3-phosphate.
8. Fructose-6-phosphate and glyceraldehyde-3-phosphate can be further catabolized through glycolysis and citric acid cycle.

### Significance of Pentose Phosphate Pathway

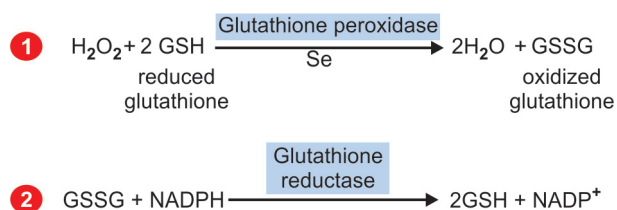
- The pentoses (ribose-5-phosphate) required for the biosynthesis of nucleotide and nucleic acids (RNA and DNA) are provided by pentose phosphate pathway.
- It provides a route for the interconversion of pentoses and hexoses.
- It generates NADPH which plays important role in several other biological processes, as given below.
  - NADPH is required for the biosynthesis of fatty acids, cholesterol, steroid hormones and neurotransmitters.
  - It is required for oxidation-reduction reactions involved in detoxification, e.g. for detoxification of drugs by microsomal **cytochrome P<sub>450</sub> mono-oxygenase** and for reduction of oxidized glutathione.
  - In RBC, NADPH is required to maintain the level of **reduced glutathione**. The reduced glutathione protects the RBC membrane from toxic effect of H<sub>2</sub>O<sub>2</sub> by reducing H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O (**Figure 12.17**).
  - NADPH also keeps iron of hemoglobin in reduced ferrous (Fe<sup>2+</sup>) state and prevents the formation of **methemoglobin**.
  - NADPH is necessary for phagocytosis carried out by white blood cell.



**Figure 12.16:** Pentose phosphate pathway, where, TPP: Thiamine pyrophosphate

**Table 12.5: Difference between glycolysis and pentose phosphate pathway**

Glycolysis	Pentose phosphate pathway
Oxidation occurs utilizing $\text{NAD}^+$ as an H-acceptor	Oxidation occurs utilizing NADP as an H-acceptor
Aerobic as well as anaerobic process	Anaerobic process
$\text{CO}_2$ is not produced at all	$\text{CO}_2$ is a characteristic product
ATP is generated, where it is a major function	ATP is not generated
Ribose phosphates are not generated	Ribose phosphates are generated
80-90% of glucose oxidized by glycolysis	10-20% glucose oxidized by pentose phosphate pathway



**Figure 12.17:** Role of NADPH in disposal of  $\text{H}_2\text{O}_2$

### Regulation of Pentose Phosphate Pathway

- The first step in the pathway, catalyzed by *glucose-6-phosphate dehydrogenase (G-6-PD)* is the rate limiting step.
- The activity of this enzyme is regulated by cellular concentration of NADPH. **NADPH is a competitive inhibitor of the enzyme G-6-PD.**
- An increased concentration of NADPH decreases the activity of G-6-PD, for example:
  - Under well-fed condition, the level of NADPH decreases and pentose phosphate pathway is stimulated.
  - However, in starvation and diabetes, the level of NADPH is high and inhibits the pathway.
- Insulin** is also involved in the regulation of pentose phosphate pathway. It enhances the pathway by inducing the enzyme G-6-PD and 6-phosphogluconolactone dehydrogenase.

### Disorders of Pentose Phosphate Pathway

#### Deficiency of Glucose-6-phosphate dehydrogenase (G-6-PD)

Glucose 6-phosphate dehydrogenase deficiency is X-linked inherited disorder, characterized by **hemolytic anemia**, due to excessive hemolysis.

- This enzyme catalyzes the first step in the pentose phosphate pathway and is needed for the formation of NADPH.
- The NADPH is required for the detoxification of  $\text{H}_2\text{O}_2$  in red blood cell (**Figure 12.17**). In deficiency of G-6-PD, the production of NADPH is inadequate both to restore the reduced glutathione level and to maintain the RBC cell membrane. The consequence is destruction of the red blood cells and severe hemolytic anemia.

- Most of the patients of G-6-PD deficiency are asymptomatic and do not show hemolytic anemia under normal condition. However, they develop severe hemolytic anemia when they are exposed to certain antibiotic, antimalarial (primaquine) or antipyretic drugs.

#### G-6-PD deficiency and resistance to malaria

The malarial parasite, *Plasmodium falciparum* infects the red blood cell, where it depends on the reduced glutathione and the products of the pentose phosphate pathway for its optimum growth. Therefore, persons with G-6-PD deficiency cannot support growth of this parasite and thus are less prone to malaria than the normal person.

#### Wernicke-Korsakoff syndrome

- This is a genetic disorder due to reduced activity of the **TPP-dependent transketolase** enzyme. The reduced activity of transketolase is due to reduced affinity for TPP, whereas the other TPP dependent enzymes are normal. Therefore, in the chronic thiamine deficiency the transketolase enzyme has a much reduced activity leading to the **Wernicke-Korsakoff syndrome**.
- The symptoms of Wernicke-Korsakoff syndrome include weakness, mental disorder, loss of memory, partial paralysis, etc.

### URONIC ACID PATHWAY (GLUCURONIC ACID CYCLE)

#### Definition

A pathway in liver for the conversion of glucose to glucuronic acid, ascorbic acid (except in humans and other primates as well as in guinea pigs) and pentoses is referred to as the uronic acid pathway. It is also an alternative oxidative pathway for glucose but does not generate ATP.

#### Reactions of Uronic Acid Pathway (Figure 12.18)

- Glucose-6-phosphate is converted to glucose-1-phosphate catalyzed by *phosphoglucomutase*.
- Glucose-1-phosphate then reacts with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDP-Glc). This reaction is catalyzed by the enzyme *UDP-glucose pyrophosphorylase*.
- UDP-Glucose is oxidized to glucuronate via UDP-glucuronate, catalyzed by an NAD dependent *UDP-Glucose dehydrogenase*.



4. Glucuronate is reduced to L-gulonate by the NADPH dependent enzyme *gulonate dehydrogenase*.
5. L-gulonate is the precursor of ascorbate (vitamin C) in those animals capable of synthesizing this vitamin. In humans and other primates, as well as in guinea pigs, ascorbic acid cannot be synthesized because of the **absence of the enzyme L-gulonolactone oxidase**.
6. L-gulonate is oxidized and decarboxylated to the pentose L-xylulose by the enzyme *L-gulonate decarboxylase*.
7. L-xylulose is reduced to xylitol catalyzed by NADPH dependent *L-xylulose dehydrogenase*.
8. Xylitol is oxidized to D-xylulose (isomer of L-xylulose) by an NAD dependent *D-xylulose dehydrogenase* enzyme.
9. D-xylulose, in turn, is phosphorylated by ATP in the presence of *xylulose kinase* to yield xylulose-5-phosphate, which is further metabolized in pentose phosphate pathway and leads to formation of glucose.

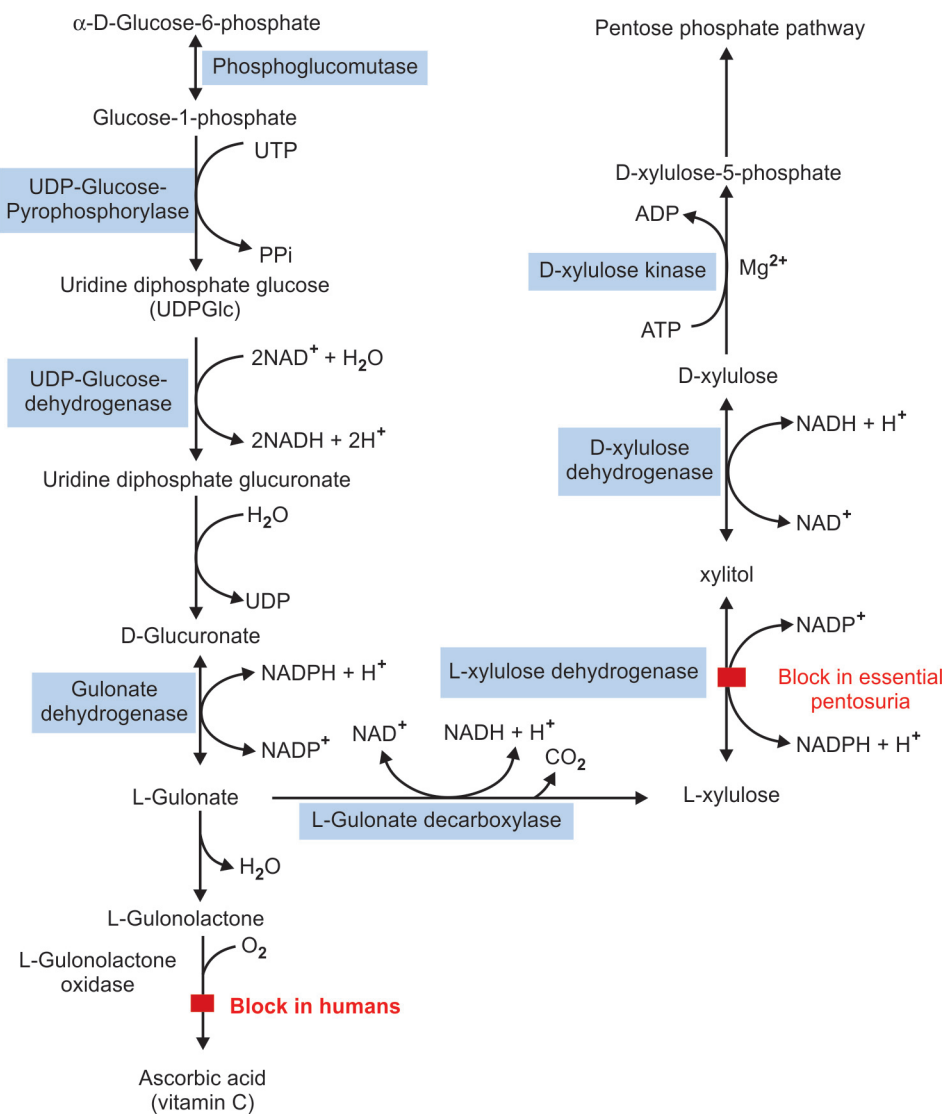


Figure 12.18: Uronic acid pathway

### Significance of Uronic Acid Pathway

- The uronic acid pathway is a source of UDP-glucuronate.
- UDP-glucuronate is a precursor in biosynthesis of proteoglycans (glycosaminoglycans) and glycoproteins.
- UDP-glucuronate is involved in detoxification reactions that occur in liver. Many naturally occurring waste substances (like bilirubin and steroid hormones) as well as many drugs (like morphine, methanol, salicylic acid, etc.) are eliminated from the body by conjugating with UDP-glucuronate (**Figure 12.19**).
- The uronic acid pathway is a source of UDP-glucose, which is used for glycogen formation.
- The uronic acid pathway provides a mechanism by which dietary D-xylulose can enter the central metabolic pathway.

### Disorder of Glucuronic Acid Pathway

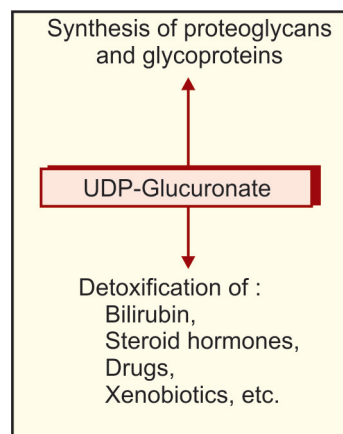
#### Essential pentosuria

It is a benign (harmless) inborn error of metabolism in which the sugar L-xylulose is excreted in the urine in excess due to defect in NADP<sup>+</sup> linked *L-xylulose dehydrogenase*, one of the enzymes in the glucuronic acid pathway (**Figure 12.18**). L-xylulose dehydrogenase is necessary to accomplish reduction of L-xylulose to xylitol.

### GALACTOSE METABOLISM AND GALACTOSEMIA

Galactose is derived from disaccharide, lactose (the milk sugar) of the diet. It is important for the formation of:

- Glycolipids
- Glycoproteins



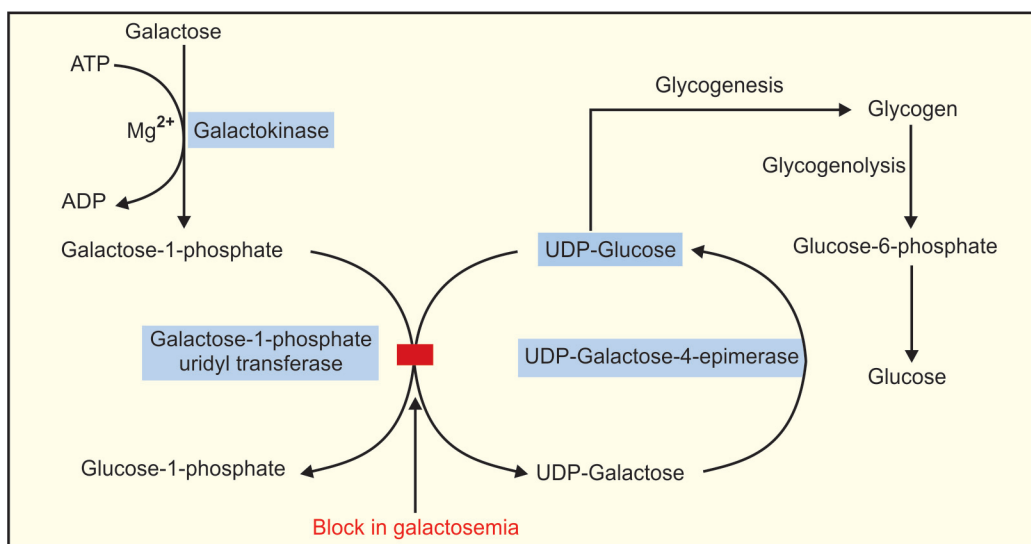
**Figure 12.19:** Metabolic significance of UDP-glucuronate

- Proteoglycans
- Lactose during lactation.

Galactose is readily converted in the liver to glucose. *The ability of the liver to convert galactose to glucose is used as a liver function test (galactose tolerance test).*

### Reactions of the Pathway (Figure 12.20)

1. The first reaction in galactose metabolism in the liver is phosphorylation of galactose to galactose-1-phosphate, by the enzyme *galactokinase*, using ATP as phosphate donor.
2. Galactose-1-phosphate reacts with UDP-glucose to form UDP-galactose and glucose-1-phosphate, catalyzed by *galactose-1-phosphate uridyl transferase*. In this reaction, galactose displaces a glucose of UDP-glucose.



**Figure 12.20:** Pathway for conversion of galactose to glucose in the liver

3. The conversion of UDP-galactose to UDP-glucose is catalyzed by an **UDP-galactose-4-epimerase**.
4. Finally, glucose is liberated from UDP-glucose via formation of glycogen by glycogenesis followed by glycogenolysis.

### Disorder of Galactose Metabolism

#### Galactosemia

- It is an inborn error of galactose metabolism, caused by deficiency of enzyme **galactose-1-phosphate uridyl transferase (Figure 12.20)**. The inherited deficiencies of **galactokinase** and **UDP-galactose-4-epimerase** also lead to minor types of galactosemia.
- They all interfere with the normal metabolism of galactose, causing a rise in blood and urine galactose.
- The commonest and most severe enzymatic defect is due to **galactose-1-phosphate uridyl transferase** deficiency which prevents conversion of galactose to glucose and leads to accumulation of galactose and galactose-1-phosphate in blood, liver, brain, kidney and eye lenses. In these organs, the galactose is reduced to galactitol (dulcitol) by the enzyme **aldose reductase**.

**Clinical findings:** The accumulation of galactitol and galactose-1-phosphate in liver, brain and eye lenses causes liver failure (hepatomegaly followed by cirrhosis), mental retardation and cataract formation respectively.

**Treatment:** Galactose in milk and milk products should be eliminated from the diet. Sufficient galactose for the body's need can be synthesized endogenously as UDP-galactose.

### METABOLISM OF FRUCTOSE

Liver is the main site of fructose metabolism.

#### Reactions of Fructose Metabolism (Figure 12.21)

- Fructose is phosphorylated to fructose-1-phosphate by **fructokinase** in the liver.
- Fructose-1-phosphate is cleaved by liver **aldolase (aldolase-B)** to dihydroxyacetonephosphate (DHAP) and D-glyceraldehyde.
- D-glyceraldehyde is phosphorylated by glyceraldehyde kinase (or triokinase) to D-glyceraldehyde-3-phosphate. These two triose phosphates (DHAP and glyceraldehyde-3-phosphate) then may enter the glycolytic pathway, gluconeogenesis or glycogenesis according to the metabolic status of the tissue.

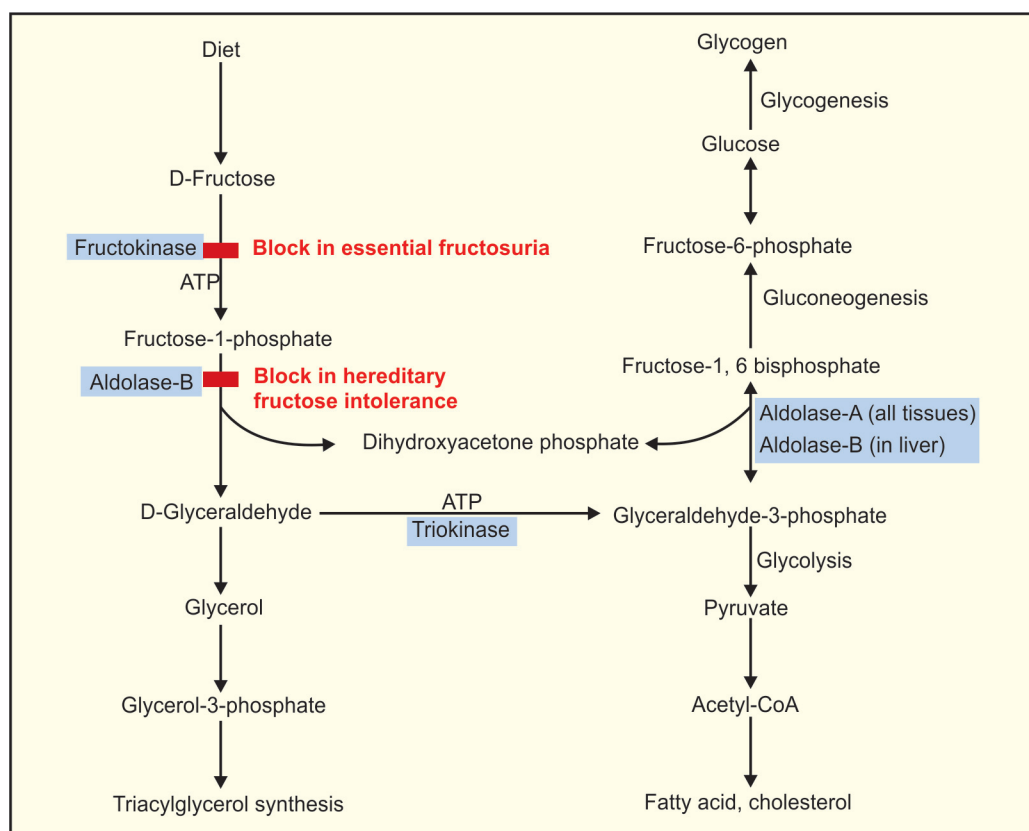


Figure 12.21: Metabolic pathway of fructose

### Sorbitol or Polyol Pathway for Formation of Fructose

The sorbitol (polyol) pathway is for formation of fructose from glucose (**Figure 12.22**).

- *Aldose reductase* reduces glucose to sorbitol (glucitol).
- In the liver, ovaries and sperm cells, there is a second enzyme, *sorbitol dehydrogenase* that can oxidize the sorbitol to fructose.
- The pathway from sorbitol to fructose in the liver provides a mechanism by which dietary sorbitol is converted into fructose.

#### The Effect of Hyperglycemia on Sorbitol Metabolism

Because insulin is not required for entry of glucose into the cells such as retina, lens and nerve, large amounts of glucose may enter these cells during hyperglycemia, e.g. in uncontrolled diabetes.

- Elevated intracellular glucose concentrations cause increase in the amount of sorbitol and therefore accumulates inside the cell.
- This causes osmotic damage, leading to cataract formation, peripheral neuropathy, retinopathy and nephropathy.

Drugs inhibiting aldose reductase improve the peripheral nerve function in diabetes.

### Disorders of Fructose Metabolism

#### Essential fructosuria

- Essential fructosuria is a rare and benign genetic disorders caused by a deficiency of the enzyme *fructokinase*.
- In this disorder, fructose cannot be converted to fructose-1-phosphate.
- This is benign because no toxic metabolites of fructose accumulate in the liver and the patient remains nearly asymptomatic with excretion of fructose in urine.

#### Hereditary fructose intolerance

- It is due to deficiency of the enzyme *Aldolase-B*.
- Fructose-1-phosphate cannot be converted to dihydroxyacetonephosphate and glyceraldehyde and therefore fructose-1-phosphate accumulates.
- This results in the inhibition of *fructokinase* and an impaired clearance of fructose from the blood.

#### Clinical findings

- Accumulation of fructose-1-phosphate leads to liver and kidney damage.
- Hypoglycemia due to inhibition of glycogenolysis and gluconeogenesis.

#### Treatment

Elimination of fructose containing foods from the diet.

### BLOOD GLUCOSE LEVEL AND ITS REGULATION

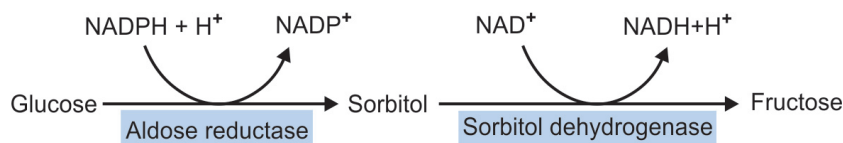
The blood glucose level must be maintained within the narrow limits of **70-100 mg/dl**. Levels above the normal range are termed *hyperglycemia*, those below are called *hypoglycemia*. After the ingestion of a carbohydrate meal, it may rise to 120-140 mg/dl.

Factors involved in the *homeostasis (regulation)* of blood glucose are:

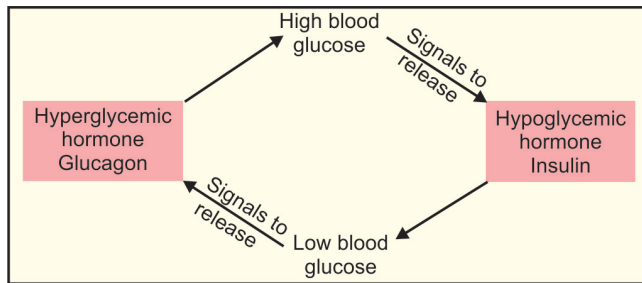
- Hormones
- Metabolic processes
- Renal mechanism.

The liver is the organ primarily responsible for controlling the concentration of glucose in the blood. It can rapidly take up and release glucose in response to the concentration of blood glucose. The uptake and release of glucose by liver is regulated by hormones. The two major hormones controlling blood glucose levels are (**Figure 12.23**):

1. Insulin (hypoglycemic hormone)
2. Glucagon (hyperglycemic hormone).



**Figure 12.22:** Sorbitol or polyol pathway



**Figure 12.23:** Regulation of blood glucose level by insulin and glucagon

### Maintenance of Glucose in Fed State (Hyperglycemic condition)

Normally, there is an increased blood glucose level shortly after each meal, a *postprandial hyperglycemia*. Increased level of blood glucose releases *insulin* by  $\beta$ -cells of the Islets of the Langerhans. This hormone reduces the blood glucose level in a number of ways (**Figure 12.24**) as follows:

1. By stimulating the active transport of glucose across cell membranes of muscle and adipose tissue but not the liver. *Glucose is rapidly taken up into liver as it is freely permeable to glucose.*
2. In the liver, insulin increases the use of glucose by *glycolysis* by inducing the synthesis of key glycolytic enzymes:
  - Glucokinase
  - Phosphofructokinase
  - Pyruvate kinase.
3. In the muscle and the liver, insulin stimulates *glycogenesis* by stimulating *glycogen synthase* and thereby leading to suppression of glycogenolysis.

4. Insulin inhibits *gluconeogenesis* by suppressing the action of key enzymes of gluconeogenesis, e.g.
  - Pyruvate carboxylase
  - Phosphoenol pyruvate carboxykinase
  - Fructose 1,6-bisphosphatase
  - Glucose-6-phosphatase.
5. In adipose tissue, glucose is converted to the glycerol-3-phosphate, needed for the formation of triacylglycerol (lipogenesis).
6. Insulin increases protein synthesis and decreases protein catabolism, thereby releasing amino acids. All these mechanisms are responsible for a drop in blood glucose level (hypoglycemia).

### Maintenance of Blood Glucose in Fasting State (Hypoglycemic Condition)

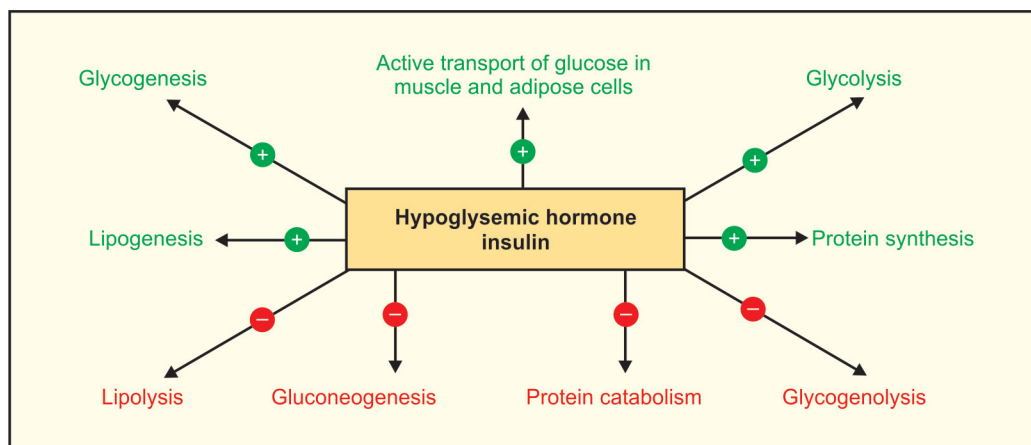
Decreased level of blood glucose, hypoglycemia causes a release of hyperglycemic hormones, e.g.

- Glucagon
- Epinephrine or adrenaline
- Glucocorticoids
- Growth hormone and adrenocorticotrophic hormone (ACTH)
- Thyroxine.

### Glucagon

Glucagon is the hormone produced by the  $\alpha$ -cells of the islets of Langerhans of the pancreas. Glucagon opposes the actions of insulin. It acts primarily in the liver as follows:

- In the liver, it stimulates glycogenolysis by activating enzyme *glycogen phosphorylase* and inhibits glycogen synthesis. Unlike epinephrine, glucagon does not have an effect on muscle phosphorylase due to lack of receptors.



**Figure 12.24:** Role of insulin in regulation of blood glucose level



- Glucagon enhances gluconeogenesis from amino acids and lactate by inducing the action of key enzymes of gluconeogenesis.
  - Alanine is the predominant amino acid released from muscle to liver by **glucose alanine cycle** (see Figure 12.9).
  - Lactate formed by oxidation of glucose in skeletal muscle is transported to the liver by **lactic acid (Cori) cycle** (see Figure 12.9).

#### Epinephrine or Adrenaline

- It is secreted by adrenal medulla.
- It stimulates **glycogenolysis** in the liver and the muscle by stimulating **glycogen phosphorylase** activity via c-AMP.
- In muscle as a result of the **absence of glucose-6-phosphatase**, glycogenolysis results with the formation of lactate, whereas in the liver, glucose is the main product, leading to increase in blood glucose.

#### Glucocorticoids

- These hormones are secreted by adrenal cortex, which causes increased:
  - Gluconeogenesis by increasing the activity of enzymes of gluconeogenesis.
  - Protein catabolism to provide glucogenic amino acid for gluconeogenesis.
  - Hepatic uptake of amino acids for gluconeogenesis.
- In addition, glucocorticoids inhibit the utilization of glucose in extrahepatic tissues. Thus, all the actions of glucocorticoids are antagonistic to insulin.

#### Growth hormone and anterior pituitary hormones

- Growth hormone and ACTH antagonise the action of insulin by elevating the blood glucose level.
- Growth hormone decreases glucose uptake in the muscle and ACTH decreases glucose utilization by the tissue.

#### Thyroxine

- It is secreted by thyroid gland.
- Thyroxine accelerates hepatic glycogenolysis with consequent rise in blood glucose.
- It may also increase the rate of absorption of hexoses from the intestine.

#### Renal Control Mechanism

- When blood glucose rises to relatively high levels, the kidney also exerts a regulatory effect. Glucose is

continuously filtered by the glomeruli but is normally reabsorbed completely in renal tubules. The capacity of the tubular system to reabsorb glucose is limited.

- If the blood glucose level is raised above **180 mg/100 ml**, complete tubular reabsorption of glucose does not occur and the extra amount appears in the urine causing **glycosuria**.
- 180 mg/100 ml is the limiting level of glucose in the blood, above which tubular reabsorption does not occur which is known as **renal threshold value for glucose**.
- Thus, by excreting extra amount of sugar in the urine during hyperglycemic state and reabsorbing sugar during the hypoglycemic state, the kidney helps in regulating the level of glucose in blood.

#### GLYCOSURIA

Normally, the urine contains about 0.05 gm% of sugar. Such a small quantity cannot be detected by Benedict's test, but under certain circumstances, a considerable amount of glucose or other sugar may be excreted in the urine.

- Excretion of detectable amount of sugar in urine is known as **glycosuria**.
- Glycosuria results from the rise of blood glucose above its renal threshold level (180 mg%)
- Glycosuria may be due to various reasons on the basis of which it is classified into the following groups:
  1. Alimentary (lag storage) glycosuria
  2. Renal glycosuria
  3. Diabetic glycosuria.

#### Alimentary (Lag Storage) Glycosuria

- The blood sugar level of some individuals after meal rises rapidly above the normal renal threshold (180 mg/dL) and results in glycosuria and known as **alimentary glycosuria**. This is due to an increased rate of absorption of glucose from the intestine. This is called alimentary glycosuria since alimentary canal (GI-tract) is involved.
- Characteristic feature of this glycosuria is that usually high blood glucose level returns to normal at 2 hours after a meal. This type of glycosuria is benign (harmless).

#### Renal Glycosuria

- This is observed due to impaired tubular reabsorption of glucose and have lowered renal threshold (may be 130-150 mg%) for glucose.



- In such cases, the blood glucose level is below 180 mg%, i.e. below normal renal threshold for glucose, but glucose appears in the urine due to lowered renal threshold.
- Renal glycosuria is a benign condition, unrelated to diabetes and it may occur temporarily in pregnancy without symptoms of diabetes.
- Renal glycosuria may result from inherited defects in the kidney or it may be acquired as a result of kidney disease.

### Diabetic Glycosuria

- Diabetic glycosuria is a pathological condition and is due to deficiency or lack of insulin which causes diabetes mellitus.
- Although the renal threshold is normal, as blood glucose level exceeds the renal threshold, the excess glucose passes into the urine to produce glycosuria.

## DIABETES MELLITUS

### Definition

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism, caused by either:

- Lack of insulin secretion or
- Decreased sensitivity of the tissues to insulin.

### Classification of Diabetes Mellitus

Diabetes mellitus is broadly divided into two groups namely:

1. Type I diabetes mellitus or insulin dependent diabetes mellitus (IDDM).
2. Type II diabetes mellitus or non-insulin dependent diabetes mellitus (NIDDM).

#### Type I diabetes mellitus

Type I diabetes is also called **insulin dependent diabetes mellitus (IDDM)** or juvenile onset diabetes.

#### Cause

It is caused by lack of insulin secretion due to destruction of pancreatic beta cells. The destructions of  $\beta$ -cells may be due to:

1. Viral infection
2. Autoimmune disorder (destruction of tissues by body's own antibodies)
3. There may be hereditary tendency for  $\beta$ -cell degeneration.

#### Onset

The usual onset of type-I diabetes occurs at about 14 years of age and for this reason it is called **juvenile diabetes mellitus** ('juvenile' means teenage in Latin).

#### Symptoms

- It develops symptoms very abruptly with:
  - Polyuria (frequent urination)
  - Polydipsia (excessive thirst)
  - Polyphagia (excessive hunger).
- These symptoms are accompanied by loss of body weight, weakness, and tiredness.
- Hyperglycemia with **glycosuria** and **ketoacidosis** are the metabolic changes.
- The patients of type-I diabetes mellitus are not obese.

#### Treatment

- Since patients of IDDM (type-I) fail to secrete insulin, administration of exogenous insulin is required.

#### Type II diabetes mellitus

Type II or non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes mellitus.

#### Cause

It is caused by decreased sensitivity of target tissues to insulin. This reduced sensitivity of insulin is often referred to as **insulin resistance**. This is perhaps due to **inadequate insulin receptors** on the cell surfaces of the target tissues. This syndrome is often found in an obese person.

#### Onset

Onset of the type II diabetes occurs after age 40 and the disorder develops gradually. Therefore, this syndrome is referred to as adult onset diabetes.

#### Symptoms

In type II diabetes mellitus, the symptoms are developed gradually which are similar to that of type-I **except ketoacidosis** is usually not present in type II diabetes mellitus.

#### Treatment

- NIDDM (type-II) can be treated in early stages by diet control, exercise and weight reduction and no exogenous insulin administration is required.
- Drugs that increase insulin sensitivity such as **thiazolidinediones** and **metformin** or drugs that cause additional release of insulin by the pancreas such as **sulfonylureas** may also be used.

- However, in the later stages insulin administration is often required to control blood glucose.

The comparison between two types of diabetes mellitus is given in **Table 12.6**.

#### Metabolic changes in diabetes mellitus

- In diabetes mellitus, the metabolic changes occur due to a deficiency of **insulin** and relative excess of **glucagon**. These changes in hormonal levels most profoundly affect metabolism in three tissues; liver, muscle and adipose tissue.
- Elevated levels of **blood glucose** and **ketone bodies** are the characteristic feature of untreated diabetes mellitus.
- The lack of insulin activity in diabetes mellitus results in failure of transfer of glucose from the blood into cells and leads to **hyperglycemia**. The body responds as it were in the fasting state (see **chapter 15**) with stimulation of:
  - Glycogenolysis
  - Gluconeogenesis
  - Lipolysis
  - Proteolysis.
- An increase in glucagon favors lipolysis in adipose tissue. Increased lipolysis leads to increased mobilization of fatty acids from adipose tissue to liver, where they are converted to **ketone bodies** ( $\beta$ -hydroxybutyrate and acetoacetate), causing the **ketoacidosis**.
- Due to lack of insulin, the synthesis of the enzyme **lipoprotein lipase**, required for the degradation of VLDL is decreased. Decreased synthesis of lipoprotein lipase leads to elevated levels of plasma VLDL, resulting in **hypertriglycerolemia**.

- The other metabolic changes that occur in diabetes mellitus is increased mobilization of amino acids due to increased rate of proteolysis. The amino acids released from muscle are converted to glucose by gluconeogenesis.

#### GLUCOSE TOLERANCE TEST (GTT)

Glucose tolerance test (GTT) is performed to assess the ability of the body to utilize glucose. GTT can be performed by two ways:

1. Oral GTT
2. Intravenous GTT.

#### Oral Glucose Tolerance Test

- The patient who is scheduled for oral GTT is instructed to eat a high carbohydrate diet for at least 3 days prior to the test, and come after an overnight fast on the day of the test.
- A fasting blood glucose sample is first drawn.
- Then 75 gm of glucose (or 1.75 gm per kg body weight) dissolved in 300 ml of water is given orally.
- After giving glucose, blood and urine specimens are collected at half hourly intervals for at least 2 hours.
- Blood glucose content is measured and urine is tested for glycosuria.
- A curve is plotted for time against blood glucose concentration and is called **glucose tolerance curve** (**Figure 12.25**).

#### Intravenous Glucose Tolerance Test

- The intravenous glucose tolerance test is often used for persons with malabsorptive disorders or previous gastric or intestinal surgery.

**Table 12.6: Comparison of two types of diabetes mellitus**

Features	Type-I: Insulin dependent diabetes mellitus	Type-II: Non-insulin dependent diabetes mellitus
Frequency	5-10%	90-95 %
Age of onset	Early during childhood or puberty usually < 20 years	Later after age of 40 years
Onset of symptoms	Abrupt and severe	Gradual, insidious
Plasma insulin	Low or absent	Normal to high
Body weight	Low to normal	Obese
Blood glucose	Increased	Increased
Insulin sensitivity	Normal	Reduced
Ketosis	Common	Rare
Acute complications	Ketoacidosis	Hyperosmolar coma
Treatment with insulin	Necessary	Usually not required

- Glucose is administered intravenously over 30 minutes using 20% glucose solution. A glucose load of 0.5 gm/kg of body weight is used.

### Types of Glucose Tolerance Curves

There are three types of Glucose Tolerance Curves:

1. Normal glucose tolerance curve
2. Decreased glucose tolerance
3. Increased glucose tolerance.

#### Normal glucose tolerance curve (Figure 12.25)

Normal response to glucose load is as follows:

- Initial fasting glucose is within the normal fasting limits (70 to 100 mg%).
- Blood glucose level rises to a peak (120 to 140 mg%) at half to 1 hour after ingestion of glucose.
- The blood glucose level then returns rapidly to the fasting normal limits in about 2 hours.
- Glucose should not be present in any of the urine specimens collected for 2 hours.

#### Decreased glucose tolerance (Figure 12.25)

Decreased glucose tolerance means decreased ability of the body to utilize glucose. In decreased glucose tolerance:

- Fasting glucose is higher than normal limits.
- The blood glucose level rises above 180 mg/100 ml (renal threshold) after ingestion of glucose.
- The blood glucose remains high for a longer time and may not return to fasting level even after 3 hours.
- The urine samples corresponding to blood glucose level over 180 mg/100 ml may show urine Benedict's test positive (glycosuria).

- Decreased glucose tolerance occurs in **diabetes mellitus** and certain endocrine disorders like:
  - Hyperthyroidism
  - Hyperpituitarism
  - Hyperadrenalism (Cushing's syndrome).

#### Increased glucose tolerance (Figure 12.25)

Increased glucose tolerance means increased ability of the body to utilize glucose. In increased glucose tolerance:

- Fasting blood glucose is lower than normal.
- Only small rise in blood glucose level may be observed (not more than 100 mg%) even after glucose administration.
- A flatter type of curve is obtained.
- No appearance of glucose in urine.
- This type of curve is obtained in endocrine hypoactivity like:
  - Hypothyroidism (myxedema, cretinism)
  - Hypoadrenalism (Addison's disease)
  - Hypopituitarism.

#### Significance of GTT

GTT is not necessary in symptomatic or in known cases of diabetic patients. In such cases, determination of fasting or postprandial glucose is usually sufficient for the diagnosis.

- GTT is most important in the investigation of asymptomatic hyperglycemia or glycosuria such as renal glycosuria and alimentary glycosuria.
- This test may give useful information in some cases of endocrine dysfunctions.
- It is also helpful in recognizing milder cases of diabetes.

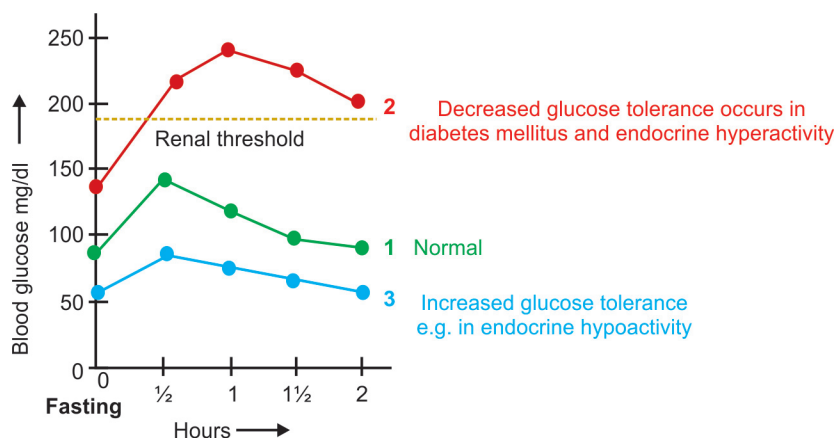


Figure 12.25: The glucose tolerance curves

### SUMMARY

- Glycolysis is the pathway found in the cytosol for the conversion of glucose into two molecules of pyruvate with generation of ATP.
- Under anaerobic condition, e.g. in exercising muscles and in erythrocytes, the pyruvate is reduced to lactate.
- In aerobic condition, cell pyruvate is oxidized to acetyl-CoA and CO<sub>2</sub> by multienzyme complex pyruvate dehydrogenase, instead of being reduced to lactate.
- In erythrocytes, the first site for generation of ATP in glycolysis may be bypassed leading to the formation of 2,3 biphosphoglycerate.
- Citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid and protein.
- The citric acid cycle is amphibolic since it has other metabolic roles in addition to oxidation. It takes part in gluconeogenesis, transamination, synthesis of heme and fatty acids.
- Gluconeogenesis is the synthesis of new glucose from noncarbohydrate sources, such as lactate, glucogenic amino acids, glycerol, and propionate. It provides glucose to the body when carbohydrate is not available from the diet.
- Glycogenesis is the synthesis of glycogen from glucose. Glycogen is broken down by a separate pathway known as glycogenolysis.
- The pentose phosphate pathway, which is present in the cytosol, generates NADPH, ribose-5-phosphate, and CO<sub>2</sub>. The pathway does not generate ATP. NADPH is used in reductive biosynthesis, e.g. lipogenesis, or steroidogenesis, whereas ribose-5-phosphate is used in the synthesis of RNA and DNA.
- In erythrocytes, the HMP pathway has a major function in preventing hemolysis by providing NADPH. Impairment of the pentose phosphate pathway due to deficiency of glucose-6-phosphate dehydrogenase leads to hemolytic anemia.
- The uronic acid pathway is the source of UDP-glucuronate which functions to detoxify (by conjugation) many endogenous and exogenous substances.
- Inability to metabolize galactose to glucose occurs in the galactosemia, which may be due to inherited defects in, uridyl transferase.
- The blood glucose level is regulated within the narrow limits of 70-100 mg/dl by metabolic, hormonal and renal mechanism.
- Insulin (hypoglycemic hormone) and glucagon (hyperglycemic hormone) play a central role in

regulating blood glucose. Other hyperglycemic hormones involved in blood glucose regulation are growth hormone, ACTH, glucocorticoids, epinephrine and thyroxine.

- Glycosuria occurs when the renal threshold for glucose is exceeded.
- Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by absolute or relative insulin deficiency and characterized by decreased glucose tolerance that leads to hyperglycemia.

### EXERCISE

#### Solve the Following

##### Case History 1

A 12-year-male had complained of abdominal discomfort, a feeling of being bloated, increased passage of urine and development of diarrhea after taking milk.

##### Questions

- Name the probable disorder.
- Cause of disorder.
- What will you suggest the patient to relieve the symptoms?

##### Case History 2

The following are the findings in a patient brought to the hospital in a coma state.

Findings	Patient	Normal
Blood sugar (Fasting)	270 mg%	70-100 mg%
Urine Benedict's test	Positive	Negative
Urine Rothera's test	Positive	Negative
Plasma pH	7.20	7.35 to 7.45

##### Questions

- Name the disorder.
- Why is patient's plasma pH lower than normal?
- What does positive Rothera's test indicate?
- What is the renal threshold value for glucose?

##### Case History 3

A 20-year-old male suffering from malaria was treated with chloroquine and manifested as hemolytic anemia. Provisional diagnosis of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency was made.

##### Questions

- Which reaction is catalyzed by the enzyme G-6-PD?

- How does deficiency of G-6-PD produce hemolytic anemia?
- Name the pathway in which this reaction occurs.

#### Case History 4

A chronically cranky, irritable and lethargic baby girl has an extended abdomen, resulting from an enlarged liver and was diagnosed of having Von Gierke's disease.

#### Questions

- Which enzyme is deficient in Von Gierke's disease?
- Name the pathways where the enzyme is required.
- Give manifestations of the disorder.

#### Case History 5

A 6-month-old infant was presented with elevated blood and urine galactose.

#### Questions

- Name the disease.
- Give the biochemical steps related to the disease and point out the metabolic defect.
- What are the clinical manifestation of the disease?

#### Case History 6

An obese person came to the hospital with complaints of polyuria, thirst, weakness and increased appetite. On investigations, he was diagnosed having diabetes mellitus.

#### Questions

- What is the cause of diabetes mellitus?
- Give names of different types of diabetes mellitus.
- What is glucosuria? Name different types of glucosuria.
- What is the normal blood sugar level?

#### Case History 7

A 28-year-old man has complained of chronic leg muscle pains and cramps during exercise. This patient suffers from McArdle syndrome.

#### Questions

- What is McArdle syndrome? To metabolism of which biomolecule is it related?
- What is the cause of this syndrome?
- Name different types of disorders related to the concerned biomolecule.

#### Case History 8

A 13-year-old diabetic boy visits a diabetic clinic for a check-up. He tells the doctor that he complies with all the dietary advice and never misses insulin. His random blood glucose level is within normal limit but his HbA<sub>1c</sub> concentration is 10% (normal 4-6%). He has no glycosuria or ketone bodies in his urine.

#### Questions

- What does normal blood glucose and urine glucose indicate?
- What does elevated level of HbA<sub>1c</sub> suggest?
- Name the type of diabetes the boy is suffering.
- What is HbA<sub>1c</sub>?

#### Case History 9

A 3-year-old patient with mild mental retardation was found to have cataract. Biochemical investigations show high blood concentrations of a sugar alcohol and galactose.

#### Questions

- Name the probable disease.
- Name enzyme most likely to be defective.
- What is the cause of development of cataracts?
- What is the treatment?

#### Multiple Choice Questions (MCQs)

- Which of the following enzymes produce a product used for synthesis of ATP by substrate level?**
  - Phosphofructokinase
  - Aldolase
  - 1,3-bisphosphate mutase
  - Enlase
- 2,3-bisphosphoglycerate is:**
  - A high energy substrate
  - Involved in substrate level phosphorylation
  - An intermediate in pentose phosphate pathway
  - An allosteric effector that decreases the O<sub>2</sub> affinity of Hb
- Muscle glycogen is not available for maintenance of blood glucose concentration because:**
  - Muscle lacks glucose-6-phosphatase activity
  - There is insufficient glycogen in muscle
  - Muscle lacks glucose transporter GLUT-4
  - Muscle lacks glucagon receptors
- The primary metabolic fate of lactate released from muscle during intense exercise is:**
  - Excretion of lactate in urine
  - Transported to liver for replenishment of blood glucose by gluconeogenesis