THOMAS ADEWUMI UNIVERSITY,
OKO, KWARA STATE
Science | Technology | Medicine

LECTURE NOTE

ON

BCM 224

CARBOHYDRATE METABOLISM

PREPARED BY: L.B. BELLO

bello.lukman@tau.edu.ng

COURSE OUTLINE

- Introduction to carbohydrates
- Anabolism and catabolism
- Anabolic processes of carbohydrates
- Catabolic processes of carbohydrates

FOCUS...

- ✓ Introduction to carbohydrates
- ✓ Anabolism and catabolism
- ✓ Anabolic processes of carbohydrates

DID YOU KNOW?

- Glucose accounts for nearly 80% of the total monosaccharides yielded from the digestion of carbohydrates.
- Glucose is the sole or major fuel source for the human brain and nervous system, as well as the erythrocytes, testes, renal medulla, and embryonic tissues.
- Out of the 160g of glucose needed by the body daily, the brain alone consumes about 120g.
- Insufficient supply of glucose to the brain may lead to coma and death.

DEFINITION OF CARBOHYDRATES

✓Carbohydrates may be defined as optically active polyhydroxy aldehydes or ketones or compounds, which produce them on hydrolysis.

✓They are primarily composed of the elements carbon, hydrogen and oxygen.

✓The name carbohydrate literally means **'hydrates of carbon'**.

✓Many (but not all) of the carbohydrates possess the empirical formula $(C.H_2O)_{n.}$

FUNCTIONS OF CARBOHYDRATES

- ✓ They are the most abundant dietary source of energy (4 Cal/g) for all organisms.
- ✓ They are precursors for many organic compounds (fats, amino acids).
- ✓ Carbohydrates (as glycoproteins and glycolipids) participate in the structure of cell membrane and cellular functions such as cell growth, adhesion and fertilization.
- ✓ They are structural components of many organisms. These include the fiber (cellulose) of plants, exoskeleton of some insects and the cell wall of microorganisms.
- ✓ They also serve as the storage form of energy (glycogen) to meet the immediate energy demands of the body.

CLASSIFICATION OF CARBOHYDRATES

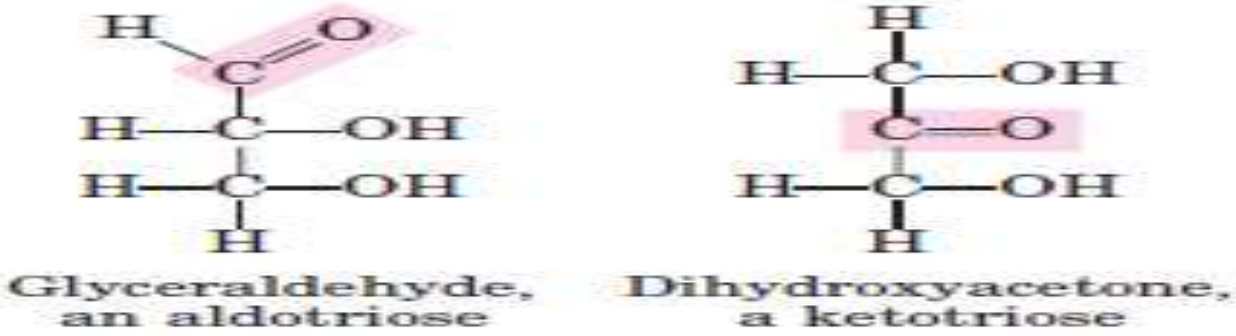
- Carbohydrates are often referred to as saccharides (Greek: *sakcharon*–sugar), and they are broadly classified into 3 major groups— monosaccharides, oligosaccharides and polysaccharides.

- This classification is based on the number of sugar units.

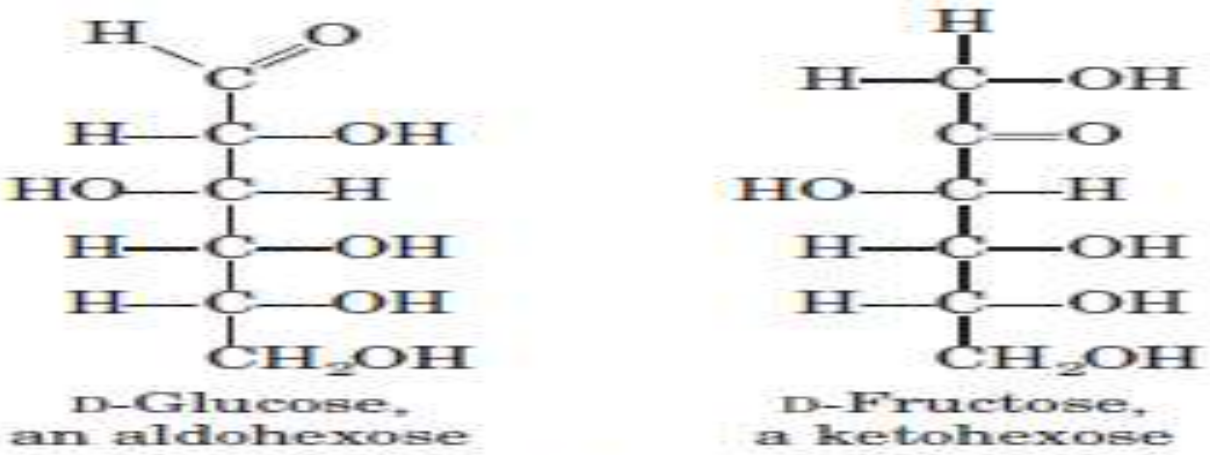
- Monosaccharides (Greek: *mono*-one), also known as simple sugars, are colourless, crystalline solids, which consist of a single polyhydroxy aldehyde or ketone unit, and are freely soluble in water but insoluble in non-polar solvents.

- Monosaccharides are divided into different categories, based on the functional group and the number of carbon atoms.

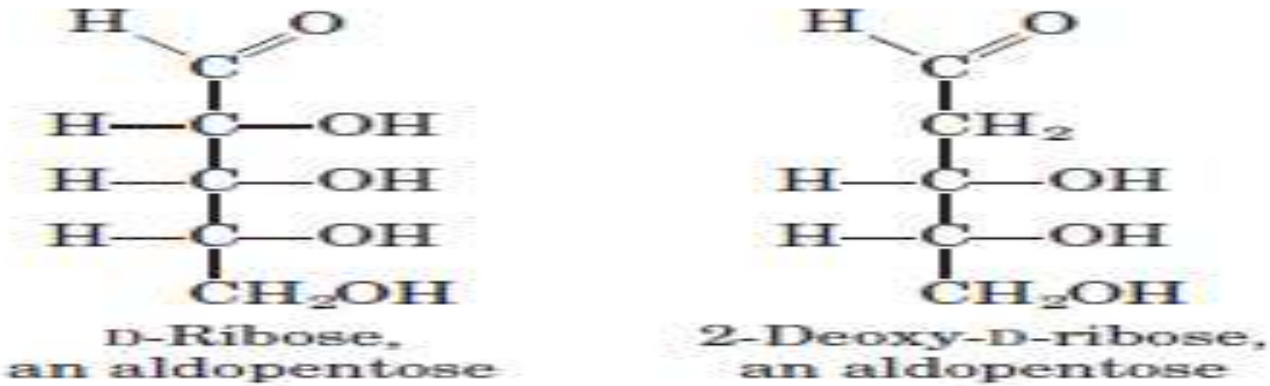
- Monosaccharides with four, five, six, and seven carbon atoms in their backbones are called, respectively, tetroses, pentoses, hexoses, and heptoses.
- These terms along with functional groups are used while naming monosaccharides.
- For instance, glucose is an aldohexose, while fructose is a ketohexose.
- Other monosaccharides include galactose, mannose, glyceraldehyde etc.
- If two monosaccharides differ from each other in their configuration around a single specific carbon (other than anomeric) atom, they are referred to as epimers to each other.


Glyceraldehyde,
an aldotriose
Dihydroxyacetone,
a ketotriose

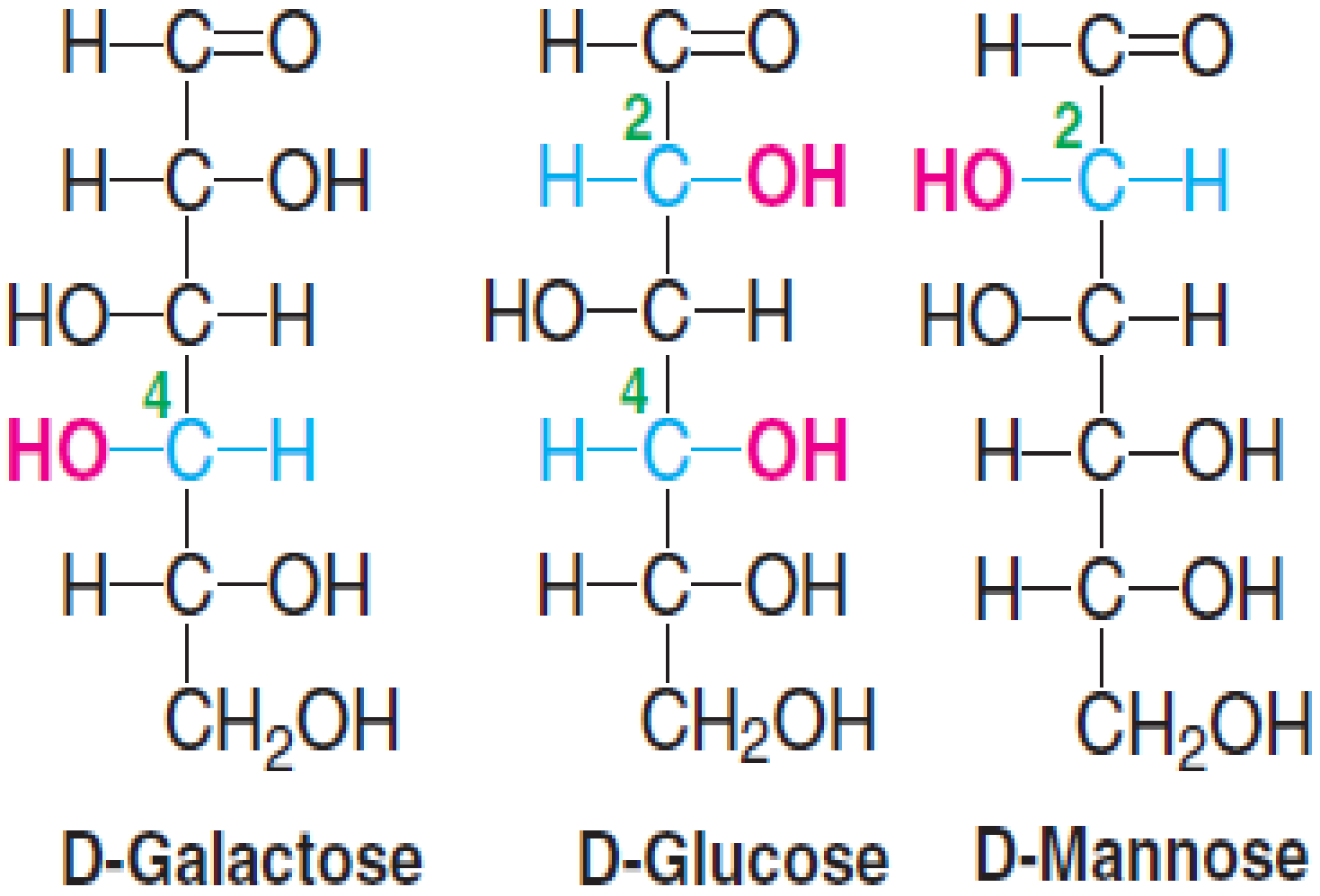
(a)


D-Glucose,
an aldohexose
D-Fructose,
a ketohexose

(b)


D-Ribose,
an aldopentose
2-Deoxy-D-ribose,
an aldopentose

(c)



Structures of epimers (glucose and galactose are C_4-epimers while glucose and mannose are C_2-epimers)

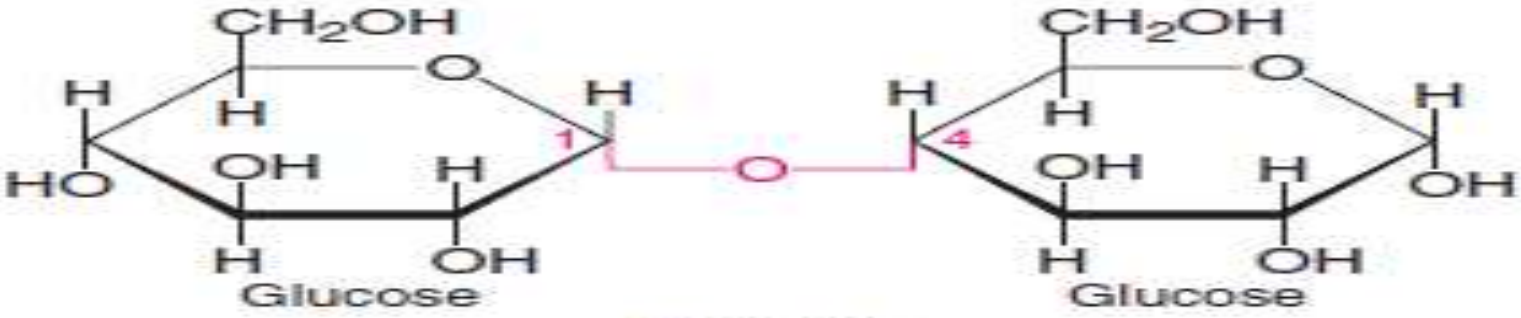
- Oligosaccharides (Greek: *oligo*-few) contain 2-10 monosaccharide units joined by glycosidic bonds.

- Based on the number of monosaccharide units present, oligosaccharides are further subdivided to disaccharides, trisaccharides etc.

- The most abundant oligosaccharides are the disaccharides.

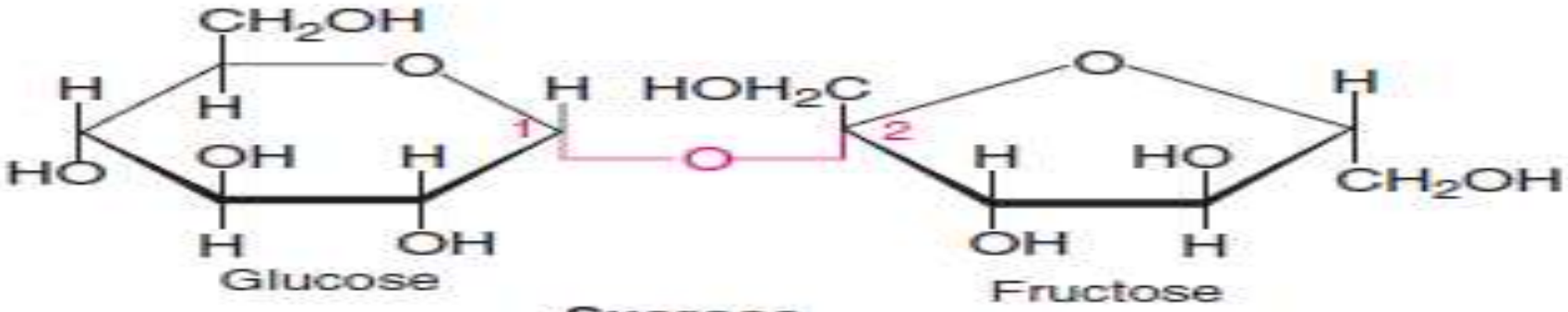
- Examples of disaccharides are lactose, sucrose, maltose etc.

- *Mono*- and *oligosaccharides* are sweet to taste, crystalline in character and soluble in water, hence they are commonly known as *sugars*.



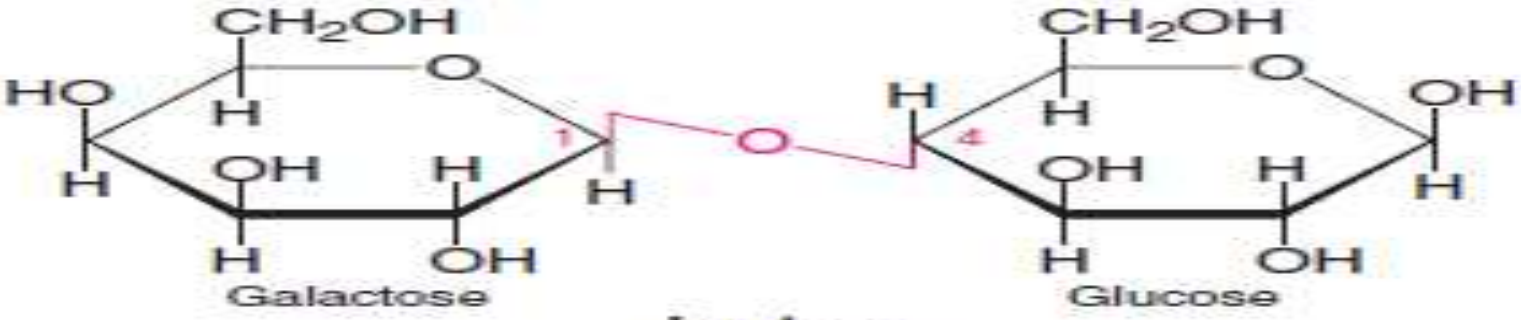
Maltose

(α-D-glucosyl (1 → 4) α-D-glucose)



Sucrose

(α-D-glucosyl (1 → 2) β-D-fructose)



Lactose

(β-D-galactosyl (1 → 4) β-D-glucose)

- Polysaccharides (Greek: *poly*-many), also called **glycans**, are polymers of monosaccharide units (or their derivatives) with high molecular weight (up to a million).
- They are usually tasteless (non-sugars) and form colloids with water.
- In the body, they play structural roles and serve as a store of energy.
- They are linear as well as branched.
- Polysaccharides are of two types:
  1. **Homopolysaccharides**: They yield only a single type of monosaccharide on hydrolysis. E.g. starch, dextrans, inulin, glycogen, cellulose, chitin.
  2. **Heteropolysaccharides**: They yield a mixture of a few monosaccharides or their derivatives on hydrolysis. E.g. mucopolysaccharides.

- **Mucopolysaccharides** are heteroglycans made up of repeating units of sugar derivatives, namely amino sugars and uronic acids.
- They are more commonly known as **glycosaminoglycans (GAG)**.
- The biologically important glycosaminoglycans along with their composition, distribution and functions are as seen below.

Glycosaminoglycan	*Composition*	*Tissue distribution*	*Function(s)*
Hyaluronic acid	D-Glucuronic acid, N-acetylglucosamine	Connective tissue, synovial fluid, vitrous humor	Serves as a lubricant, and shock absorber. Promotes wound healing
Chondroitin sulfate	D-Glucuronic acid, N-acetylgalactosamine 4-sulfate	Cartilage, bone, skin, blood vessel walls	Helps to maintain the structure and shapes of tissues
Heparin	D-Glucuronate 2-sulfate, N-sulfoglucosamine 6-sulfate	Blood, lung, liver, kidney, spleen	Acts as an anticoagulant
Dermatan sulfate	L-Iduronic acid, N-acetyl-galactosamine 4-sulfate	Blood vessel valves, heart valves, skin	Maintains the shapes of tissues
Keratan sulfate	D-Galactose, N-acetyl-glucosamine 6-sulfate	Cartilage, cornea, connective tissues	Keeps cornea transparent

Source: Satyanarayana and Chakrapani, 2013

METABOLISM

- Metabolism refers to the entire spectrum of chemical reactions occurring in the living system.
- It is broadly divided into two: catabolism and anabolism.
- **Catabolism** is the breakdown of complex molecules to simpler ones, with a concomitant release of energy, while a**nabolism** refers to the biosynthetic reactions involving the formation of complex molecules from simple precursors.
- **Amphibolism** is a term used to refer to reactions which are both catabolic and anabolic in nature.
- A **metabolic pathway** is a series of enzymatic reactions that produces specific products.
- The term **metabolite** refers to a substrate, an intermediate or a product in a metabolic pathway.

CARBOHYDRATE METABOLISM

- Glucose is a central molecule in carbohydrate metabolism because all the major pathways of carbohydrate metabolism are connected with it.

- Effectively, glucose is either a starting material, intermediate or end product in carbohydrate metabolism.

- Glucose is utilized as a source of energy (ATP and NADH), and can be synthesized from non-carbohydrate precursors and stored as glycogen to release glucose as and when the need arises.

- Fructose, galactose and mannose are other monosaccharides important in carbohydrate metabolism.

- The major pathways of carbohydrate metabolism include glycolysis, tricarboxylic acid (TCA) cycle, pentose phosphate pathway, glycogenesis, gluconeogenesis, glycogenolysis, uronic acid pathway etc.

- **Glycogenesis** and **gluconeogenesis** are the main anabolic processes of carbohydrates.

- The entry of glucose into the cell is not by simple diffusion, but by two known specific transport systems:

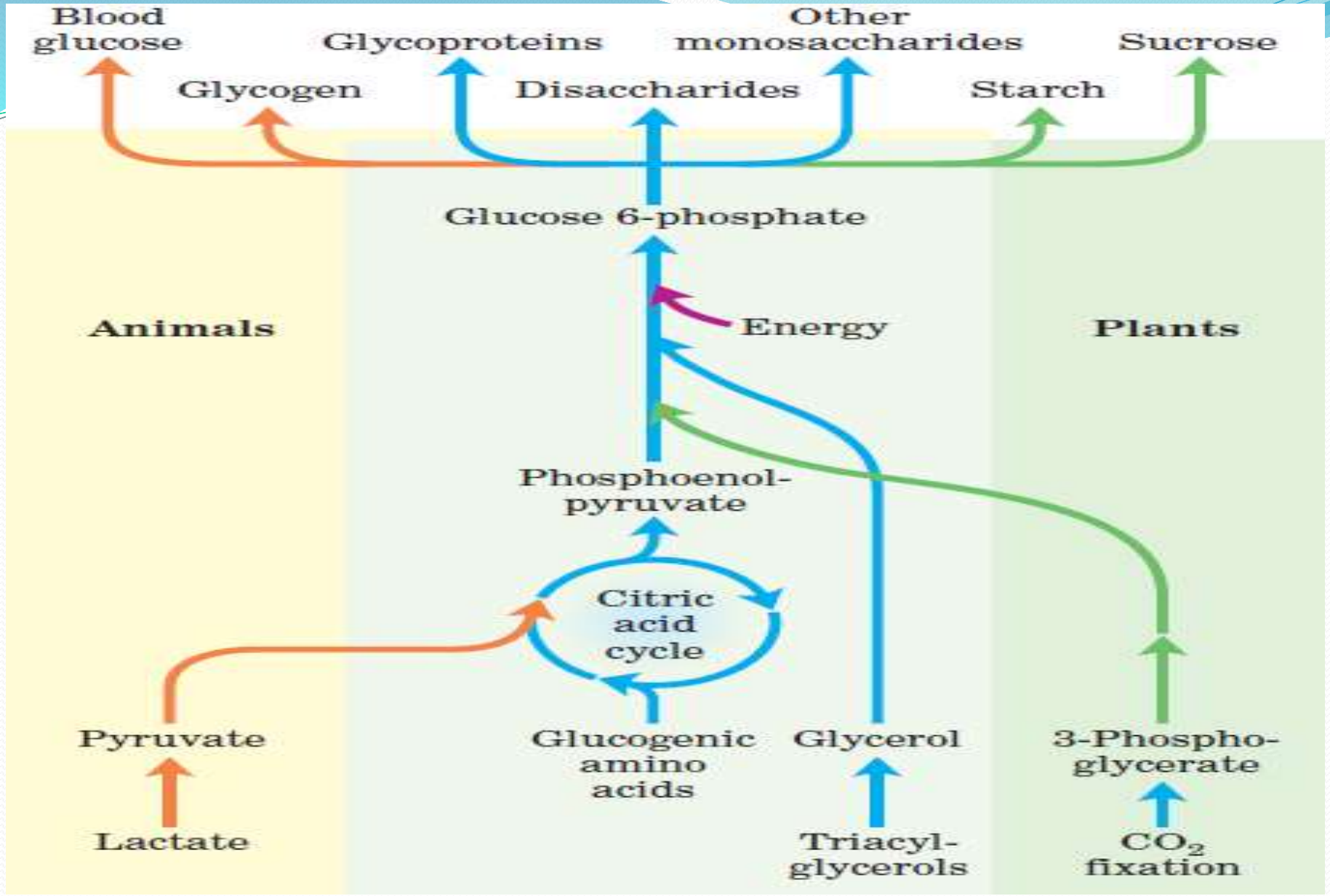
- The **insulin-independent transport system** of glucose (operative in hepatocytes, erythrocytes and brain) is a carrier-mediated uptake of glucose which is not dependent on the hormone insulin.

- **Insulin-dependent transport system** occurs in muscle and adipose tissue, and is dependent on the hormone insulin.

GLUCOSE TRANSPORTERS

- At least six glucose transporters (GLUT-1 to GLUT-5 and GLUT-7) in the cell membranes have been identified.
- These transporters exhibit tissue specificity.
- GLUT-1 is abundant in erythrocytes
- GLUT-4 is abundant in skeletal muscle and adipose tissue.
- Insulin increases the number of GLUT-4 and promotes its activity in skeletal muscle and adipose tissue.

ANABOLIC PROCESSES OF CARBOHYDRATES



Carbohydrate synthesis from simple precursors

Source: Nelson and Cox, 2016

GLYCOGENESIS

- Glycogenesis is the synthesis of **glycogen** from glucose.
- Glycogen is a polysaccharide made up of glucose.
- Like starch in plants, glycogen is the storage form of glucose in animals.
- It is stored mostly in liver (6-8%) and muscle (1-2%).
- Due to more muscle mass, the quantity of glycogen in muscle (250g) is about three times higher than that in the liver (75g).
- Glycogen is stored as granules in the cytosol, where most of the enzymes of glycogen synthesis and breakdown are present.

- The main function of liver glycogen is to provide glucose to other cells and maintain the blood glucose level in normal amounts, particularly between meals.

- Muscle glycogen serves as readily available source of glucose for glycolysis in the muscle during vigorous exercise.

- Glycogen is more preferred (than fat) as the fuel reserve of the body because it can be rapidly mobilized and can generate energy in the absence of oxygen (through anaerobic glycolysis).

- Also, the brain depends on continuous glucose supply, which mostly comes from glycogen.

- Glycogen metabolism includes glycogenesis (synthesis) and glycogenolysis (breakdown).

REACTIONS OF GLYCOGENESIS

1. The enzymes **hexokinase** (in muscle) and **glucokinase** (in liver) convert glucose to glucose 6-phosphate.

2. **Phosphoglucomutase** catalyses the conversion of glucose 6-phosphate to glucose 1-phosphate.

3. Uridine diphosphate glucose (UDPG) is synthesized from glucose 1-phosphate and UTP by **UDP-glucose pyrophosphorylase**.

   To initiate glycogen synthesis, a small fragment of pre-existing glycogen must act as a '**primer**'.

   However, it has been found that in the absence of a glycogen primer, a specific protein called '**glycogenin**' can accept glucose from UDPG.

   The hydroxyl group of the amino acid tyrosine of glycogenin is the site at which the initial glucose unit is attached.

4. The enzyme **glycogen initiator synthase** transfers the first molecule of glucose to glycogenin. Then glycogenin itself takes up a few glucose residues to form a fragment of primer, which serves as an acceptor for the rest of the glucose molecules.

5. **Glycogen synthase** transfers the glucose from UDP-glucose to the non-reducing end of glycogen to form α-1,4 glycosidic linkages.

6. The formation of branches is brought about by the action of a branching enzyme called **glucosyl α-4-6 transferase** (amylo α-1,4 $\longrightarrow$ α-1,6 transglucosidase).

   This enzyme transfers a small fragment of five to eight glucose residues from the non-reducing end of glycogen chain (by breaking α-1,4 linkages) to another glucose residue where it is linked by α-1,6 bond.

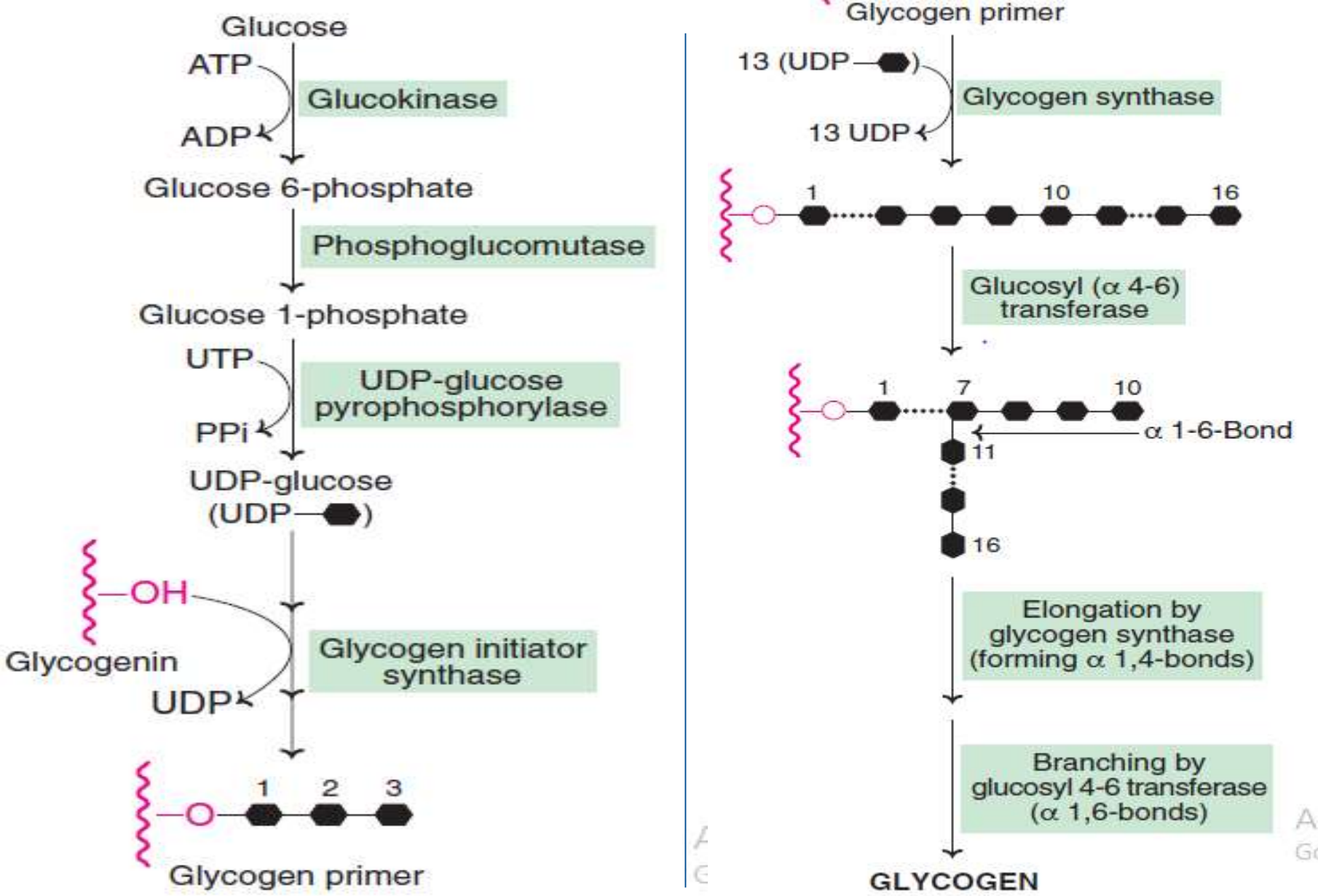
   This leads to the formation of a new non-reducing end, besides the existing one.

7. Glycogen is further elongated and branched, respectively, by the enzymes **glycogen synthase** and **glucosyl 4-6 transferase**.

The overall reaction of the glycogen synthesis for the addition of each glucose residue is

$$(Glucose)_n + Glucose + 2ATP \longrightarrow (Glucose)_{n+1} + 2ADP + Pi$$

One of the two ATP utilized is required for the phosphorylation of glucose, while the other is needed for conversion of UDP to UTP.



Glycogen synthesis from glucose(glycogenesis)

Source: Satyanarayana and Chakrapani, 2013

REGULATION OF GLYCOGENESIS

- A good coordination and regulation of glycogen synthesis and its degradation are essential to maintain the blood glucose levels.

- Glycogenesis is controlled by the enzyme **glycogen synthase** through allosteric and hormonal regulations.

- The **allosteric regulation** of glycogen synthase is carried out in such a way that glycogen synthesis is increased when substrate availability and energy levels are high.

- In a well-fed state, the availability of **glucose 6-phosphate** is high which allosterically activates glycogen synthase for more glycogen synthesis.

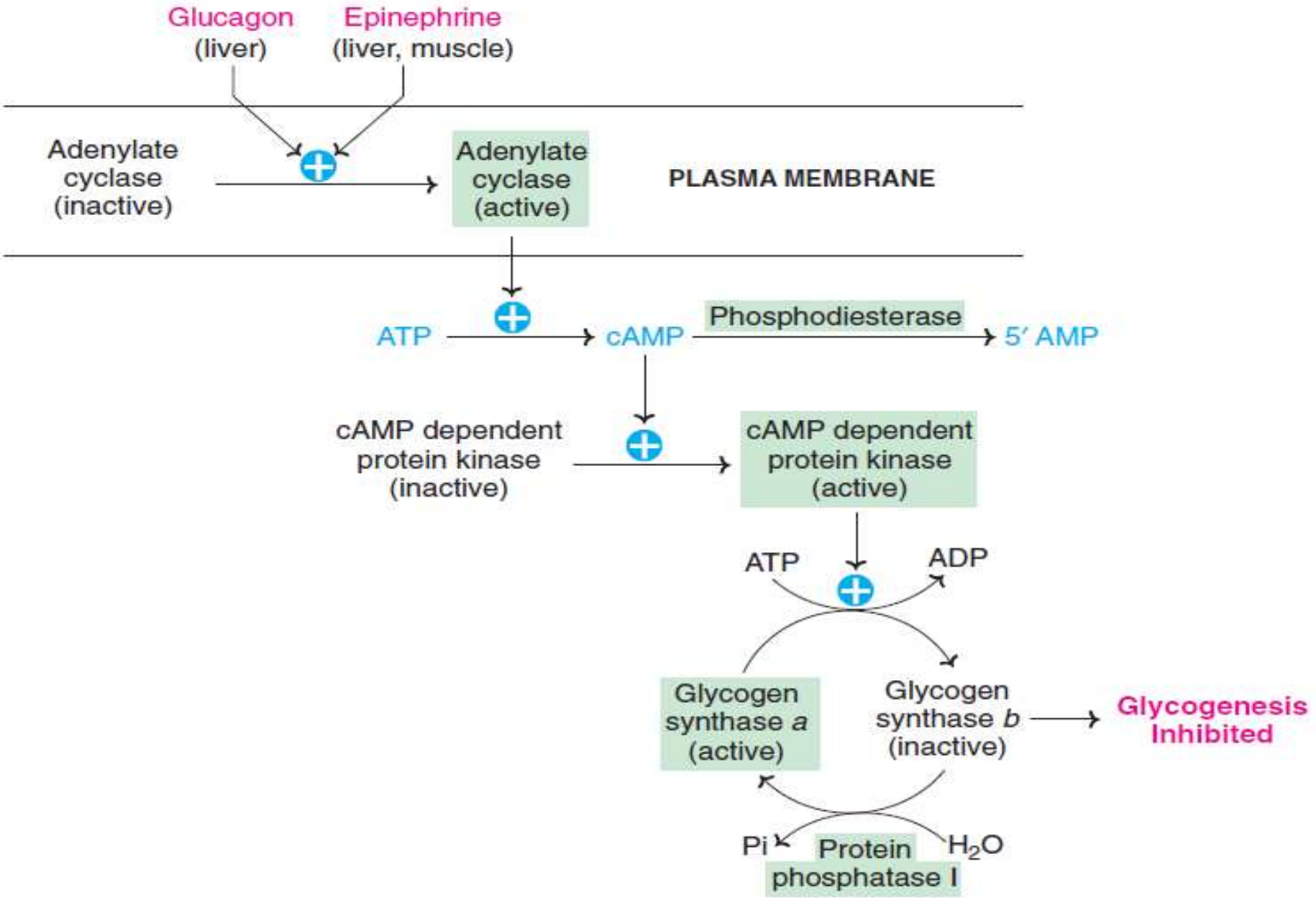
- **Hormonal regulation of glycogenesis:** Hormones like epinephrine and norepinephrine, and glucagon (in liver) activate adenylate cyclase to increase the production of cAMP.

- **Phosphodiesterase** breaks down cAMP, and insulin increases the phosphodiesterase activity in liver and lowers the cAMP levels.

- Glycogen synthase, which regulate glycogenesis, exists in two forms—glycogen synthase 'a'—which is unphosphorylated and active, and secondly, glycogen synthase 'b' as phosphorylated inactive form.

- Glycogen synthase 'a' can be converted to 'b' form (inactive) by phsophorylation, and the degree of phosphorylation is proportional to the inactive state of the enzyme.

- The inhibition of glycogen synthesis brought by epinephrine (also norepinephrine) and glucagon through cAMP by converting active glycogen synthase 'a' to inactive synthase 'b' is shown below.



Hormonal regulation of glycogenesis

Source: Satyanarayana and Chakrapani, 2013

GLYCOGEN STORAGE DISEASES

- Glycogen storage diseases refer to the metabolic defects concerned with glycogen synthesis and degradation.
- These disorders are characterized by deposition of normal or abnormal type of glycogen in one or more tissues.
- A summary of glycogen metabolism along with the defective enzymes in the glycogen storage disorders is depicted in the figure below.
- The biochemical lesions and the characteristic features of the disorders are also given.

Glycogen storage diseases – biochemical lesions and characteristic features

Type	Name	Enzyme defect	Organ(s) involved	Characteristic features
I	von Gierke's disease (type I glycogenosis)	Glucose 6-phosphatase	Liver, kidney and intestine	Glycogen accumulates in hepatocytes and renal cells, enlarged liver and kidney, fasting hypoglycemia, lactic acidemia; hyperlipidemia; ketosis; gouty arthritis.
II	Pompe's disease	Lysosomal α-1,4 glucosidase (acid maltase)	All organs	Glycogen accumulates in lysosomes in almost all the tissues; heart is mostly involved; enlarged liver and heart, nervous system is also affected; death occurs at an early age due to heart failure.
III	Cori's disease (limit dextrinosis, Forbe's disease)	Amylo α-1,6-glucosidase (debranching enzyme)	Liver, muscle, heart, leucocytes	Branched chain glycogen accumulates; liver enlarged; clinical manifestations are similar but milder compared to von Gierke's disease.
IV	Anderson's disease (amylopectinosis)	Glucosyl 4-6 transferase (branching enzyme)	Most tissues	A rare disease, glycogen with only few branches accumulate; cirrhosis of liver, impairment in liver function.
V	McArdle's disease (type V glycogenosis)	Muscle glycogen phosphorylase	Skeletal muscle	Muscle glycogen stores very high, not available during exercise; subjects cannot perform strenous exercise; suffer from muscle cramps; blood lactate and pyruvate do not increase after exercise; muscles may get damaged due to inadequate energy supply.
VI	Her's disease	Liver glycogen phosphorylase	Liver	Liver enlarged; liver glycogen cannot form glucose; mild hypoglycemia and ketosis seen.
VII	Tarui's disease	Phosphofructokinase	Skeletal muscle, erythrocytes	Muscle cramps due to exercise; blood lactate not elevated; hemolysis occurs.

Rare glycogen disorders VIII, IX, X and XI have been identified. They are due to defects in the enzymes concerned with activating and deactivating liver phosphorylase.

Source: Satyanarayana and Chakrapani, 2013

GLUCONEOGENESIS

- Gluconeogenesis is the synthesis of glucose from non-carbohydrate compounds.
- It literally means ***formation of new sugar***.
- It occurs in all animals, plants and microorganisms.
- The major precursors for gluconeogenesis are lactate, pyruvate, glucogenic amino acids, propionate and glycerol.
- Gluconeogenesis in mammals takes place mainly in the liver (about 1 kg glucose synthesized daily) and to a lesser extent, in kidney matrix (about one-tenth of liver capacity).

IMPORTANCE OF GLUCONEOGENESIS

- The continuous supply of glucose is absolutely essential to the body for a variety of functions.
- The brain and central nervous system, as well as the erythrocytes, testes, renal medulla, and embryonic tissues are dependent on glucose for continuous supply of energy.
- Glucose is the only source that supplies energy to the skeletal muscle, under anaerobic conditions.
- In fasting more than a day, gluconeogenesis must occur to meet the basal requirements of the body for glucose and to maintain the intermediates of citric acid cycle, which is essential for the survival of humans and other animals.
- Gluconeogenesis effectively clears certain metabolites produced in the tissues, which accumulate in the blood, e.g. lactate, glycerol, propionate etc.

REACTIONS OF GLUCONEOGENESIS

- In animals, both glycolysis and gluconeogenesis occur largely in the cytosol, making their regulation reciprocal and coordinated.
- Although gluconeogenesis looks like the reversed pathway of glycolysis, it is not a complete reversal of glycolysis.
- The glycolytic pathway has seven reversible reactions and three irreversible ones.
- The seven reversible reactions are common for both glycolysis and gluconeogenesis.
- The three irreversible steps of glycolysis are catalyzed by the enzymes, namely hexokinase, phosphofructokinase and pyruvate kinase.
- These three irreversible steps are bypassed in gluconeogenesis by a separate set of enzymes.

1. Conversion of pyruvate to phosphoenolpyruvate

This takes place in two steps.

Pyruvate carboxylase is a biotin-dependent mitochondrial enzyme that converts pyruvate to oxaloacetate in the presence of ATP and CO_2.

This enzyme regulates gluconeogenesis and requires acetyl CoA for its activity.

Oxaloacetate is synthesized in the mitochondrial matrix, and has to be transported to the cytosol to be used in gluconeogenesis, where the rest of the pathway occurs.

Due to membrane impermeability, oxaloacetate cannot diffuse out of the mitochondria, so it is converted to malate and then transported to the cytosol.

Within the cytosol, oxaloacetate is regenerated.

The reversible conversion of oxaloacetate and malate is catalysed by malate dehydrogenase, an enzyme present in both mitochondria and cytosol.

In the cytosol, **phosphoenolpyruvate carboxykinase** converts oxaloacetate to phosphoenolpyruvate.

GTP or ITP (not ATP) is used in this reaction and the CO_2 (fixed by carboxylase) is liberated.

For the conversion of pyruvate to phosphoenol pyruvate, 2 ATP equivalents are utilized, which is in contrast to only one ATP that is liberated in glycolysis for this reaction.

2. Conversion of fructose 1,6-bisphosphate to fructose 6-phosphate:

Phosphoenolpyruvate undergoes the reversal of glycolysis until fructose 1,6-bisphosphate is produced.

The enzyme **fructose 1,6-bisphosphatase** converts fructose 1,6-bisphosphate to fructose 6-phosphate.

This enzyme requires Mg^{2+} ions.

Fructose 1,6-bisphosphatase is absent in smooth muscle and heart muscle.

It is also a regulatory enzyme in gluconeogenesis.

3. Conversion of glucose 6-phosphate to glucose

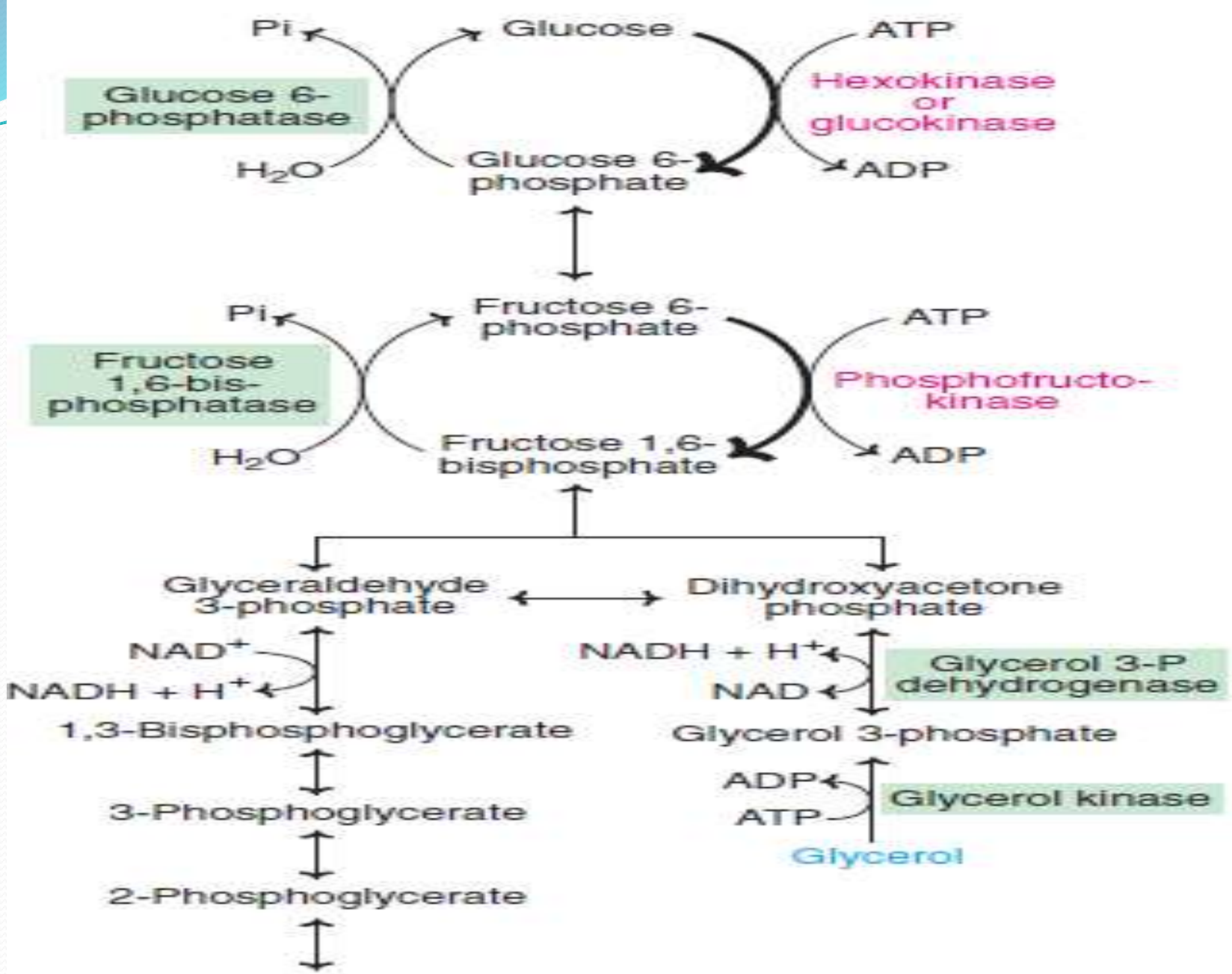
Glucose 6-phosphatase catalyses the conversion of glucose 6-phosphate to glucose.

The presence or absence of this enzyme in a tissue determines whether the tissue is capable of contributing glucose to the blood or not.

It is mostly present in liver and kidney but absent in muscle, brain and adipose tissue.

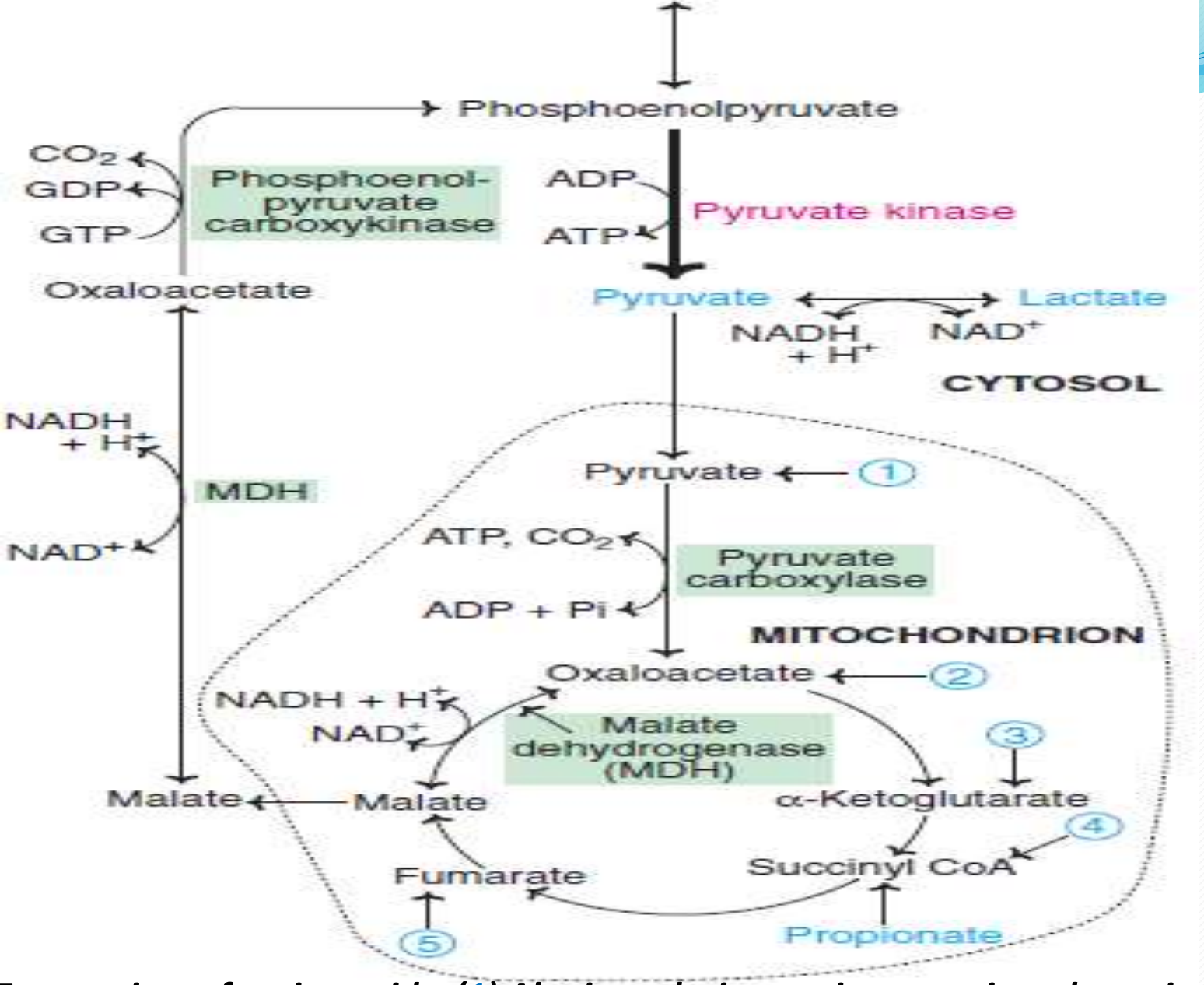
The overall summary of gluconeogenesis for the conversion of pyruvate to glucose is as follows:

$$2\ \text{Pyruvate} + 4\text{ATP} + 2\text{GTP} + 2\text{NADH} + 2\text{H}^+ + 6\text{H}_2\text{O} \longrightarrow \text{Glucose} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{GDP} + 6\text{Pi} + 6\text{H}^+$$



Source: Satyanarayana and Chakrapani, 2013

The pathway of gluconeogenesis [The enzymes catalyzing irreversible steps in glycolysis are shown in red. The important enzymes participating in gluconeogenesis are shown in shaded green]



Source: Satyanarayana and Chakrapani, 2013

Entry points of amino acids: (1) Alanine, glycine, serine, cysteine, threonine and tryptophan; (2) Aspartate and asparagine; (3) Arginine, glutamate, glutamine, histidine, proline; (4) Isoleucine, methionine, valine; (5) Phenylalanine, tyrosine]

PRECURSORS FOR GLUCONEOGENESIS

- **Amino acids:** The carbon skeleton of glucogenic amino acids (all except leucine and lysine) results in the formation of pyruvate or the intermediates of citric acid cycle, which ultimately result in the synthesis of glucose.

- **Glycerol:** It is liberated mostly in the adipose tissue by the hydrolysis of fats (triacylglycerols).

  The enzyme **glycerokinase** (found in liver and kidney, but absent in adipose tissue) activates glycerol to glycerol 3-phosphate.

  The latter is converted to dihydroxyacetone phosphate by **glycerol 3-phosphate dehydrogenase**.

  Dihydroxyacetone phosphate is an intermediate in glycolysis which can be conveniently used for glucose production.

- **Propionate:** Oxidation of odd chain fatty acids and the breakdown of some amino acids (methionine, isoleucine) yields a three carbon propionyl CoA.

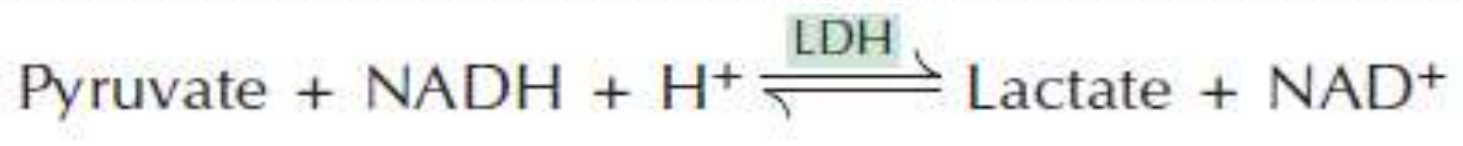
- **Propionyl CoA carboxylase** acts on propionyl CoA in the presence of ATP and biotin and converts it to methyl malonyl CoA.

- This is then converted to succinyl CoA in the presence of B_{12} coenzyme (5-Deoxyadenosyl cobalamin).

- Succinyl CoA formed from propionyl CoA enters gluconeogenesis via the TCA cycle.

CORI CYCLE

- One of the major precursors for gluconeogenesis is lactate produced by active skeletal muscle.
- The cycle involving the synthesis of glucose in liver from the skeletal muscle lactate and the reuse of the synthesized glucose by the muscle for energy is known as **Cori cycle**.
- Under anaerobic conditions, pyruvate from glycolysis is reduced to lactate by lactate dehydrogenase (LDH).



- However, lactate is a dead end in glycolysis, since it must be reconverted to pyruvate for its further metabolism.

- The very purpose of lactate production is to regenerate NADH so that glycolysis proceeds uninterrupted in skeletal muscle, especially during rigorous exercise.

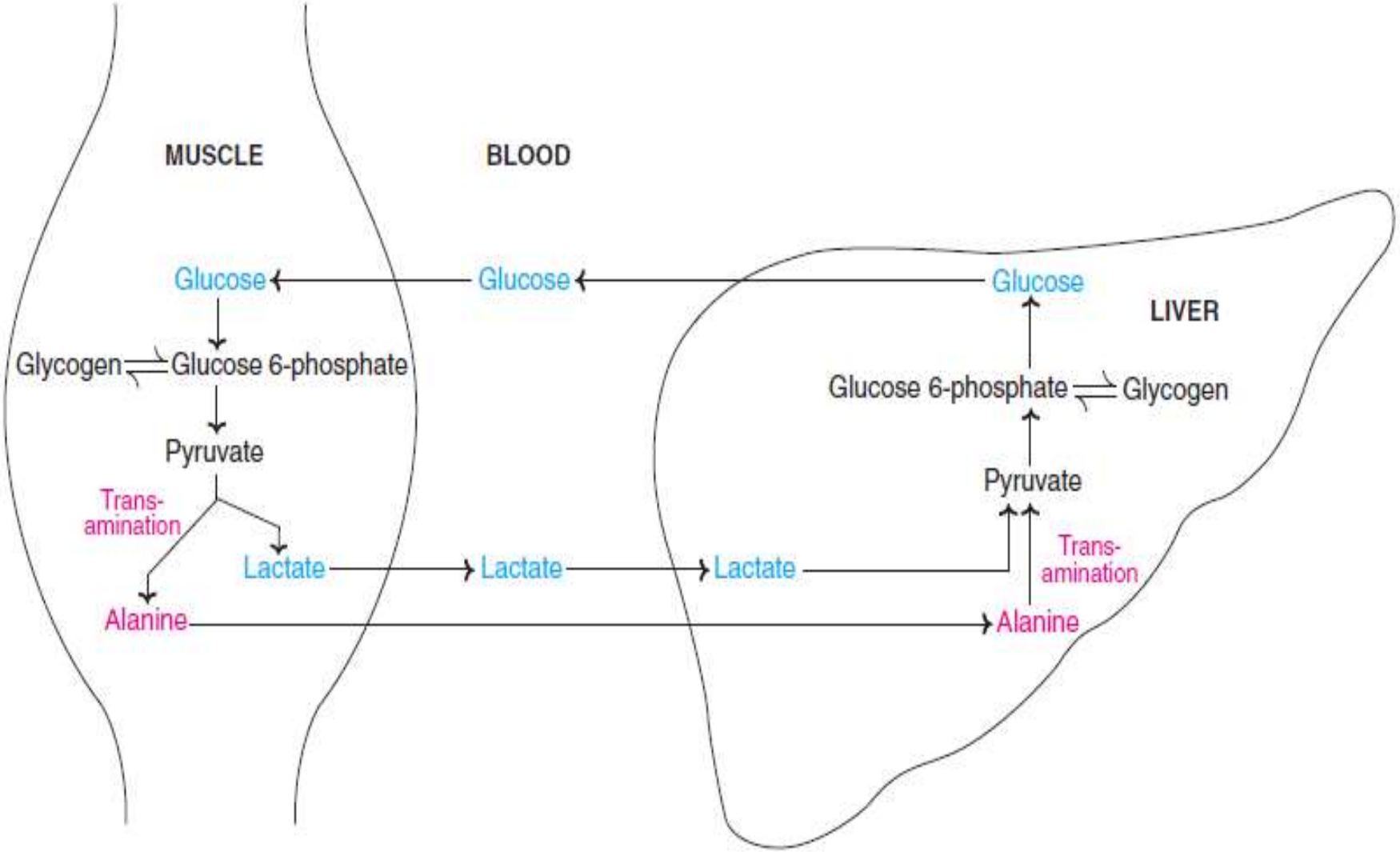
- Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of glucose due to the absence of the key enzymes of gluconeogenesis (glucose 6-phosphatase and fructose 1,6-bisphosphatase).

- The plasma membrane is freely permeable to lactate, so lactate is carried from the skeletal muscle through the blood and handed over to liver, where it is oxidized to pyruvate.

- Pyruvate, so produced, is converted to glucose by gluconeogenesis, which is then transported to the skeletal muscle.

GLUCOSE-ALANINE CYCLE

- There is a continuous transport of amino acids from muscle to liver, which predominantly occurs during starvation.
- Alanine dominates among the transported amino acids.
- It is postulated that pyruvate in skeletal muscle undergoes transamination to produce alanine.
- Alanine is transported to liver and used for the synthesis of glucose via gluconeogenesis.
- Glucose is then transported to the muscle where it results in the synthesis of alanine, which is transported back to the liver.
- This cycle is referred to as the glucose-alanine cycle.



Source: Satyanarayana and Chakrapani, 2013

Cori cycle (blue) and glucose-alanine cycle (red)
(other reactions are common for both cycles)

REGULATION OF GLUCONEOGENESIS

- Gluconeogenesis is mainly regulated by the hormone glucagon and the availability of substrates.

- Glucagon is a hormone secreted by α-cells of the pancreatic islets.

- Glucagon stimulates gluconeogenesis by two mechanisms:

  1. Active form of pyruvate kinase is converted to inactive form through the mediation of cyclic AMP, brought about by glucagon.

  Decreased pyruvate kinase results in the reduced conversion of phosphoenol pyruvate to pyruvate and the former is diverted for the synthesis of glucose.

  2. Glucagon reduces the concentration of fructose 2,6-bisphosphate, which is a compound that allosterically inhibits phosphofructokinase and activates fructose 1,6-bisphosphatase.

  Both enzymes favour increased gluconeogenesis.

- **Availability of substrates:** Among the various substrates, glucogenic amino acids have stimulating influence on gluconeogenesis.

- This is particularly important in a condition like diabetes mellitus (decreased insulin level) where amino acids are mobilized from muscle protein for the purpose of gluconeogenesis.

- Due to excessive lipolysis in adipose tissue during starvation, acetyl CoA accumulates in the liver.

- Acetyl CoA allosterically activates pyruvate carboxylase resulting in enhanced glucose production.

- Hence, acetyl CoA promotes gluconeogenesis.

ALCOHOL AND GLUCONEOGENESIS

- Ethanol oxidation in the liver to acetaldehyde by the enzyme **alcohol dehydrogenase** utilizes NAD^+.

- The excess NADH produced in the liver interferes with gluconeogenesis as illustrated below.

$$\text{Ethanol} + NAD^+ \longrightarrow \text{Acetaldehyde} + NADH + H^+$$

$$\text{Pyruvate} + NADH + H^+ \leftrightarrow \text{Lactate} + NAD^+$$

$$\text{Oxaloacetate} + NADH + H^+ \leftrightarrow \text{Malate} + NAD^+$$

- From the above reactions, pyruvate and oxaloacetate, which are the predominant substrates for gluconeogenesis, are made unavailable by alcohol intoxication.

- This is due to the overconsumption of NAD^+ and excessive production of NADH by alcohol, hence, alcohol consumption increases the risk of hypoglycemia (reduced plasma glucose).

- Hypoglycemia is frequently observed in diabetic patients (particularly on insulin treatment), and undernourished persons consuming alcohol.

PENTOSE PHOSPHATE PATHWAY

- The pentose phosphate pathway (PPP) is also called ***hexose monophosphate (HMP) pathway***, ***hexose monophosphate (HMP) shunt*** or ***phosphogluconate pathway.***

- It is an alternative pathway to glycolysis and TCA cycle for the oxidation of glucose.

- However, it is also anabolic in nature, since it is concerned with the biosynthesis of NADPH and pentoses.

- HMP shunt starts with glucose 6-phosphate, so no ATP is directly utilized or produced in the pathway.

- It is a unique multifunctional pathway, since there are several interconvertible substances produced, which may proceed in metabolic directions.

- The enzymes of HMP shunt are located in the cytosol.

- Tissues such as liver, adipose tissue, adrenal gland, erythrocytes, testes and lactating mammary gland are highly active in HMP shunt.

- Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

- The PPP is important for the generation of **pentoses** and **NADPH** needed for biosynthetic reactions and other functions.

IMPORTANCE OF PENTOSES

- In the HMP shunt, hexoses are converted into pentoses, the most important being ribose 5-phosphate.
- This pentose or its derivatives are useful for the synthesis of nucleic acids (RNA and DNA) and many nucleotides such as ATP, NAD^+, FAD and CoA.
- Skeletal muscle is capable of synthesizing pentoses, although only the first few enzymes of HMP shunt are active.
- It, therefore, appears that the complete pathway of HMP shunt may not be required for the synthesis of pentoses.

IMPORTANCE OF NADPH

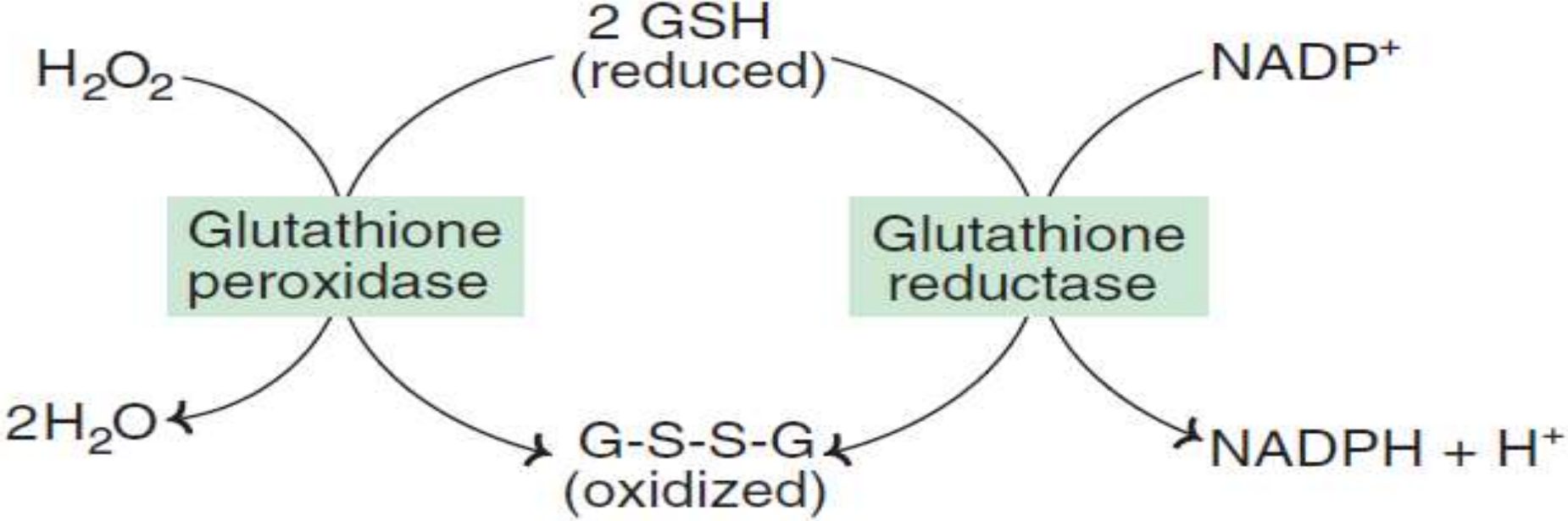
1. NADPH is required for the reductive biosynthesis of fatty acids and steroids, hence HMP shunt is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

2. NADPH is used in the synthesis of certain amino acids involving the enzyme glutamate dehydrogenase.

3. There is a continuous production of H_2O_2 in the living cells, which can chemically damage unsaturated lipids, proteins and DNA.

   This is, however, prevented to a large extent through antioxidant (free radical scavenging) reactions involving NADPH.

   Glutathione-mediated reduction of H_2O_2 is illustrated below.



4. Microsomal cytochrome P_{450} system (in liver) brings about the detoxification of drugs and foreign compounds by hydroxylation reactions involving NADPH.

5. Phagocytosis, which is the engulfment of foreign particles, including microorganisms, carried out by white blood cells, requires the supply of NADPH.

6. **Special functions of NADPH in RBC:** NADPH produced in erythrocytes maintains the concentration of reduced glutathione, which is essentially required to preserve the integrity of RBC membrane.

   NADPH is also necessary to keep ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

7. High concentration of NADPH in the lens of eyes is necessary to preserve the transparency of the lenses.

REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY

The reactions of HMP shunt are divided into two phases: oxidative and non-oxidative.

OXIDATIVE PHASE:

1. **Glucose 6-phosphate dehydrogenase** (G6PD) is an NADP-dependent enzyme that converts glucose 6-phosphate to 6-phosphogluconolactone.

2. 6-phosphogluconolactone is hydrolyzed by **gluconolactone hydrolase** to 6-phosphogluconate.

3. The next reaction involving the synthesis of NADPH is catalyzed by **6-phosphogluconate dehydrogenase**, which decarboxylate 6-phosphogluconate to give ribulose 5-phosphate.

NON-OXIDATIVE PHASE:

The non-oxidative reactions are concerned with the inter-conversion of three, four, five and seven carbon monosaccharides.

5. Ribulose 5-phosphate is acted upon by a **ribulose 5-phosphate epimerase** to produce xylulose 5-phosphate, while **ribose 5-phosphate ketoisomerase** converts ribulose 5-phosphate to ribose 5-phosphate.

6. The enzyme **transketolase** catalyzes the transfer of two carbon moiety from xylulose 5-phosphate to ribose 5-phosphate to give a 3-carbon glyceraldehyde 3-phosphate and a 7-carbon sedoheptulose 7-phosphate.

   Transketolase is dependent on the coenzyme thiamine pyrophosphate (TPP) and Mg^{2+} ions.

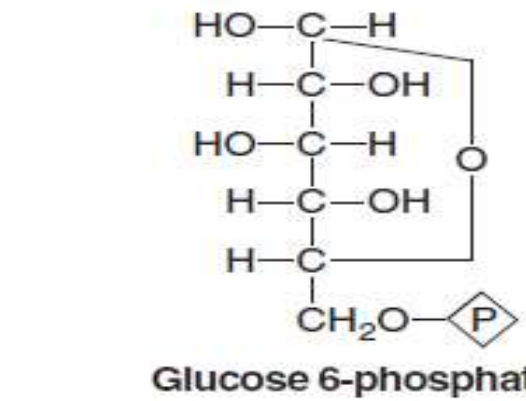
7. **Transaldolase** brings about the transfer of a 3-carbon fragment (active dihydroxyacetone) from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to give fructose 6-phosphate and four carbon erythrose 4-phosphate.

8. **Transketolase** acts on xylulose 5-phosphate and transfers a 2-carbon fragment from it to erythrose 4-phosphate to generate fructose 6-phosphate and glyceraldehyde 3-phosphate.

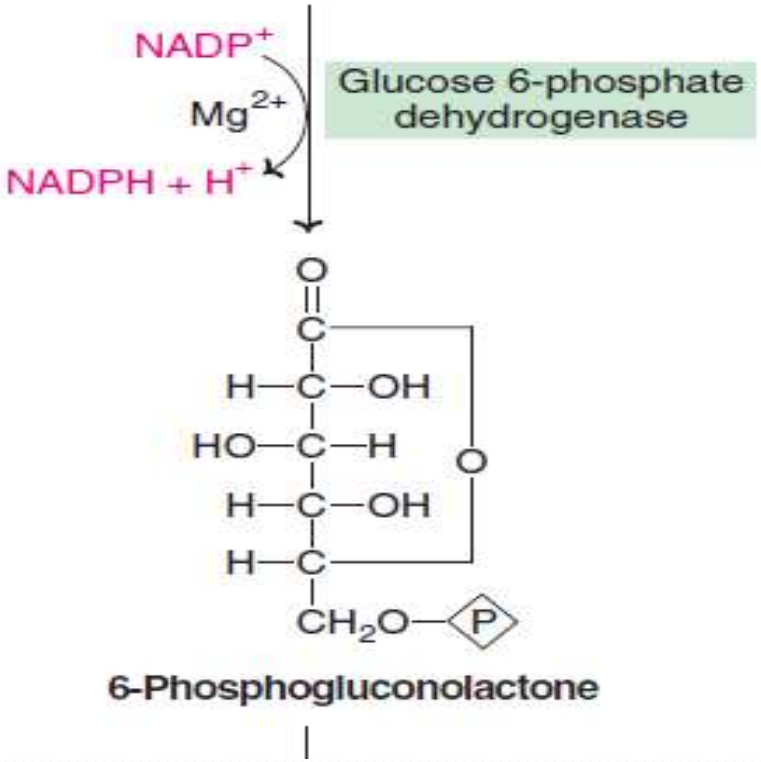
Fructose 6-phosphate and glyceraldehyde 3-phosphate can be further catabolized through glycolysis and citric acid cycle, glucose may also be synthesized from these two compounds.

For the complete oxidation of glucose 6-phosphate to $6CO_2$, the pathway has to start with 6 molecules of glucose 6-phosphate, and 5 molecules of glucose are regenerated with the production of 12 NADPH.

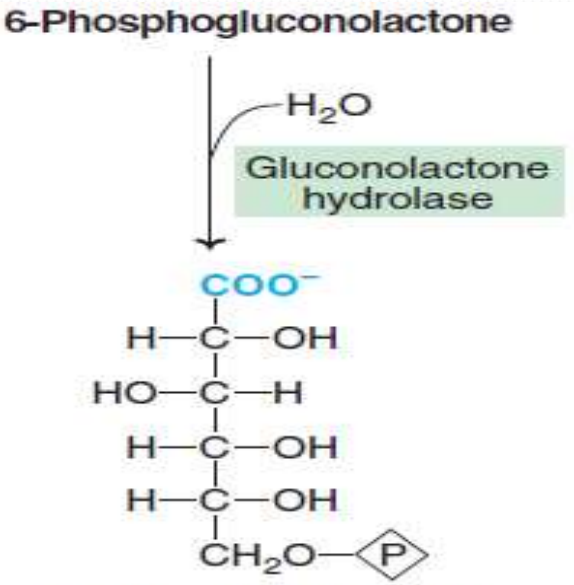
$$6\text{ Glucose 6-phosphate} + 12NADP^+ + 6H_2O \longrightarrow 6CO_2 + 12NADPH + 12H^+ + 5\text{Glucose 6-phosphate}$$



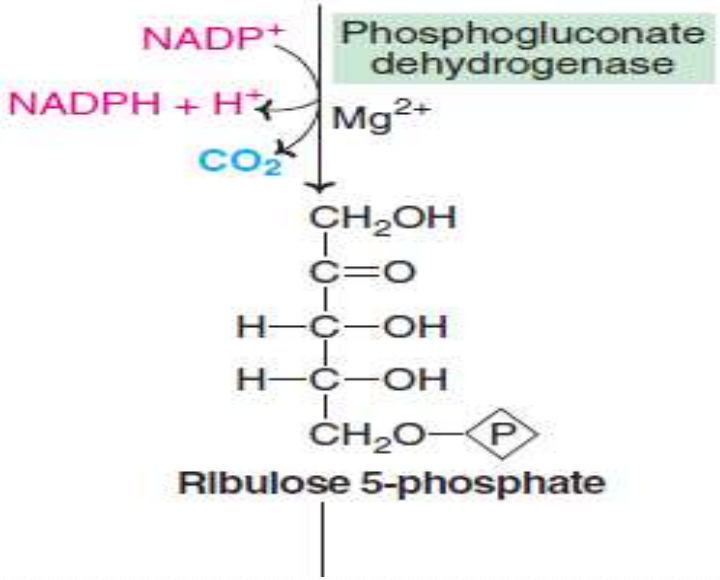
Glucose 6-phosphate



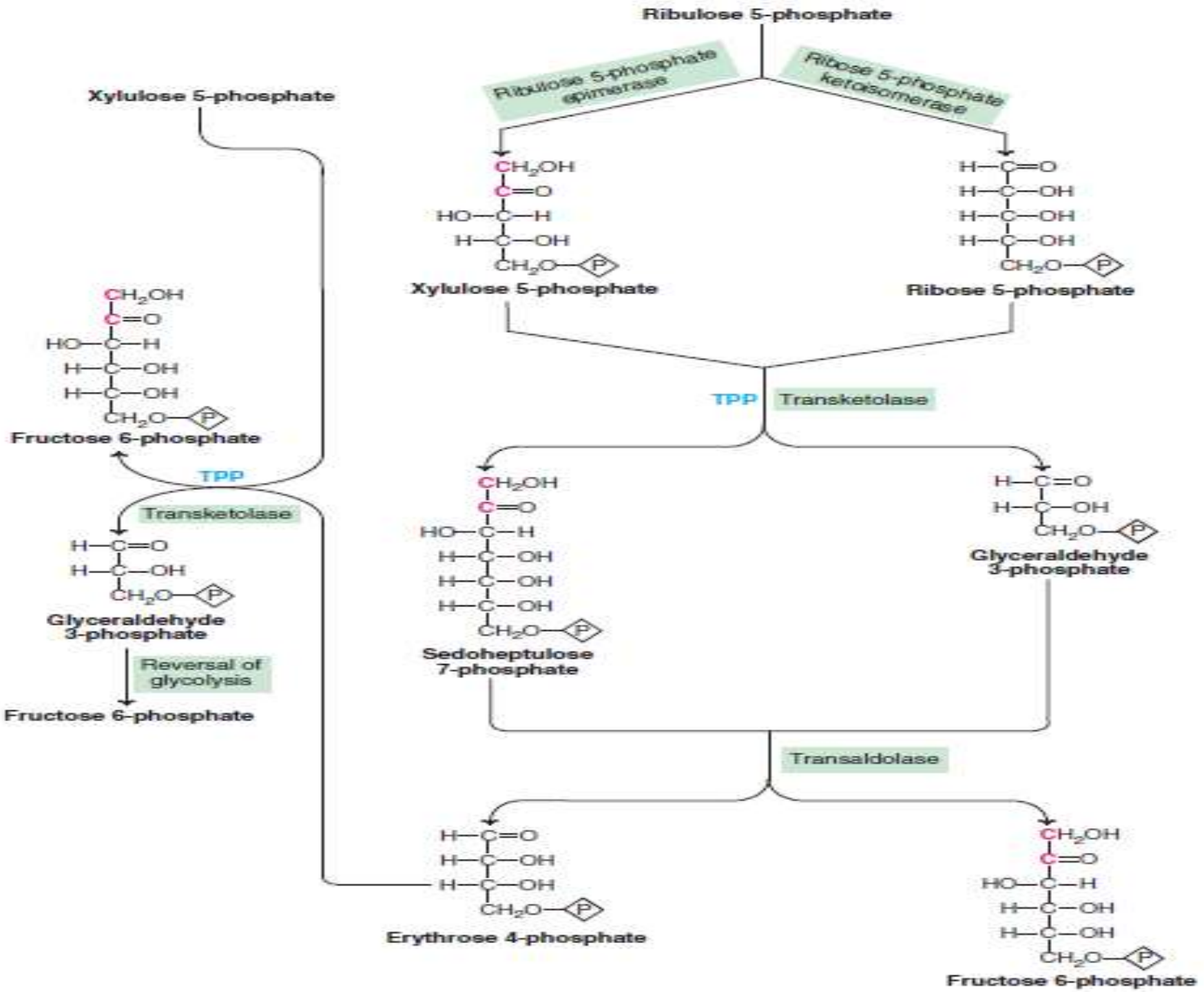
6-Phosphogluconolactone



6-Phosphogluconate



Ribulose 5-phosphate


Ribulose 5-phosphate
Ribulose 5-phosphate epimerase
Ribose 5-phosphate ketoisomerase
Xylulose 5-phosphate
Xylulose 5-phosphate
Ribose 5-phosphate
TPP
Transketolase
Fructose 6-phosphate
TPP
Transketolase
Glyceraldehyde 3-phosphate
Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
Reversal of glycolysis
Fructose 6-phosphate
Transaldolase
Erythrose 4-phosphate
Fructose 6-phosphate

REGULATION OF HMP SHUNT

- Glucose 6-phosphate dehydrogenase, which catalyzes the first reaction, regulates the HMP shunt.
- This enzyme catalyzes an irreversible reaction.
- NADPH competitively inhibits glucose 6-phosphate dehydrogenase.
- It is the ratio of NADPH/NAD^+ that ultimately determines the flux of this pathway.

GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

- G6PD deficiency is an inherited sex-linked trait, affecting the pentose phosphate pathway.
- The deficiency occurs in all the cells of the affected individuals, but it is more severe in RBC.
- HMP shunt is the only means of providing NADPH in the erythrocytes, so the decreased activity of G6PD impairs the synthesis of NADPH in RBC.
- This results in the accumulation of methemoglobin and peroxides in erythrocytes leading to hemolysis.
- Clinical manifestations in G6PD deficiency: Most of the patients with G6PD deficiency do not

CLINICAL MANIFESTATIONS OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY

- Most of the patients with G6PD deficiency do not usually exhibit clinical symptoms, but some of them develop hemolytic anemia if they are administered oxidant drugs or exposed to a severe infection.

- Drugs such as primaquine (antimalarial), acetanilide (antipyretic), sulfamethoxazole (antibiotic) or ingestion of fava beans (favism) produce hemolytic jaundice in these patients.

- Severe infection results in the generation of free radicals (in macrophages), which can enter the RBC and cause hemolysis (due to decreased NADPH and reduced GSH).

GLUCOSE 6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND RESISTANCE TO MALARIA

- Interestingly, G6PD deficiency is associated with resistance to malaria (caused by *Plasmodium falciparum*).
- This is explained from the fact that the parasites that cause malaria are dependent on HMP shunt and reduced glutathione for their optimum growth in RBC.
- Hence, G6PD deficiency, which is seen frequently in Africans, protects them from malaria, a common disease in the region.
- It is regarded as an adaptability of the people living in malaria-infected regions of the world.
- Biochemical diagnosis can be done by detecting reduced activity of G6PD in RBC.
- The management of G6PD deficiency includes avoiding oxidative stress and symptomatic treatment of hemolysis.

WERNICKE-KORSAKOFF SYNDROME

- The Wernicke-Korsakoff syndrome is a genetic disorder associated with HMP shunt.
- An alteration in transketolase activity that reduces its affinity (by about tenfold) with thiamine pyrophosphate is the biochemical lesion.
- The symptoms of Wernicke-Korsakoff syndrome include mental disorder, loss of memory and partial paralysis.
- The symptoms are manifested in vitamin-deficient alcoholics.

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