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

LECTURE NOTE

ON

BCH 308

METABOLISM OF CARBOHYDRATES

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COURSE OUTLINE

- Degradation and digestion of carbohydrates - sugars, storage polysaccharides and cell walls
- Reactions of sugars
- Glycolysis
- Citric acid cycle
- Pentose phosphate pathway
- Uronic (glucuronic) acid pathway
- Glyoxylate cycle
- Calvin cycle
- Gluconeogenesis
- Cori cycle
- Glycogenesis
- Glycogenolysis
- Disorders of carbohydrate metabolism

DID YOU KNOW?

- Glucose accounts for nearly 80% of the total monosaccharides yielded from the digestion of carbohydrates.
- Out of the 160g of glucose needed by the body daily, the brain alone consumes about 120g.
- About 65-70% of the ATP produced in the body is synthesized in the TCA cycle.
- TCA cycle utilizes about two-third of the total oxygen consumed by the body.

DEGRADATION AND DIGESTION OF CARBOHYDRATES

- The digestion of carbohydrates starts briefly from the mouth and ends in the small intestine.

- Before that, polysaccharides are hydrated during heating, which enhances digestion.

- During mastication in the mouth, starch is randomly acted upon by salivary **α-amylase** (ptyalin), which cleaves α- 1,4-glycosidic bonds to produce α-limit dextrins, maltotriose and maltose.

- The hydrolysis of glycosidic bonds is carried out by a group of enzymes generally called **glycosidases**

- Interestingly, carbohydrates are not digested in the stomach due to the inactivation of salivary amylase by high acidity in the stomach.
- However, the acidic dietary contents of the stomach are neutralized in the small intestine by bicarbonate produced by pancreas.
- Hence, the pancreatic α-amylase acts on starch and continues the digestion process, producing disaccharides (maltose, isomaltose) and oligosaccharides.
- Amylase specifically acts on α-1,4-glycosidic bonds and not on α-1,6 bonds.
- Di- and oligosaccharides are finally digested to monosaccharides at the mucosal lining of the upper jejunum.

- This is carried out by **oligosaccharidases** (e.g. glucoamylase acting on amylose) and disaccharidases (e.g. maltase, sucrase, lactase).

- The digestion of carbohydrates mainly yields the monosaccharides glucose, fructose and galactose.

- Glucose accounts for nearly 80% of the total monosaccharides yielded from the digestion of carbohydrates.

- The absorption of sugars mostly takes place in the duodenum and upper jejunum of the small intestine.

- Galactose is the most efficiently absorbed among the monosaccharides, followed by glucose and fructose.

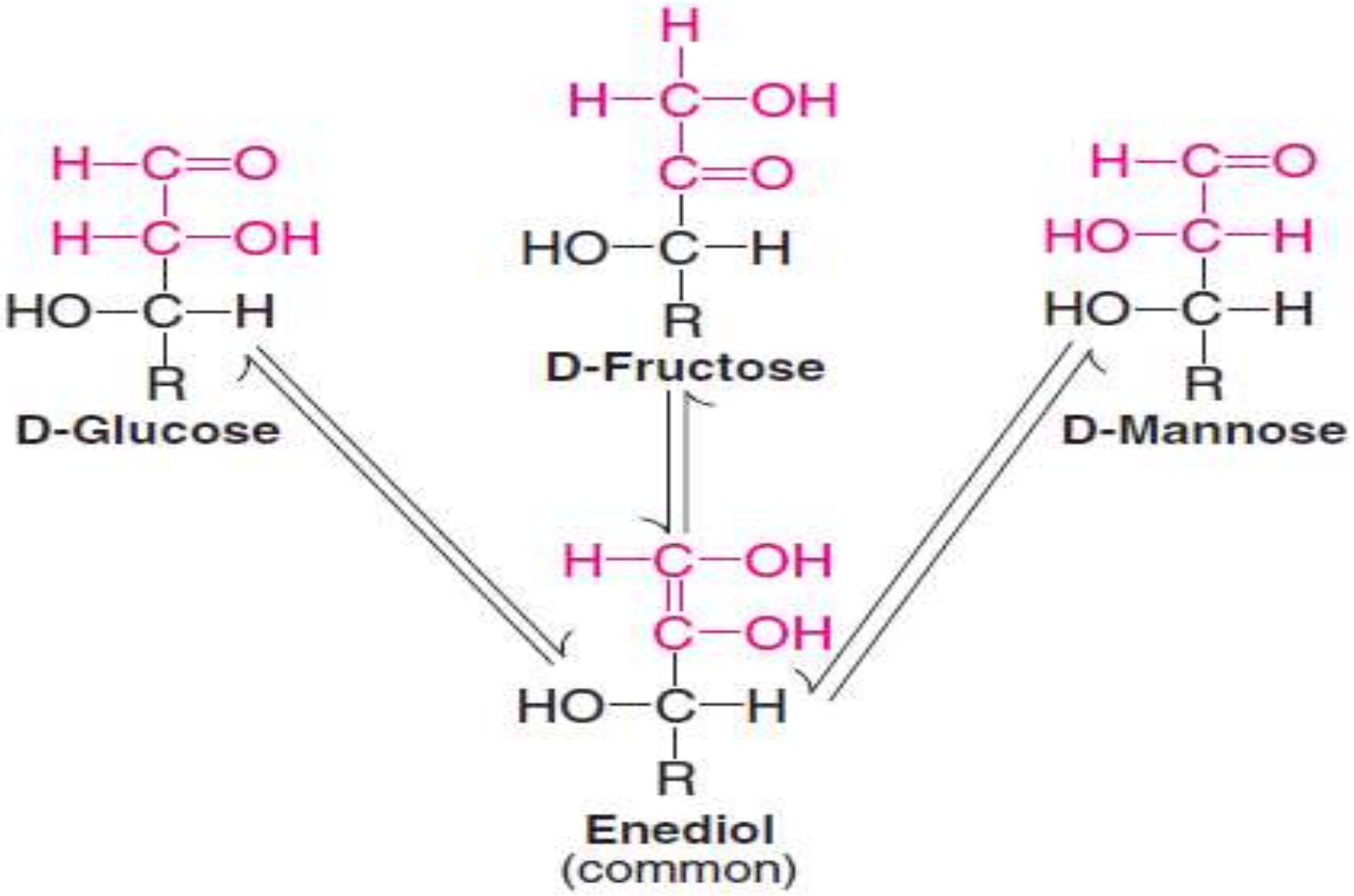
REACTIONS OF SUGARS

- **Tautomerization or enolization:** The process of shifting a hydrogen atom from one carbon atom to another to produce enediols. Sugars possessing anomeric carbon atom undergo tautomerization in alkaline solutions.

When glucose is kept in alkaline solution for several hours, it undergoes isomerization to form D-fructose and D-mannose.

This reaction— known as the *Lobry de Bruyn-von Ekenstein transformation*—results in the formation of a common intermediate—namely enediol—for all the three sugars.

The enediols are highly reactive, hence sugars in alkaline solution are powerful reducing agents.


H
H—C—OH
C=O
HO—C—H
R
D-Fructose
H—C=O
H—C—OH
HO—C—H
R
D-Glucose
H—C=O
HO—C—H
HO—C—H
R
D-Mannose
H—C—OH
C—OH
HO—C—H
R
Enediol
(common)

- **Reducing properties** (owing to the free aldehyde or keto group of anomeric carbon): Sugars are classified as reducing or non-reducing. Many laboratory tests such as Benedict's test, Fehling's test, Barfoed's test etc. are employed to identify the reducing action of sugars.

- **Oxidation:** Depending on the oxidizing agent used, the terminal aldehyde (or keto) or the terminal alcohol or both the groups may be oxidized.

- **Reduction:** When treated with reducing agents such as sodium amalgam, the aldehyde or keto group of monosaccharide is reduced to its corresponding alcohol.

- **Dehydration**: When treated with concentrated sulfuric acid, monosaccharides undergo dehydration with an elimination of 3 water molecules.

- **Osazone formation**: When boiled with phenylhydrazine in acetic acid, reducing sugars form **osazones**. Reducing disaccharides also give osazones.

- **Formation of esters**: The alcoholic groups of monosaccharides may be esterified by non-enzymatic or enzymatic reactions.

- **Glycosides**: They are formed when the hemiacetal or hemiketal hydroxyl group of a carbohydrate reacts with a hydroxyl group of another carbohydrate or a non-carbohydrate (e.g. methyl alcohol, phenol, glycerol). The bond so formed is known as **glycosidic bond** and the non-carbohydrate moiety (when present) is referred to as **aglycone**.

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, citric acid cycle, pentose phosphate pathway, gluconeogenesis, glycogenesis, glycogenolysis, uronic acid pathway etc.

- 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.

GLYCOLYSIS

- Glycolysis (Greek *glykys* – sweet and *lysis* – splitting) is defined as the sequence of enyzme-catalyzed reactions converting glucose to pyruvate, with the production of ATP.
- The complete pathway of glycolysis was elucidated around 1940, making it the first metabolic pathway to be elucidated, and probably the best understood.
- It is often referred to as Embden-Meyerhof pathway (E.M. pathway) in honour of the two biochemists who made a major contribution to the knowledge of glycolysis.
- Glycolysis is a universal pathway that takes place in all cells of the body, and its enzymes are present in the cytosomal fraction of the cell.
- It occurs in the absence of oxygen (anaerobic) or in the presence of oxygen (aerobic).

- Under anaerobic condition, lactate is the end product, while in aerobic condition, pyruvate is formed, which is then oxidized to CO_2 and H_2O.
- Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes, cornea, lens etc.
- It is very essential for the brain, which is dependent on glucose for energy.
- Interestingly, the human brain consumes about 120g of glucose per day out of the 160g needed by the body.
- Insufficient supply of glucose to the brain may lead to coma and death.
- Glycolysis is a central metabolic pathway with many of its intermediates providing branch point to other pathways.

REACTIONS OF GLYCOLYSIS

- The glycolytic pathway can be divided into three phases: A (Energy investment phase or priming stage), B (Splitting phase) and C (Energy generation or payoff phase).

A. Energy investment phase

1. Glucose is phosphorylated to glucose 6-phosphate by **hexokinase** or **glucokinase**. It is an irreversible reaction dependent on ATP and Mg^{2+}.

2. **Phosphohexose isomerase** catalyzes the isomerization of glucose 6-phosphate to fructose 6-phosphate in the presence of Mg^{2+}.

3. Fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate by **phosphofructokinase** (PFK). This is an irreversible and a regulatory step in glycolysis.

B. Splitting phase

4. Fructose 1,6-bisphosphate is split (hence the name glycolysis) to two 3-carbon compounds, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate by the enzyme **aldolase** (fructose 1,6-bisphosphate aldolase).

5. **Phosphotriose isomerase** catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Hence, two molecules of glyceraldehyde 3-phosphate are obtained from one molecule of glucose.

C. Energy generation phase

6. Glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate by **glyceraldehyde 3-phosphate dehydrogenase.** Iodoacetate and arsenate inhibit the enzyme.

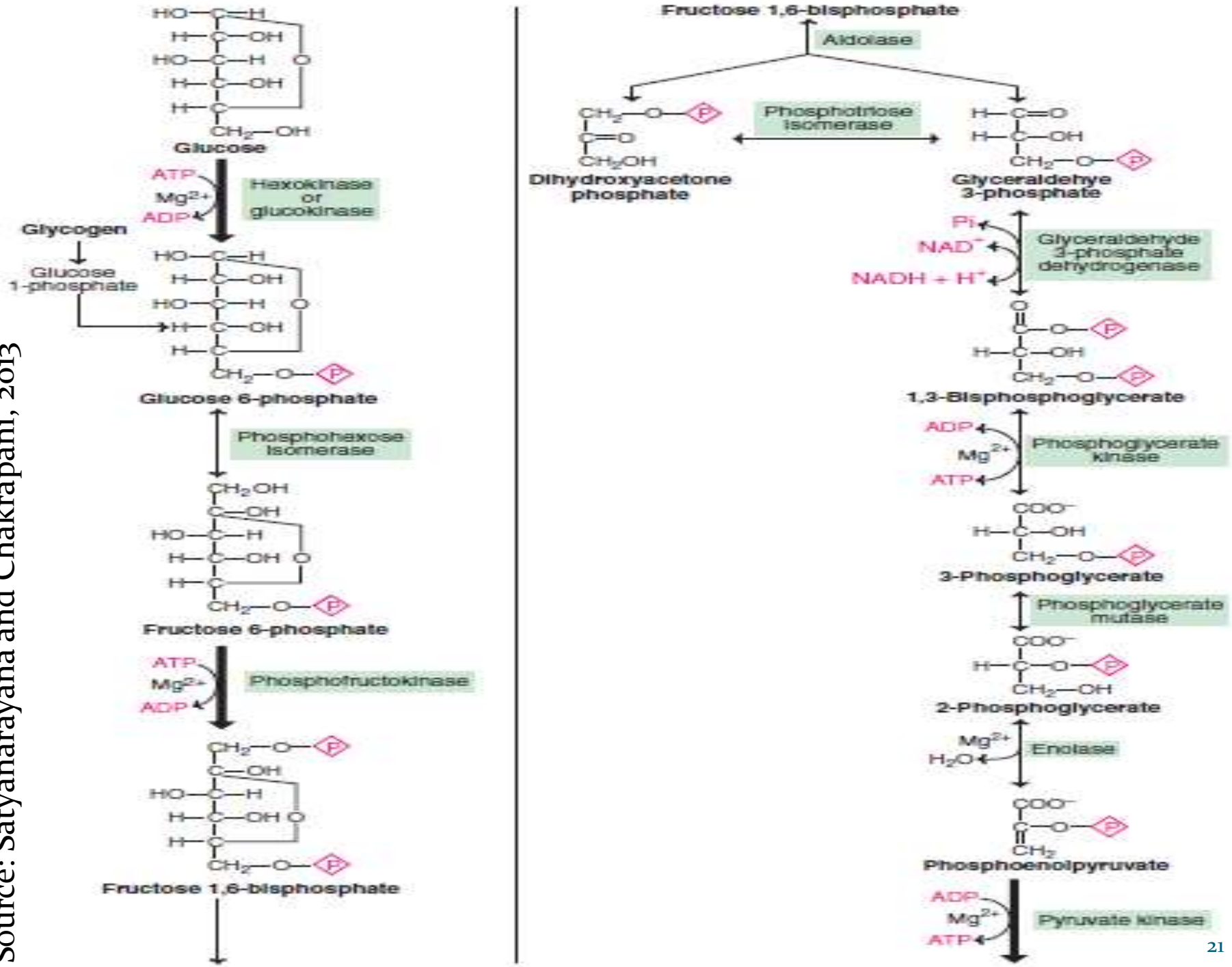
   NADH + H^+ is also formed, and in aerobic condition, it passes through the electron transport chain and 6 ATP (2 x 3 ATP) are synthesized by oxidative phosphorylation. inhibit the enzyme glyceraldehyde 3-phosphate dehydrogenase

7. **Phosphoglycerate kinase** acts on 1,3-bisphosphoglycerate resulting in the synthesis of ATP and formation of 3-phosphoglycerate – a good example of substrate level phosphorylation. This reaction is reversible, which is a rare example among the kinase reactions.

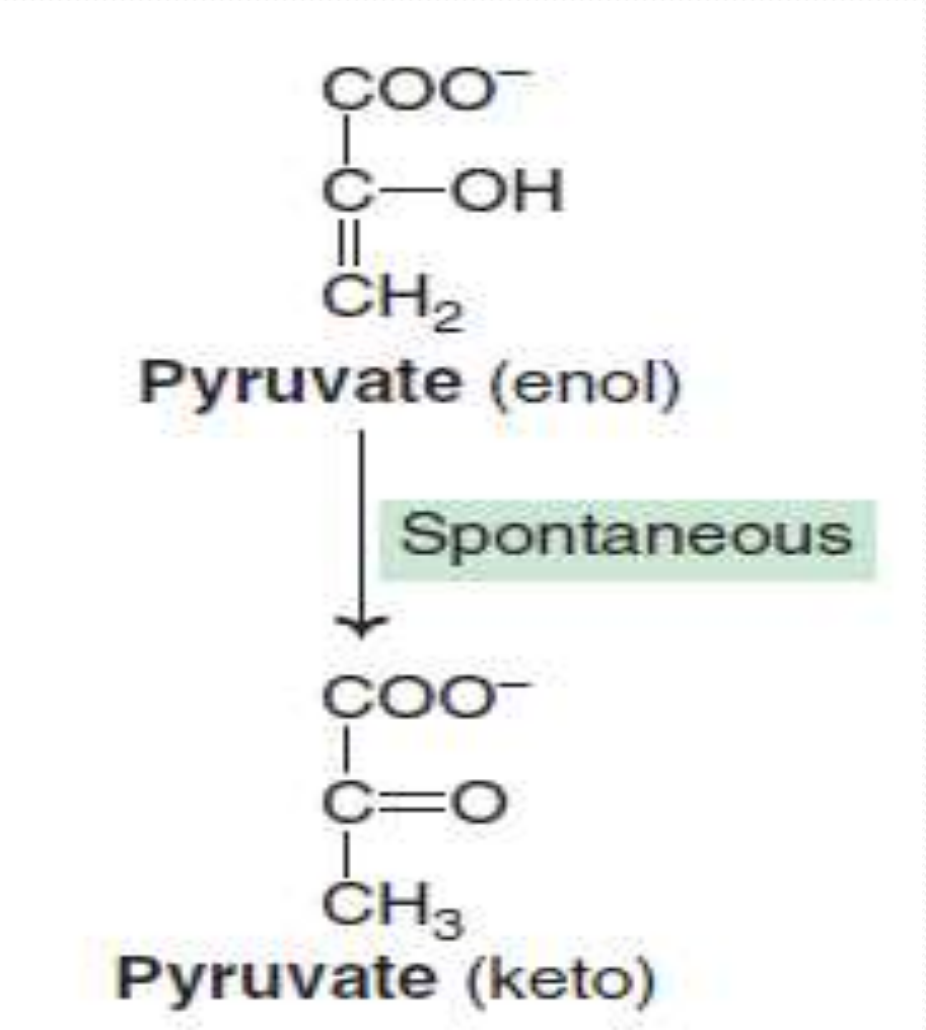
8. **Phosphoglycerate mutase** catalyzes the isomerization of 3-Phosphoglycerate to 2-phosphoglycerate.

9. Dependent on Mg^{2+} or Mn^{2+}, and inhibited by fluoride, the enzyme **enolase** generates phosphoenol pyruvate from 2-phosphoglycerate.

10. In another substrate level phosphorylation, the enzyme **pyruvate kinase** catalyses the irreversible transfer of high energy phosphate from phosphoenol pyruvate to ADP, leading to the formation of ATP and pyruvate.

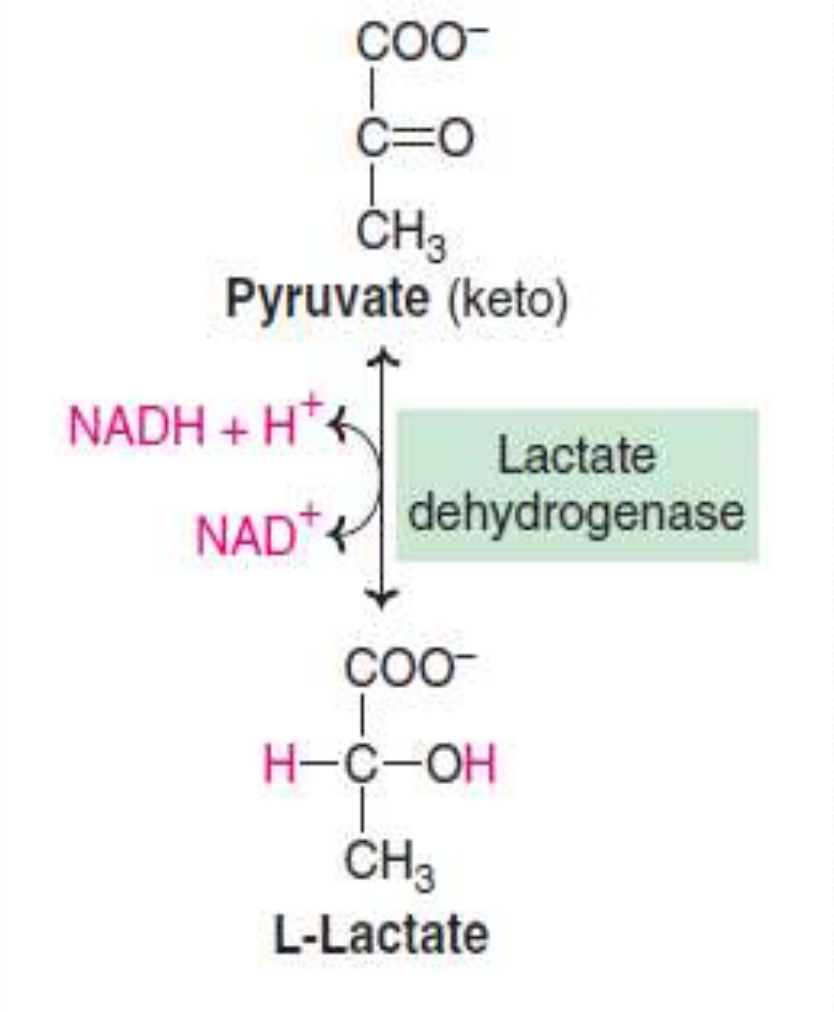


Source: Satyanarayana and Chakrapani, 2013


COO⁻
C—OH
CH2
Pyruvate (enol)
Spontaneous
COO⁻
C=O
CH3
Pyruvate (keto)

CONVERSION OF PYRUVATE TO LACTATE

- Under anaerobic conditions, pyruvate is reduced by NADH to lactate in presence of the enzyme lactate dehydrogenase (competitive inhibitor—oxamate).
- The NADH utilized in this step is obtained from the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase.
- The formation of lactate allows the regeneration of NAD^+ which can be reused by glyceraldehyde 3-phosphate dehydrogenase so that glycolysis proceeds even in the absence of oxygen to supply ATP.
- This is very essential in skeletal muscle during strenuous exercise where oxygen supply is very limited.
- Glycolysis in the erythrocytes leads to lactate production, since mitochondria, which are the sites for aerobic oxidation—are absent.



Conversion of pyruvate to lactate

ENERGETICS OF GLYCOLYSIS

- Under anaerobic conditions, 4 ATP are synthesized while, under aerobic conditions, 10 or 8 ATP are synthesized, depending on the shuttle pathway that operates for oxidative phosphorylation.

- If the cytosolic NADH uses malate-aspartate shuttle, 3 ATP are generated from each molecule of NADH, while only 2 ATP are produced via the glycerolphosphate shuttle.

- **When the 2 ATP used in the priming stage are subtracted, the net ATP produced will be 2 and 8 (or 6) respectively under anaerobic and aerobic conditions.**

- When glycolysis occurs from glycogen, one more ATP is generated because no ATP is consumed for the activation of glucose, as glycogen directly produces glucose 1-phosphate which forms glucose 6-phosphate).

- Thus, in anaerobic glycolysis, 3 ATP are produced from glycogen.

REGULATION OF GLYCOLYSIS

- The enzymes namely **hexokinase** (and glucokinase), **phosphofructokinase** and **pyruvate kinase**, catalysing the irreversible reactions regulate glycolysis.

- **Hexokinase:** It is inhibited by glucose 6-phosphate, and prevents the accumulation of glucose 6-phosphate due to product inhibition.

- Glucokinase, which specifically phosphorylates glucose, is an inducible enzyme.

- The substrate (glucose), probably through the involvement of insulin, induces glucokinase.

- **Phosphofructokinase (PFK):** It is the most important regulatory enzyme in glycolysis.

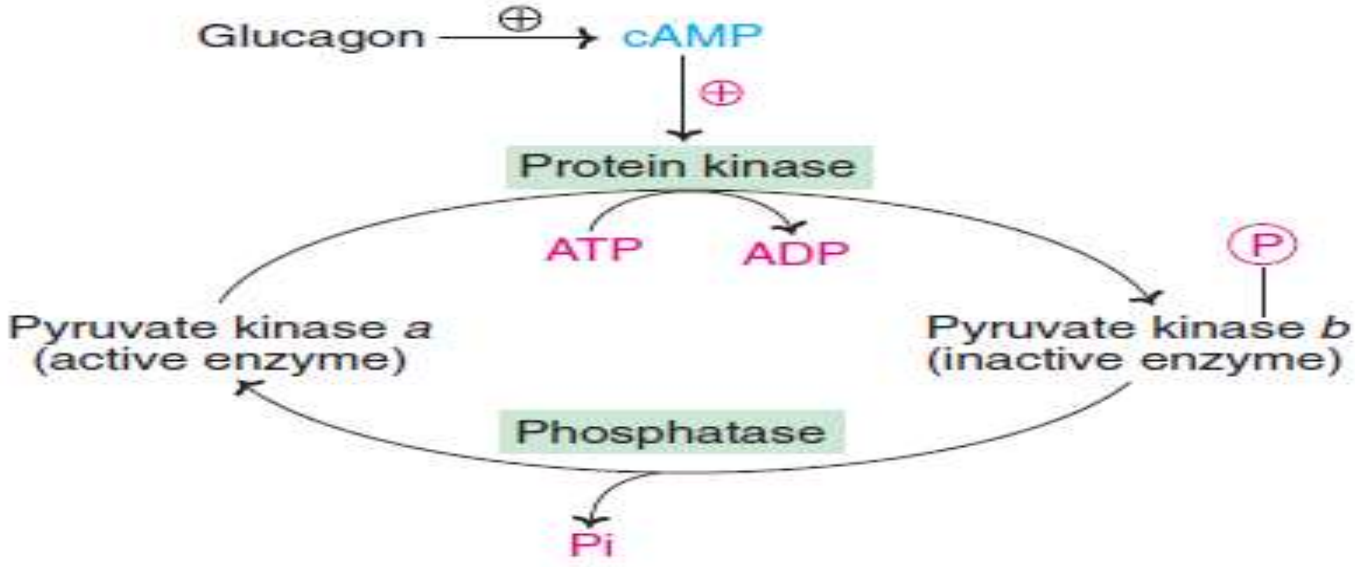
- This enzyme catalyzes the *rate-limiting committed step* in glycolysis.

- PFK is an allosteric enzyme regulated by allosteric effectors.

- ATP, citrate and H^+ ions (low pH) are the most important allosteric inhibitors of PFK.

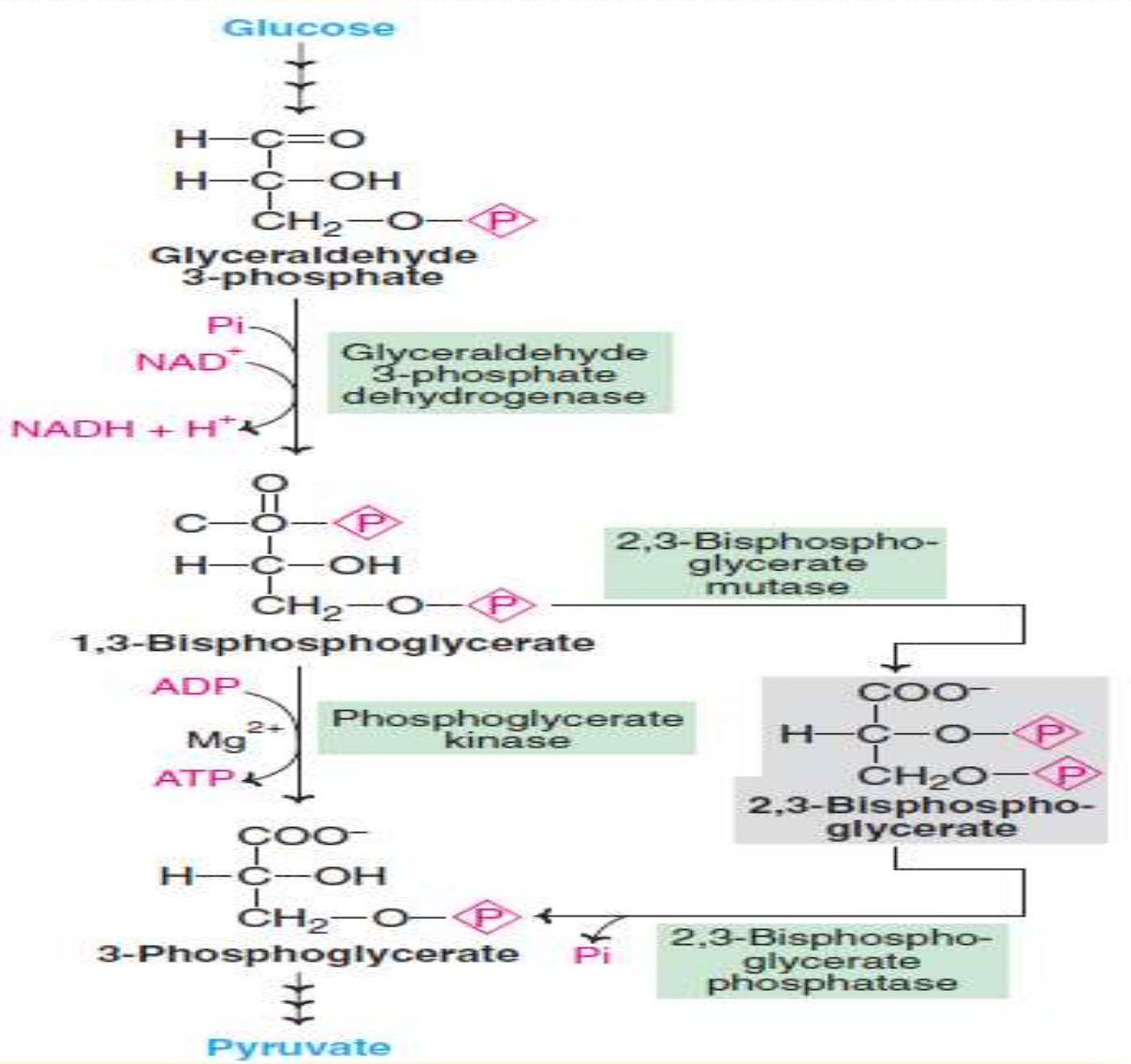
- Fructose 2,6-bisphosphate, ADP, AMP and Pi are the allosteric activators PFK.

- **Pyruvate kinase:** This enzyme is inhibited by ATP and activated by F1,6-BP.
- Pyruvate kinase is active (a) in dephosphorylated state and inactive (b) in phosphorylated state.
- Inactivation of pyruvate kinase by phosphorylation is brought about by cAMP-dependent protein kinase.
- The hormone *glucagon* inhibits hepatic glycolysis by this mechanism as illustrated below.



RAPAPORT-LEUBERING CYCLE

- The Rapaport-Leubering cycle is a supplementary pathway to glycolysis, which is operative in the erythrocytes of man and other mammals.
- It is mainly concerned with the synthesis of 2,3-bisphosphoglycerate (2,3-BPG) in the red blood cell (RBC).
- 1,3-Bisphosphoglycerate (1,3-BPG) produced in glycolysis is converted to 2,3-BPG by the enzyme **2,3-bisphosphoglycerate mutase**.
- 2,3-BPG is hydrolyzed to 3-phosphoglycerate by **bisphosphoglycerate phosphatase**.
- It is now believed that bisphosphoglycerate mutase is a bifunctional enzyme with mutase and phosphatase activities catalyzed by two different sites present on the same enzyme.
- About 15-25% of the glucose that gets converted to lactate in erythrocytes goes via 2,3-BPG synthesis.



Rapaport-Leubering Cycle

SIGNIFICANCE OF 2,3-BPG

1. Production of 2,3-BPG allows glycolysis to proceed without the synthesis of ATP.

   The Rapaport-Leubering cycle, therefore is a shunt pathway of glycolysis to dissipate or waste the energy not needed by erythrocytes.

2. 2,3-BPG is not a waste molecule in RBC, as it combines with hemoglobin (Hb) and reduces Hb affinity with oxygen.

   Therefore, in the presence of 2,3-BPG, oxyhemoglobin unloads more oxygen to the tissues.

   Increase in erythrocyte 2,3-BPG is observed in hypoxic condition, high altitude, fetal tissues, anemic conditions etc.

   In all these cases, 2,3-BPG will enhance supply of oxygen to tissues.

3. Glycolysis in the erythrocytes is linked with 2,3-BPG production and oxygen transport.

In hexokinase deficiency, glucose is not phosphorylated, hence the synthesis and concentration of 2,3-BPG are low in RBC.

Hemoglobin exhibits high oxygen affinity in hexokinase-deficient patients.

On the other hand, in the patients with pyruvate kinase deficiency, the level of 2,3-BPG in erythrocytes is high, resulting in low oxygen affinity.

CONVERSION OF PYRUVATE TO ACETYL CoA

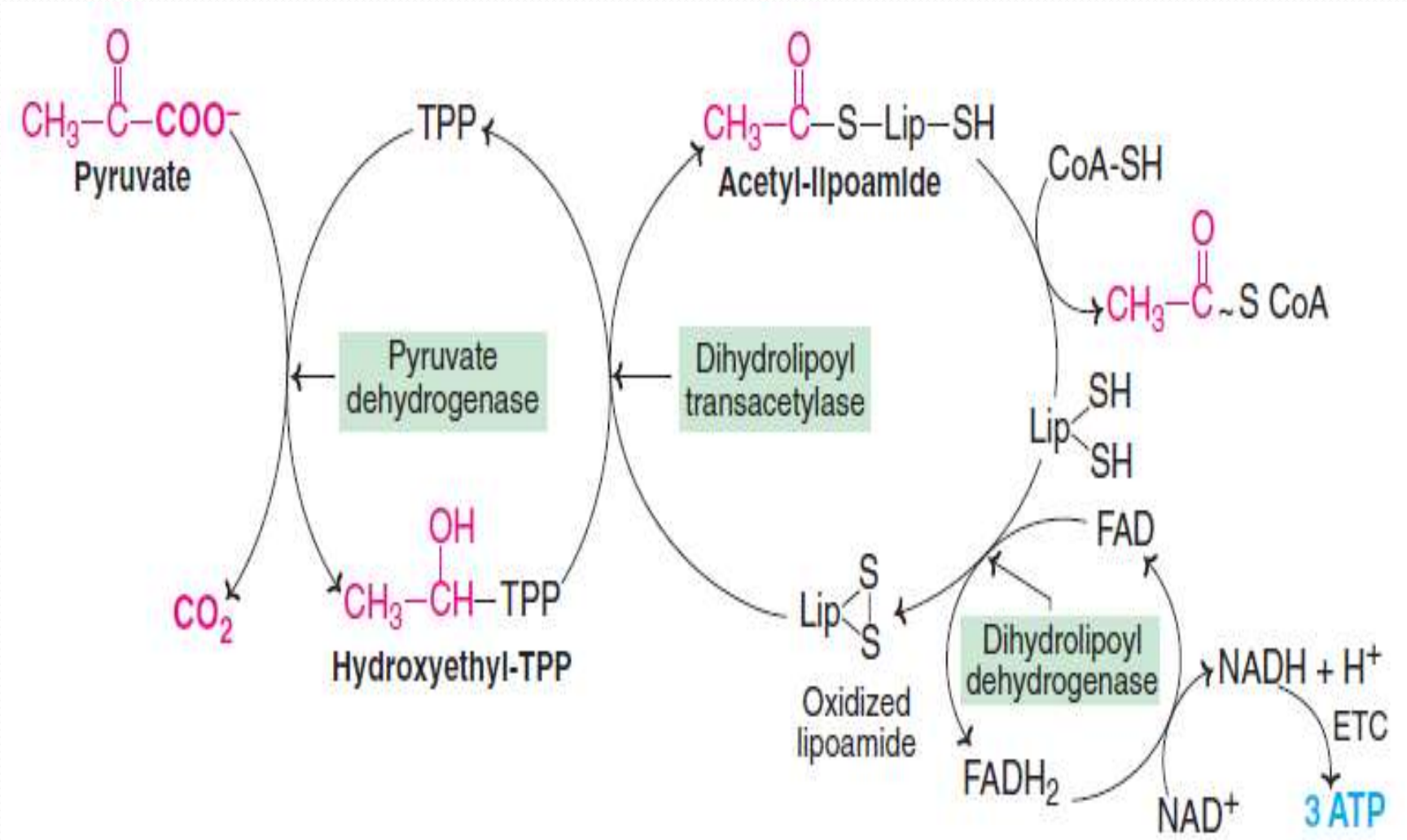
- Pyruvate produced via glycolysis in the cytosol moves to the mitochondria where it undergoes an irreversible oxidative decarboxylation to acetyl CoA.

- The reaction is catalyzed by the enzyme ***pyruvate dehydrogenase complex*** (PDH), which is a multi-enzyme complex found only in the mitochondria.

- This reaction is the connecting link between glycolysis and TCA cycle.

- High activities of PDH are found in cardiac muscle and kidney.

- PDH complex consists of three enzymes (dihydrolipoyltransacetylase, pyruvate dehydrogenase and dihydrolipoyl dehydrogenase) and requires five coenzymes, namely—TPP, lipoamide, FAD, coenzyme A and NAD^+ as shown below.



The mechanism of action of pyruvate dehydrogenase complex

- The intermediates of the PDH-catalyzed reaction are not free but bound to the enzyme complex.

- PDH is similar to α-ketoglutarate dehydrogenase complex of citric acid cycle which catalyzes the oxidative decarboxylation of α-ketoglutarate to succinyl CoA.

- Both enzymes are inhibited by arsenite and mecuric ion, which bind to thiol (-SH) groups of lipoic acid in lipoamide and makes it unavailable to serve as cofactor.

- PDH is regulated by end product (acetyl CoA, NADH) inhibition.

- It is also regulated by phosphorylation and dephosphorylation.

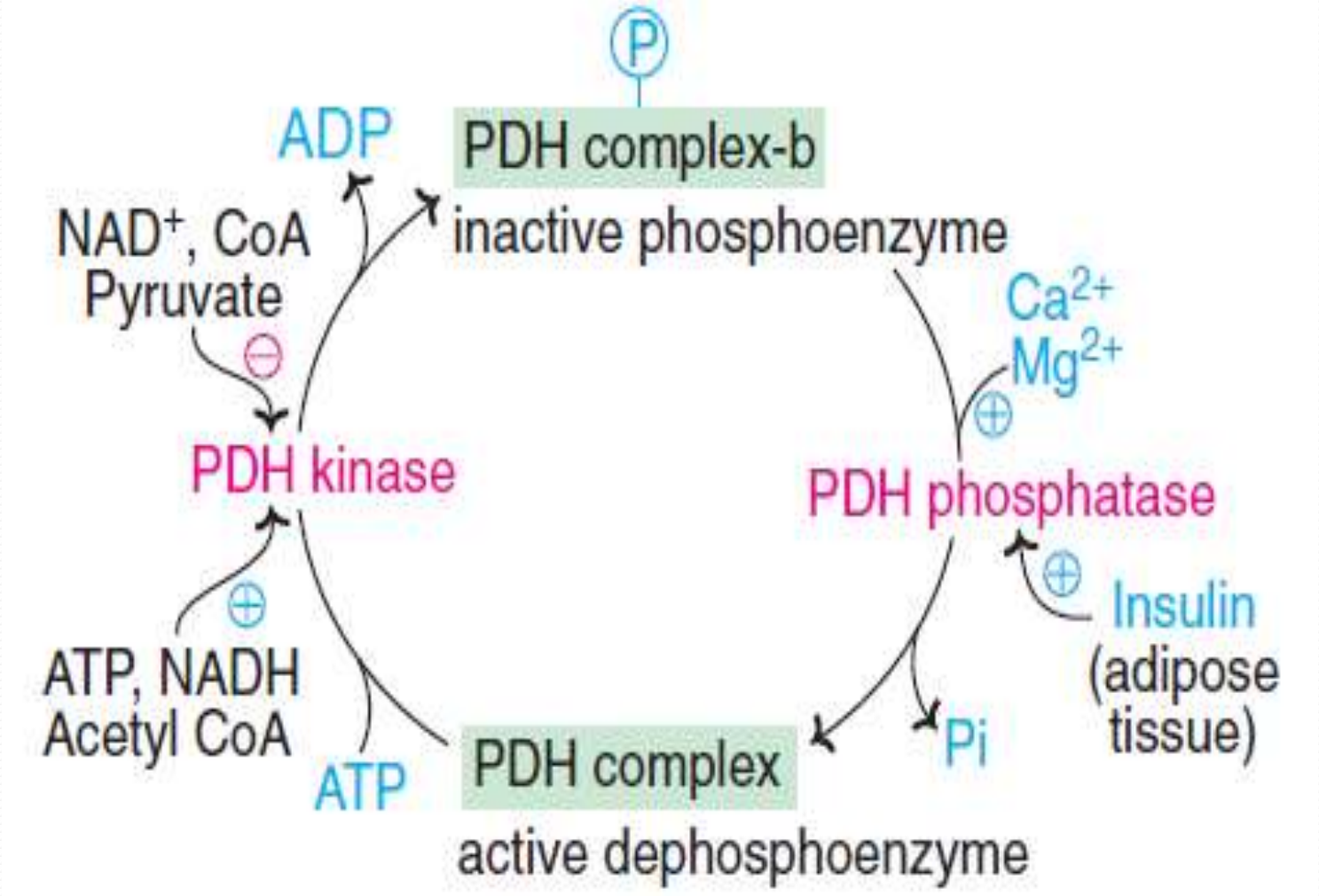
- PDH is active as a dephosphoenzyme while it is inactive as a phosphoenzyme.

- PDH phosphatase activity is promoted by Ca^{2+}, Mg^{+} and insulin (in adipose tissue).

- Interestingly, calcium released during muscle contraction stimulates PDH (by increasing phosphatase activity) for energy production.

- PDH kinase (responsible to form inactive PDH) is promoted by ATP, NADH and acetyl CoA, while it is inhibited by NAD^{+}, CoA and pyruvate.

- The net result is that in the presence of high energy signals (ATP, NADH), the PDH is turned off.



Regulation of pyruvate dihydrogenase (PDH) complex

ASSIGNMENT

Write on the following:

- ✓ Lactic acidosis
- ✓ The role of fructose 2,6-bisphosphate in glycolysis
- ✓ Pasteur effect
- ✓ Crabtree effect
- ✓ Glycolysis and cancer
- ✓ Glycolysis and dental caries

CITRIC ACID CYCLE

- The citric acid cycle (Krebs cycle or tricarboxylic acid—TCA cycle) is the most important metabolic pathway for the energy supply to the body.
- The cycle was proposed by Hans Adolf Krebs in 1937, and named in his honour (Nobel Prize for Physiology and Medicine in 1953).
- About 65-70% of the ATP is synthesized in the TCA cycle.
- It is the final common oxidative pathway for carbohydrates, fats and amino acids.
- It essentially involves the oxidation of acetyl CoA to CO_2 and H_2O.
- This cycle operates only aerobically and utilizes about two-third of the total oxygen consumed by the body.

- This cycle not only supplies energy but also provides many intermediates required for the synthesis of amino acids, glucose, heme etc.

- Krebs cycle is the most important central pathway connecting almost all the individual metabolic pathways (either directly or indirectly).

- At the outset of the cycle, tricarboxylic acids (citrate, cisaconitate and isocitrate) participate, hence, the name TCA cycle.

- The enzymes of TCA cycle are located in the mitochondrial matrix, in close proximity to the electron transport chain.

- This enables the synthesis of ATP by oxidative phosphorylation without any hindrance.

REACTIONS OF CITRIC ACID CYCLE

1. **Formation of Citrate:** The first reaction of the cycle is the condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by **citrate synthase**.

2. **Formation of Isocitrate via cis-Aconitate:** The enzyme **aconitase** (more formally, aconitate hydratase) catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of the tricarboxylic acid cis-aconitate, which normally does not dissociate from the active site.

3. **Oxidation of Isocitrate to α-Ketoglutarate:** **Isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.

   The formation of NADH and the liberation of CO_2 occur at this stage.

   There are two forms of isocitrate dehydrogenase in all cells: one requires NAD^+ as electron acceptor and the other requires $NADP^+$.

4. **Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2:** α-ketoglutarate is converted to succinyl-CoA and CO_2 by the action of the **α-ketoglutarate dehydrogenase** complex.

   This enzyme is similar to PDH, and also requires five coenzymes.

5. **Conversion of Succinyl-CoA to Succinate:** **Succinyl-CoA synthetase** or succinate thiokinase catalyzes the conversion of succinyl-CoA to succinate.

   This is a substrate level phosphorylation, as GTP is formed.

   GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

6. **Oxidation of Succinate to Fumarate:** The succinate formed from succinyl-CoA is oxidized to fumarate by the flavoprotein **succinate dehydrogenase**.

   This reaction results in the production of $FADH_2$ and not NADH.

7. **Hydration of Fumarate to Malate:** The reversible hydration of fumarate to L-malate is catalyzed by **fumarase** (formally, fumarate hydratase).

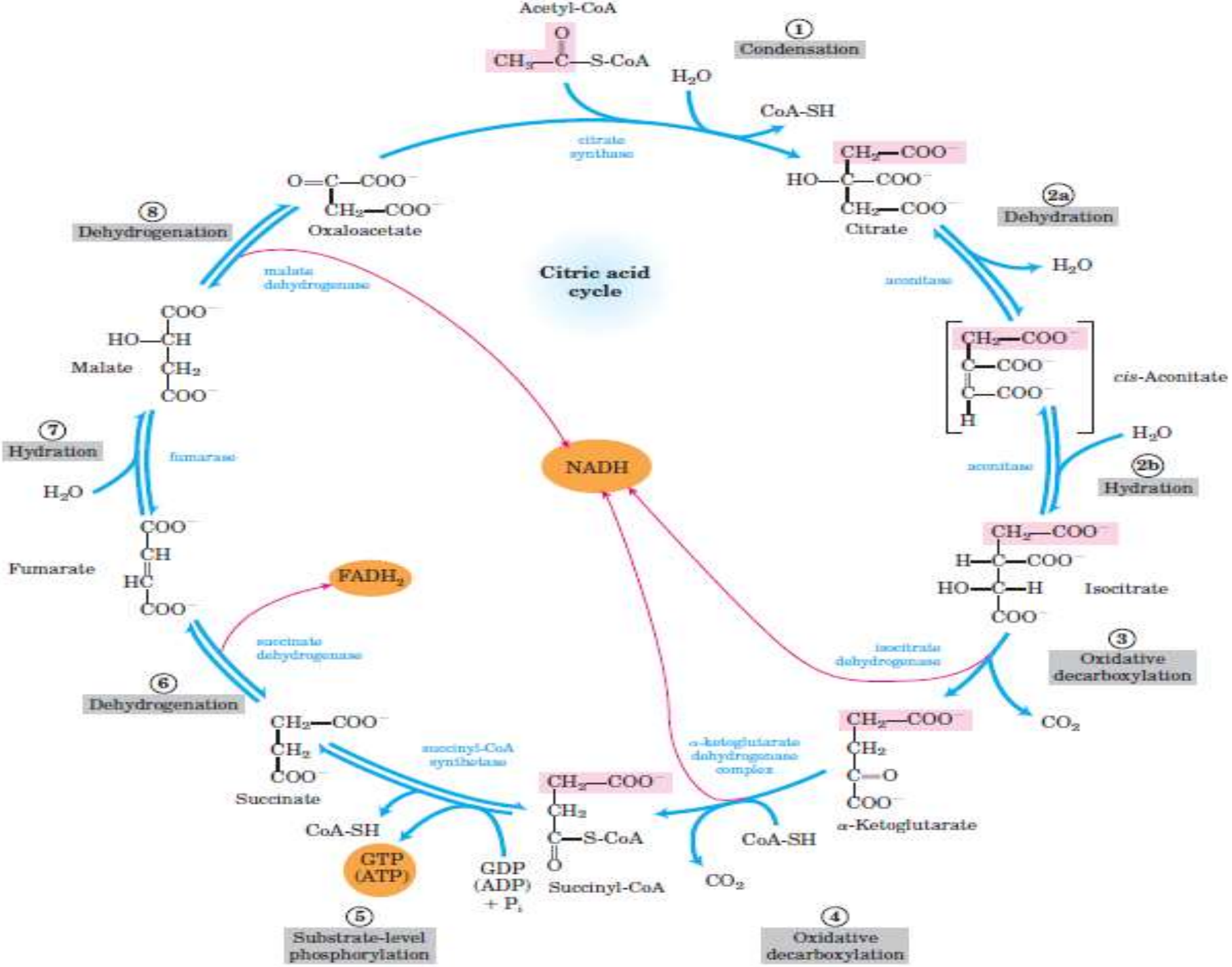
8. **Oxidation of Malate to Oxaloacetate:** In the last reaction of the citric acid cycle, NAD-linked **malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate.

The final synthesis of NADH occurs at this stage.

Oxaloacetate is regenerated and can combine with another molecule of acetyl CoA, and continue the cycle.

Summary of citric acid cycle:

$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + Pi + 2H_2O \longrightarrow 2CO_2 + 3NADH + 3H^+ + FADH_2 + GTP + CoA$$


Citric acid cycle
Acetyl-CoA
CH3—C—S-CoA
O
H2O
CoA-SH
1
Condensation
citrate synthase
Oxaloacetate
Citrate
2a
Dehydration
aconitase
H2O
cis-Aconitate
aconitase
H2O
2b
Hydration
Isocitrate
3
Oxidative decarboxylation
isocitrate dehydrogenase
CO2
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
CoA-SH
CO2
Succinyl-CoA
4
Oxidative decarboxylation
succinyl-CoA synthetase
CoA-SH
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
Fumarate
7
Hydration
fumarase
H2O
Malate
8
Dehydrogenation
malate dehydrogenase
NADH

ENERGETICS OF TCA CYCLE

- During the process of oxidation of acetyl CoA via citric acid cycle, 4 reducing equivalents (3 NADH and 1 $FADH_2$) are produced.

- Oxidation of 3 NADH by the electron transport chain coupled with oxidative phosphorylation results in the synthesis of 9 ATP, and $FADH_2$ leads to the formation of 2 ATP.

- There is also one substrate level phosphorylation.

- Thus, a total of 12 ATP (10 as per recent evidence) are produced from one acetyl CoA, and 24 ATP from two (2 molecules of acetyl CoA from 1 molecule of glucose).

Summary of the complete oxidation of glucose to CO_2 and H_2O via glycolysis and TCA cycle:

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38Pi \longrightarrow 6CO_2 + 6H_2O + 38ATP$$

Generation of ATP in glucose metabolism

Pathway	Enzyme (method of ATP synthesis)	Number of ATP synthesized
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6(5)
	Phosphoglycerate kinase (substrate level phosphorylation)	2
	Pyruvate kinase (substrate level phosphorylation)	2
	Two ATP are consumed in the reactions catalysed by hexokinase and phosphofructokinase	–2
	Net ATP synthesis in glycolysis in aerobic condition	***8(7)***
	Pyruvate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6(5)
Citric acid cycle	Isocitrate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6(5)
	α-Ketoglutarate dehydrogenase	6(5)
	Succinate thiokinase (substrate level phosphorylation)	2
	Succinate dehydrogenase (2 $FADH_2$, ETC, oxidative phosphorylation)	4(3)
	Malate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	6(5)
	Total ATP per mole of glucose under aerobic condition	***38(32)***
	Total ATP per mole of glucose under anaerobic condition	2

Note : Values in brackets in red colour represent ATP synthesized as per the new P:O ratios of 2.5 for NADH and 1.5 for $FADH_2$.

Source: Satyanarayana & Chakrapani, 2013

VITAMINS IN TCA CYCLE

Four B-complex vitamins are essential for Krebs cycle, and thus energy generation:

1. Thiamine (as TPP) is a coenzyme for α-ketoglutarate dehydrogenase.

2. Riboflavin (as FAD) is a coenzyme for succinate dehydrogenase.

3. Niacin (as NAD^+) is an electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase.

4. Pantothenic acid (as coenzyme A) is attached to active carboxylic acid residues i.e. acetyl CoA, succinyl CoA.

INHIBITORS OF TCA CYCLE

- The important enzymes of TCA cycle inhibited by the respective inhibitors are listed in the table below.

Enzyme	Inhibitor
Aconitase	Fluoroacetate (non-competitive)
α-Ketoglutarate dehydrogenase	Arsenite (non-competitive)
Succinate dehydrogenase	Malonate (competitive)

- Fluoroacetate, which is ordinarily a harmless substrate, is activated to fluoroacetyl CoA which then condenses with oxaloacetate to form the toxic fluorocitrate, which inhibits TCA cycle (enzyme-aconitase).

- This is a suicide reaction committed by the cell, and thus, fluoroacetate is regarded as a ***suicide substrate***

REGULATION OF TCA CYCLE

- The energy demands of the cells are crucial in controlling the rate of the TCA cycle.
- The regulation is brought about either by enzymes or the levels of ADP.
- The three enzymes catalyzing the irreversible reactions, namely citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, regulate the TCA cycle.
- **Citrate synthase** is inhibited by ATP, NADH, acetyl CoA and succinyl CoA.
- **Isocitrate dehydrogenase** is activated by ADP, and inhibited by ATP and NADH.
- **α-Ketoglutarate dehydrogenase** is inhibited by succinyl CoA and NADH.

- The **availability of ADP** is very important for the TCA cycle to proceed.

- This is because unless sufficient levels of ADP are available, oxidation (coupled with phosphorylation of ADP to ATP) of NADH and $FADH_2$ through electron transport chain stops.

- The accumulation of NADH and $FADH_2$ will lead to inhibition of the enzymes (as stated above) and also limit the supply of NAD^+ and FAD, which are essential for TCA cycle to proceed.

TCA CYCLE AS AN AMPHIBOLIC PATHWAY

- The TCA cycle provides various intermediates for the synthesis of many compounds needed by the body.
- It is both catabolic and anabolic in nature, hence regarded as amphibolic.
- TCA cycle is actively involved in gluconeogenesis, transamination and deamination.
- **Oxaloacetate and α-ketoglutarate**, respectively, serve as precursors for the synthesis of aspartate and glutamate, which in turn, are required for the synthesis of other non-essential amino acids, purines and pyrimidines.
- **Succinyl CoA** is used for the synthesis of porphyrins and heme.
- Mitochondrial **citrate** is transported to the cytosol, where it is cleaved to provide acetyl CoA for the biosynthesis of fatty acids, sterols etc.

ANAPLEROSIS

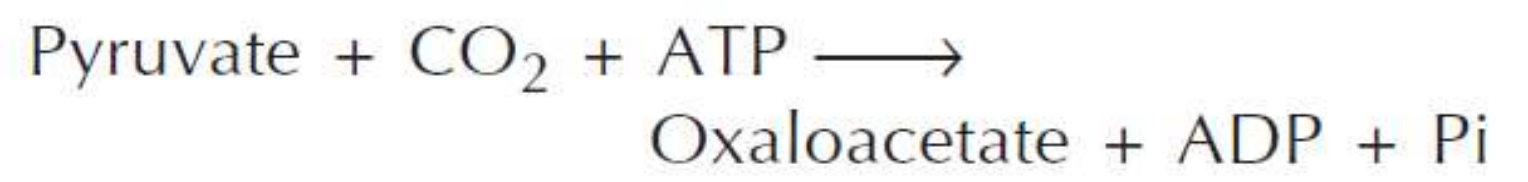
- The offshoot synthetic reactions of TCA cycle deplete its intermediates.

- The cycle will cease to operate unless the intermediates drawn out are replenished.

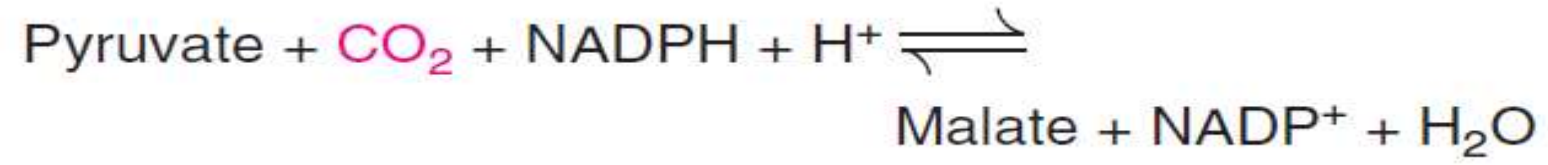
- The reactions concerned to replenish or to fill up the intermediates of TCA cycle are called anaplerosis (Greek: fill up) or **anaplerotic reactions**.

- The salient features of important anaplerotic reactions are described below

1. Pyruvate carboxylase catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate.

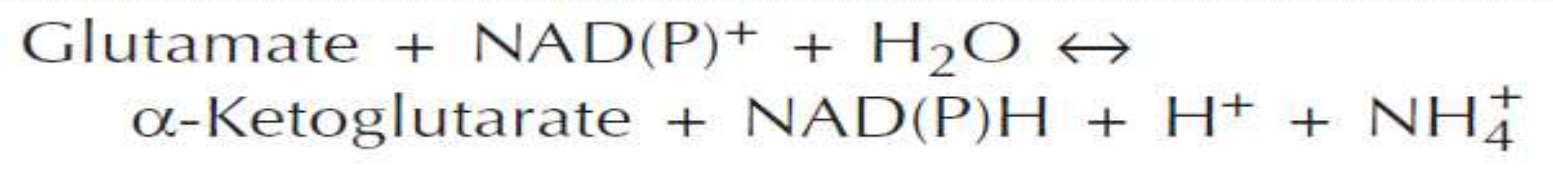


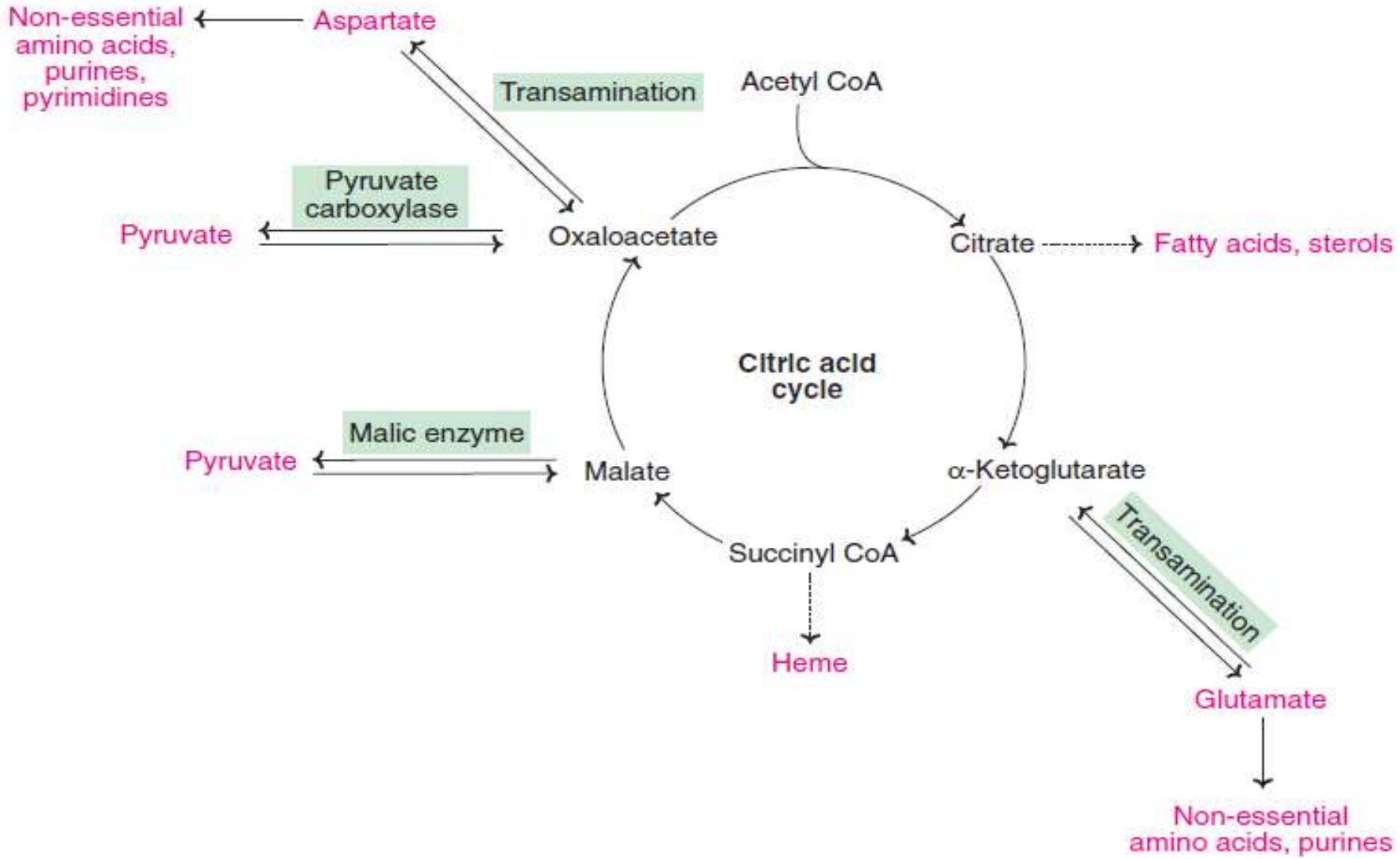
2. Pyruvate is converted to malate by $NADP^+$ dependent malate dehydrogenase (malic enzyme).



3. The formation of α-ketoglutarate and oxaloacetate occurs by transamination.

4. α-Ketoglutarate can also be synthesized from glutamate by glutamate dehydrogenase action.





Major synthetic and anaplerotic pathways of the intermediates of citric acid cycle

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

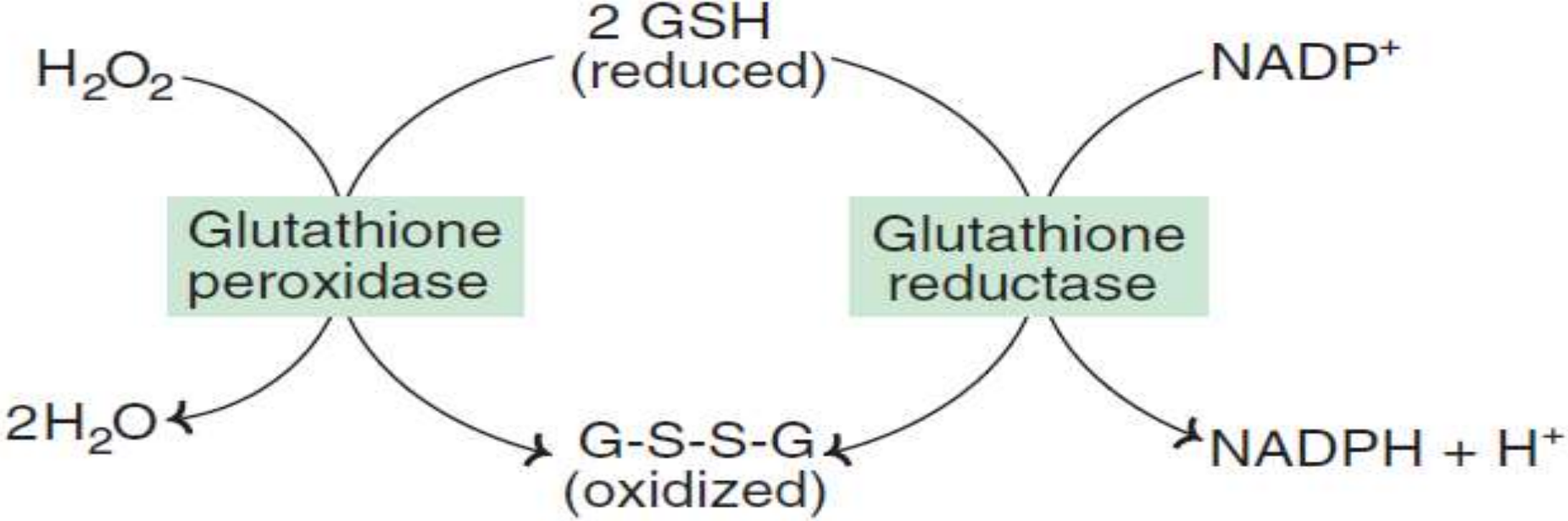
1. NADPH is required for the reductive biosynthesis of fatty acids and steroids, hence HMP shunt is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

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.

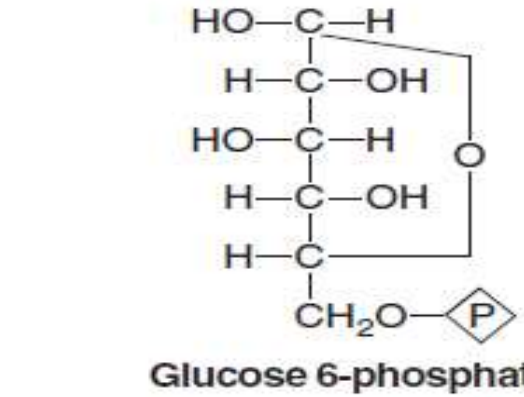
7. **Transaldolase** brings about the transfer of a 3-carbon fragment (active dihydroxyacetone) from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate to give fructose 6-phosphate and four carbon erythrose 4-phosphate.

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.

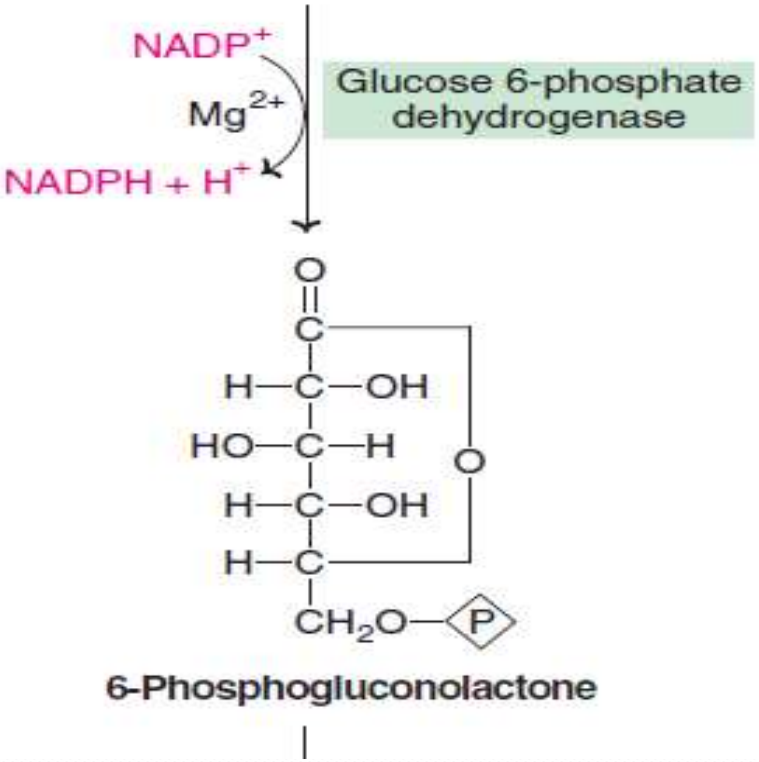
Fructose 6-phosphate and glyceraldehyde 3-phosphate can be further catabolized through glycolysis and citric acid cycle, glucose may also be synthesized from these two compounds.

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.

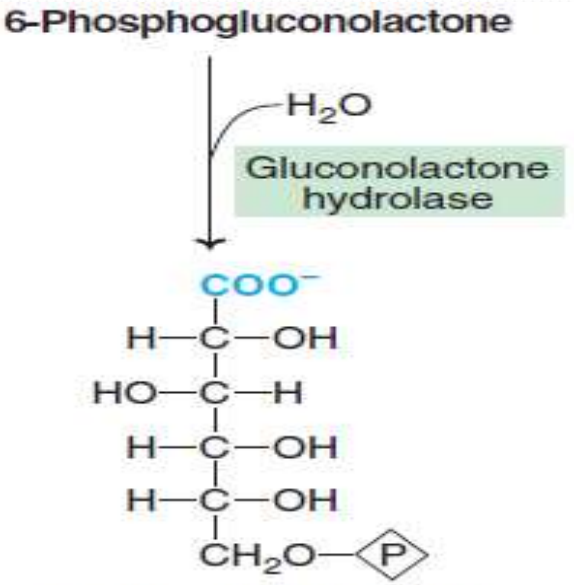
$$6\text{ Glucose 6-phosphate} + 12NADP^+ + 6H_2O \longrightarrow 6CO_2 + 12NADPH + 12H^+ + 5\text{Glucose 6-phosphate}$$



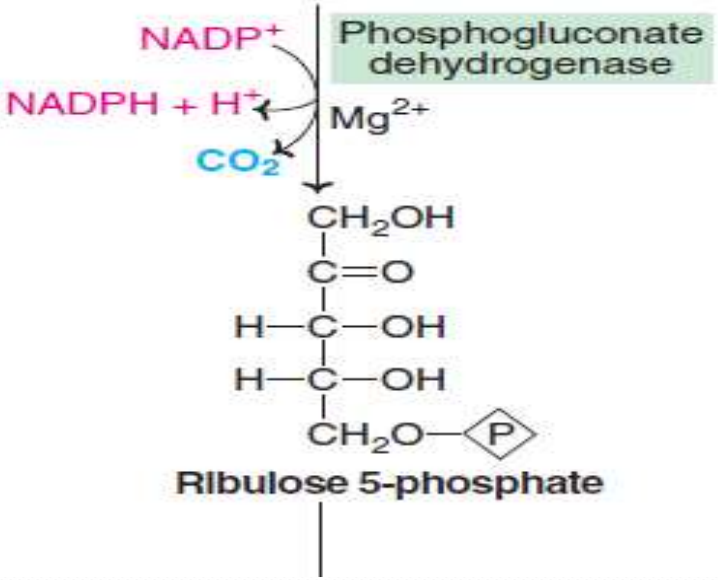
Glucose 6-phosphate



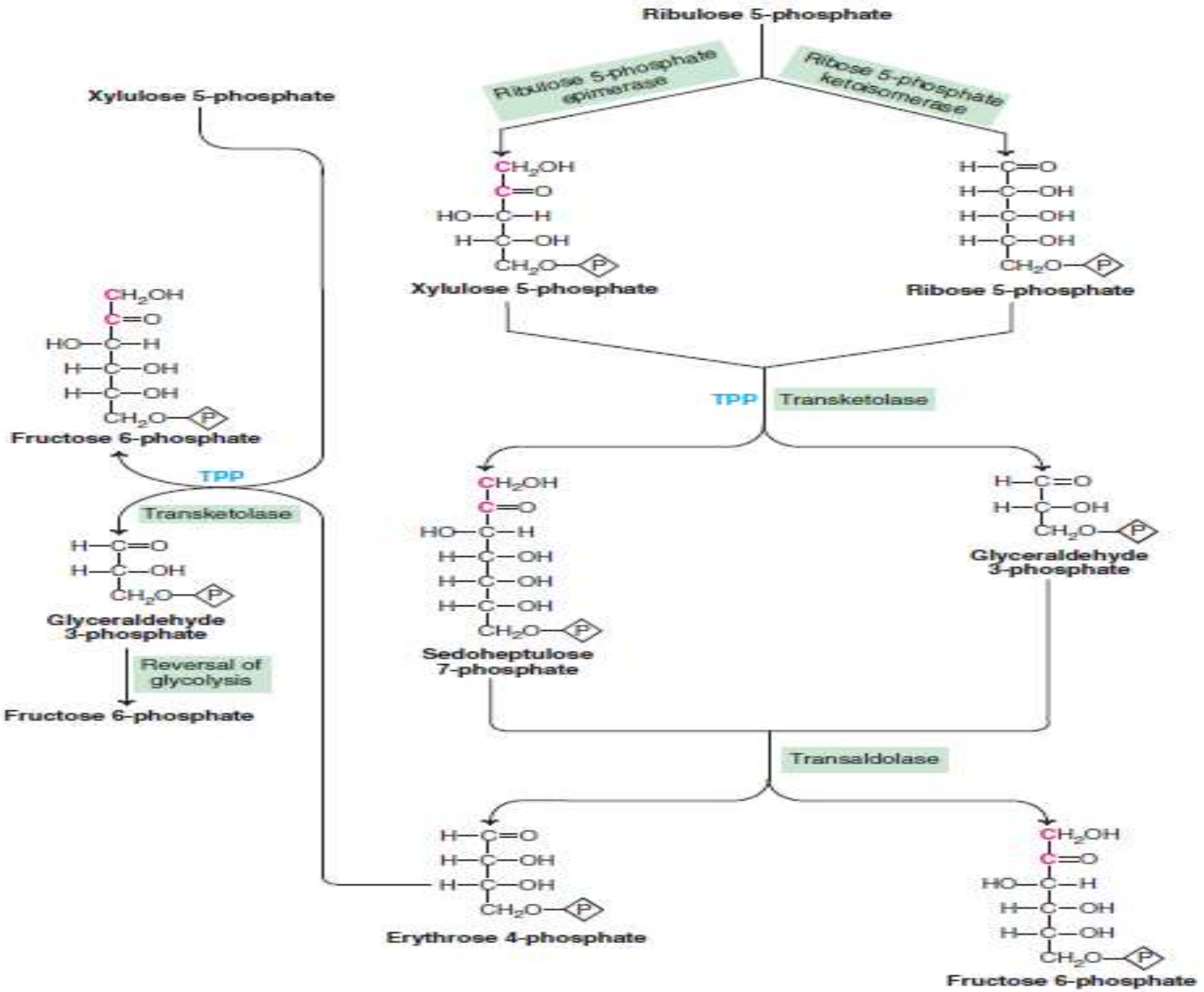
6-Phosphogluconolactone



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.

URONIC ACID PATHWAY

- The uronic acid pathway is an alternative oxidative pathway for glucose.
- It is also known as ***glucuronic acid pathway***.
- The pathway is concerned with the synthesis of glucuronic acid, pentoses and vitamins, ascorbic acid (except in primates and guinea pigs).
- Dietary xylulose enters the uronic acid pathway through which it can participate in other metabolisms.

REACTIONS OF URONIC ACID PATHWAY

Formation and importance of UDPglucuronate

- Glucose 6-phosphate is first converted to glucose 1-phosphate by **phosphoglucomutase**.
- UDP-glucose is then synthesized from glucose 1-phosphate by **UDP-glucose pyrophosphorylase**.
- **UDP-glucose dehydrogenase** oxidizes UDP-glucose to UDP-glucuronate.
- UDP-glucuronate is the metabolically active form of glucuronate, which is utilized for conjugation with many substances like bilirubin, steroid hormones and certain drugs.
- Several insoluble compounds such as drugs, are detoxified by being converted to soluble compounds through conjugation.
- UDP-glucuronate is also required for the synthesis of glycosaminoglycans and proteoglycans.

Conversion of UDP-glucuronate to L-gulonate

- UDP-glucuronate loses its UDP moiety in a hydrolytic reaction catalyzed by **glucuronidase** to release D-glucuronate.

- D-glucuronate is then reduced to L-gulonate by an NADPH-dependent reaction.

Synthesis of ascorbic acid in some animals

- L-Gulonate is the precursor for the synthesis of ascorbic acid (vitamin C) in many animals.

- The enzyme **L-gulonolactone oxidase**, which converts gulonate to ascorbic acid, is absent in man, other primates and guinea pigs.

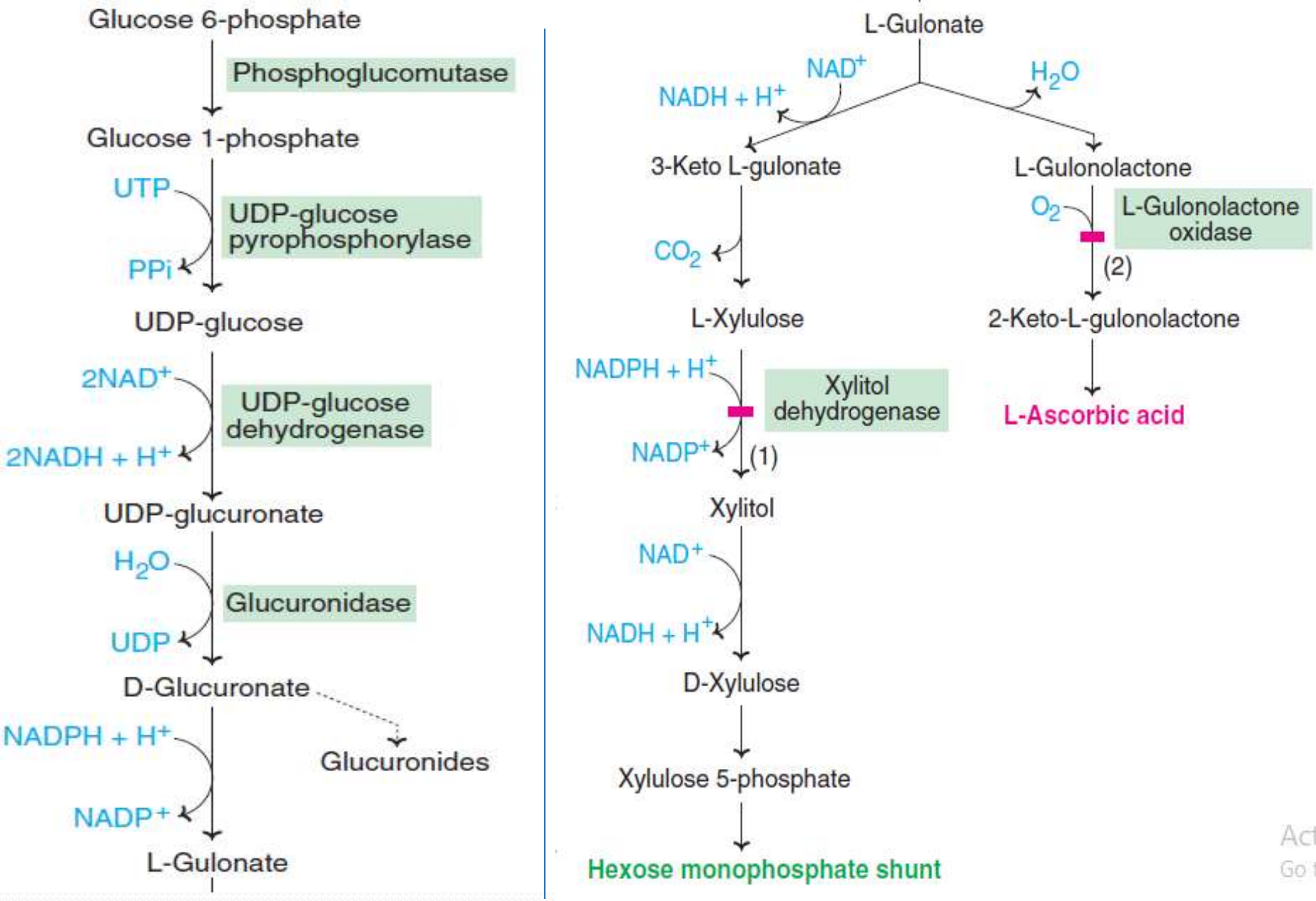
- Hence, vitamin C has to be supplemented in the diet for these animals.

Oxidation of L-gulonate

- L-Gulonate is oxidized to 3-ketogulonate and then decarboxylated to L-xylulose.

- L-Xylulose is converted to D-xylulose via xylitol by reduction (NADPH-dependent) followed by oxidation (NAD^+-dependent) reaction.

- This is necessary since the D-xylulose (and not L-form)—after getting phosphorylated—can enter the hexose monophosphate shunt, for further metabolism.



Uronic acid pathway

(1) Blocked in essential pentosuria (2) Enzyme absent in primates (including man) and guinea pigs

Source: Satyanarayana & Chakrapani, 2013

EFFECT OF DRUGS ON URONIC ACID PATHWAY

- Administration of drugs (barbital, chlorobutanol etc.) significantly increases the uronic acid pathway to achieve more synthesis of glucuronate from glucose.

- Certain drugs (aminopyrine, antipyrine) were found to enhance the synthesis of ascorbic acid in rats.

GLYOXYLATE CYCLE

- Animals (including man) cannot carry out the net synthesis of carbohydrate from fat.
- However, plants and many microorganisms can convert fat into carbohydrates through the ***glyoxylate cycle***.
- The pathway is very significant in germinating seeds where the stored triacylglycerol (fat) is converted to sugars to meet the energy needs.
- The glyoxylate cycle occurs in the ***glyoxysome***.
- Glyoxysomes are specialized cellular organelles, where fatty acid oxidation is also operative.

REACTIONS OF THE GLYOXYLATE CYCLE

- The glyoxylate cycle is regarded as an anabolic variant of the citric acid cycle.

1. Acetyl CoA produced from fatty acid oxidation condenses with oxaloacetate to give citrate – catalyzed by **citrate synthase**.

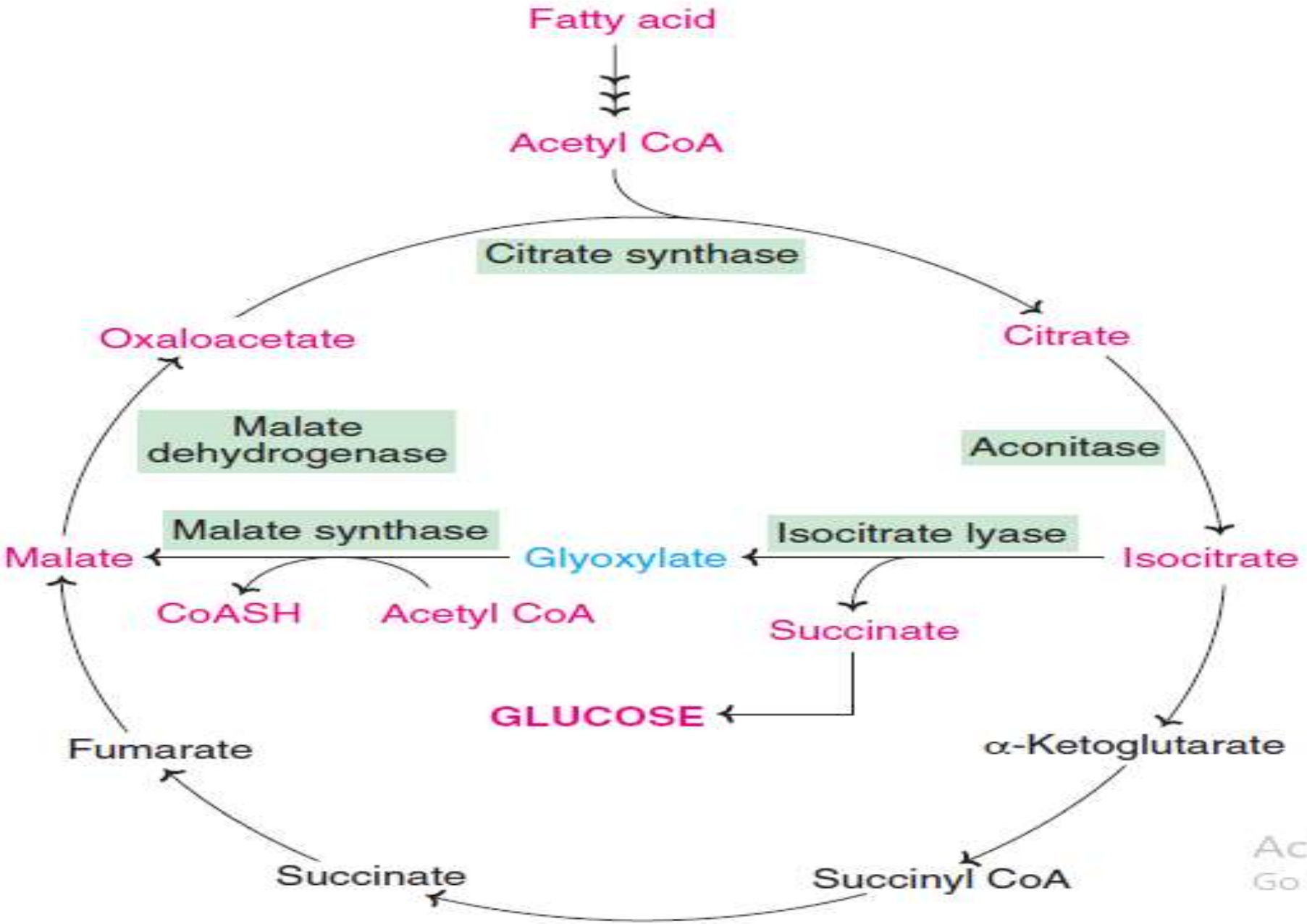
2. Citrate is then converted to isocitrate by **aconitase**.

3. At this stage, isocitrate bypasses the citric acid cycle and is cleaved by **isocitrate lyase** to succinate and glyoxylate.

   The succinate formed is converted to oxaloacetate and then finally to glucose involving the reactions of gluconeogenesis.

4. Another molecule of acetyl CoA combines with glyoxylate to form malate - catalyzed by **malate synthase**.

   The malate so formed enters the citric acid cycle.



The glyoxylate cycle (enzymes represented in green shade)

Source: Satyanarayana & Chakrapani, 2013

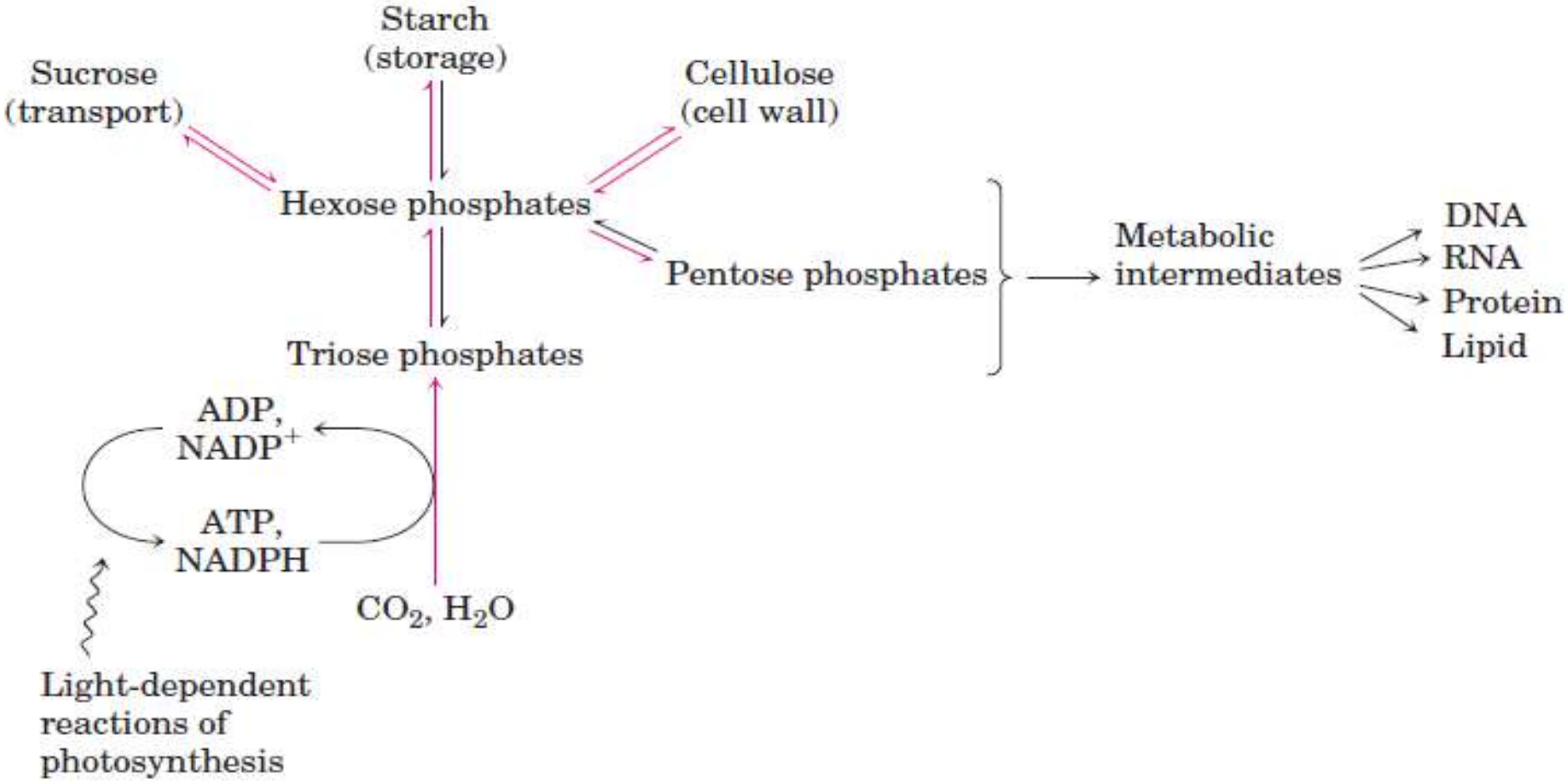
CALVIN CYCLE

- The synthesis of carbohydrates in animals always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO_2.
- However, plants and photosynthetic microorganisms can synthesize carbohydrates from CO_2 and water, by reducing CO_2, using the energy (ATP) and reducing power (NADPH) that are generated by the light-dependent reactions of photosynthesis as illustrated below.
- Green plants contain in their chloroplasts, unique enzymatic machinery that catalyzes the conversion of CO_2 to simple (reduced) organic compounds, a process called **CO_2 assimilation**.
- This process is also been called **CO_2 fixation** or **carbon fixation**.
- Carbon dioxide is assimilated via a cyclic pathway called the **Calvin cycle** or **photosynthetic carbon reduction cycle**.

- The terms **CO_2 fixation** or **carbon fixation** are also used for the specific reaction in which CO_2 is incorporated (fixed) into a three-carbon organic compound, the triose phosphate, **3-phosphoglycerate,** which is eventually reduced to **glaceraldehyde 3-phosphate**.

- This simple product of photosynthesis is the precursor of more complex biomolecules, including sugars, polysaccharides, and the metabolites derived from them, all of which are synthesized by metabolic pathways similar to those of animal tissues.

- The Calvin cycle was elucidated in the early 1950s by Melvin Calvin, Andrew Benson, and James A. Bassham.



Assimilation of CO_2 into the biomass in plants

Source: Nelson and Cox, 2016

REACTIONS OF CALVIN CYCLE

- CO_2 assimilation takes place in the chloroplast, and in three stages.

Stage 1: Fixation of CO_2 into 3-Phosphoglycerate

- The assimilation of CO_2 into biomolecules is the **carbon-fixation reaction**, in which **ribulose 1,5-bisphosphate carboxylase/oxygenase (**shortened to **rubisco)** catalyzes the condensation of CO_2 with a five-carbon acceptor, **ribulose 1,5-bisphosphate**, to form two molecules of **3-phosphoglycerate**.

Stage 2: Conversion of 3-Phosphoglycerate to Glyceraldehyde 3-Phosphate

- The 3-phosphoglycerate formed in stage 1 is converted to glyceraldehyde 3-phosphate in two steps that are essentially the reversal of the corresponding steps in glycolysis, with one exception: the nucleotide cofactor for the reduction of 1,3-bisphosphoglycerate is NADPH rather than NADH.

- The chloroplast stroma contains all the glycolytic enzymes except phosphoglycerate mutase.

- In the first step of stage 2, the stromal **3-phosphoglycerate kinase** catalyzes the transfer of a phosphoryl group from ATP to 3-phosphoglycerate, yielding 1,3-bisphosphoglycerate.

- Then NADPH donates electrons in a reduction catalyzed by the chloroplast-specific isozyme of **glyceraldehyde 3-phosphate dehydrogenase**, producing glyceraldehyde 3-phosphate and Pi.

- **Triose phosphate isomerase** then interconverts glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

- Overall, three molecules of CO_2 are fixed to three molecules of **ribulose 1,5-bisphosphate** to form six molecules of **glyceraldehyde 3-phosphate** (18 carbons) in equilibrium with dihydroxyacetone phosphate.

- Most of the triose phosphate thus produced is used to regenerate ribulose 1,5-bisphosphate.

- The rest is either converted to starch in the chloroplast and stored for later use or immediately exported to the cytosol and converted to sucrose for transport to growing regions of the plant.

- In developing leaves, a significant portion of the triose phosphate may be degraded by glycolysis to provide energy.

Stage 3: Regeneration of Ribulose 1,5-Bisphosphate from Triose Phosphates

- Five of the six molecules of triose phosphate (15 carbons) are used to regenerate three molecules of ribulose 1,5-bisphosphate (15 carbons), which is the starting material.

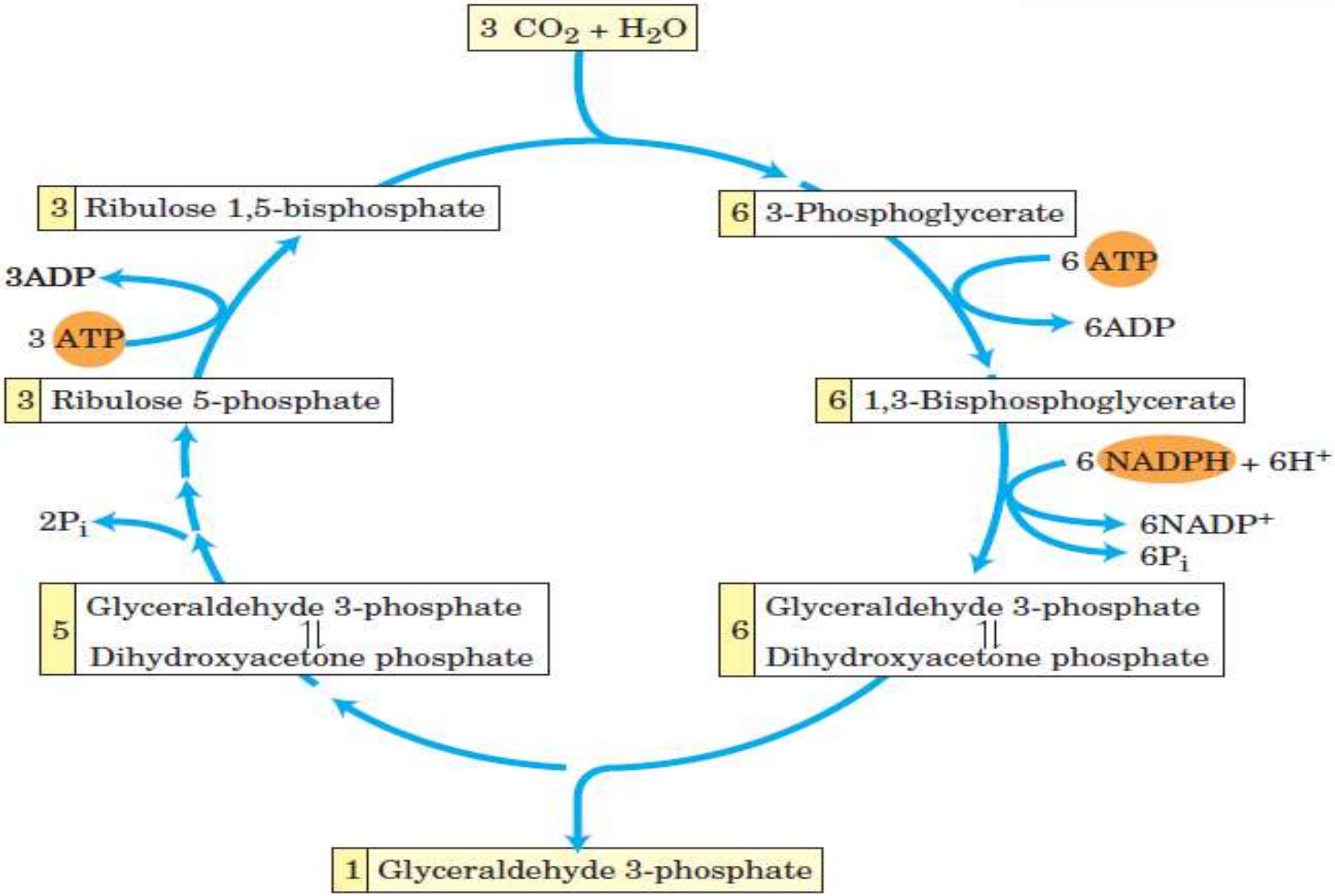
- The sixth molecule of triose phosphate, can be used to make hexoses for fuel and building materials.

- Thus, the overall process is cyclical, with the continuous conversion of CO_2 to triose and hexose phosphates.

- For every three CO_2 molecules fixed, one molecule of triose phosphate (glyceraldehyde 3-phosphate) is produced and nine ATP and six NADPH are consumed.

- Fructose 6-phosphate is a key intermediate in stage 3 of CO_2 assimilation, as it stands at a branch point, leading either to the regeneration of ribulose 1,5-bisphosphate or to the synthesis of starch.

- The pathway from hexose phosphate to pentose bisphosphate involves many of the same reactions used in animal cells for the conversion of pentose phosphates to hexose phosphates during the non-oxidative phase of the pentose phosphate pathway.



The Calvin Cycle

Source: Nelson and Cox, 2016

REGULATION OF THE CALVIN CYCLE

- As the catalyst for the first step of photosynthetic CO_2 assimilation, **rubisco** is a prime target for regulation.
- The enzyme is inactive until carbamoylated on the ε-amino group of Lys^{201}.
- Ribulose 1,5-bisphosphate inhibits carbamoylation by binding tightly to the active site and locking the enzyme in the "closed"conformation, in which Lys^{201} is inaccessible.
- **Rubisco activase** overcomes the inhibition by promoting ATP-dependent release of the ribulose 1,5-bisphosphate, exposing the Lys amino group to non-enzymatic carbamoylation by CO_2.
- This is followed by Mg^{2+} binding, which activates the rubisco.
- Rubisco activase in some species is activated by light through a redox mechanism.

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

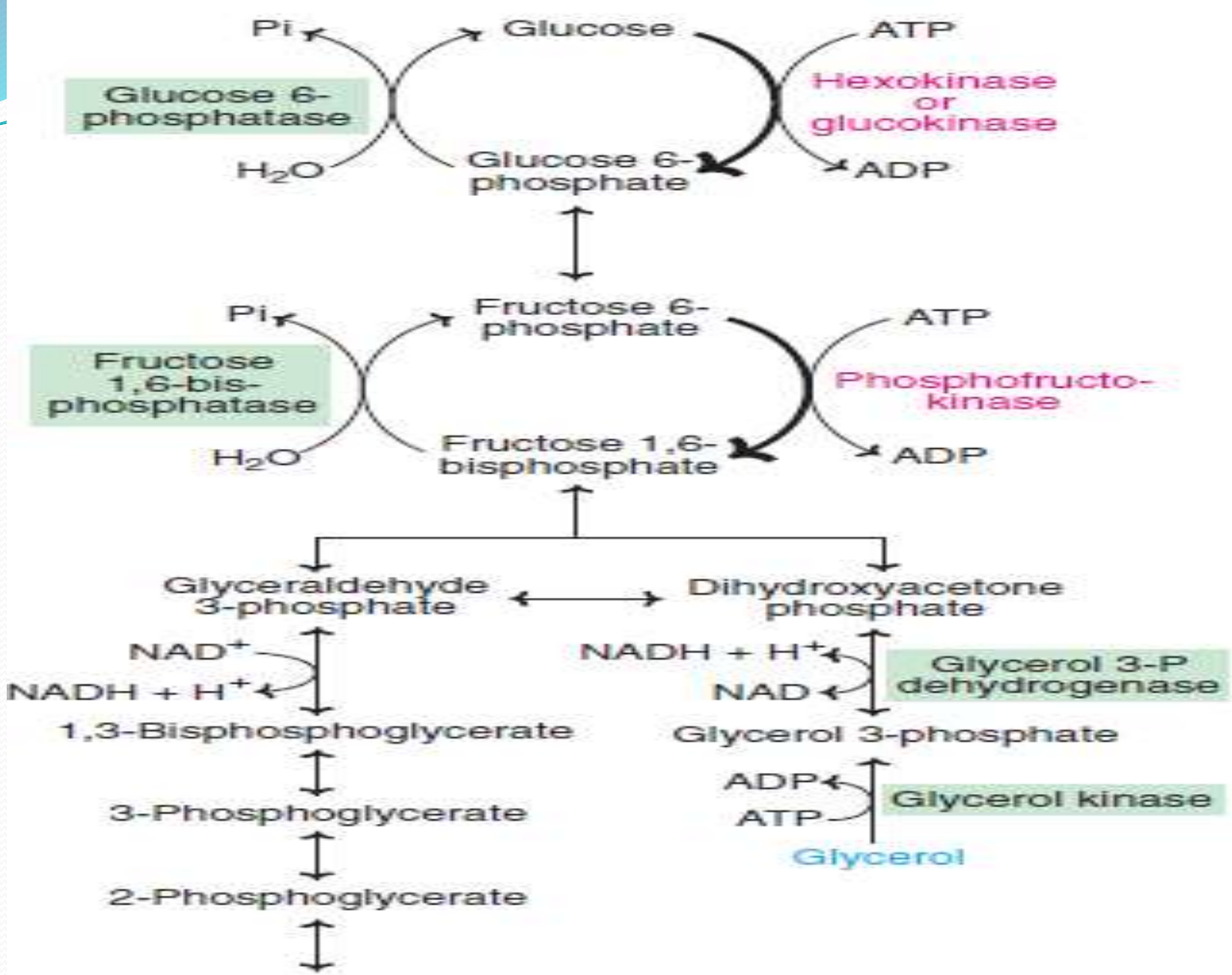
Glucose 6-phosphatase catalyses the conversion of glucose 6-phosphate to glucose.

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.

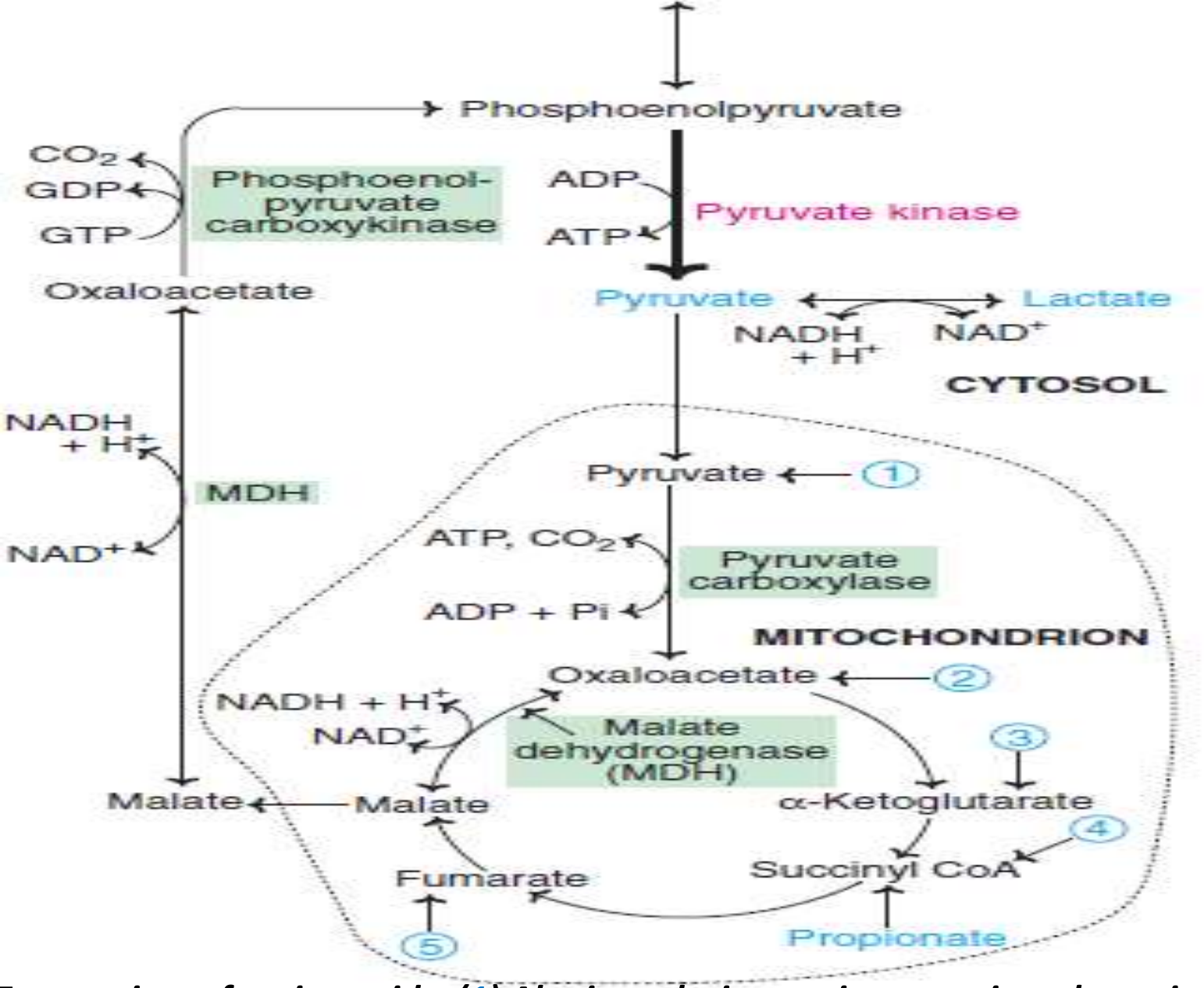
The overall summary of gluconeogenesis for the conversion of pyruvate to glucose is as follows:

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



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.

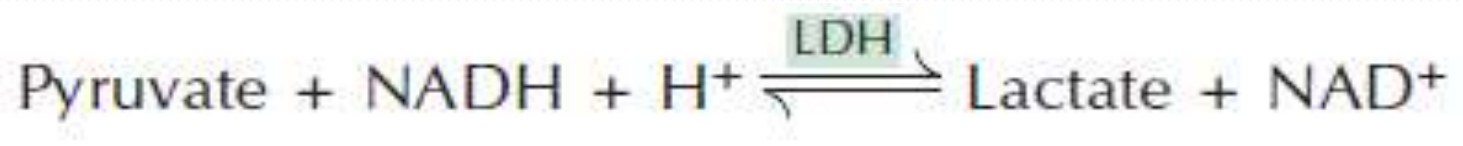
- **Propionyl CoA carboxylase** acts on propionyl CoA in the presence of ATP and biotin and converts it to methyl malonyl CoA.

- 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.

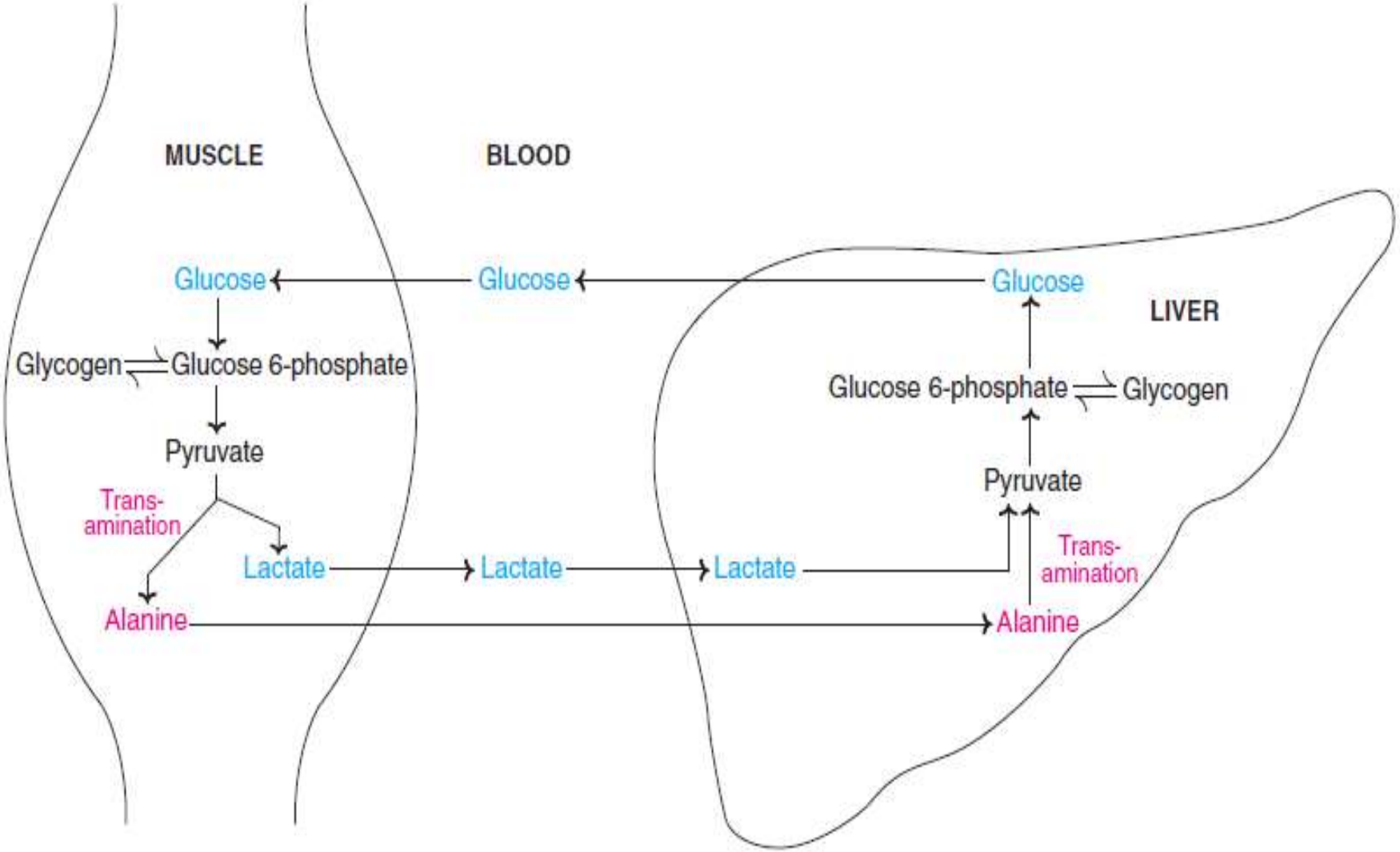
- Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of glucose due to the absence of the key enzymes of gluconeogenesis (glucose 6-phosphatase and fructose 1,6-bisphosphatase).

- 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.

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.

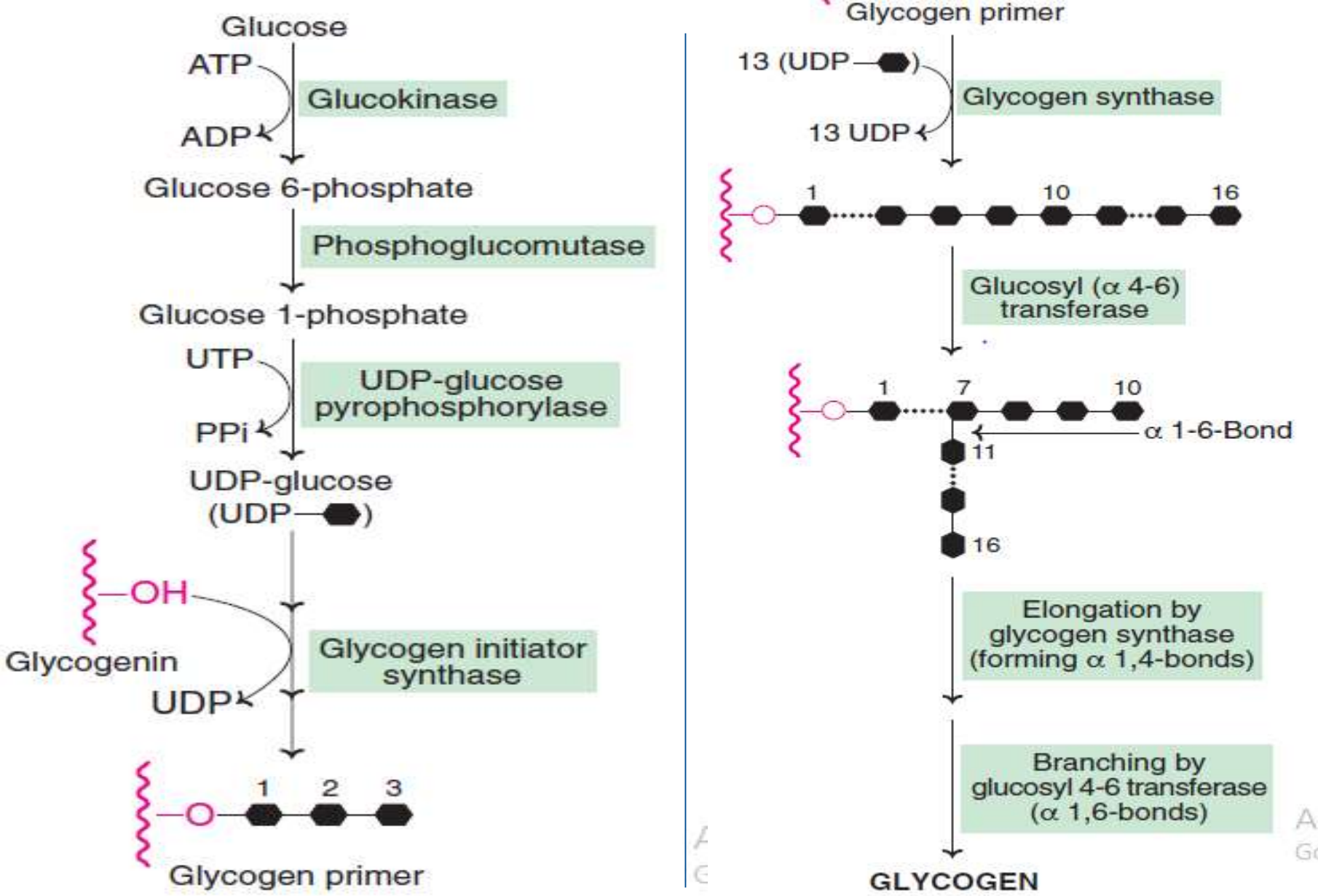
   This leads to the formation of a new non-reducing end, besides the existing one.

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

GLYCOGENOLYSIS

- Glycogenolysis is the degradation of stored glycogen in liver and muscle.
- The pathways for the synthesis and degradation of glycogen are not reversible.
- Hence, an independent set of enzymes present in the cytosol carry out glycogenolysis.
- Glycogen is degraded by breaking the α-1,4- and α-1,6-glycosidic bonds

REACTIONS OF GLYCOGENOLYSIS

1. Action of glycogen phosphorylase:

The α-1,4-glycosidic bonds (from the non-reducing ends) of glycogen are cleaved sequentially by the enzyme **glycogen phosphorylase** to yield glucose 1-phosphate.

This process—called phosphorolysis—continues until four glucose residues remain on either side of branching point (α-1,6-glycosidic link).

The glycogen so formed is known as ***limit dextrin*** which cannot be further degraded by phosphorylase.

Glycogen phosphorylase possesses a molecule of pyridoxal phosphate, covalently bound to the enzyme.

2. Action of debranching enzyme:

The branches of glycogen are cleaved by two enzyme activities present on a single polypeptide called **debranching enzyme** (formally called **oligo (α1⟶ 6) to (α1⟶ 4) glucan transferase**), hence it is a bifunctional enzyme.

A fragment of three or four glucose residues attached at a branch is removed and transferred to another chain.

Here, one α-1,4 bond is cleaved and the same α-1,4 bond is made, but at a different point.

Amylo α-1,6-glucosidase breaks the α-1,6 bond at the branch with a single glucose residue and releases a free glucose.

The remaining molecule of glycogen is again available for the action of phosphorylase and debranching enzyme to repeat the reactions stated in 1 and 2.

3. Formation of glucose 6-phosphate and glucose:

Through the combined action of glycogen phosphorylase and debranching enzyme, glucose 1-phosphate and free glucose in a ratio of 8:1 are produced.

Glucose 1-phosphate is converted to glucose 6-phosphate by **phosphoglucomutase**.

The fate of glucose 6-phosphate depends on the tissue.

The liver, kidney and intestine contain **glucose 6-phosphatase** that cleaves glucose 6-phosphate to glucose, but this enzyme is absent in muscle and brain, hence free glucose cannot be produced from glucose 6-phosphate in muscle and brain.

Therefore, liver is the major glycogen storage organ to provide glucose into the circulation to be utilized by various tissues.

- In the peripheral tissues, glucose 6-phosphate produced by glycogenolysis will be used for glycolysis.

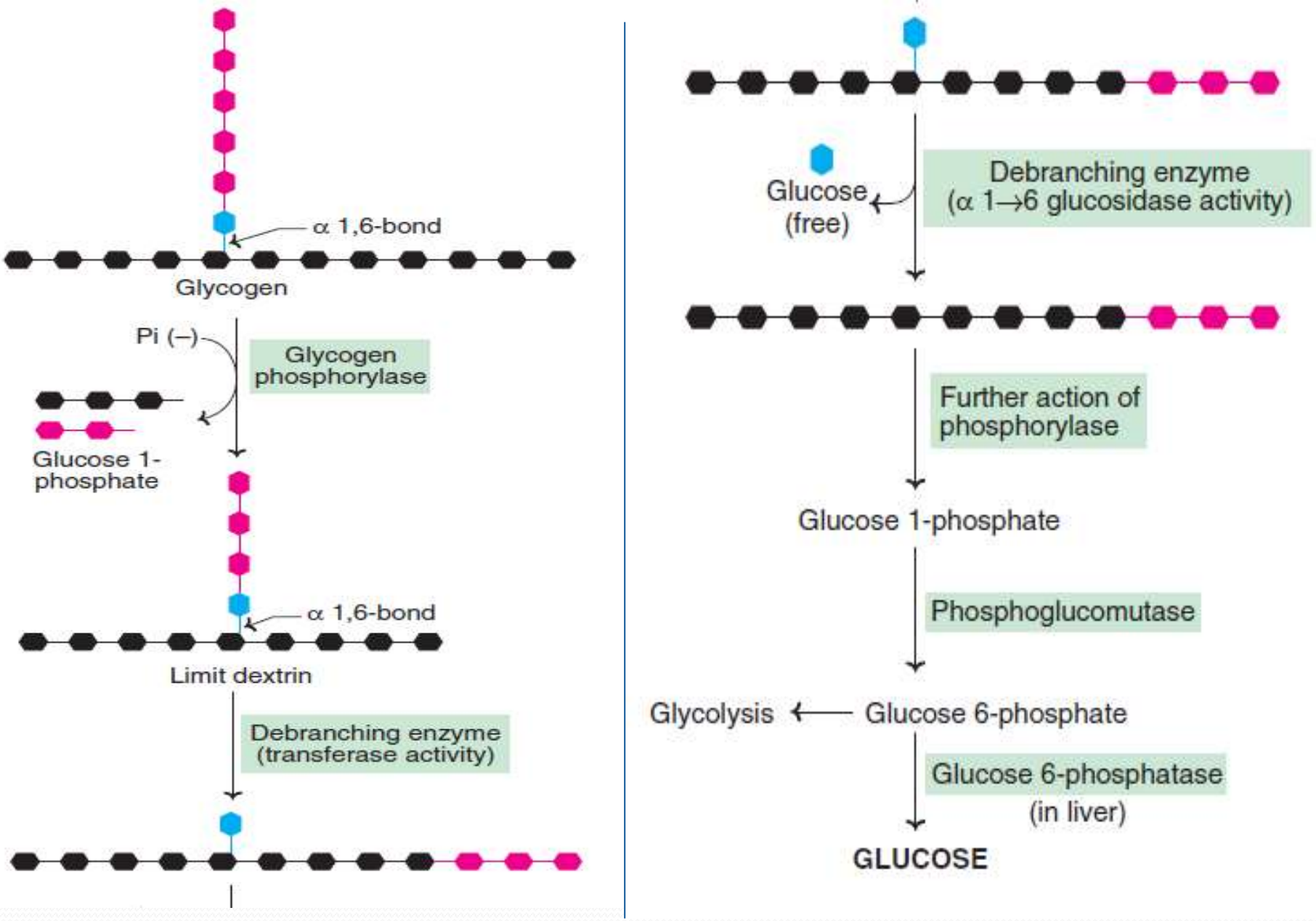
- It may be noted that though glucose 6-phosphatase is absent in muscle, some amount of free glucose (8-10% of glycogen) is produced in glycogenolysis due to the action of debranching enzyme (α-1,6-glucosidase activity).

Lysosomal acid maltase

- Lysosomal acid maltase or α-1,4-glucosidase continuously degrades a small quantity of glycogen.

- The significance of this pathway is not very clear, but it has been observed that the deficiency of lysosomal acid maltase results in glycogen accumulation.

- This accumulation causes glycogen storage disease type II (i.e. Pompe's disease).



Glycogenolysis

Source: Satyanarayana and Chakrapani, 2013

REGULATION OF GLYCOGENESIS AND GLYCOGENOLYSIS

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

- Glycogenesis and glycogenolysis are respectively controlled by the enzyme **glycogen synthase** and **glycogen phosphorylase** through allosteric and hormonal regulations, as well as the influence of calcium.

- 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., while glycogen phosphorylase (hence glycogenolysis) is enhanced when glucose concentration and energy levels are low.

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

- Glucose 6-phosphate and ATP allosterically inhibit glycogen phosphorylase, while free glucose in liver acts as an allosteric inhibitor of glycogen phosphorylase.

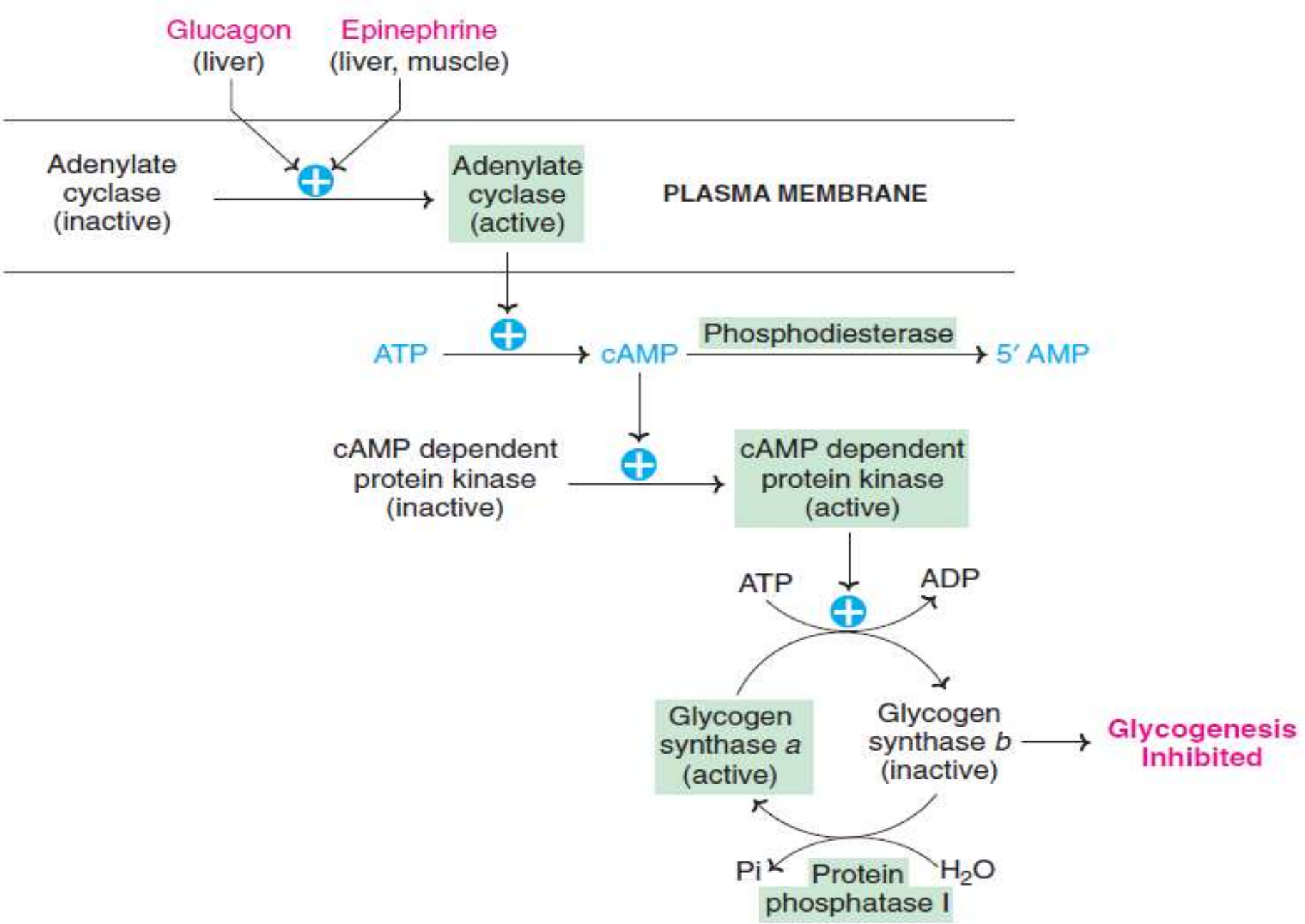
- **Hormonal regulation of glycogenesis:** Hormones like epinephrine and norepinephrine, and glucagon (in liver) activate adenylate cyclase to increase the production of cAMP.

- **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

- **Hormonal regulation of glycogenolysis:** Epinephrine and glucagon bring about glycogenolysis by their action on glycogen phosphorylase through cAMP.

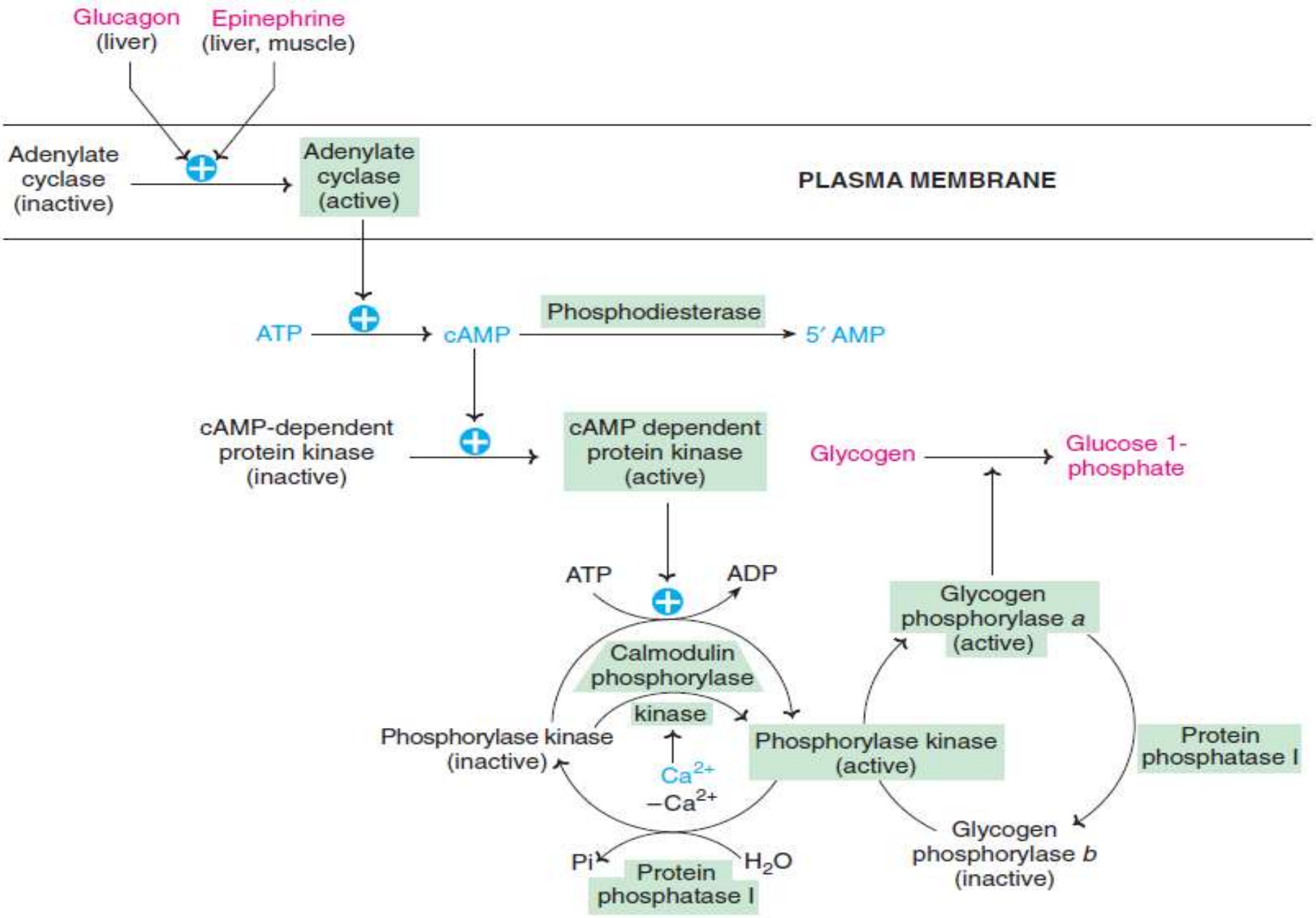
- Glycogen phosphorylase exists in two forms, an active ‘a’ form and inactive form ‘b’.

- The cAMP—formed due to hormonal stimulus—activates cAMP dependent protein kinase.

- This active protein kinase phosphorylates inactive form of glycogen phsophorylase kinase to active form, while the enzyme protein phosphatase removes phosphate and inactivates phosphorylase kinase.

- The active phosphorylase kinase phosphorylates inactive glycogen phosphorylase ‘b’ to active glycogen phosphorylase ‘a’ which degrades glycogen.

- The enzyme protein phosphatase I can dephosphorylate and convert active glycogen phosphorylase ‘a’ to inactive ‘b’ form.



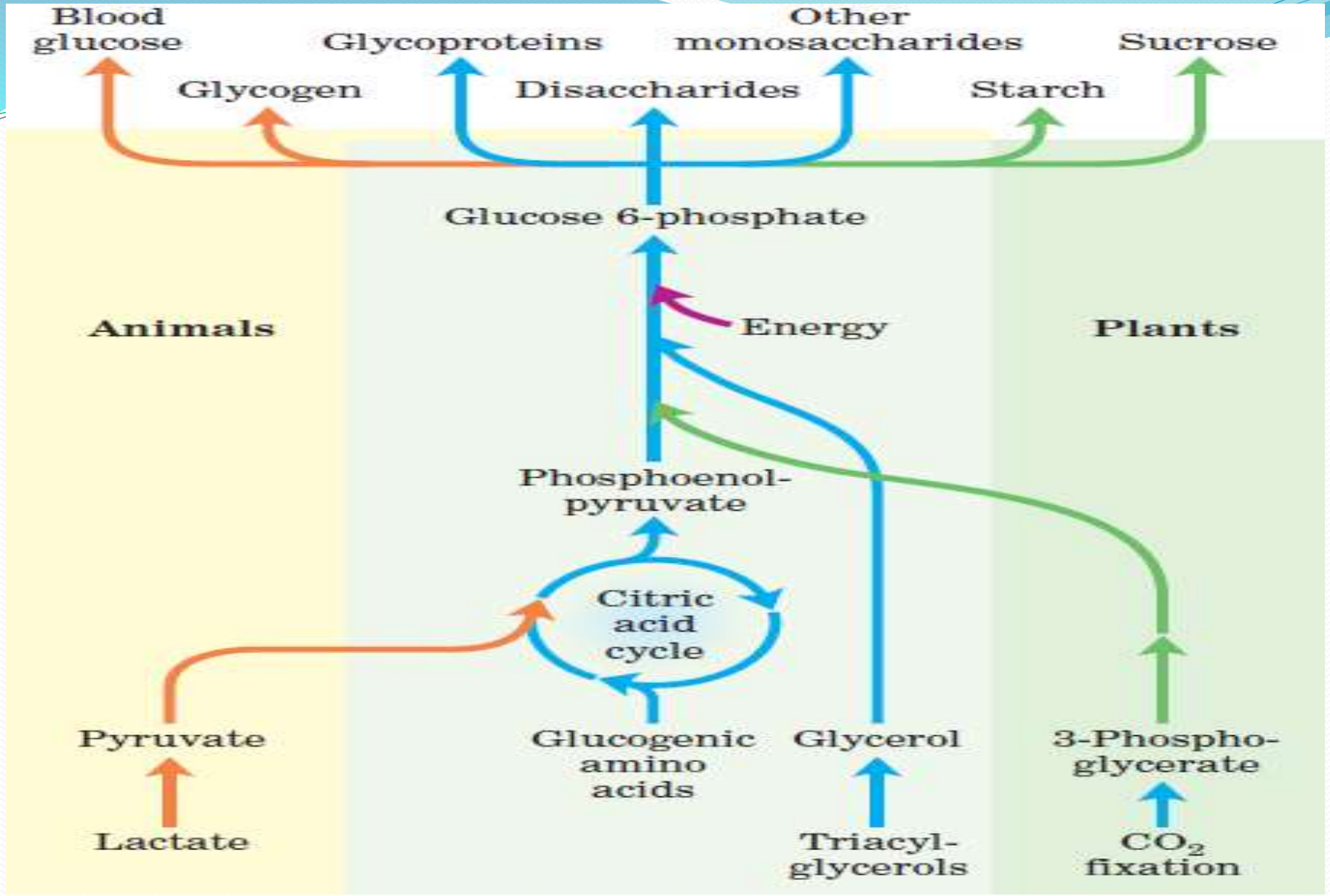
Hormonal regulation of glycogenolysis

- **Effect of Ca^{2+} ions on glycogenolysis:** When the muscle contracts, Ca^{2+} ions are released from the sarcoplasmic reticulum.

- Ca^{2+} binds to calmodulin-calcium modulating protein and directly activates phosphorylase kinase without the involvement of cAMP-dependent protein kinase.

- The overall effect of hormones on glycogen metabolism is that an elevated glucagon or epinephrine level increases glycogen degradation.

- On the other hand, an elevated insulin results in increased glycogen synthesis.



Carbohydrate synthesis from simple precursors

Source: Nelson and Cox, 2016

DISORDERS OF CARBOHYDRATE METABOLISM

DIABETES MELLITUS

- Diabetes mellitus (Greek: *diabetes*—a siphon or running through; *mellitus*—sweet) is a metabolic disorder characterized by increased blood glucose level (hyperglycemia) due to insufficient or inefficient insulin.

- Consequently, the blood glucose level is elevated which spills over into urine.

- Diabetes mellitus is the third leading cause of death (after heart disease and cancer) in many developed countries, and it affects about 6 to 8% of the general population.

- The complications of diabetes affect the eye, kidney and nervous system.

- It is a major cause of blindness, renal failure, amputation, heart attacks and stroke.

- The term diabetes, whenever used, refers to diabetes mellitus, however, **diabetes insipidus** is another disorder characterized by large volumes of urine excretion due to antidiuretic hormone deficiency.

- An important feature of diabetes is that the body cells are starved of glucose despite its very high concentration around i.e. scarcity in plenty.

- Insulin is required for the uptake of glucose by muscle (skeletal, cardiac and smooth), adipose tissue, leukocytes and mammary glands.

- Surprisingly, about 80% of glucose uptake in the body is not dependent on insulin.

- Tissues into which glucose can freely enter include brain, kidney, erythrocytes, retina, nerve, blood vessels and intestinal mucosa.

- As regards liver, glucose entry into hepatocytes does not require insulin.

- However, insulin stimulates glucose utilization in liver and, thus, indirectly promotes its uptake.

CLASSIFICATION OF DIABETES MELLITUS

- Diabetes mellitus is broadly divided into 2 groups:
- **Insulin-dependent diabetes mellitus (IDDM):** This type of diabetes is also known as type I diabetes or (less frequently) juvenile onset diabetes.
- It mainly occurs in childhood (particularly between ages 12-15), and it accounts for about 10 to 20% of the known diabetics.
- It is characterized by almost total deficiency of insulin due to destruction of β-cells of the pancreas.
- The β-cell destruction may be caused by drugs, viruses, or autoimmunity.
- Usually, the symptoms of diabetes appear when 80-90% of the β-cells have been destroyed, and the pancreas ultimately fails to secrete insulin in response to glucose ingestion.
- The patients of IDDM require insulin therapy.

- **Non-insulin dependent diabetes mellitus (NIDDM):** This type is also called type II diabetes or (less frequently) adult-onset diabetes.

- It is the most common, accounting for 80 to 90% of the diabetic population, and it occurs in adults (usually above 35 years) and is less severe than IDDM.

- The causative factors of NIDDM include genetic and environmental factors, and it more commonly occurs in obese individuals.

- Overeating coupled with underactivity leading to obesity is associated with the development of NIDDM.

- Obesity acts as a diabetogenic factor and leads to a decrease in insulin receptors on the insulin responsive (target) cells, but the patients have either normal or even increased insulin levels.

- Many a times, weight reduction by diet control alone is often sufficient to correct NIDDM.

Comparison of two types of diabetes mellitus

Character	Insulin-dependent diabetes mellitus (IDDM)	Non-insulin dependent diabetes mellitus (NIDDM)
General		
Prevalence	10-20% of diabetic population	80-90% of diabetic population
Age at onset	Usually childhood (<20 yrs)	Predominantly in adults (>30yrs)
Body weight	Normal or low	Obese
Genetic predisposition	Mild or moderate	Very strong
Biochemical		
Defect	Insulin deficiency due to destruction of β-cells	Impairment in the production of insulin by β-cells and/or resistance of target cells to insulin
Plasma insulin	Decreased or absent	Normal or increased
Auto antibodies	Frequently found	Rare
Ketosis	Very common	Rare
Acute complications	Ketoacidosis	Hyperosmolar coma
Clinical		
Duration of symptoms	Weeks	Months to years
Diabetic complications at diagnosis	Rare	Found in 10-20% cases
Oral hypoglycemic drugs	Not useful for treatment	Suitable for treatment
Administration of insulin	Always required	Usually not necessary

Source: Satyanarayana and Chakrapani, 2013

METABOLIC CHANGES IN DIABETES

- Diabetes mellitus is associated with several metabolic alterations.

- Most important among them are hyperglycemia, ketoacidosis and hypertriglyceridemia.

1. **Hyperglycemia:** Elevation of blood glucose concentration is the hallmark of uncontrolled diabetes.

Hyperglycemia is primarily due to reduced glucose uptake by tissues and its increased production via gluconeogenesis and glycogenolysis.

When the blood glucose level goes beyond the renal threshold, glucose is excreted into urine (glycosuria).

2. **Glucose toxicity:** High concentrations of glucose can be harmful causing osmotic effects/hypertonic effects (water drawn from cells into extracellular fluid and excreted into urine, resulting in dehydration), β-cell damage by free radicals (due to enhanced oxidative phosphorylation, oxidative stress, and increased free radicals) and glycation of proteins (associated with diabetic complications like neuropathy, nephropathy, retinopathy etc.).

3. **Ketoacidosis:** Increased mobilization of fatty acids results in overproduction of ketone bodies, which often leads to ketoacidosis.

4. **Hypertriglyceridemia:** Conversion of fatty acids to triacylglycerols and the secretion of VLDL and chylomicrons is comparatively higher in diabetics.

   Also, the activity of the enzyme lipoprotein lipase is low in diabetic patients, so the plasma levels of VLDL, chylomicrons and triacylglycerols are increased.

   Hypercholesterolemia is also frequently seen in diabetics.

LONG TERM EFFECTS OF DIABETES

- Hyperglycemia is directly or indirectly associated with several complications.
- These include atherosclerosis, retinopathy, nephropathy and neuropathy.
- The biochemical basis of these complications is not clearly understood.
- However, it is believed that at least some of them are related to microvascular changes caused by glycation of proteins.

MANAGEMENT OF DIABETES

- The diagnosis of diabetes can be made on the basis of individual's response to oral glucose load, through the oral glucose tolerance test (OGTT).

- Diet, exercise, drug and, insulin administration are the management options in diabetics.

- Approximately, 50% of the new cases of diabetes can be adequately controlled by diet alone, 20-30% need oral hypoglycemic drugs while the remaining 20-30% require insulin.

- **Dietary management:** Diabetic patients are advised to consume low calories (i.e. low carbohydrate and fat), high protein and fiber rich diet.

- Carbohydrates should be taken in the form of starches and complex sugars, and as far as possible, refined sugars (sucrose, glucose) should be avoided.

- Fat intake should be drastically reduced so as to meet the nutritional requirements of unsaturated fatty acids.

- Diet control and exercise will help, to a large extent, obesed NIDDM patients.

- **Hypoglycemic drugs:** Oral hypoglycemic drugs are broadly of two categories: sulfonylureas and biguanides.

- The latter are less commonly used these days due to side effects, but sulfonylureas such as acetohexamide, tolbutamide and glibenclamide are frequently used.

- They promote the secretion of endogenous insulin and thus help in reducing blood glucose level.

- **Management with insulin:** Two types of insulin preparations are commercially available: short acting and long acting.

- The short acting insulins are unmodified and their action lasts for about 6 hours, while the long acting insulins are modified ones (such as adsorption to protamine) and act for several hours, which depends on the type of preparation.

- The advent of genetic engineering is a boon to diabetic patients since bulk quantities of insulin can be produced in the laboratory.

HYPOGLYCEMIA

- Hypoglycemia is a condition of low blood glucose level.
- When the concentration of blood glucose falls below 45 mg/dl, the symptoms of hypoglycemia appear, and the manifestations include headache, anxiety, confusion, sweating, slurred speech, seizures and coma, and, if not corrected, death.
- All these symptoms are directly and indirectly related to the deprivation of glucose supply to the central nervous system (particularly the brain) due to a fall in blood glucose level.
- The mammalian body has developed a well regulated system for an efficient maintenance of blood glucose concentration, so hypoglycemia is not commonly observed.
- However, there are three types of hypoglycemia encountered by physicians.

TYPES OF HYPOGLYCEMIA

1. **Post-prandial hypoglycemia:** This is also called reactive hypoglycemia, and is observed in subjects with an elevated insulin secretion following a meal.

   This causes transient hypoglycemia and is associated with mild symptoms.

   The patient is advised to eat frequently rather than the 3 usual meals.

2. **Fasting hypoglycemia:** Low blood glucose concentration in fasting is not very common.

However, fasting hypoglycemia is observed in patients with pancreatic β-cell tumor and hepatocellular damage.

3. **Hypoglycemia due to alcohol intake:** Alcohol consumption may cause hypoglycemia in some individuals who are starved or engaged in prolonged exercise,.

   This is due to the accumulation of NADH (during the course of alcohol metabolism by alcohol dehydrogenase), which diverts the pyruvate and oxaloacetate (substrates of gluconeogenesis) to form lactate and malate respectively.

   The net effect is that gluconeogenesis is reduced due to alcohol consumption.

4. **Hypoglycemia due to insulin overdose:** The most common complication of insulin therapy in diabetic patients is hypoglycemia.

   This is particularly observed in patients who are on intensive treatment regime.

5. **Hypoglycemia in premature infants:** Premature and underweight infants have smaller stores of liver glycogen, and are susceptible to hypoglycemia.

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.

ESSENTIAL PENTOSURIA

- Essential pentosuria is a rare genetic disorder associated with the uronic acid pathway.
- It is related to the deficiency of an NADP-dependent enzyme, xylitol dehydrogenase.
- Due to this enzyme defect, L-xylulose cannot be converted to xylitol, so the affected individuals excrete large amounts of L-xylulose in urine.
- Essential pentosuria is asymptomatic and the individuals suffer from no ill-effects.
- It has been reported that the administration of drugs aminopyrine and antipyrine increases the excretion of L-xylulose in pentosuric patients.

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

ASSIGNMENT

Read on the following:

- ✓ Metabolism of galactose
- ✓ Metabolism of fructose
- ✓ Metabolism of amino sugars
- ✓ Futile cycles

BIBLIOGRAPHY AND SUGGESTED FURTHER READING

1. Satyanarayana U., Chakrapani U. (2006): Biochemistry, 3rd ed. Kolkata: Arunabha Sen Books and Allied Ltd.
2. David Nelson and Michael Cox (2016). Lehninger Principles of Biochemistry,7th ed. McGrawHill education.
3. Victor Rodwell, David Bender, Kathleen Botham, Peter Kennelly, and Anthony Weil (2018). Harper's Illustrated Biochemistry. McGrawHill education lange.
4. Reginald Garrett and Charles Grisham (2010). Biochemistry. Brooks/Cole, Cengage Learning.