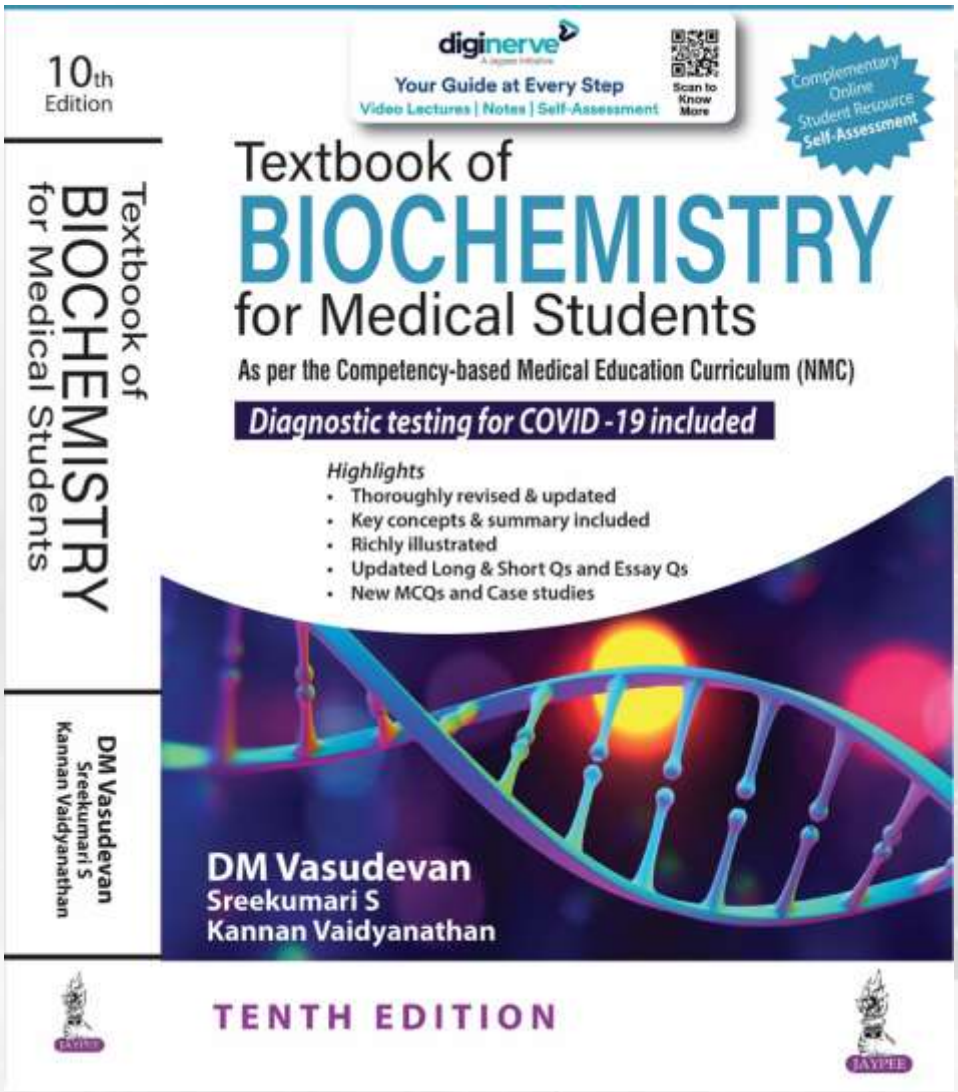


Chapter 8B:

Glycogen HMP Shunt Pathway Polyol Pathway

Textbook of
BIOCHEMISTRY
for Medical Students

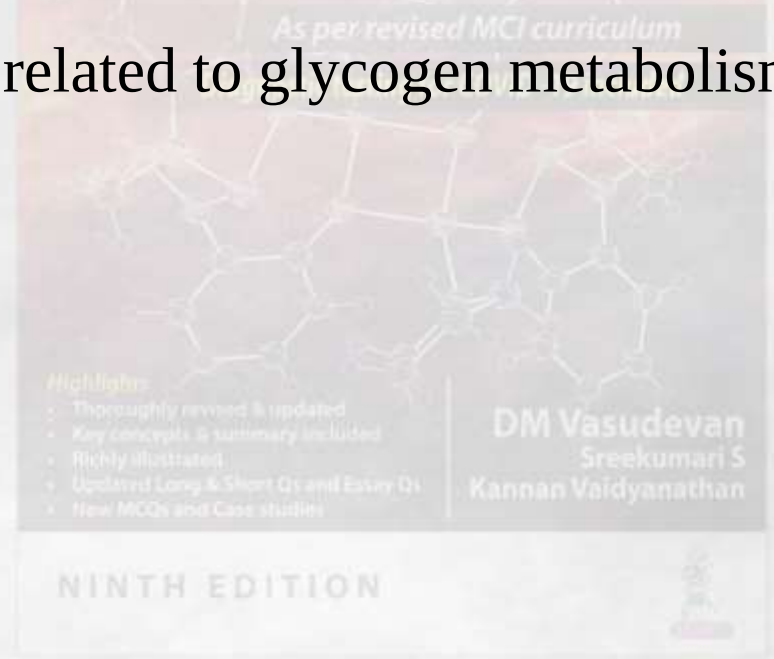
By DM Vasudevan, *et al.*

TENTH EDITION

Functions of Glycogen

- Glycogen is the storage form of carbohydrates in the human body.
- The major sites of storage are liver and muscle.
- The major function of **liver glycogen is to provide glucose during fasting.**
- The glycogen content of liver (10 gm/100 gm tissue) is more than in the skeletal muscle (1–2 gm/100 gm).
- But the total quantity of muscle glycogen is more than liver glycogen because of the larger muscle mass.
- When blood glucose level falls, liver glycogen is broken down and helps to maintain blood glucose level.
- After taking food, blood glucose tends to rise, which causes glycogen deposition in liver.
- About 5 hours after taking food, the blood glucose tends to fall.
- But, glycogen is lysed to glucose so that the energy needs are met.

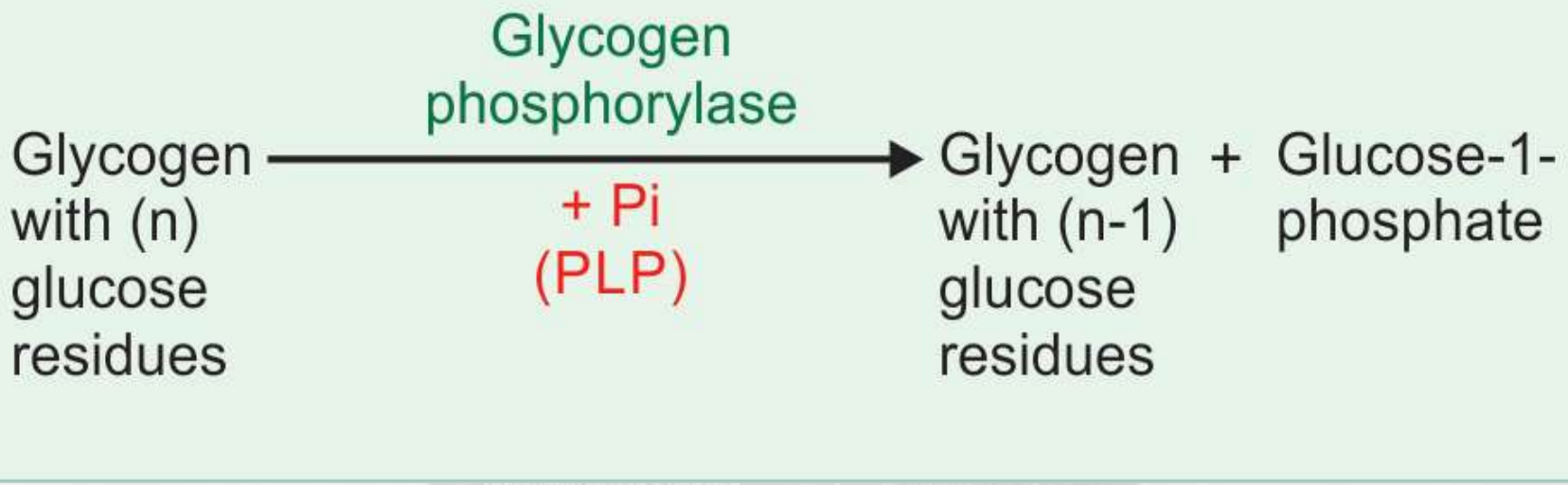
- After about 18 hours fasting, most of the liver glycogen is depleted, when depot fats are hydrolysed and energy requirement is met by fatty acid oxidation.
- **The function of muscle glycogen is to act as reserve fuel for muscle contraction**.
- All the enzymes related to glycogen metabolism are cytoplasmic.



Degradation of Glycogen (Glycogenolysis)

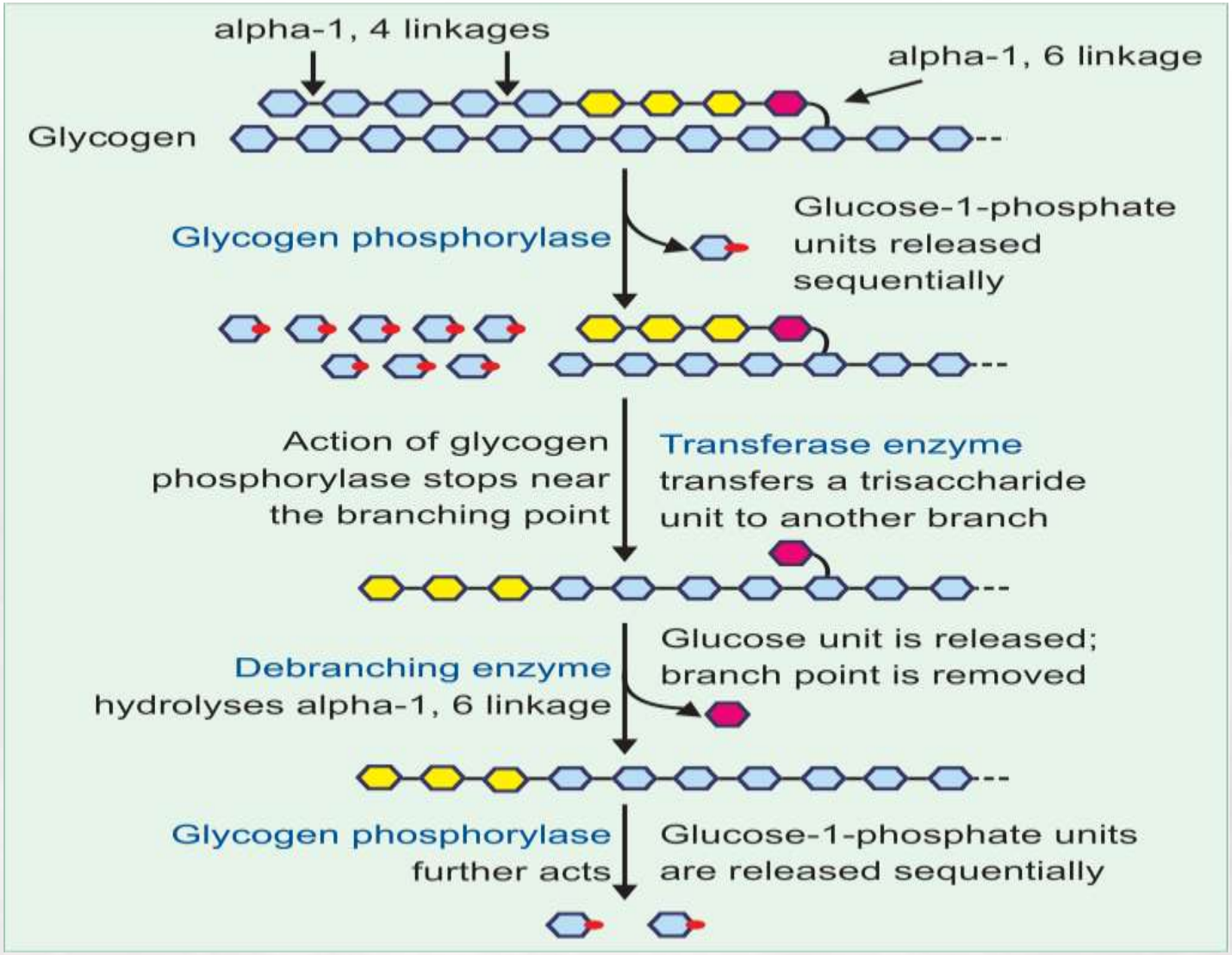
1. Glycogen Phosphorylase

- Glycogen phosphorylase removes glucose as glucose-1-phosphate from glycogen (phosphorolysis).
- It contains **pyridoxal phosphate (PLP) as a prosthetic group.**
- The alpha-1,4 linkages in the glycogen are cleaved.
- It removes glucose units one at a time.
- Enzyme sequentially hydrolyses alpha-1,4 glycosidic linkages, till it reaches a glucose residue, 3-4 glucose units away from a branch point.
- It cannot attack the 1,6 linkage at branch point.
- If glycogen phosphorylase alone acts on a glycogen molecule, the final product is a highly branched molecule; it is called **limit dextrin.**


Glycogen with (n) glucose residues
Glycogen phosphorylase
+ Pi
(PLP)
Glycogen with (n-1) glucose residues
+
Glucose-1-phosphate

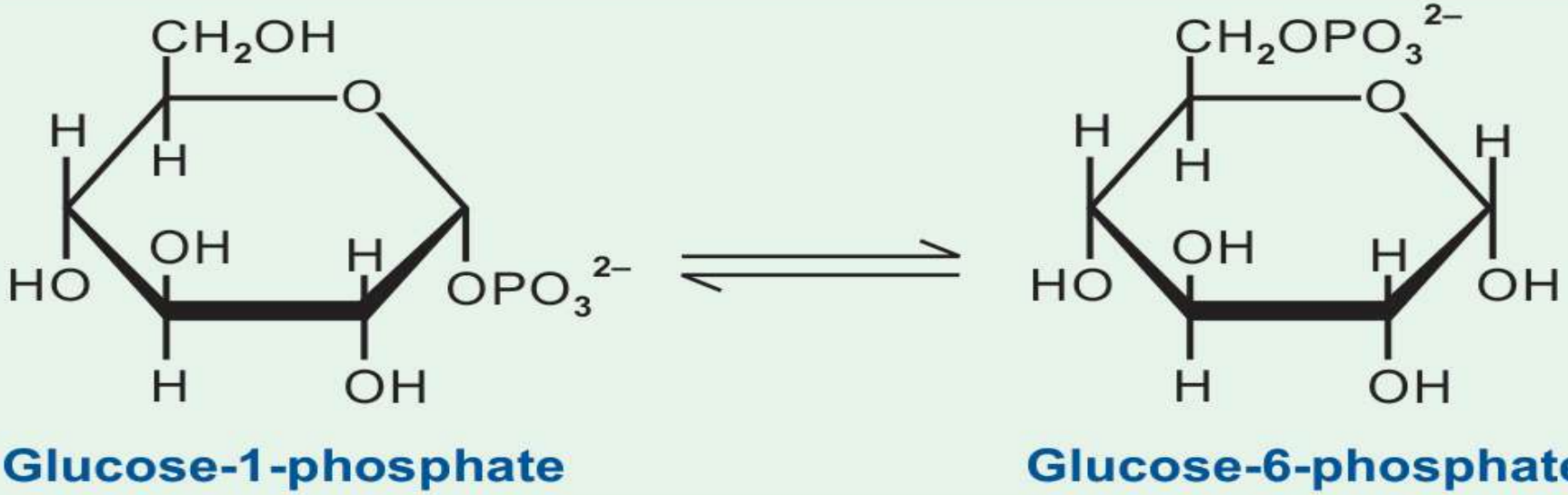
2. Debranching by bifunctional (two) Enzymes

- Then a block of 3 glucose residues (trisaccharide unit) are transferred from the branching point to another branch.
- This enzyme is alpha-1,4 ® alpha-1,4 glucan transferase.
- Now the branch point is free.
- Then alpha- 1,6- glucosidase (debranching enzyme) can hydrolyse the remaining glucosyl unit held in alpha-1,6 linkage at the branch point.
- This glucose residue is released as **free glucose.**
- At this stage, the ratio of glucose-1- phosphate to free glucose is about 8:1.
- The transferase and alpha-1,6-glucosidase will together convert the branch point to a linear one.
- With the removal of the branch point, phosphorylase can proceed with its action.


alpha-1, 4 linkages
alpha-1, 6 linkage
Glycogen
Glycogen phosphorylase
Glucose-1-phosphate units released sequentially
Action of glycogen phosphorylase stops near the branching point
Transferase enzyme transfers a trisaccharide unit to another branch
Debranching enzyme hydrolyses alpha-1, 6 linkage
Glucose unit is released; branch point is removed
Glycogen phosphorylase further acts
Glucose-1-phosphate units are released sequentially

3. Phosphogluco mutase

- Phosphorylase reaction produces glucose-1- phosphate while debranching enzyme releases glucose.
- The glucose-1-phosphate is converted to glucose-6-phosphate by phosphoglucomutase

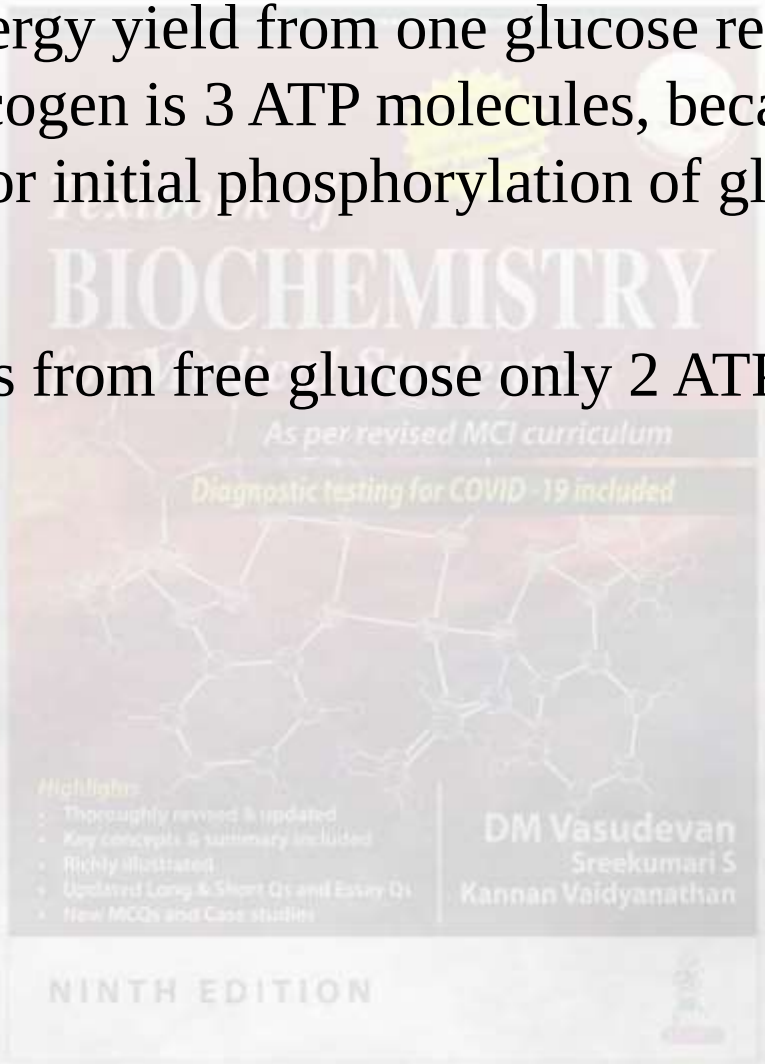


4. Glucose-6-phosphatase in Liver

- Next, hepatic glucose-6-phosphatase hydrolyses glucose-6-phosphate to glucose.
- The free glucose is released to the blood stream.
- Muscle will not release glucose to the blood stream, because muscle tissue does not contain glucose-6-phosphatase.
- Instead, in muscle, glucose-6-phosphate undergoes glycolysis to produce ATP for muscle contraction.

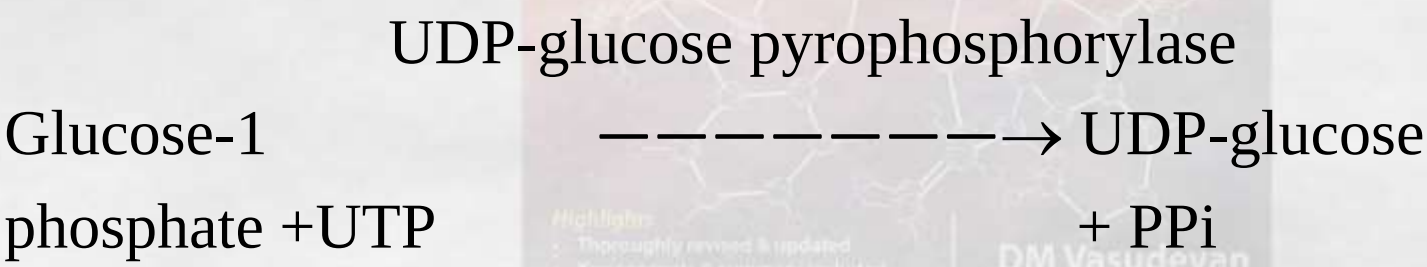
Energetics

- In muscle, the energy yield from one glucose residue derived from glycogen is 3 ATP molecules, because no ATP is required for initial phosphorylation of glucose (step 1 of glycolysis).
- If glycolysis starts from free glucose only 2 ATPs are produced.



Glycogen Synthesis (Glycogenesis)

- The glycogen synthesis occurs by a pathway distinctly different from the reversal of glycogen breakdown, which would prevent the operation of futile cycles.
- UDP glucose is formed from glucose-1-phosphate and UTP (uridine triphosphate) by the enzyme **UDP glucose pyrophosphorylase.**



Glycogen Synthase

- The glucose moiety from UDP-glucose is transferred to a glycogen primer (glycogenin) molecule.
- The primer is essential as the acceptor of the glycosyl unit.
- The primer is a protein-carbohydrate complex.
- It is a dimeric protein, having two identical monomers.
- An oligosaccharide chain of 7 glucose units is added to each monomer.

- In the next step, activated glucose units are sequentially added by the enzyme glycogen synthase.

Glycogen synthase

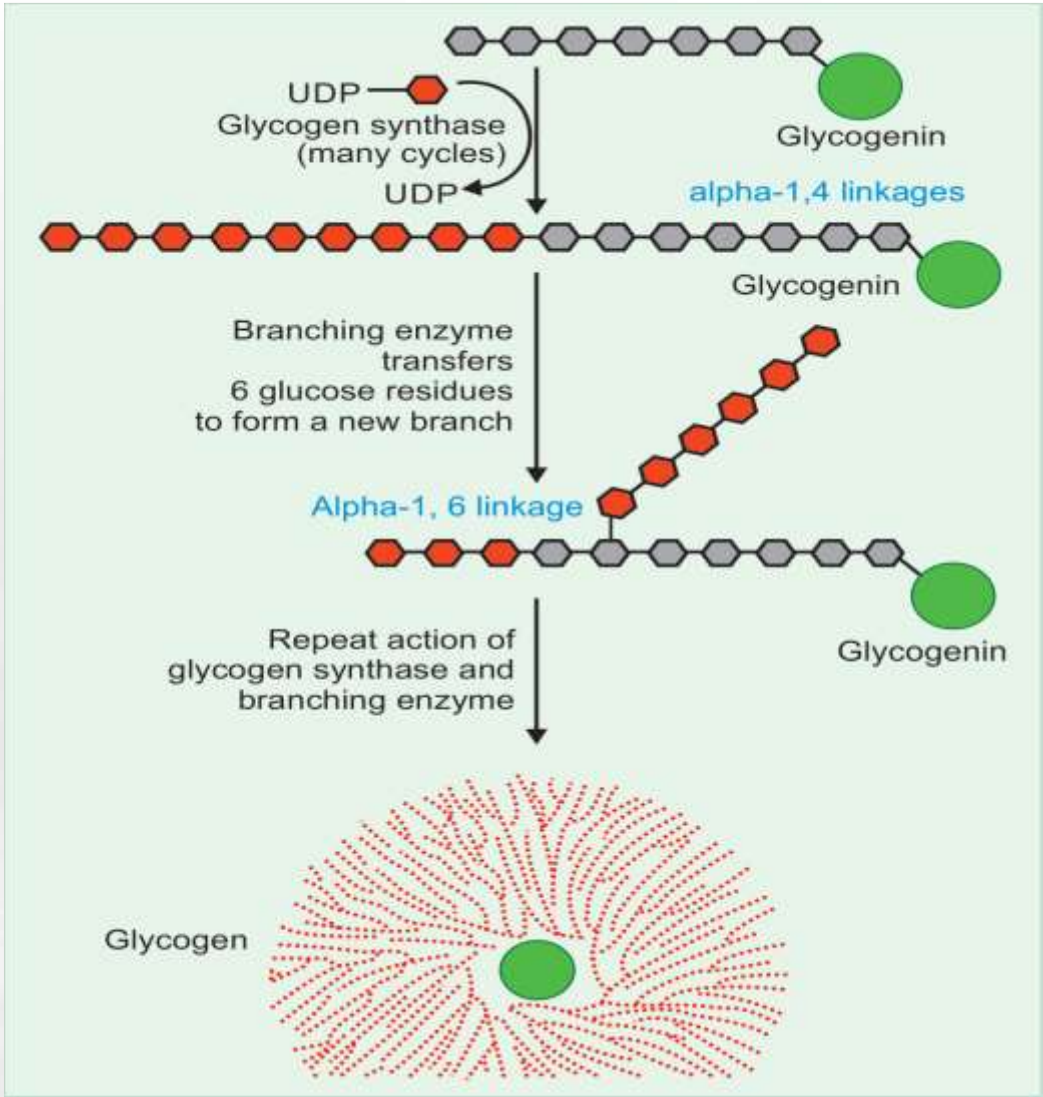
Glycogen primer (n) ——→ Glycogen (n+1)

+ UDP-glucose + UDP

- The glucose unit is added to the nonreducing (outer) end of the glycogen primer to form an alpha-1,4 glycosidic linkage and UDP is liberated.

Branching Enzyme

- The glycogen synthase can add glucose units only in alpha-1,4 linkage.
- A branching enzyme is needed to create the alpha-1,6 linkages.
- When the chain is lengthened to 11 - 12 glucose residues, the branching enzyme will transfer a block of 6 to 8 glucose residues from this chain to another site on the growing molecule.
- The enzyme amylo-[1,4]→[1,6]-transglucosidase (**branching enzyme)** forms this alpha-1,6 linkage.
- To this newly created branch, further glucose units can be added in alpha-1,4 linkage by glycogen synthase.


UDP
Glycogen synthase
(many cycles)
UDP
Glycogenin
alpha-1,4 linkages
Glycogenin
Branching enzyme
transfers
6 glucose residues
to form a new branch
Alpha-1, 6 linkage
Glycogenin
Repeat action of
glycogen synthase and
branching enzyme
Glycogen

Regulation of Glycogen Metabolism

- The synthetic and degradative pathways are reciprocally regulated to prevent **futile cycles.**
- **The phosphorylated form of glycogen phosphorylase is active; but glycogen synthase becomes inactive on phosphorylation.**
- The covalently modified phosphorylase is active even without AMP.
- The covalent modification of glycogen phosphorylase and synthase is by a cyclic AMP mediated cascade.
- Specific protein kinases bring about phosphorylation and protein phosphatases cause dephosphorylation.

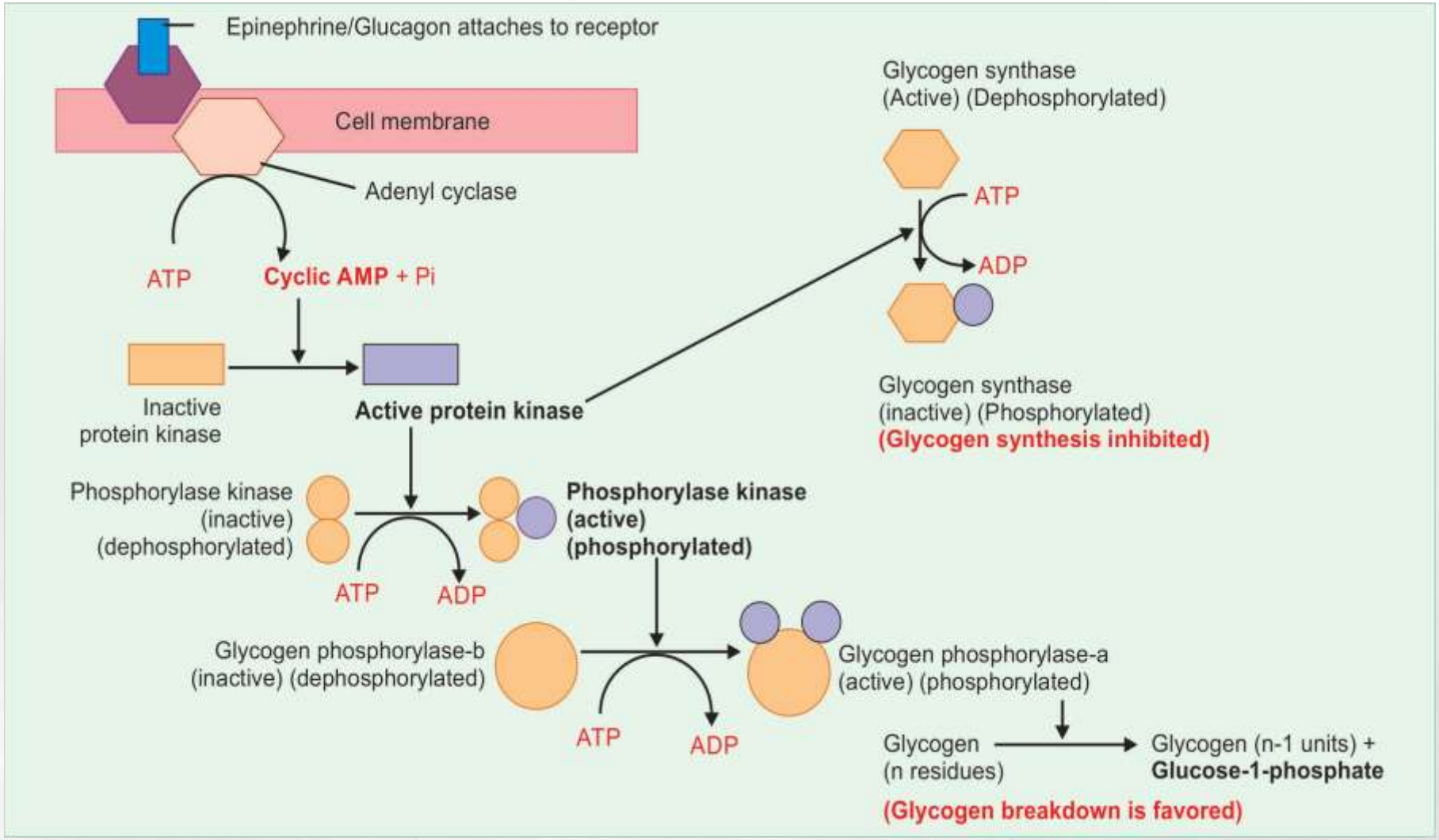
Generation of Cyclic AMP (cAMP)

- Both liver and muscle phosphorylases are activated by a cyclic AMP mediated activation cascade triggered by the hormonal signal.
- The hormones **epinephrine and glucagon** can activate liver glycogen phosphorylase but glucagon has no effect on the muscle.
- When the hormone binds to a specific receptor on the plasma membrane, the enzyme adenyl cyclase is activated which converts ATP to cyclic AMP (cAMP).
- When level of cyclic AMP rises, it will activate a protein kinase.
- The protein kinase is inactive when the catalytic (C) and regulatory (R) subunits are associated with each other.

Phosphorylase Kinase Activation

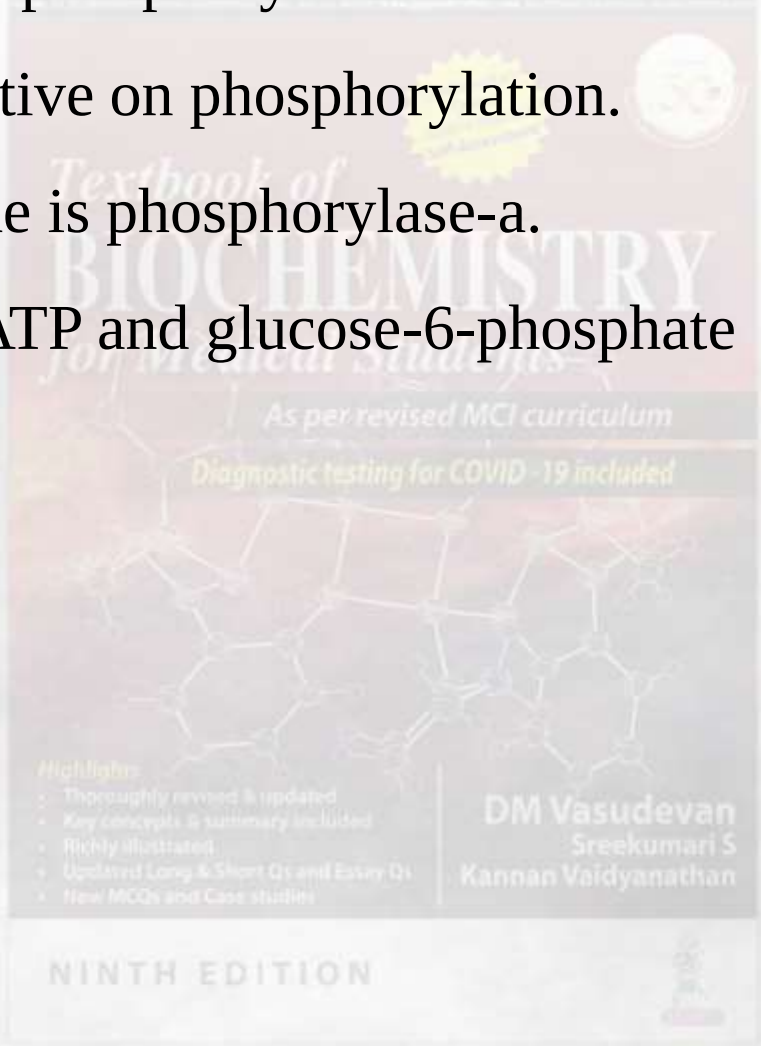
- The active protein kinase converts the phosphorylase kinase to an active phosphorylated form
- Active phosphorylase kinase converts phosphorylase-b to phosphorylase- a.

Cyclic AMP Mediated Activation Cascade



Glycogen Phosphorylase in Liver and Muscle

1. **Liver:** The liver phosphorylase-b is the inactive form.
   - It becomes active on phosphorylation.
   - Active enzyme is phosphorylase-a.
   - Inhibited by ATP and glucose-6-phosphate



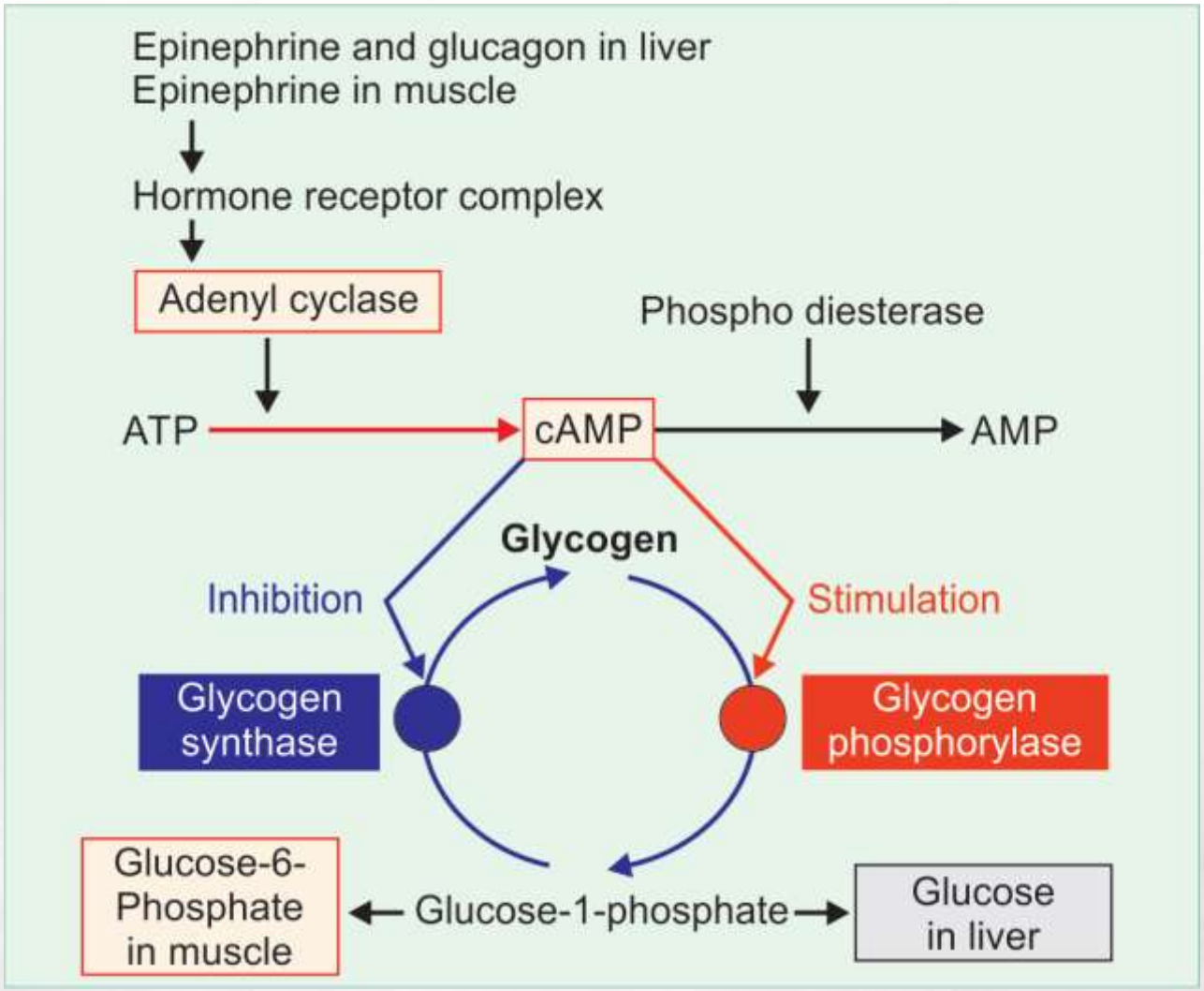
2. Muscle: Skeletal muscle glycogen is degraded only when the demand for ATP is high.

- The regulation of glycogenolysis in skeletal muscle is by epinephrine.
- Glucagon has no effect on muscle glycogenolysis.
- AMP formed by degradation of ATP during muscle contraction is an allosteric activator of phosphorylase b.

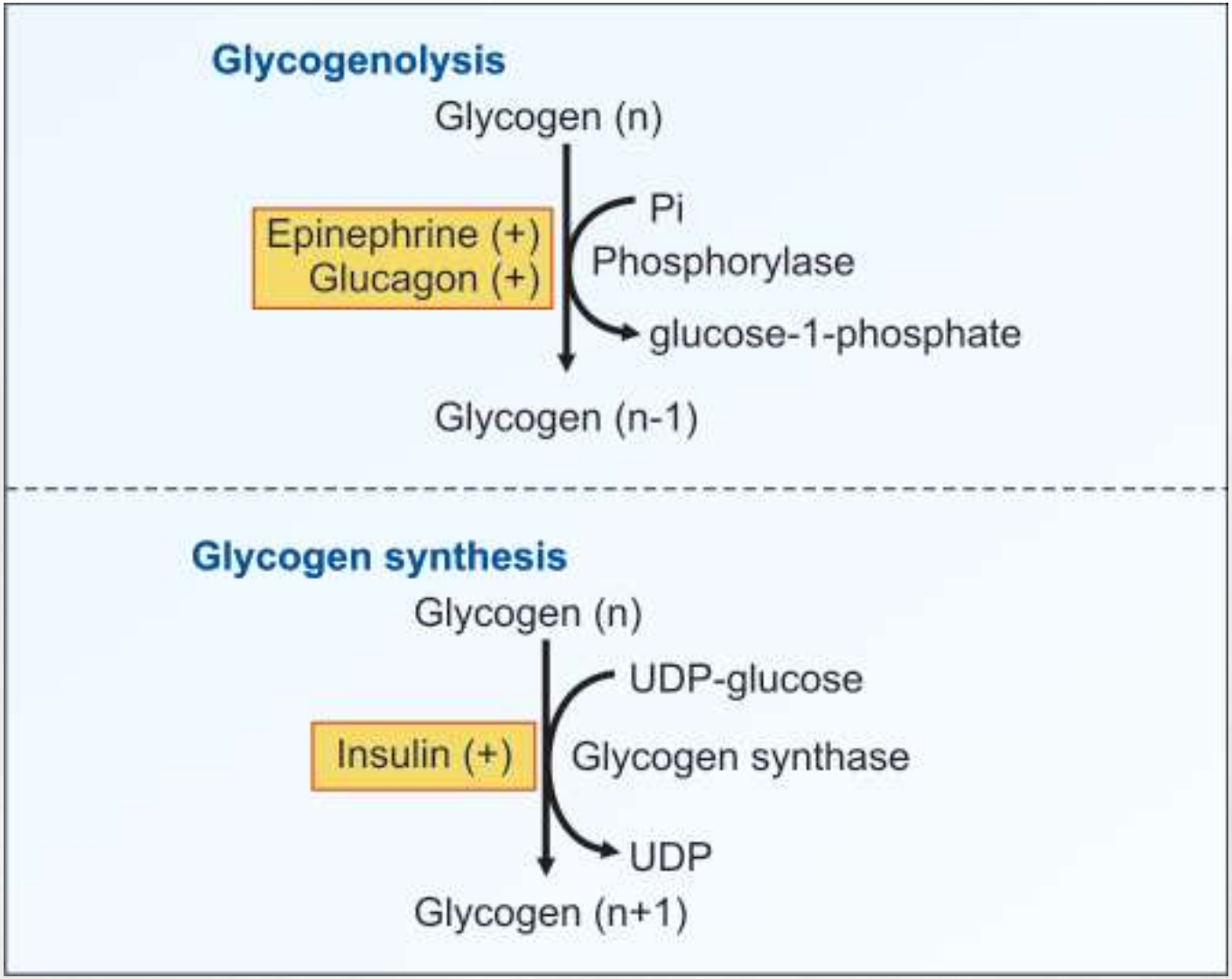
Glycogen Synthase

- Glycogen synthase and phosphorylase activities are **reciprocally regulated.**
- The same protein kinase, which phosphorylates the phosphorylase kinase would also phosphorylate glycogen synthase.
- The activity of the glycogen synthase is markedly decreased on phosphorylation.
- Insulin promotes glycogen synthesis by favoring dephosphorylation.
- The reciprocal regulation of glycogenolysis and glycogenesis is by covalent modification (phosphorylation and dephosphorylation).
- Insulin and glucagon are the major regulatory hormones, although epinephrine has stimulatory effect on glycogenolysis in both liver and muscle.

Reciprocal Regulation of Glycogenolysis and Glycogen Synthesis by Cyclic AMP



Effects of Hormones on Glycogen



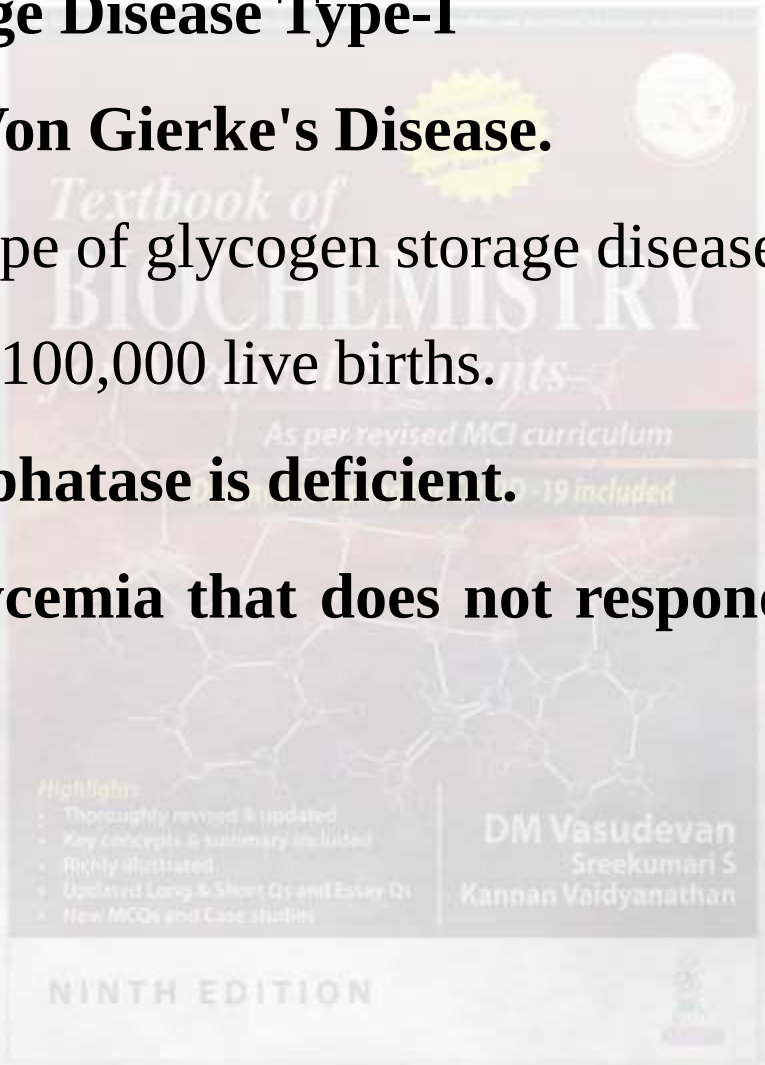
Regulators of glycogen metabolism				
Effectors	Glycogen phosphory -lase-Liver	Glycogen synthase-Liver	Glycogen Phospho-rylase-Muscle	**Glycogen synthase-Muscle**
ATP	Inhibition	Inhibition	Inhibition	**Inhibition**
AMP	Activation		Activation	**Inhibition**
Glucose-6Phosphate	Inhibition	Activation	Inhibition	**Activation**
Ca++	**Activation**		**Activation**	

Summary of Regulation

- The key enzyme for glycogenolysis is phosphorylase, which is activated by glucagon and adrenaline, under the stimulus of hypoglycemia.
- The key enzyme for glycogen synthesis is glycogen synthase, the activity of which is decreased by adrenaline but is enhanced by insulin, under the stimulus of hyperglycemia.
- Glycogen metabolism is regulated by coordinated regulation of glycogen synthase and glycogen phosphorylase.
- The regulatory mechanisms include allosteric control as well as hormonal control by covalent modification of enzymes.
- The allosteric effectors are ATP, glucose-6-phosphate and AMP.

Glycogen Storage Diseases

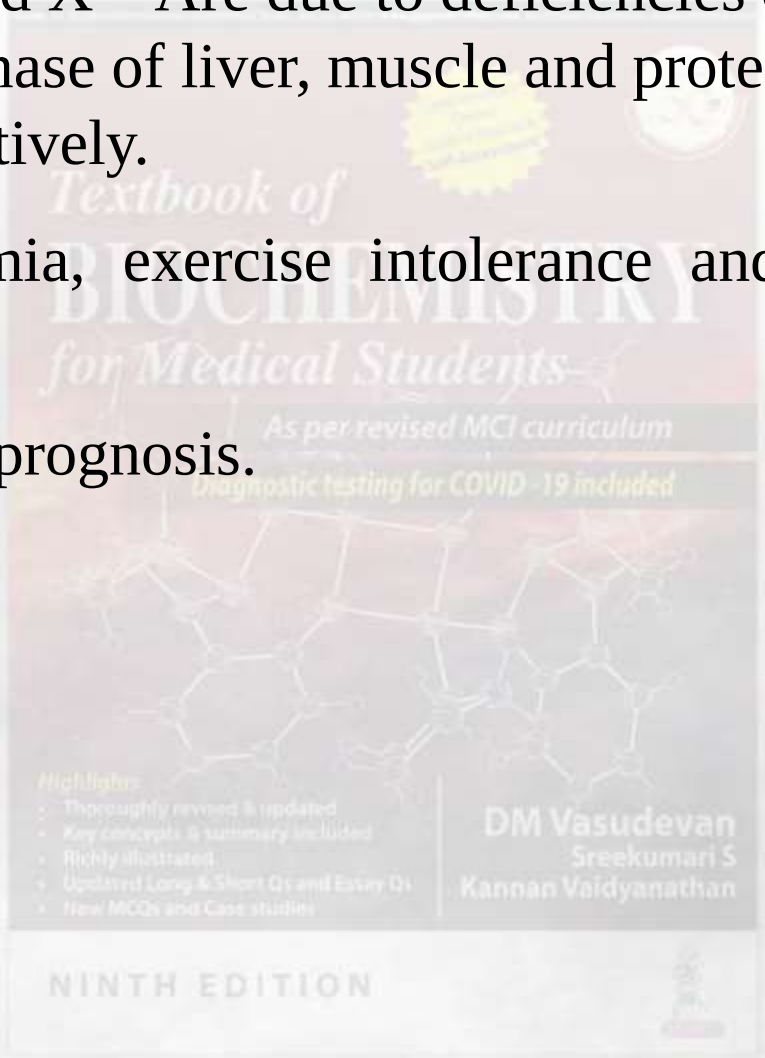
- **Glycogen Storage Disease Type-I**
- It is also called **Von Gierke's Disease.**
- Most common type of glycogen storage disease is type I.
- Incidence is 1 in 100,000 live births.
- **Glucose-6-phosphatase is deficient.**
- **Fasting hypoglycemia that does not respond to stimulation by adrenaline.**



- The glucose cannot be released from liver during over night fasting.
- Hyperlipidemia, lactic acidosis and ketosis.
- Glucose-6-phosphate is accumulated, so it is channeled to HMP shunt pathway producing more ribose and more nucleotides.
- Purines are then catabolized to uric acid, leading to **hyperuricemia.**
- Glycogen gets deposited in liver.
- Massive liver enlargement may lead to **cirrhosis.**
- Children usually die in early childhood.
- Treatment is to give small quantity of food at frequent intervals.

Type	Name	Enzyme	Clinical features
Ia	Von Gierke's disease	Glucose 6 phosphatase	Fasting hypoglycemia, hepatomegaly
II	Pompe's disease	Lysosomal maltase	Glycogen accumulates in liver, muscle and heart. Death by 2 yrs
III	Limit dextrinosis, Cori's disease	Debranching enzyme	Fasting hypoglycemia, hepatomegaly
IV	Amylopectinosis, Anderson's disease	Branching enzyme	Mild hypoglycemia, hepatosplenomegaly
V	McArdle's disease	Muscle phosphorylase	Exercise intolerance
VI	Hers' disease	Liver phosphorylase	Mild hypoglycemia, hepatomegaly, better prognosis
VII	Tarui's disease	Muscle PFK	Exercise intolerance, hemolytic anemia

- Types VIII, IX and X – Are due to deficiencies of phosphorylase kinase of liver, muscle and protein kinase A deficiency respectively.
- Mild hypoglycemia, exercise intolerance and hepatomegaly are seen.
- They have better prognosis.



Hexose Monophosphate Shunt Pathway

"**Dickens-Horecker pathway**".

"**Shunt pathway**".

"**Hexose monophosphate (HMP) pathway**".

"**Pentose phosphate pathway**".

"**Direct oxidative pathway**" or

"Phosphogluconate oxidative pathway"

About 10% of glucose molecules per day are entering in this pathway. The liver and RBC metabolize about 30% of glucose by this pathway. The major purpose of this pathway is generation of NADPH and pentose phosphates.

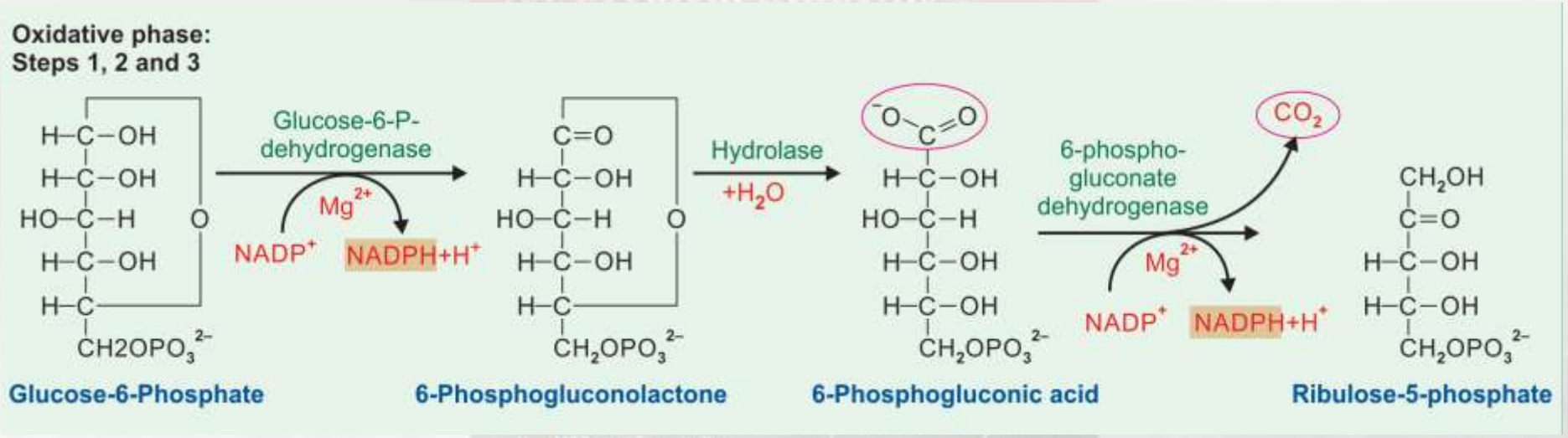
NAD and NADP are Different

NADH is used for reducing reactions in catabolic pathways, e.g. pyruvate to lactate. NADH enters the electron transport chain, and ATP is generated.

NADPH is used for reductive biosynthetic reactions, e.g. de novo synthesis of fatty acid, synthesis of cholesterol, etc. NADPH is generated mainly by the hexose monophosphate shunt pathway. NADPH is not entering the electron transport chain; and NADPH will not generate ATP.

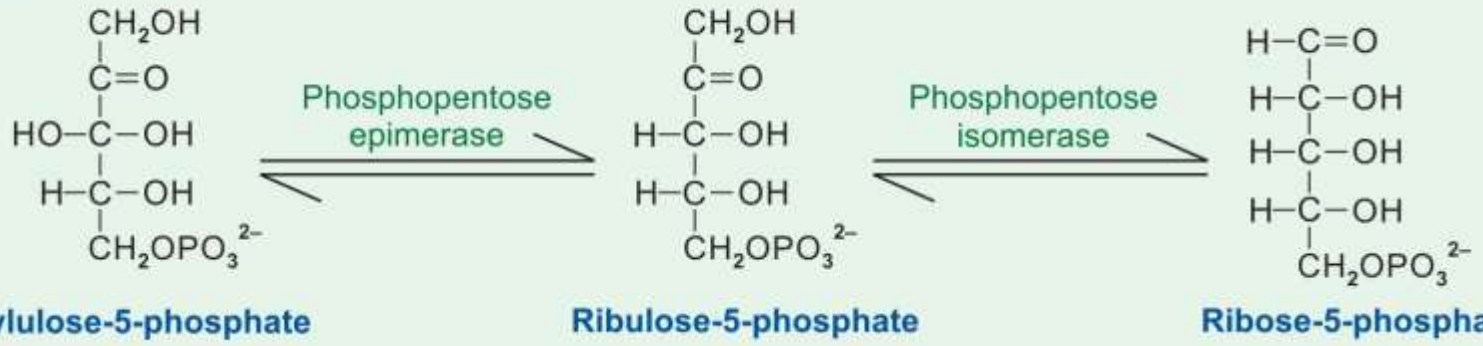
NADP differs from NAD in having an additional phosphate group. These two coenzymes are specific for enzymes; they are not interchangeable.

During the **oxidative phase**, (Step 1, 2 and 3) glucose-6-phosphate is oxidized with the generation of 2 molecules of NADPH, and one molecule of pentose phosphate, with the liberation of one molecule of CO2.0



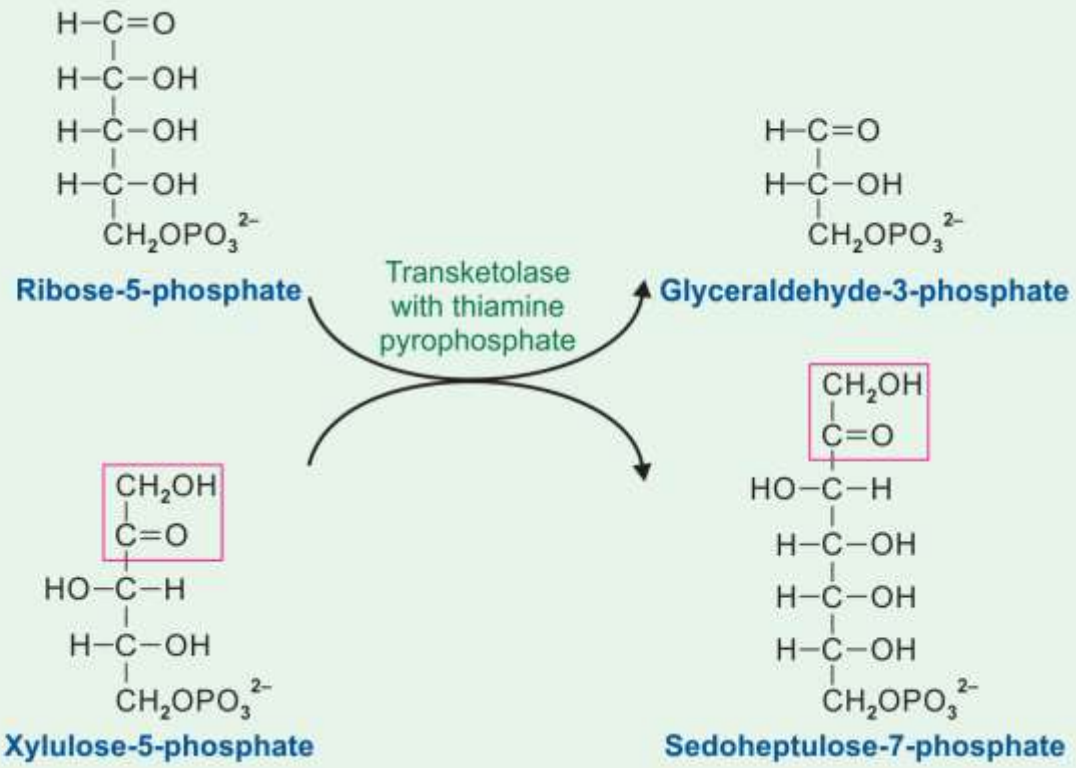
Step 4: Isomerization

Non-oxidative phase:
Step 4



Xylulose-5-phosphate **Ribulose-5-phosphate** **Ribose-5-phosphate**

Step 5: First transketolase reaction



Summary : 5C + 5C = 7C + 3C

Step 6: Transaldolase Reaction

Transfer of a 3 carbon unit, from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate.

Summary: **7C + 3C = 6C +4C**

Step 7: Second Transketolase Reaction

One 2C unit is transferred from xylulose-5-phosphate to erythrose-4-phosphate to form fructose-6-phosphate and glyceraldehyde-3-phosphate.

Summary: **5C + 4C = 6C +3C**

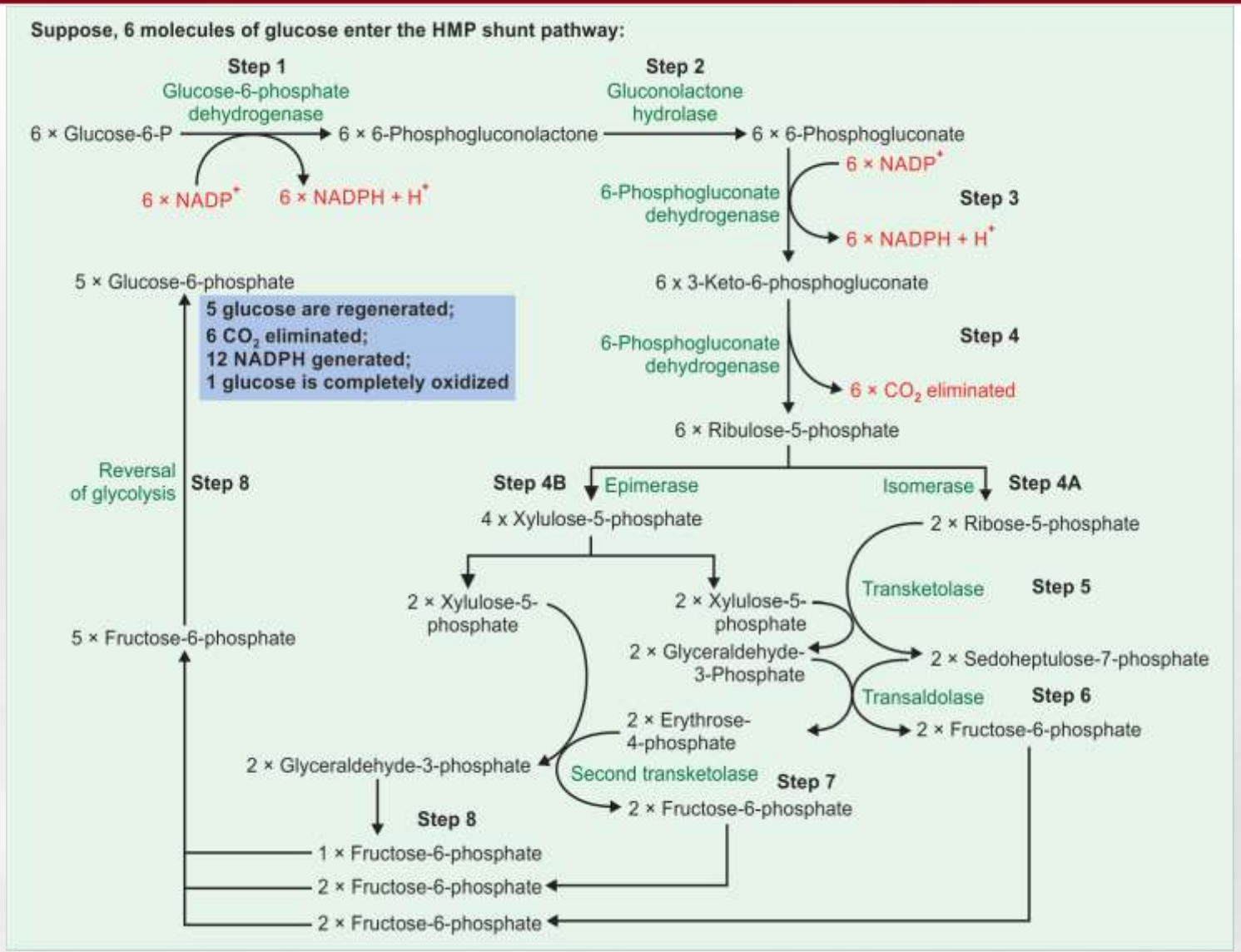
Step 8: Regeneration of Glucose-6-Phosphate

Two molecules of glyceraldehyde-3-phosphate formed in step 7 are condensed to form one fructose-6-phosphate and then converted to glucose-6-phosphate

Summary of Shunt Pathway

Suppose, 6 molecules of glucose (6 × 6 = 36 carbons) are entering in this pathway. The first carbon atoms of all 6 glucose molecules are removed as 6 molecules of CO2. (This is equivalent to complete oxidation of 1 molecule of glucose). In this process, 12 NADPH are generated.

The remaining 6 molecules of 5-carbon pentoses (6 × 5 = 30C) are interchanged in such a way that 5 molecules of glucose (5 × 6 = 30C) are regenerated.


Suppose, 6 molecules of glucose enter the HMP shunt pathway:
Step 1
Glucose-6-phosphate dehydrogenase
6 × Glucose-6-P
6 × 6-Phosphogluconolactone
6 × NADP+
6 × NADPH + H+
Step 2
Gluconolactone hydrolase
6 × 6-Phosphogluconate
6-Phosphogluconate dehydrogenase
6 × NADP+
Step 3
6 × NADPH + H+
6 x 3-Keto-6-phosphogluconate
6-Phosphogluconate dehydrogenase
Step 4
6 × CO2 eliminated
6 × Ribulose-5-phosphate
Step 4B
Epimerase
Isomerase
Step 4A
4 x Xylulose-5-phosphate
2 × Ribose-5-phosphate
2 × Xylulose-5-phosphate
2 × Xylulose-5-phosphate
Transketolase
Step 5
2 × Glyceraldehyde-3-Phosphate
2 × Sedoheptulose-7-phosphate
Transaldolase
Step 6
2 × Erythrose-4-phosphate
2 × Fructose-6-phosphate
2 × Glyceraldehyde-3-phosphate
Second transketolase
Step 7
2 × Fructose-6-phosphate
Step 8
1 × Fructose-6-phosphate
2 × Fructose-6-phosphate
2 × Fructose-6-phosphate
5 × Fructose-6-phosphate
Reversal of glycolysis
Step 8
5 × Glucose-6-phosphate
5 glucose are regenerated;
6 CO2 eliminated;
12 NADPH generated;
1 glucose is completely oxidized

Regulation of HMP Shunt Pathway

The pathway is mainly regulated by the level of NADP+.

The first reaction catalyzed by **GPD is the rate-limiting** step and it is inhibited by NADPH.

The oxidative phase is therefore controlled by the level of NADP+ and nonoxidative phase by the requirement of pentoses.

Insulin will induce GPD and therefore will increase the overall pathway.

When more NADPH is needed, the pathway proceeds to completion.

Physiological Significance of the Pathway

The main purpose of the shunt pathway is to produce ribose phosphate and to generate NADPH. The significance of the NADPH in metabolism is shown in Box 8.10.

The oxidative phase of the pathway is seen in the following organs, where NADPH generation is required for lipid synthesis or steroid synthesis.

Liver

Adipose tissue

Adrenal cortex

Mammary glands

Testes and ovaries

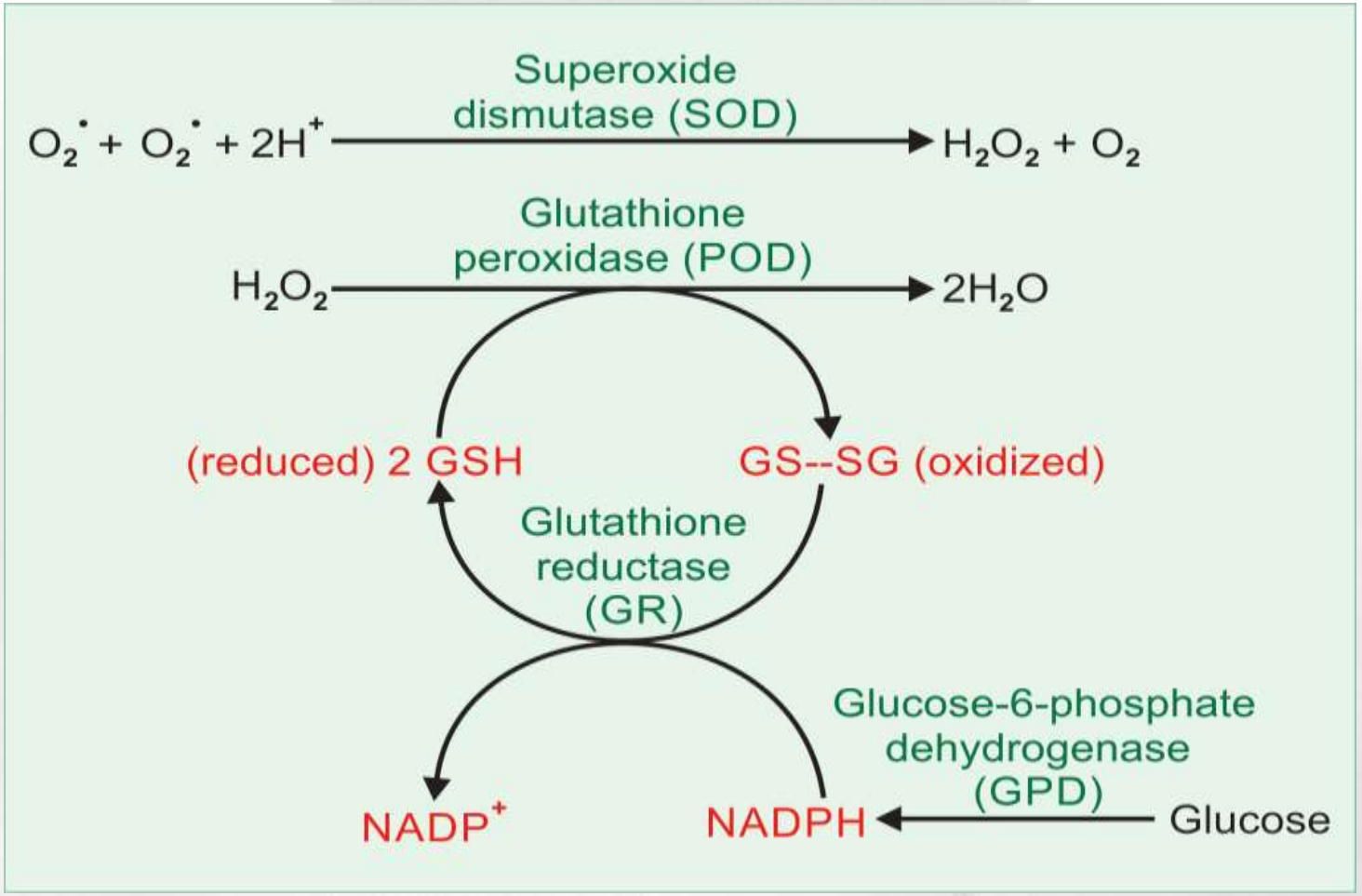
Red blood cells

Lens of eye.

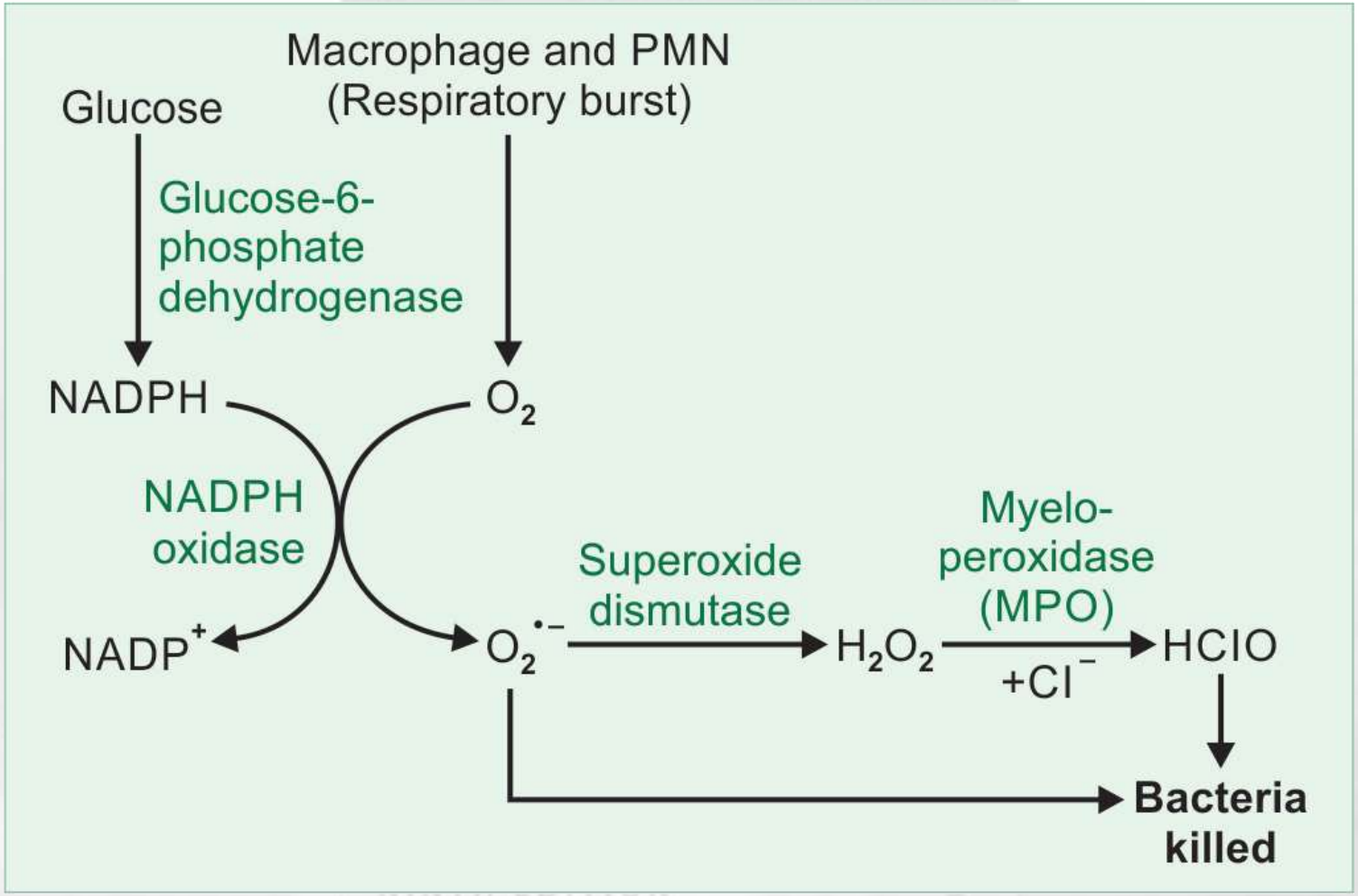
The nonoxidative phase is present in all tissues, and so synthesis of ribose is possible in all tissues of the body.

Significance of Hexose Monophosphate Shunt Pathway

1. To produce NADPH which is required for:
   i. Reductive biosynthesis—fatty acids, cholesterol and steroid hormones
   ii. Free radical scavenging
   iii. Maintain RBC membrane integrity by keeping GSH in reduced state
   iv. Prevention of methemoglobin formation
   v. Detoxification by hydroxylation
   vi. Maintain the transparency of lens
   vii. Bactericidal activity of macrophages
2. To produce ribose and deoxyribose for DNA and RNA synthesis
3. Clinical importance of the shunt pathway
   i. Glucose-6-phosphate dehydrogenase deficiency
   ii. Drug-induced hemolytic anemia
   iii. Methemoglobinemia
   iv. Thiamine deficiency leads to reduced transketolase activity



Free radical scavenging enzymes. GSH: glutathione.



Generation of reactive oxygen species in macrophages

Clinical Significance of Shunt Pathway

GPD Deficiency

The enzyme glucose-6-phosphate dehydrogenase (GPD) may be deficient in some persons. It is the most common enzyme deficiency seen in clinical practice. The defect is transmitted as an **X-linked recessive** trait.

The deficiency will lead to **drug-induced hemolytic anemia**.

The deficiency is manifested only when exposed to certain drugs or toxins, e.g. intake of **antimalarial drugs** like primaquine. Primaquin stimulates peroxide formation inside RBC. In GPD deficient cells, the level of NADPH is low; hence further production of peroxides will lead to cell lysis. Similarly, ingestion of toxic glycosides present in fava beans may have similar effect (**Favism**). **Sulfa drugs** and furadantin may also precipitate the hemolysis. This will lead to jaundice and severe anemia.

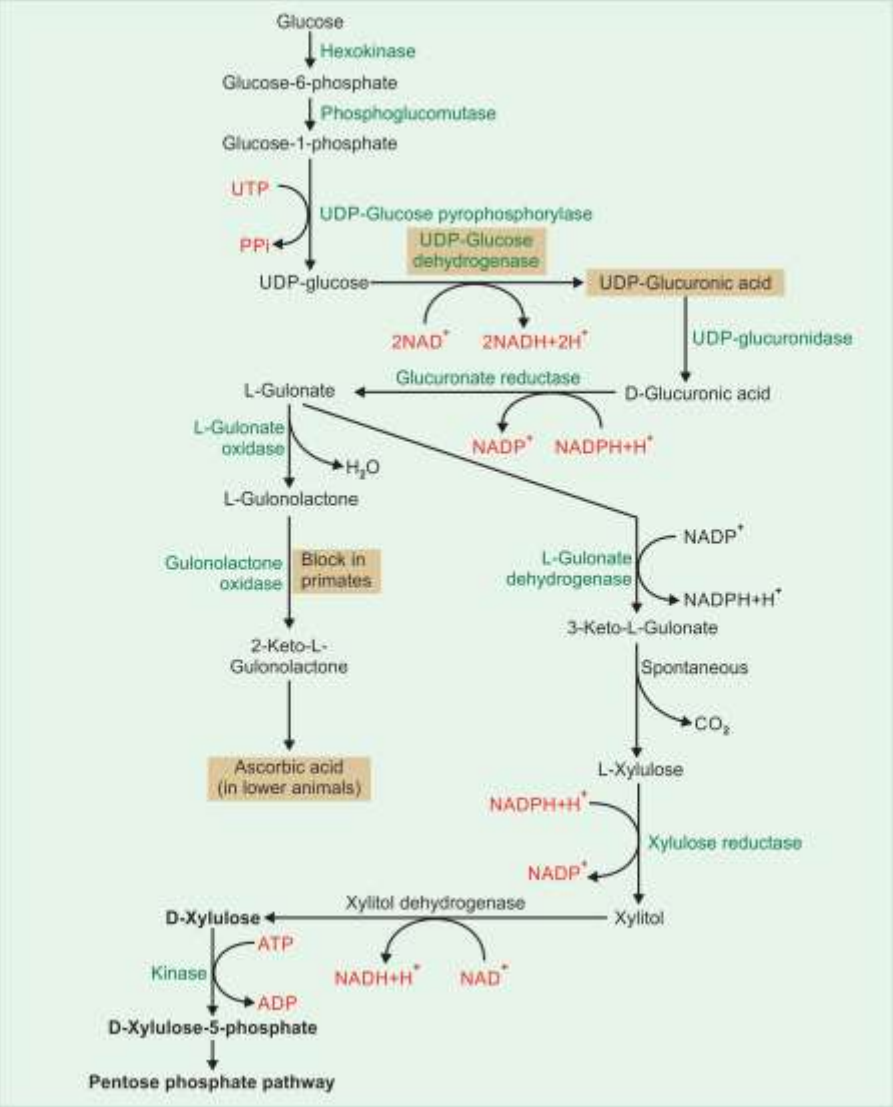
Glucuronic Acid Pathway

It provides **UDP-glucuronic acid**, which is the active form of glucuronic acid. It is used for the following purposes:

- Conjugation of bilirubin
- Conjugation of steroids
- Conjugation of various drugs which will make them more water soluble and more easily excretable.
- Synthesis of glycosaminoglycans (GAG).

Site

The pathway is operating mainly in liver.


Glucose
Hexokinase
Glucose-6-phosphate
Phosphoglucomutase
Glucose-1-phosphate
UTP
UDP-Glucose pyrophosphorylase
PPi
UDP-Glucose dehydrogenase
UDP-glucose
UDP-Glucuronic acid
2NAD+
2NADH+2H+
UDP-glucuronidase
Glucuronate reductase
L-Gulonate
D-Glucuronic acid
L-Gulonate oxidase
NADP+
NADPH+H+
H2O
L-Gulonolactone
NADP+
Gulonolactone oxidase
Block in primates
L-Gulonate dehydrogenase
NADPH+H+
3-Keto-L-Gulonate
2-Keto-L-Gulonolactone
Spontaneous
CO2
Ascorbic acid (in lower animals)
L-Xylulose
NADPH+H+
Xylulose reductase
NADP+
Xylitol dehydrogenase
D-Xylulose
Xylitol
ATP
Kinase
NADH+H+
NAD+
ADP
D-Xylulose-5-phosphate
Pentose phosphate pathway

Vitamin C Synthesis in Lower Animals

The enzyme **L-gulonolactone oxidase is absent in human beings**, primates, guinea pigs and bats. Hence ascorbic acid cannot be synthesized by human beings.

Essential Pentosuria

It is an inborn error of metabolism. In the pathway, L-xylulose is converted to D-xylulose by two enzymes, **xylitol dehydrogenase** and **xylulose reductase**. Absence of any of these enzymes leads to pentosuria.

L-xylulose is excreted in urine and gives a positive Benedict's test. **Barbiturates**, aminopyrine, etc. will induce uronic acid pathway and will increase xylulosuria in such patients. This condition does not produce any harm; but it should be differentiated from diabetes mellitus.

Polyol Pathway of Glucose

Reduction of glucose by aldose reductase to sorbitol, which can then be oxidized to fructose. This would amount to the interconversion of glucose to fructose.

Sorbitol, cannot diffuse out of the cell easily and gets trapped there. Sorbitol is normally present in lens of eyes. But in **diabetes mellitus**, when glucose level is high, the sorbitol concentration also increases in the lens. This leads to osmotic damage of the tissue and development of **cataract**. Galactitol also causes cataract.

Fructose is present in semen in large quantities. It is produced by the polyol pathway. The polyol pathway is active in eye, kidney, peripheral nerves and seminal vesicles. This pathway is inactive in liver.

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