2.5 Pentose phosphate pathway

Hexose monophosphate shunt (HMP) or pentose phosphate pathway or phosphogluconate pathway is an alternative pathway to glucose oxidation. The enzymes of HMP shunt are located in the cytosol and in plastids. Hexose monophosphate shunt has two primary functions:

1. To provide NADPH for reductive biosynthesis and;
2. To provide ribose-5-phosphate for nucleotide and nucleic acid biosynthesis.

The sequence of reactions of HMP shunt is divided into two phases–oxidative and non-oxidative phase.

Oxidative phase (Irreversible)

Glucose 6-phosphate dehydrogenase (G6PD) is an NADP-dependent enzyme that converts glucose 6-phosphate to 6-phosphogluconolactone. The latter is then hydrolysed by the gluconolactonase to 6-phosphogluconate. The next reaction involving the synthesis of NADPH is catalyzed by 6-phosphogluconate dehydrogenase to produce 3-keto 6-phosphogluconate which then undergoes decarboxylation to give ribulose 5-phosphate. The net result of the oxidative phase is generation of 2 moles of NADPH, oxidation of one carbon to CO_2, and synthesis of 1 mole of pentose phosphate.

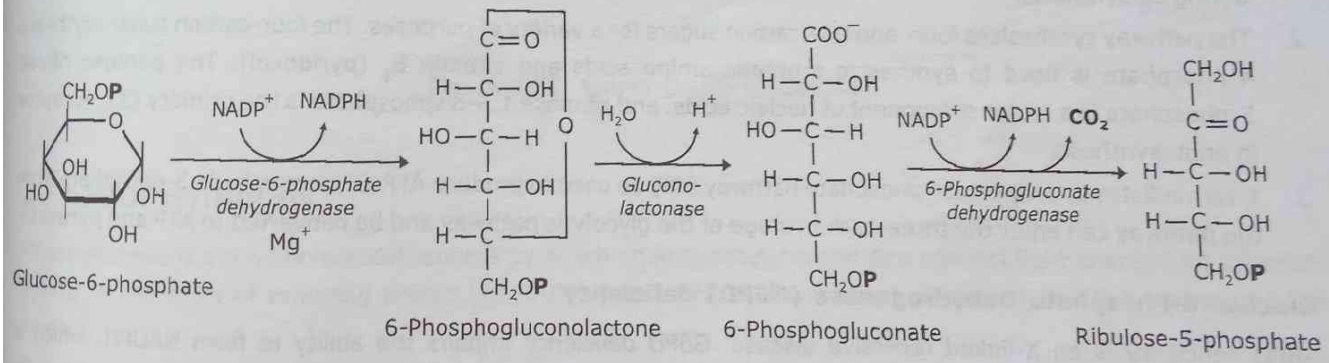


Figure 2.28 Oxidative phase of the pentose phosphate pathway. The three reactions of the oxidative phase include two oxidations, which produce NADPH.

$$3 \times \text{Glucose 6-phosphate} \xrightarrow{6\ NADP^+ \;\; 6\ NADPH} 3 \times \text{Ribulose 5-phosphate} + 3 \times CO_2$$

Note: In pentose phosphate pathway, oxidation of glucose-6-Phosphate utilizes $NADP^+$ rather than NAD^+ and produces CO_2, whereas glycolytic process utilizes NAD^+ and does not produce CO_2. Similarly, no ATP is generated in the pentose phosphate pathway, whereas it is a major product of glycolysis.

Non-oxidative phase (Reversible)

In oxidative phase, three molecules of glucose-6-phosphate (G6P) give rise to three molecules of CO_2 and three five carbon sugars. These are rearranged to regenerate two molecules of glucose-6-phosphate and one molecule of glyceraldehyde-3-phosphate (G3P) in non-oxidative phase.

The non-oxidative reactions are concerned with the interconversion of three, four, five and seven carbon monosaccharides. Ribulose 5-phosphate (Ru5P) is acted upon by an *epimerase* to produce xylulose 5-phosphate (Xu5P) while *phospho-pentose isomerase* converts ribulose 5-phosphate to ribose 5- phosphate (R5P). Two enzymes unique to the pentose phosphate pathway act in interconversions of three, four, five and seven carbon monosaccharides: *transketolase* and *transaldolase*. Transketolase and transaldolase catalyze transfer of 2-C and 3-C molecular fragments respectively, in each case from a ketose donor to an aldose acceptor. However, the names of these enzymes should be changed, since transketolase (alternative name glycoaldehyde transferase) actually transfers an aldol moiety and transaldolase actually transfers a ketol moiety. However, the traditional enzyme names are used here. Transketolase employs thiamine pyrophosphate as a coenzyme.

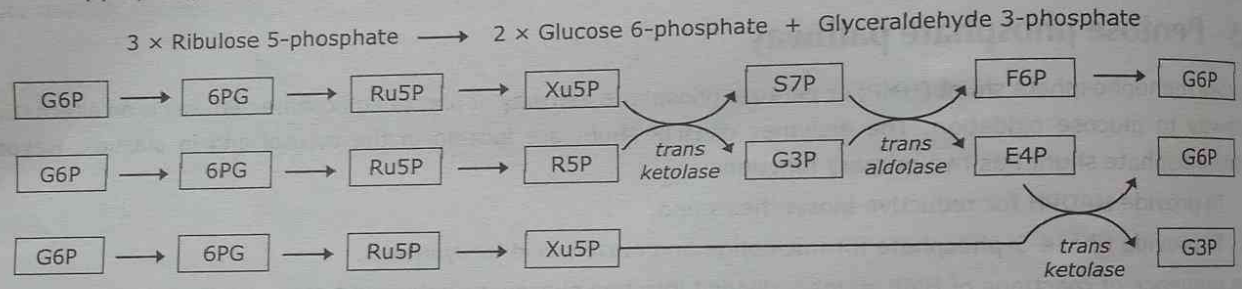


Figure 2.29 Flowchart of pentose phosphate pathway.

G6P (Glucose 6-phosphate), 6PG (6-Phosphogluconate), Ru5P (Ribulose-5-phosphate), Xu5P (Xylulose 5-phosphate), R5P (Ribose 5-phosphate), G3P (Glyceraldehyde 3-phosphate), E4P (Erythrose 4-phosphate), S7P (sedoheptulose 7-phosphate).

Function of pentose phosphate pathways

1. NADPH from the pentose phosphate pathway serves as a source of electrons for the reduction of molecules during biosynthesis.
2. The pathway synthesizes four- and five-carbon sugars for a variety of purposes. The four-carbon sugar erythrose 4-phosphate is used to synthesize aromatic amino acids and vitamin B_6 (pyridoxal). The pentose ribose 5-phosphate is a major component of nucleic acids, and ribulose 1,5-bisphosphate is the primary CO_2 acceptor in photosynthesis.
3. Intermediates in the pentose phosphate pathway may be used to produce ATP. Glyceraldehyde 3-phosphate from the pathway can enter the three-carbon stage of the glycolytic pathway and be converted to ATP and pyruvate.

Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency

G6PD deficiency is an X-linked recessive disease. G6PD deficiency impairs the ability to form NADPH, which is required for several reductive processes in addition to lipid biosynthesis. For example, erythrocyte membrane integrity requires a plentiful supply of reduced glutathione (GSH), a *Cys*-containing tripeptide (γ-glutamyl-cysteinylglycine).

A major function of GSH in the erythrocyte is to eliminate H_2O_2 and organic hydroperoxides. H_2O_2, a toxic product of various oxidative processes, reacts with double bonds in the fatty acid residues of the erythrocyte cell membrane to form organic hydroperoxides. These, in turn, result in premature cell lysis. Peroxides are eliminated through the action of glutathione peroxidase, yielding glutathione disulfide (GSSG). So, G6PD deficiency results in hemolytic anemia caused by the inability to detoxify oxidizing agents.

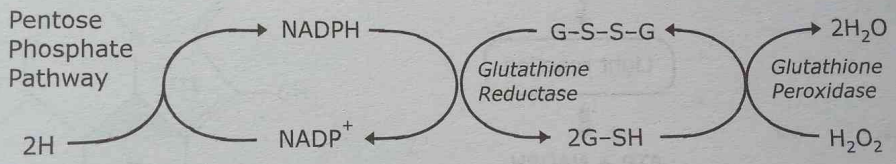


Figure 2.30 Role of the pentose phosphate pathway in the reduction of oxidized glutathione.

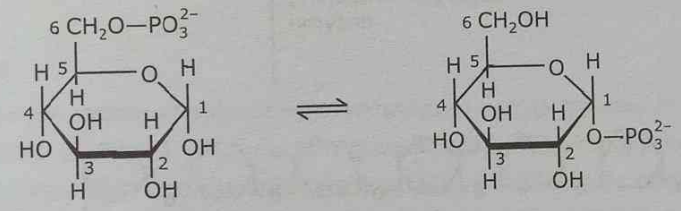
2.9.2 Glycogen metabolism

Glycogen is a highly branched, very large polymer of glucose molecules linked along its main line by α-1,4-glycosidic linkages; branches arise by α-1,6-glycosidic bonds at about every tenth residue. Glycogen occurs in the cytosol as granules, which also contain the enzymes that catalyze its formation and use.

Muscle and liver are the major sites for the storage of glycogen, and although its concentration in the liver is higher, the much greater mass of skeletal muscle stores a greater total amount of glycogen. Liver can mobilize its glycogen for the release of glucose to the rest of the body, but muscle can only use its glycogen for its own energy needs.

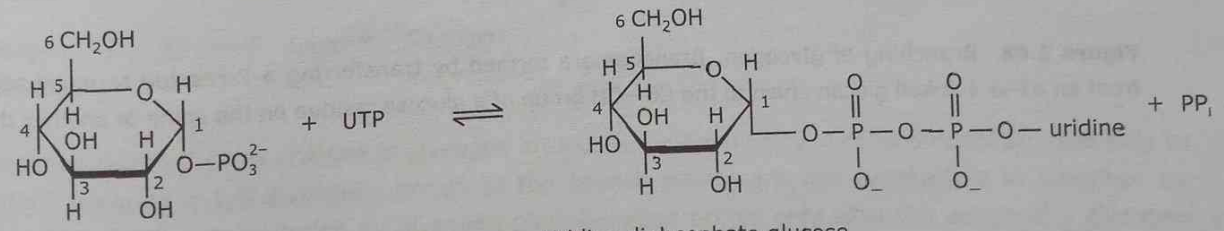
Glycogen synthesis (Glycogenesis)

Glycogen is synthesized from glucose 6-phosphate (G6P) mainly in the muscle and liver and stored within these tissues as glycogen granules. The first step in glycogen synthesis is the formation of glucose 1-phosphate (G1P), catalyzed by *phosphoglucomutase*. G6P is isomerized to glucose 1-phosphate.



Glucose 6-phosphate ⇌ Glucose 1-phosphate

The glucose 1-phosphate is then activated to enable its incorporation into glycogen. The activated form is uridine diphosphate glucose (UDP-glucose). UDP-glucose acts as a precursor of glycogen and is formed from glucose 1-phosphate and uridine triphosphate (UTP).



Glucose 1-phosphate + UTP ⇌ Uridine diphosphate glucose + PP_i

The reaction is catalyzed by *UDP-glucose pyrophosphorylase*. The C-1 carbon of the glucosyl unit being esterified to the diphosphate moiety of UDP.

Action of glycogen synthase

New glucosyl units are added to the non-reducing terminal residues of glycogen. The activated glucosyl unit, UDP-glucose is transferred to the hydroxyl group at a C-4 terminus of glycogen to form an α-1,4-glycosidic linkage. In the process of elongation, UDP is displaced by the terminal hydroxyl group of the growing glycogen molecule. This reaction is catalyzed by *glycogen synthase*, the key regulatory enzyme in glycogen synthesis.

$$\text{UDP-glucose} + \underset{N \text{ residues}}{\text{Glycogen}} \longrightarrow \text{UDP} + \underset{N+1 \text{ residues}}{\text{Glycogen}}$$

Glycogen synthase can add glucosyl residues only if the polysaccharide chain already contains more than four residues. Thus, glycogen synthesis requires a *primer*. This priming function is carried out by *glycogenin*, a protein composed of two identical subunits, each bearing an oligosaccharide made up of few glucose units linked by α-1,4 glycosidic linkage. Carbon 1 of the first unit of this chain, the reducing end, is covalently attached to the specific tyrosine in each glycogenin subunit. How is this primer formed? Each subunit of glycogenin catalyzes the addition of few glucose units to its partner in the glycogenin dimer. UDP-glucose is the donor in this autoglycosylation. After the synthesis of oligosaccharide (a primer), glycogen synthase takes over to extend the glycogen molecule.

Formation of branch chains

Glycogen synthase catalyzes only α1→4 glycosidic bond formation to yield α-amylose. Branching is accomplished by a separate enzyme called *branching enzyme*. Branching enzyme (also known as amylo-1,4→1,6 transglycosylase) moves a 7-unit segment of α1→4 residues from a glycogen chain to a C-6 hydroxyl group of a glucosyl residue, that is at least four residues away from an existing branch. Branching increases the solubility of glycogen.

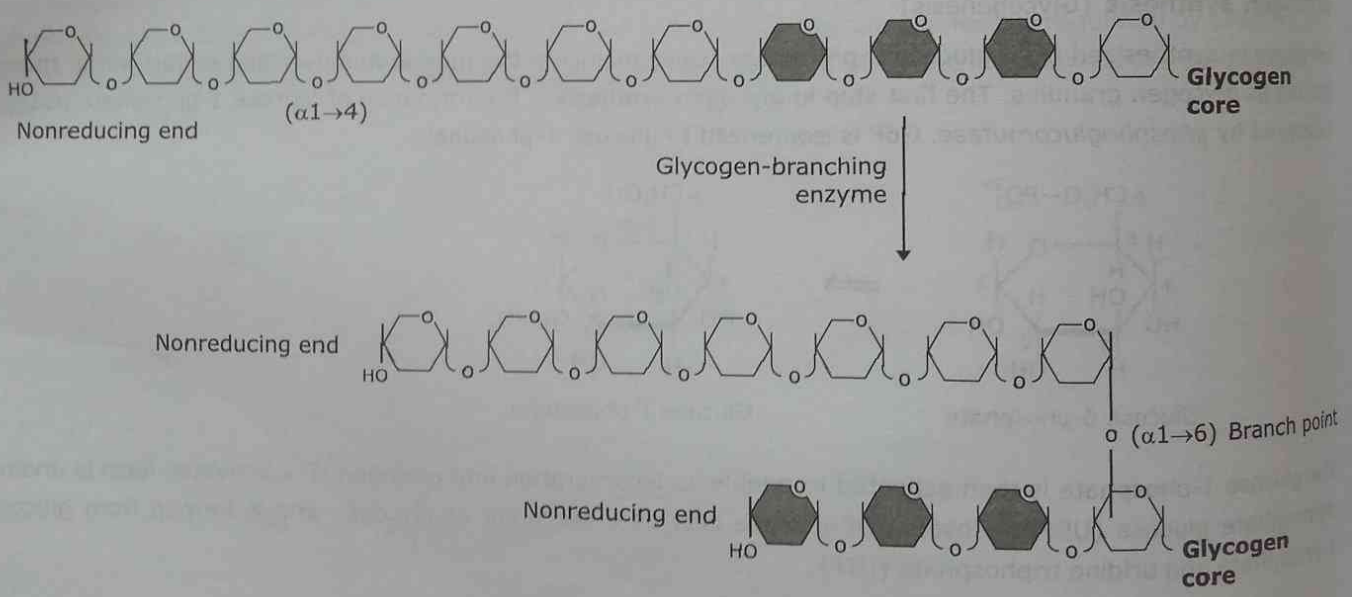


Figure 2.68 Branching of glycogen. Branches are formed by transferring a 7-residue terminal segment from an α1 → 4 linked glucan chain to the C6—OH group of a glucose residue on the same or another chain.

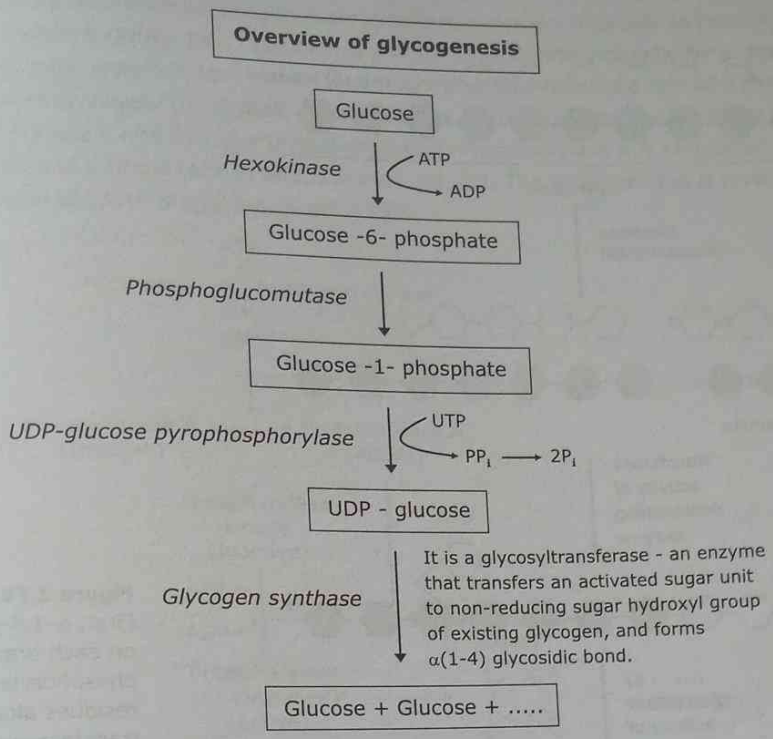


Figure 2.69 An overview of glycogen synthesis in which glucose is activated to UDP glucose that acts as precursor for glycogen.

Glycogenolysis

During glycogenolysis, terminal glucose residue removes as α-D-glucose-1-phosphate (G1P) from the non-reducing end (Phosphorolytic cleavage). This reaction is catalyzed by enzyme *glycogen phosphorylase,* a dimer of identical 842 residue subunits. Glycogen phosphorylase attacks exoglycosidic bonds. *Pyridoxal phosphate is an essential cofactor in the glycogen phosphorylase reaction.* Each catalytic site includes a pyridoxal-phosphate group, linked to lysine^{680} of the enzyme. Its phosphate group acts as a general acid catalyst, promoting attack by P_i on the glycosidic bond. Glycogen phosphorylase acts repetitively on the non-reducing end of glycogen until it reaches a point 4 glucose residues away from an (α1→6) branch point. It is an allosteric enzyme. ATP, G6P and glucose act as allosteric inhibitors and AMP acts as allosteric activator. It is also regulated by covalent modification.

$$\underset{N \text{ residues}}{\text{Glycogen}} + P_i \longrightarrow G1P + \underset{N-1 \text{ residues}}{\text{Glycogen}}$$

Glycogen phosphorylase is the key enzyme in glycogen breakdown but can carry out this process by itself only to a limited extent because α-1,6-glycosidic bonds at the branch points are not susceptible to cleavage by phosphorylase. Thus further degradation by glycogen phosphorylase occurs only after the action of a glycogen debranching enzyme (α1,4 → α1,4) *glucantransferase*, which show two different activities.

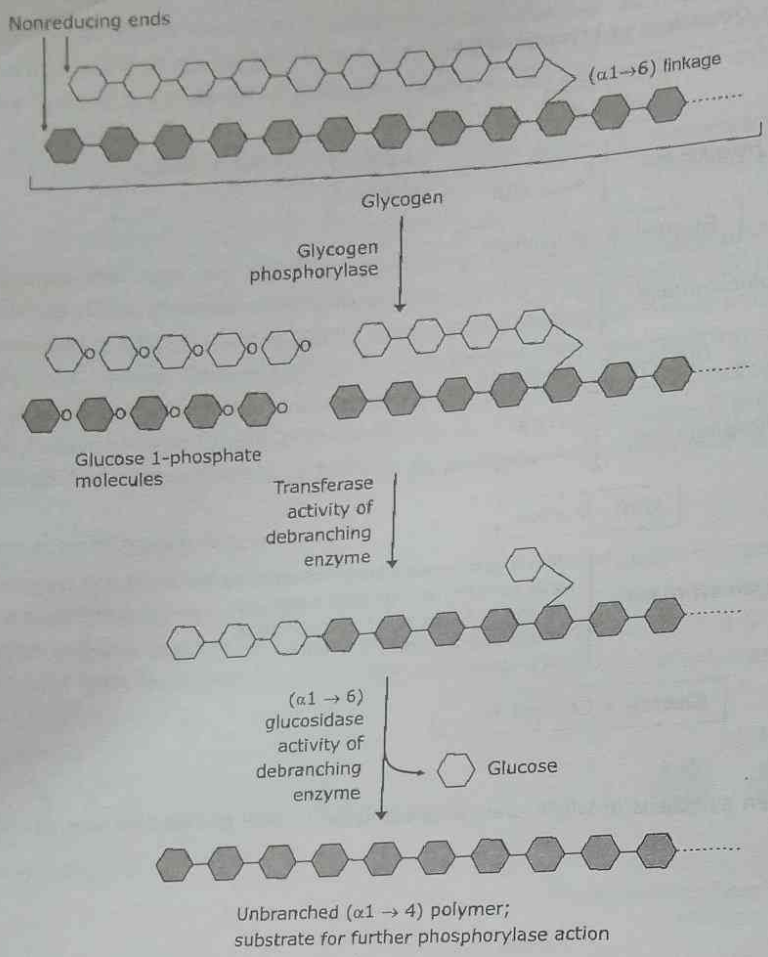


Figure 2.70
First, α-1,4-glycosidic bonds on each branch are cleaved by phosphorylase, leaving four residues along each branch. The transferase shifts a block of three glycosyl residues from one outer branch to the other. The glucose residue is then removed by α-1,6-glucosidase, leaving a linear chain with all alpha-1,4 linkages, suitable for further cleavage by phosphorylase.

First is the *transferase activity*, in which the enzyme removes terminal 3 of remaining glucose residues and transfers this trisaccharide moiety intact to the non-reducing end of another branch.

Second, the remaining glucose residue, which is still attached to the main chain is removed by an α(1→6) *glucosidase activity* of the same debranching enzyme. Thus, about 92% of glycogen's glucose residues are converted to G1P. The remaining about 8%, those at the branch points, are converted to glucose.

Effect of glucagon/epinephrine on glycogenolysis

Glucagon and epinephrine markedly stimulate the breakdown of glycogen in muscle and liver. The liver is more responsive to glucagon whereas muscle is more to epinephrine. Glycogen phosphorylase, a key enzyme involved in glycogenolysis, is regulated by reversible phosphorylation, which is responsive to hormones such as epinephrine and glucagon.

Glucagon is a polypeptide hormone secreted by the α-cells of the pancreatic islets. Glucagon opposes many of the actions of insulin. Most importantly glucagon acts to maintain blood glucose levels by activation of hepatic glycogenolysis and gluconeogenesis. Glucagon is composed of 29 amino acids arranged in a single polypeptide chain. Unlike insulin, the amino acid sequence of glucagon is the same in all mammalian species examined to date. Glucagon is synthesized as a large precursor molecule that is converted to glucagons through a series of selective proteolytic cleavages.

Epinephrine (adrenaline) is synthesized from the amino acid tyrosine and secreted from the adrenal medulla. It is secreted on stimulation by a sympathetic nervous system for meeting an emergency or stress condition, like

injury, pain, fear, accident, grief, fall in blood pressure etc. Hence called *emergency hormone*. It increases the sugar level in the blood by stimulating glycogenolysis in liver and skeletal muscles. In muscle, epinephrine causes activation of protein kinase A (PKA). PKA promotes glycogen degradation indirectly by phosphorylating and thus activating a kinase, *glycogen phosphorylase kinase*, that in turn phosphorylates and activates *glycogen phosphorylase*, the enzyme that degrades glycogen. The dimeric skeletal muscle *glycogen* phosphorylase exists in two interconvertible forms: *active* phosphorylase *a* and *inactive* phosphorylase *b*. Phosphorylase *b* is converted into phosphorylase *a* when it is phosphorylated at a single serine residue in each subunit. The entire process is reversed when epinephrine is removed and the level of cAMP drops, inactivating PKA.

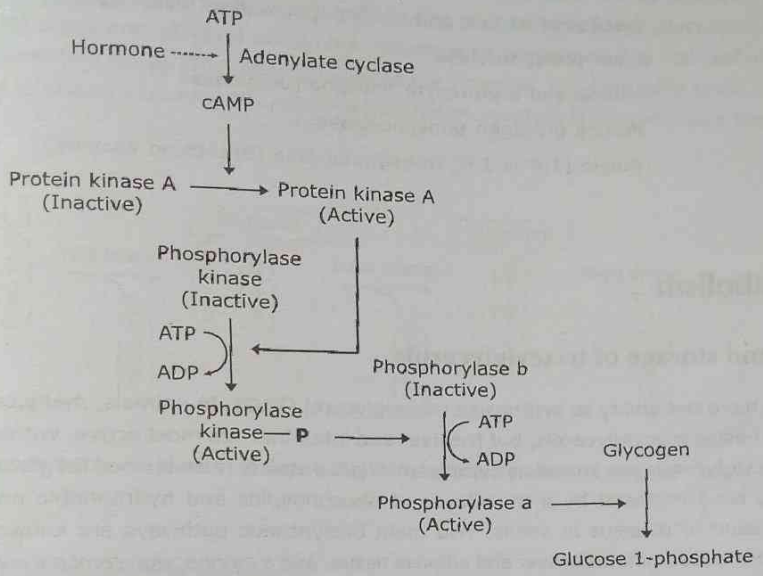


Figure 2.71 The epinephrine-stimulated increase in cAMP and subsequent activation of PKA enhance the conversion of glycogen to glucose 1-phosphate by stimulating glycogen degradation. PKA phosphorylates and thus activates an intermediate kinase, glycogen phosphorylase kinase, that in turn phosphorylates and activates glycogen phosphorylase, the enzyme that degrades glycogen. The entire process is reversed when epinephrine is removed and the level of cAMP drops, inactivating PKA.

Role of insulin in carbohydrate metabolism

Insulin is a polypeptide hormone produced by the beta-cells of the islets of Langerhans. It favors the synthesis of glycogen, triacylglycerols, and protein. Insulin is composed of 51 amino acids arranged in two polypeptide chains, designated A and B, which are linked together by two disulfide bridges.

Insulin acts to *reduce* glucose concentrations in the blood and therefore stimulates the conversion of glucose to fats, proteins, ribulose 5-phosphate and glycogen. In the liver, insulin decreases the production of glucose by inhibiting gluconeogenesis and the breakdown of glycogen. In muscle and liver, insulin increases glycogen synthesis. In muscle, insulin increases glucose uptake by increasing the number of glucose transporters in the cell membrane. Epinephrine and glucagon have the opposite effects to insulin. It acts to *increase the glucose concentration* in the blood. They, therefore, stimulate the conversion of fats, glycogen and pyruvate to glucose.

Table 2.16 Comparisons of effect of insulin, glucagon and epinephrine on carbohydrate metabolism

Pathway	*Effect of Insulin*	*Effect of Glucagon/Epinephrine*
Glycogenesis	Stimulates	Inhibits
Glycogenolysis	Inhibits	Stimulates
Gluconeogenesis	Inhibits	Stimulates
Glycolysis	Stimulates	Inhibits

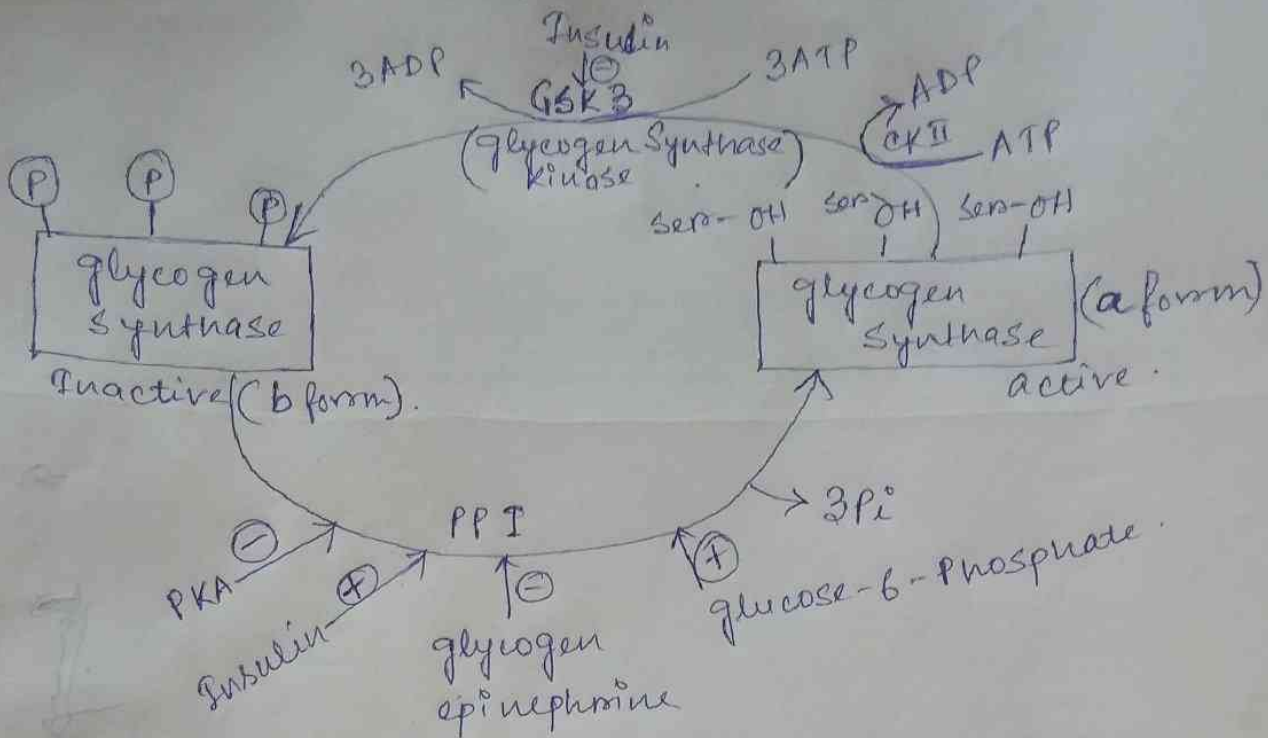
Glycogen storage diseases

Glycogen storage diseases are caused by a genetic deficiency of one or another of the enzymes of glycogen metabolism. Many diseases have been characterized that result from an inherited deficiency of the enzyme. These defects are listed in the table.

Table 2.17 Glycogen storage diseases

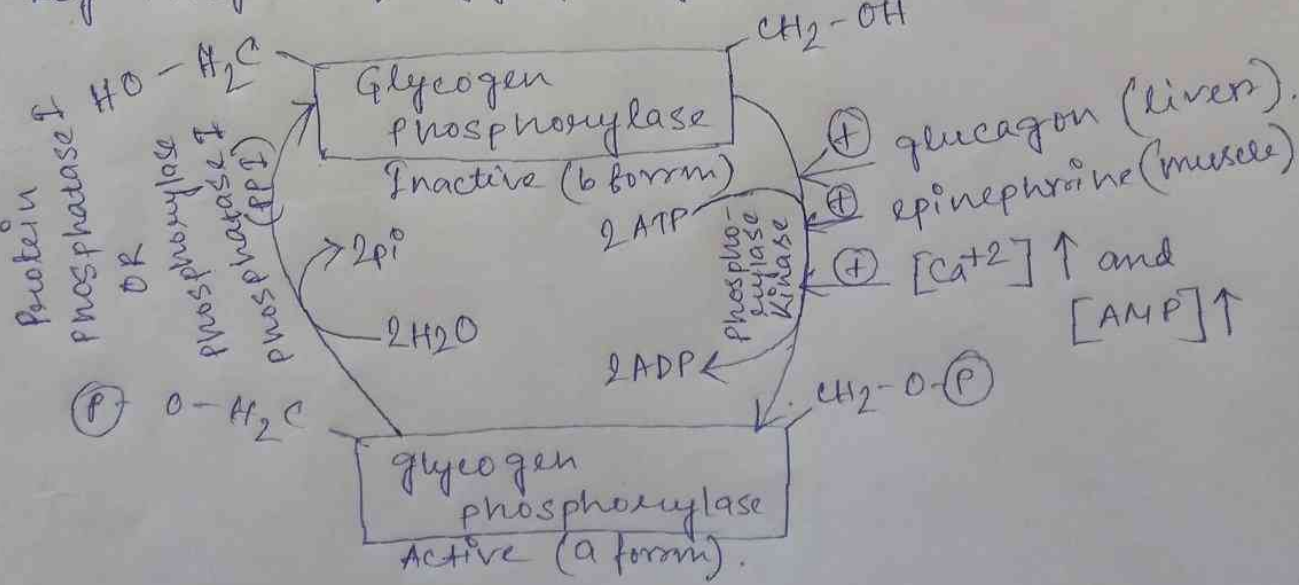
Name	*Enzyme deficiency*
Von Gierke's disease	Liver glucose-6-phosphatase
Pompe's disease	Lysosomal $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$ glucosidase (acid maltase)
Hers' disease	Liver phosphorylase
Tarui's disease	Muscle and erythrocyte phosphofructokinase 1
McArdle's disease	Muscle glycogen phosphorylase
Andersen's disease	Amylo (1,4 → 1,6) transglycosylase (Branching enzyme)

① Glycogen Synthase is regulated by hormones, allosteric controls, and covalent modification.



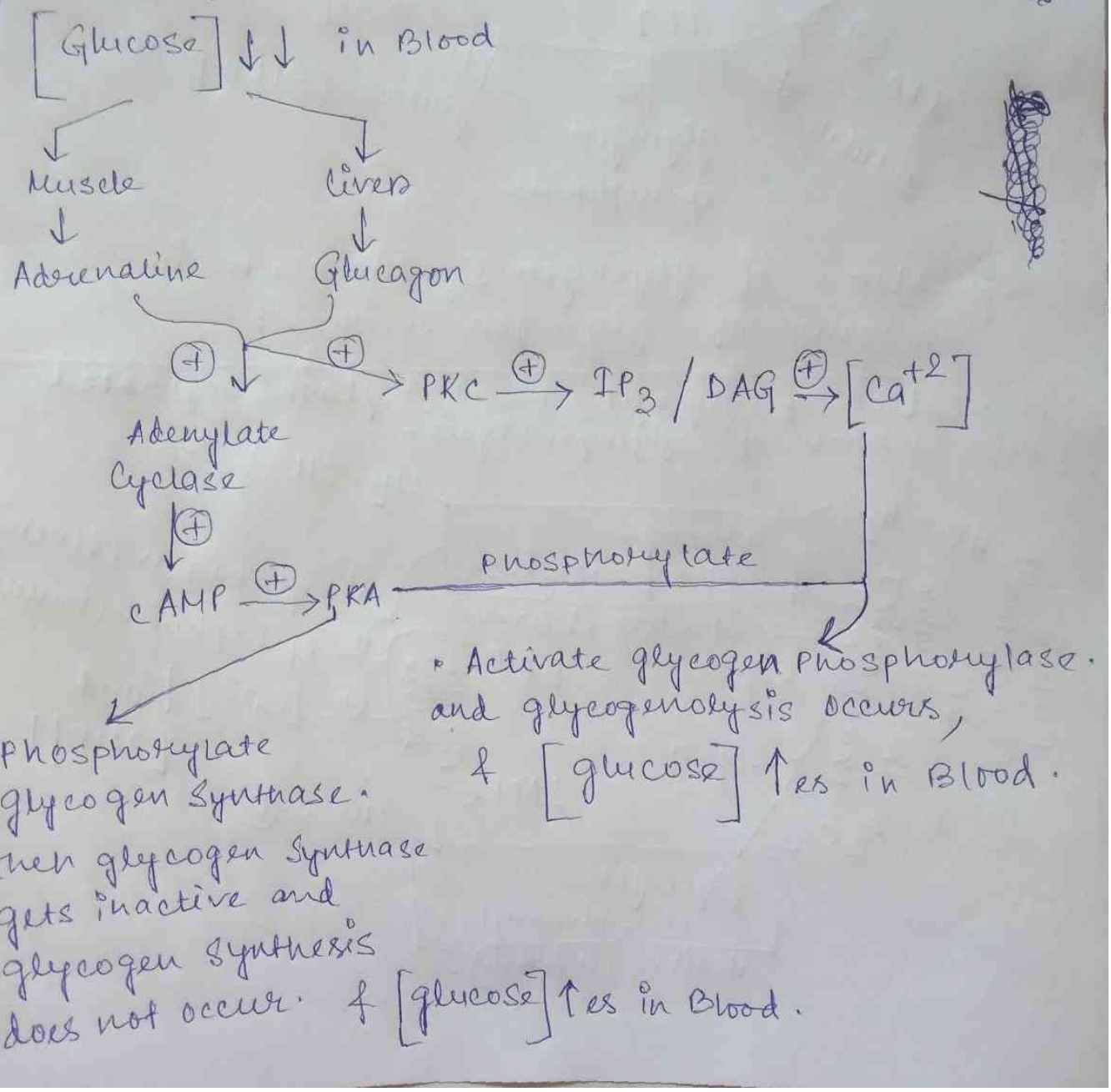
Regulation of glycogen Synthase, main enzyme of glycogen Synthesis.

② Regulation of glycogen phosphorylase, ~~regulated~~ Key enzyme of glycogenolysis —



Blood Glucose Regulation

(1)



(2)

[Glucose] ↑↑es in Blood.

(+) → Insulin → (−) → GSK 3 → glycogen systhase does not get phosphorylated and gets active, then glycogen synthesis occurs.

(−) → Adrenaline & Glucagon → ⁂ → glycogen phosphorylase gets inactive. → glycogen breakdown does not occur.