A study material for M.Sc. Biochemistry (Semester: II) Students

on the topic (CC-6; Unit II)

Glycogen Metabolism

The synthesis and breakdown of our stored carbohydrate

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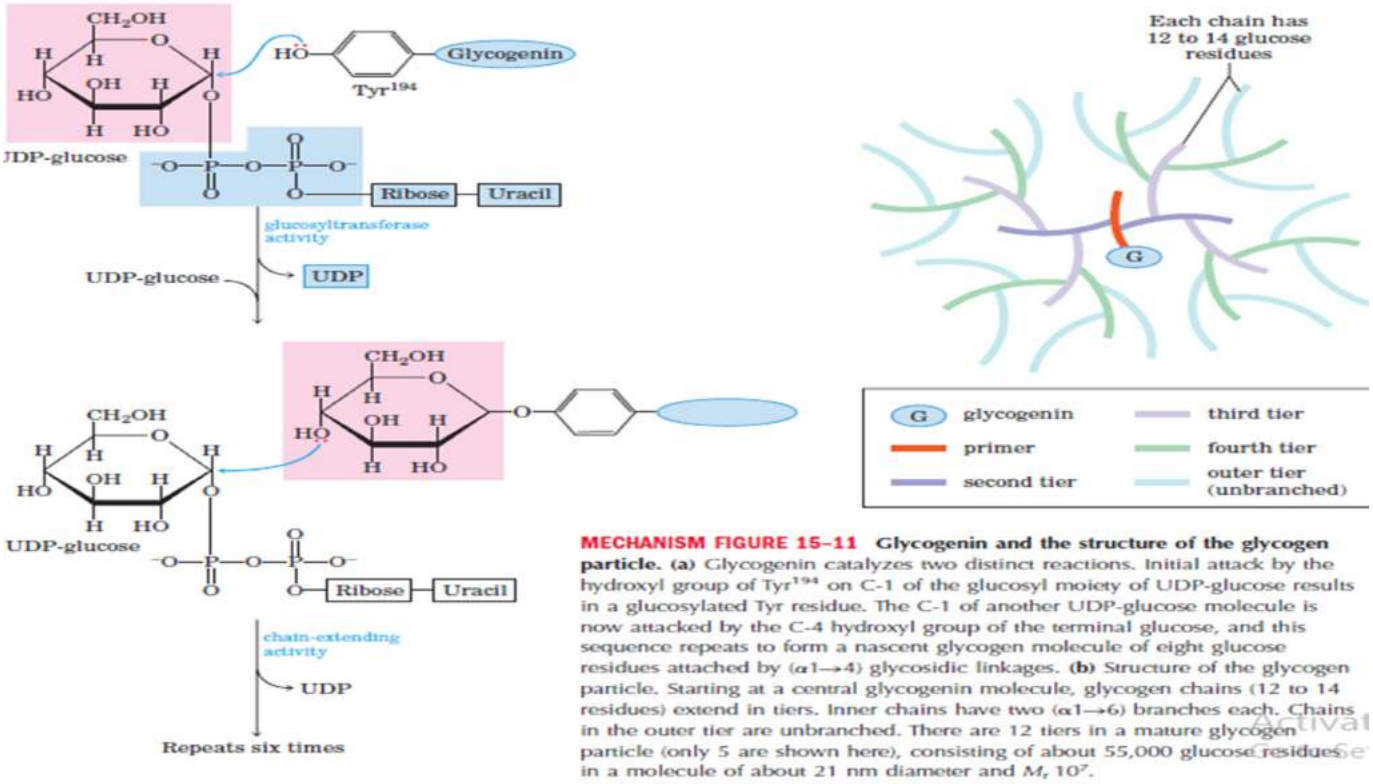
Glycogen: Glycogen is the stored carbohydrate in case of mammals. It is a branched homo – polysaccharide. The monomers of glycogen are glucose. Glycogen acts as energy reserve for short period of time and we can say that it is our currency kept in locker while the fatty acid acts as reserve energy material for a longer period of time and we can say it is our currency kept in bank. For our energy requirements, we are dependent on the amount of glucose running in our blood. If this amount drops, then the breakdown of glycogen takes place and if we have excess energy or after a meal we get too much carbohydrate in our blood, then the synthesis of glycogen takes place. But the breakdown of fat only takes place when the glycogen storage depletes. So we can say that glycogen has an important role to maintain the amount of glucose in our blood or blood glucose homeostasis.

Glycogen contains several branches that helps during breakdown of the glycogen as it provides so many number of reducing ends. Hence the process of breakdown of glycogen becomes fast and when needed the synthesis of glycogen also becomes fast fast due to the presence of several number of non - reducing ends. But as the glycogen is a branched carbohydrate, so we can keep few number of glycogen in a large number of spaces. Therefore, with respect to fat molecule, the amount of glycogen that can be stored is low. It also requires water for its storage. The sites of storage of glycogen are liver and muscles. The synthesis of glycogen from glucose molecule is known as glycogenesis, while the breakdown of glycogen into glucose molecules is known as glycogenolysis. This glycogenesis and glycogenolysis are all together known as glycogen metabolism.



MECHANISM FIGURE 15–11 **Glycogenin and the structure of the glycogen particle.** **(a)** Glycogenin catalyzes two distinct reactions. Initial attack by the hydroxyl group of Tyr^{194} on C-1 of the glucosyl moiety of UDP-glucose results in a glucosylated Tyr residue. The C-1 of another UDP-glucose molecule is now attacked by the C-4 hydroxyl group of the terminal glucose, and this sequence repeats to form a nascent glycogen molecule of eight glucose residues attached by (α1→4) glycosidic linkages. **(b)** Structure of the glycogen particle. Starting at a central glycogenin molecule, glycogen chains (12 to 14 residues) extend in tiers. Inner chains have two (α1→6) branches each. Chains in the outer tier are unbranched. There are 12 tiers in a mature glycogen particle (only 5 are shown here), consisting of about 55,000 glucose residues in a molecule of about 21 nm diameter and M_r 10^7.

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

Let us discuss one by one:

Glycogenesis

Glycogenesis or glycogen synthesis is divided into seven steps:

1. First step is the conversion of glucose into glucose 6 phosphate. We have already studied in glycolysis that this process takes place with the help of the enzyme Hexokinase. There are several isozymes of hexokinase. Hexokinase I and II are found in muscle while hexokinase IV is found in liver. This process utilizes ATP. Glucose 6 phosphate may have more than one fates.

2. With the help of enzyme Phospho-glucomutase, the conversion of Glucose 6 phosphate into Glucose 1 phosphate takes place. Now, this Glucose 1 phosphate acts as precursor of glycogen. Hence, the conversion of Glucose 6 phosphate into Glucose 1 phosphate takes it in the direction of glycogenesis. It is committing step of Glycogenesis.

3. Later on Glucose 1 phosphate convert into UDP-Glucose with the help the enzyme UDP-Glucose phosphorylase. This uses one UTP per glucose molecule. Hence we can say that, the glycogen synthesis is an expensive process.

   The formation of nucleotide - glucose makes this process irreversible. During this process Inorganic Pyro-phosphate (PPi) is removed and this is highly exergonic process. The ΔG^0 of this reaction is -19.2 KJ/mole. This integration of Nucleotide in glucose molecule has several benefits, such as it pulls the reaction in forward direction. The nucleotides sugar associate with the enzymes is an excellent living group hence it facilitates nucleophilic attack on sugar carbon and by tagging some hexose with nucleotidyl group cell can set them aside in a pool for one purpose that is glycogen synthesis.

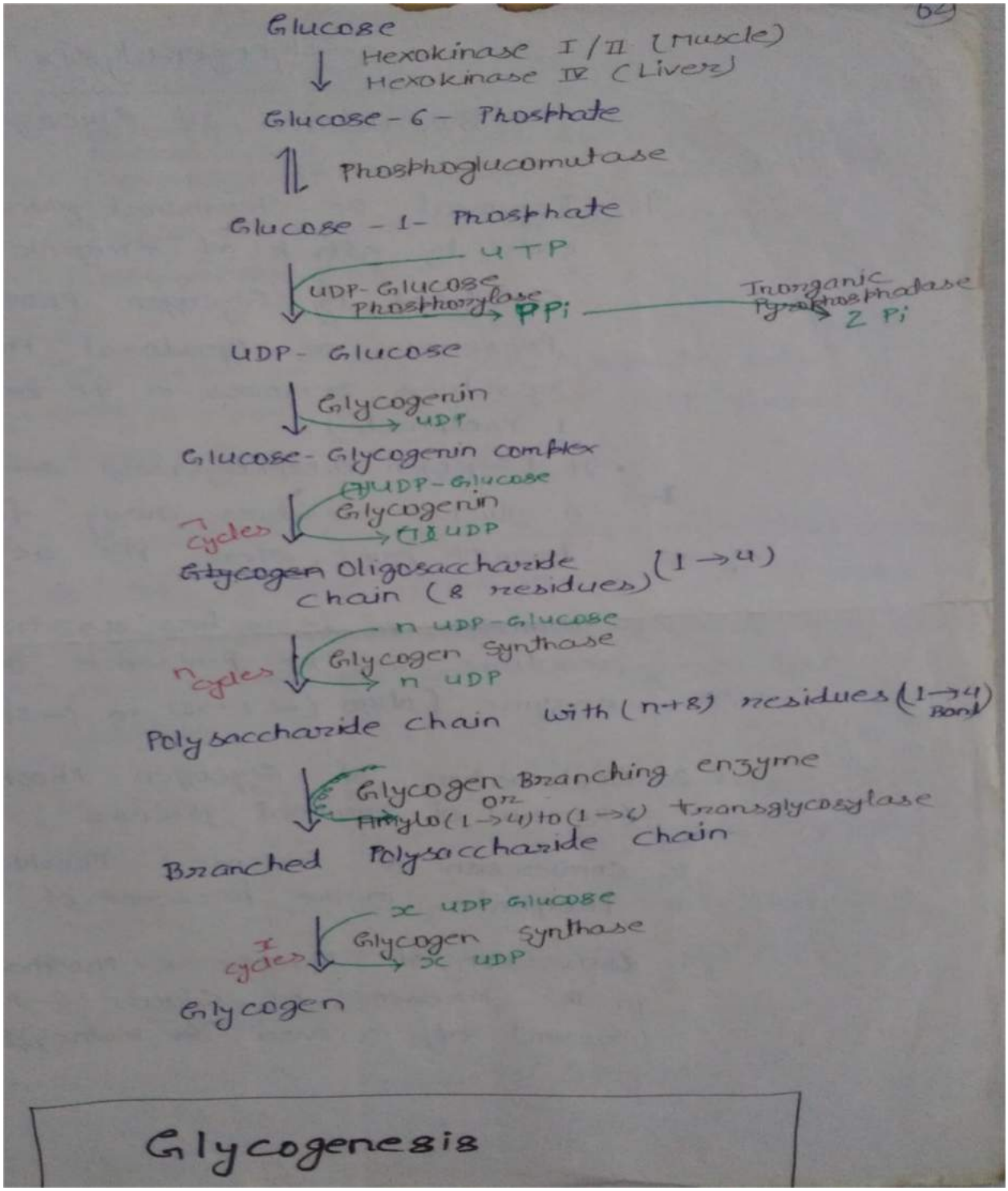
4. Now, the main enzyme of glycogen synthesis is glycogen synthase but it has a drawback. It cannot synthesize the glycogen De-novo, that is, it cannot synthesize from the starting point. It needs a primer and here the function of the primer and the initial extension of the chain is done by another enzyme. This enzyme is Glycogenin.

   This glycogenin act both as primer and as a base on which the initial synthesis of glycogen starts and its extension takes place. It adds glucose from one UDP-Glucose to its Tyrosine Residue (at number 194). In next step it transfer glucose molecule from UDP - Glucose to the first glucose molecule and so on, up to 8 residues.

5. When the initial glycogen chain forms of eight glucose residues, then this chain is transfered to another enzyme, that is, the main enzyme of glycogen synthesis Glycogen Synthase. Now, the glycogen synthase transfer several glucose molecules from UDP-Glucose to the non - reducing end of the glycogen molecule.

6. One more enzyme is involved in the process that is Glycogen Branching Enzyme. This is also known as Amylo α (1→4) to α (1→6) transglycosylase or Glycosyl (4→ 6) transferase. This enzyme transfers terminal fragment of 6 to 7 glucose residues from the non - reducing end on a glycogen having at least 11 residues. It breaks α (1→4) Bond and transfer it at α (1→6) hydroxyl group of a glucose residue and does it creates a new branch.

7. Now, again glycogen synthase adds up glucose residue at both non reducing ends that is one at the main chain and another at the branched chain. After sometime another branch can be created either in the main chain or the pre-existing branch. The biological cause of branching is to make the glycogen molecule more soluble and to increase the number of non-reducing ends, so that the rate of its synthesis and breakdown process maybe enhanced.



Glycogenolysis

Glycogenolysis is the breakdown of glycogen in glucose molecules. It takes place in three steps. There are two enzymes involved in the whole process, one is Glycogen Phosphorylase which is the main enzyme for the breakdown of glycogen and the another enzyme is the Branching Enzyme.

1. The first step is the removal of terminal glucose at the non-reducing ends by attack of inorganic phosphate. This reaction is catalysed by the enzyme glycogen phosphorylase, in the presence of pyridoxal phosphate. The glucose molecules removes are in the form of α-D-Glucose 1 phosphate.

   There are several non - reducing ends in a glycogen molecule and hence this process starts simultaneously from all the non - reducing ends. This point validates that as much as the number of branches in glycogen has, it can breakdown with a much faster speed and hence it can fulfill the glucose demand of the blood quickly.

2. Now, by removing glucose molecules from non - reducing ends the Glycogen Phosphorylase reaches at a point, which is four glucose residue away from the branch point. At this step, the action of Glycogen Phosphorylase stops. Now, the transfer of branch is done with the help of the Branching Enzyme that is Oligo α (1→6) to α (1→ 4) glucose transferase in 2 steps.

   a. First step is the transferase activity when the De-branching enzyme transfers three glucose residues from the branch to its main chain.

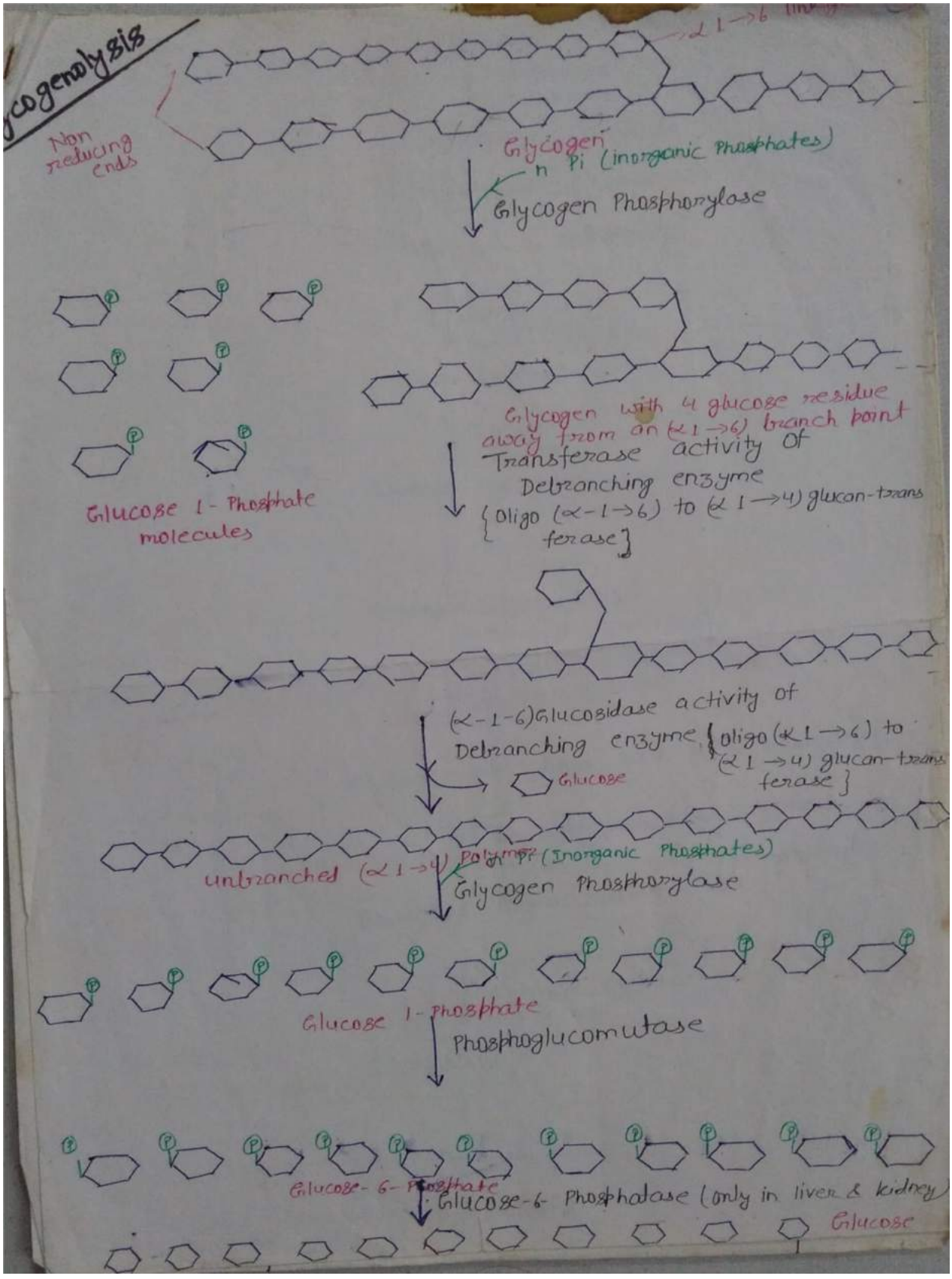
   b. In second step at the branch point, the only remaining glucose molecule at the branch is removed by the glucosidase activity of the de-branching enzyme.

3. Again Glycogen Phosphorylase breaks glucose molecules from the non - reducing ends till the next branch point comes. In this way several Glucose 1 phosphate molecules are released. Now these Glucose 1 phosphate molecules are converted back into Glucose 6 phosphate molecule with the help of the enzyme phosphoglucomutase.

At last, if necessary, the Glucose 6 phosphate molecule can convert into glucose molecules only in liver or kidney with the help of the enzyme Glucose 6 phosphatase and the glucose molecules can now be sent in the bloodstream.

The whole process can be illustrated as follows:

ycogenolysis



Conversion of Glucose 6 Phosphate into Glucose:

The glucose 6-phosphate formed from glycogen in skeletal muscle can enter glycolysis and serve as an energy source to support muscle contraction. In liver, glycogen breakdown serves a different purpose: to release glucose into the blood when the blood glucose level drops, as it does between meals. This requires an enzyme, glucose 6-phosphatase, that is present in liver and kidney but not in other tissues. The enzyme is an integral membrane protein of the endoplasmic reticulum, predicted to contain nine trans-membrane helices, with its active site on the lumenal side of the ER. Glucose 6-phosphate formed in the cytosol is transported into the ER lumen by a specific transporter (T1) and hydrolyzed at the lumenal surface by the glucose 6-phosphatase. The resulting Pi and glucose are thought to be carried back into the cytosol by two different transporters (T2 and T3), and the glucose leaves the hepatocyte via yet another transporter in the plasma membrane (GLUT2). Notice that by having the active site of glucose 6-phosphatase inside the ER lumen, the cell separates this reaction from the process of glycolysis, which takes place in the cytosol and would be aborted by the action of glucose 6-phosphatase. Because muscle and adipose tissue lack glucose 6-phosphatase, they cannot convert the glucose 6- phosphate formed by glycogen breakdown to glucose, and these tissues therefore do not contribute glucose to the blood.

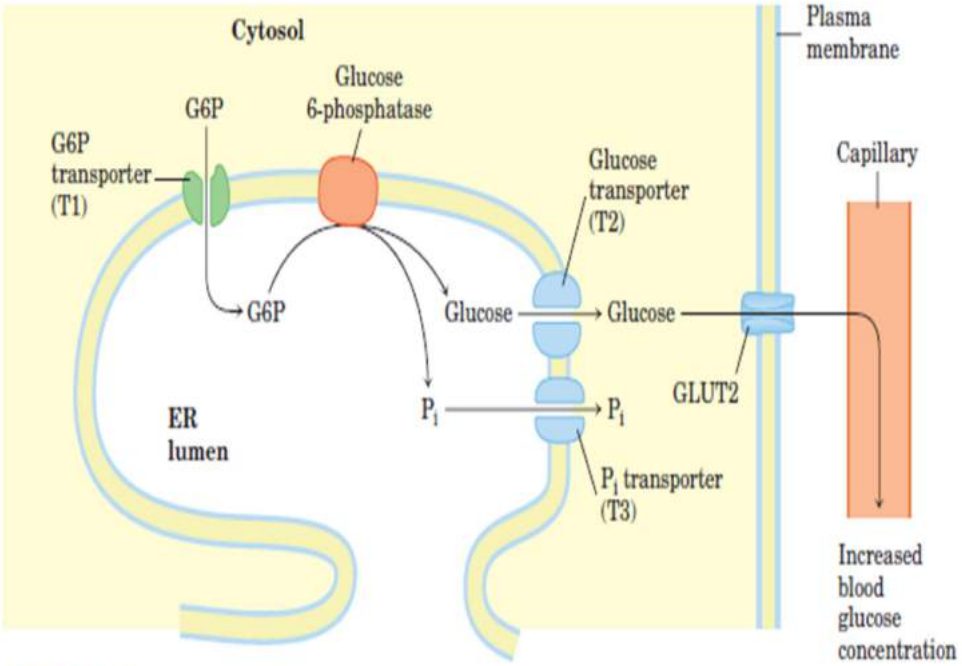


FIGURE 15-6 Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase of the ER. The catalytic site of glucose 6-phosphatase faces the lumen of the ER. A glucose 6-phosphate (G6P) transporter (T1) carries the substrate from the cytosol to the lumen, and the products glucose and P_i pass to the cytosol on specific transporters (T2 and T3). Glucose leaves the cell via the GLUT2 transporter in the plasma membrane.

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

Glycogen Storage Diseases:

Genetic defects in either glucose 6-phosphatase or T1 lead to serious derangement of glycogen metabolism, resulting in type Ia glycogen storage disease. The different defects and different Glycogen Storage diseases caused by them are listed in the table below:

TABLE 1 Glycogen Storage Diseases of Humans

Type (name)	*Enzyme affected*	*Primary organ affected*	*Symptoms*
Type 0	Glycogen synthase	Liver	Low blood glucose, high ketone bodies, early death
Type Ia (von Gierke's)	Glucose 6-phosphatase	Liver	Enlarged liver, kidney failure
Type Ib	Microsomal glucose 6-phosphate translocase	Liver	As in Ia; also high susceptibility to bacterial infections
Type Ic	Microsomal P_i transporter	Liver	As in Ia
Type II (Pompe's)	Lysosomal glucosidase	Skeletal and cardiac muscle	Infantile form: death by age 2; juvenile form: muscle defects (myopathy); adult form: as in muscular dystrophy
Type IIIa (Cori's or Forbes's)	Debranching enzyme	Liver, skeletal and cardiac muscle	Enlarged liver in infants; myopathy
Type IIIb	Liver debranching enzyme (muscle enzyme normal)	Liver	Enlarged liver in infants
Type IV (Andersen's)	Branching enzyme	Liver, skeletal muscle	Enlarged liver and spleen, myoglobin in urine
Type V (McArdle's)	Muscle phosphorylase	Skeletal muscle	Exercise-induced cramps and pain; myoglobin in urine
Type VI (Hers's)	Liver phosphorylase	Liver	Enlarged liver
Type VII (Tarui's)	Muscle PFK-1	Muscle, erythrocytes	As in V; also hemolytic anemia
Type VIb, VIII, or IX	Phosphorylase kinase	Liver, leukocytes, muscle	Enlarged liver
Type XI (Fanconi-Bickel)	Glucose transporter (GLUT2)	Liver	Failure to thrive, enlarged liver, rickets, kidney dysfunction

(List Source: Lehninger's Biochemistry, Fifth Edition)

Regulation of Glycogen Metabolism

The regulation of glycogen metabolism is associated with the regulation of carbohydrate metabolism as well as the regulation of fatty acid metabolism. They cannot be separated. Let us take the example of blood glucose level, if the level of glucose in blood drops down glycogen breakdown starts, glycogen maintains the level of glucose in the blood by releasing glucose molecules. If all the reserve glycogen depletes, then the breakdown of fatty acid starts and our body switches over to fatty acid for its energy needs.

Now, after a meal when blood glucose level elevates then the synthesis of glycogen starts and if there is excess energy and excess amount of glucose is present in the blood then fatty acid synthesis starts. But at any time the two antagonistic processes cannot take place simultaneously suppose the cell is in in a need of energy, so the glucose has to break down through glycolysis and as the glucose is breaking down so the storage of glucose in the form of glycogen cannot take place. Glycolysis (at high rate) along with glycogen synthesis cannot take place simultaneously.

Hence, when the cells are in a need of glucose then glycogenesis and fat synthesis cannot take place. So, we can say that they all are connected. Now, in this situation strong regulation over the cycles is necessary. We cannot separate the regulation of glycogen with the metabolism of Carbohydrate (Glucose) and lipids, but on a broad spectrum, we can say that the glycogen is regulated by three methods:

1. Enzymatic regulation
2. Hormonal regulation
3. Regulation by different ions such as calcium

1. Enzymatic regulation

One of the famous examples of enzymatic regulation of glycogen metabolism is the activation and deactivation of glycogen phosphorylase enzyme. Glycogen phosphorylase or popularly written as phosphorylase is found in two forms: glycogen phosphorylase a and glycogen phosphorylase b. Glycogen phosphorylase b is less active while glycogen phosphorylase a is its active form. Glycogen phosphorylase b convertes into Glycogen phosphorylase a through phosphorylation.

Phosphorylation and dephosphorylation are the basic way of enzymes activation and deactivation. When a phosphate group is added on the enzyme it becomes phosphorylated and active and this phosphate group is added by another enzyme Phosphorylase b kinase. It converts Glycogen phosphorylase in its active form, if glycogenolysis is needed and if it is not needed then another enzyme Phosphorylase a Phosphatase converts the active Phosphorylate kinase a into inactive Phosphorylase kinase b form.

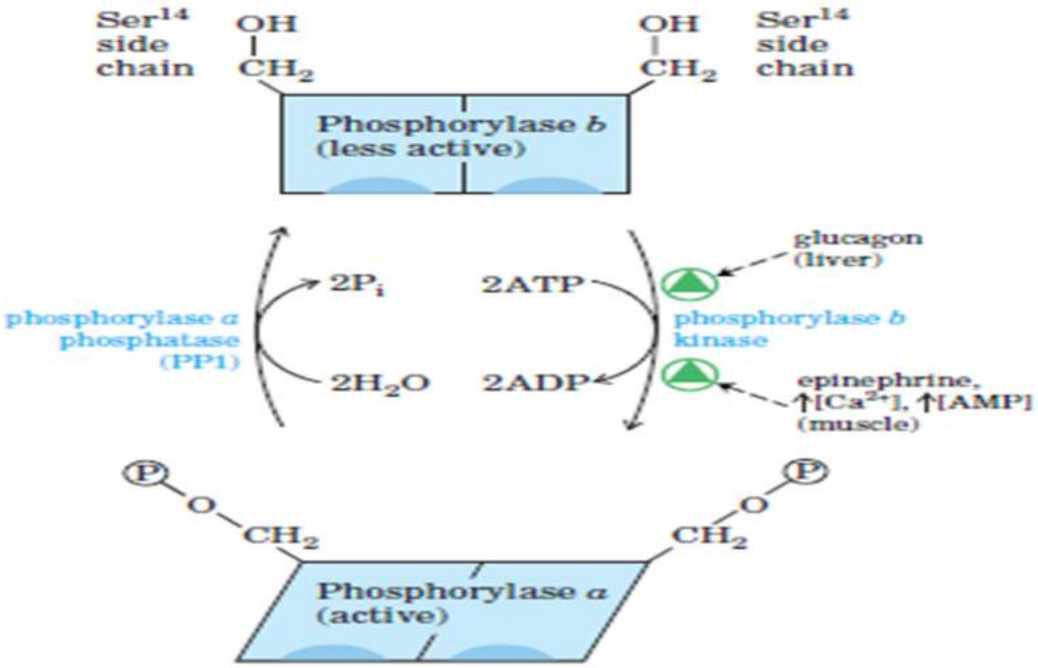


FIGURE 15–24 Regulation of muscle glycogen phosphorylase by covalent modification. In the more active form of the enzyme, phosphorylase *a*, Ser^{14} residues, one on each subunit, are phosphorylated. Phosphorylase *a* is converted to the less active form, phosphorylase *b*, by enzymatic loss of these phosphoryl groups, catalyzed by phosphorylase *a* phosphatase (PP1). Phosphorylase *b* can be reconverted (reactivated) to phosphorylase *a* by the action of phosphorylase *b* kinase.

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

Here we see that the activation and deactivation of glycogen phosphorylated depends on two another enzymes Phosphorylase b kinase and Phosphorylase a Phosphatease. Now, another question arises: who will regulate these two enzymes? When will they activated and inactivated ? How can they know that when they have to activate glycogen phosphorylase or inactivate glycogen phosphorylase? The search of answer of thse questions lead us towards another regulatory mechanism that is hormonal regulation.

Hormonal Regulation

There are two main hormones which regulate glycogen metabolism along with glucose metabolism and fat metabolism, they are insulin and glucagon. They are antagonistic in nature. However another hormone epinephrine has similar function as that of glucagon but its function is much faster. Hormones are biomolecules which are present in very less concentration but are most effective. They control all the metabolic pathways. How glucagon and epinephrine regulate carbohydrate metabolism and glycogen metabolism, let us see this.

The hormones perform their function through a cascade of mechanism. This cascade is the activation of a molecule from another activated molecule and that activated molecule is previously activated by another molecule and this process amplifies the message as given by the hormone for thousands of folds.

The process of activation is depicted in the following figure:

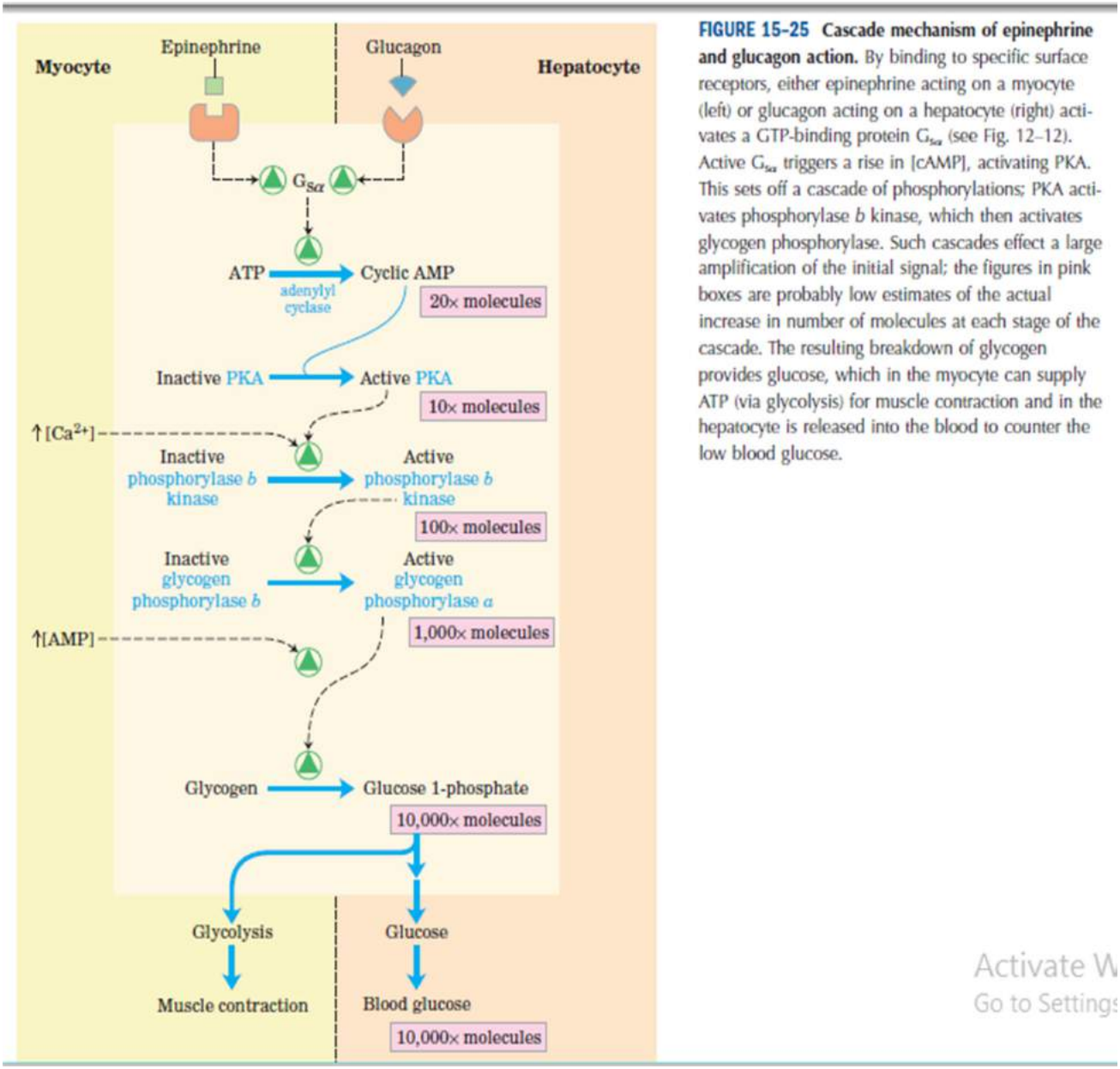


FIGURE 15-25 Cascade mechanism of epinephrine and glucagon action. By binding to specific surface receptors, either epinephrine acting on a myocyte (left) or glucagon acting on a hepatocyte (right) activates a GTP-binding protein $G_{s\alpha}$ (see Fig. 12–12). Active $G_{s\alpha}$ triggers a rise in [cAMP], activating PKA. This sets off a cascade of phosphorylations; PKA activates phosphorylase *b* kinase, which then activates glycogen phosphorylase. Such cascades effect a large amplification of the initial signal; the figures in pink boxes are probably low estimates of the actual increase in number of molecules at each stage of the cascade. The resulting breakdown of glycogen provides glucose, which in the myocyte can supply ATP (via glycolysis) for muscle contraction and in the hepatocyte is released into the blood to counter the low blood glucose.

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

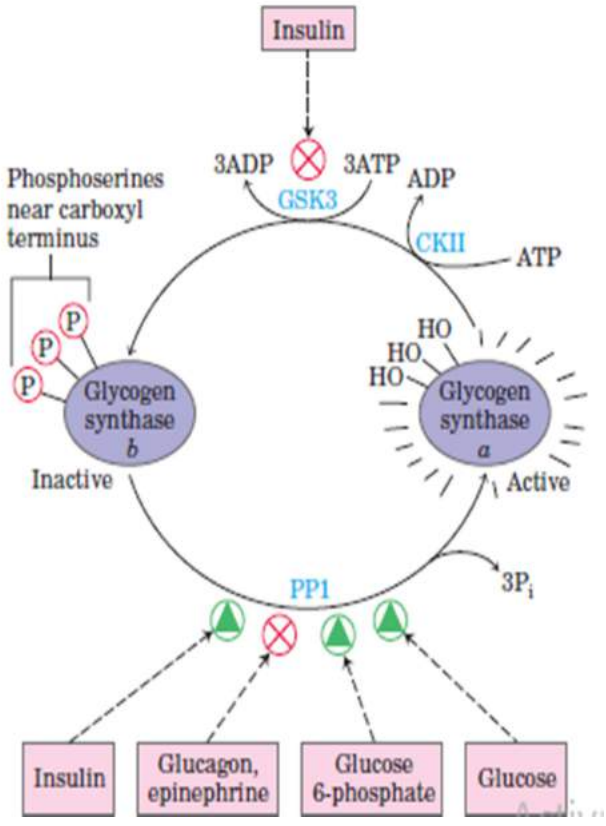
We can see in the above figure that, the receptor of epinephrine or glucagon receives the hormone as their signal through the G protein coupled receptor. The change in G-Protein Coupled Receptor configuration activates an enzyme Adenylyl Cyclase. This adenylyl cyclase converts ATP into cyclic

AMP. Cyclic AMP acts as secondary messenger. As the concentration of cyclic AMP increases in a Cell it further activates the inactive Phosphorylase Kinase a. Activated Phosphorylase kinase a further activates the inactive phosphorylase b kinase. As we have seen earlier that this Phosphorylase Kinase a further, activates Glycogen Phosphorylase b into Glycogen Phosphorylase a and the glycogen phosphorylase a which is its active form performs the glycogenolysis process, i.e., the breakdown of glycogen into glucose molecules. Hence the glucose molecules are liberated in a large amount.

There are several other ions and molecules which further enhance this process, such as concentration of Calcium ions and the concentration of AMP. In the above, figure we can see that the increase in calcium level in myocytes increases the activity of Phosphorylate Kinase and the increased AMP level increases the activity of glycogen phosphorylase. These molecules also plays important role in the activation and deactivation of the enzymes such as glycogen phosphorylase and hence they regulate glycogen metabolism.

Similarly the activation and deactivation of glycogen synthase through different activators and deactivator such as Casein Kinase and different enzymes and different hormones such as Insulin, Glucagon and Epinephrine takes place. This is explained in the following figure:

FIGURE 15-27 Effects of GSK3 on glycogen synthase activity. Glycogen synthase *a*, the active form, has three Ser residues near its carboxyl terminus, which are phosphorylated by glycogen synthase kinase 3 (GSK3). This converts glycogen synthase to the inactive (*b*) form (GS*b*). GSK3 action requires prior phosphorylation (priming) by casein kinase (CKII). Insulin triggers activation of glycogen synthase *b* by blocking the activity of GSK3 (see the pathway for this action in Fig. 12–8) and activating a phosphoprotein phosphatase (PP1 in muscle, another phosphatase in liver). In muscle, epinephrine activates PKA, which phosphorylates the glycogen-targeting protein GM (see Fig. 15–30) on a site that causes dissociation of PP1 from glycogen. Glucose 6-phosphate favors dephosphorylation of glycogen synthase by binding to it and promoting a conformation that is a good substrate for PP1. Glucose also promotes dephosphorylation; the binding of glucose to glycogen phosphorylase *a* forces a conformational change that favors dephosphorylation to glycogen phosphorylase *b*, thus relieving its inhibition of PP1 (see Fig. 15–29).



(Figure Source: Lehninger's Biochemistry, Fifth Edition)

Glycogen synthase is the main enzyme of glycogenesis, that is the synthesis of glycogen. Synthesis of glycogen takes place when the level of glucose is high in our blood and no extra need of energy is there. Glycogen synthase is found in two different forms: one is glycogen synthase a which is active form and another is glycogen synthase b which is inactive form. The inactive form of glycogen synthase b converts into active form glycogen synthase a with the help of enzyme Phosphoprotein Phosphatase, due to de-phosphorylation. This phosphoprotein Phosphatase is further activated by the hormone insulin, while it is inhibited by glucagon and epinephrine. The increased level of glucose and glucose 6 phosphate in the blood also affect positively to this enzyme. Hence when the blood glucose level increases insulin is released and due to these two phenomenons Phosphoprotein Phosphatase becomes active. It activates glycogen synthase b into glycogen synthase a due to de-phosphorylation

In another case, when the Glucose level is very low, glycogen synthase kinase 3 becomes activate and with the help of another enzyme Casein kinase it inactivates glycogen synthase a into glycogen synthase b due to phosphorylation. It uses ATP. Insulin inhibits this glycogen synthase kinase 3 enzyme. It means when Glucose level is high, Insulin is released and one side this insulin activates Phosphoprotein Phosphatase to further active Glycogen synthase and enhance Glycogenesis, while in another side this insulin inhibits Glycogen Synthase Kinase enzyme, so that it is unable to inactivate Glycogen Synthase and in this way Glycogenesis may be enhanced to lower the blood glucose level.

Now, how insulin inactivates Glycogen Synthase Kinase enzyme is depicted in the following figure:

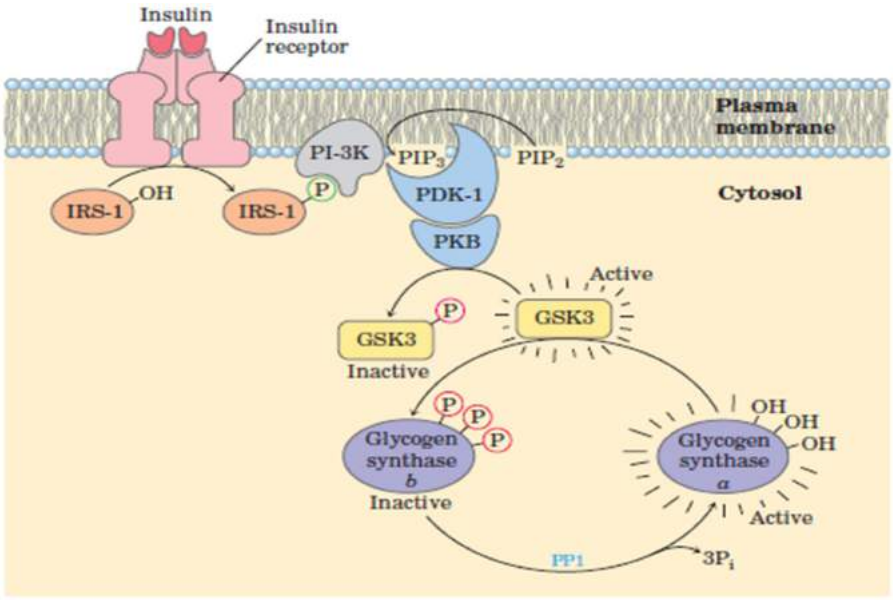


FIGURE 15–29 The path from insulin to GSK3 and glycogen synthase. Insulin binding to its receptor activates a tyrosine protein kinase in the receptor, which phosphorylates insulin receptor substrate-1 (IRS-1). The phosphotyrosine in this protein is then bound by phosphatidylinositol 3-kinase (PI-3K), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) in the membrane to phosphatidylinositol 3,4,5-trisphosphate (PIP_3). A protein kinase (PDK-1) that is activated when bound to PIP_3 activates a second protein kinase (PKB), which phosphorylates glycogen synthase kinase 3 (GSK3) in its pseudosubstrate region, inactivating it by the mechanisms shown in Figure 15–28b. The inactivation of GSK3 allows phosphoprotein phosphatase 1 (PP1) to dephosphorylate glycogen synthase, converting it to its active form. In this way, insulin stimulates glycogen synthesis. (See Fig. 12–8 for more details on insulin action.)

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

Homeostasis of blood glucose level:

We have already learnt that the hormones insulin and glucagon regulate the level of glucose in blood. Insulin activates glycogen synthase and it inactivates glycogen phosphorylase. Hence in one way it enhances the synthesis of glycogen and in another way it stops the glycogen breakdown. So, we can say that when glycogen breakdown stops, that is no further glucose is added and the pre-existing glucose are used up either in glycolysis or in glycogen synthesis. Hence after some time the blood glucose level decreases and comes within the limit.

In second case, when the level of blood glucose dropdown, glucagon is secreted. It uses cyclic AMP as its second messenger, as we have already seen in the previous section. Glucagon slows down the glycolysis process so that the use of glucose in cells can be stopped. It also lowers the glycogen synthesis process and enhances the glycogen breakdown process, so that maximum number of glucose molecules can be available in the blood after the breakdown of glycogen. Due to these processes, the blood glucose level increases and comes within the limit.

The whole process can be depicted in the following figure:

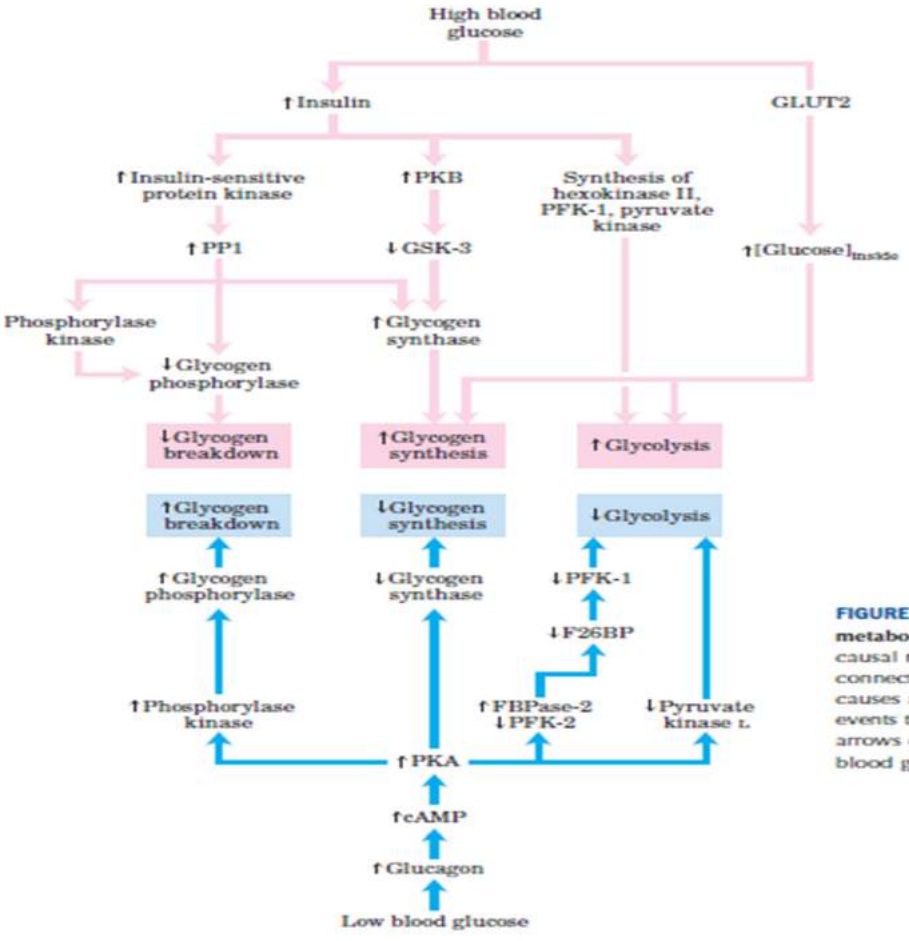


FIGURE 15–31 Regulation of carbohydrate metabolism in the hepatocyte. Arrows indicate causal relationships between the changes they connect. ↓A → ↑B means that a decrease in A causes an increase in B. Pink arrows connect events that result from high blood glucose; blue arrows connect events that result from low blood glucose.

(Figure Source: Lehninger's Biochemistry, Fifth Edition)

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