MSC ZOOLOGY BZ 402- UNIT II: CARBOHYDRATE METABOLISM

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- Glucose is the major form of sugar moiety present in blood and other body fluids. The digestion of food carbohydrates, such as starch, sucrose, and lactose produces the monosaccharides glucose, fructose and galactose, which pass into the blood stream. The study of synthesis (Anabolism) and degradation (Catabolism) of biomolecules is biochemically termed as metabolism.

Anabolism + Catabolism = *Metabolism*

(Synthesis) (Degradation)

- Since glucose is the most important carbohydrate existing in physiological amounts in the body and is easily absorbed from the diet, the metabolism of carbohydrate resolves it self to the study of the metabolism of glucose and its main derivatives. The monosaccharides galactose and fructose are converted to glucose in the liver. All the monosaccharides are completely absorbed in the small intestine.
- The glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body and used for the production of energy. Normally carbohydrate metabolism supplies more than half of the energy requirements of the body. In fact the brain largely depends upon carbohydrate metabolism as a source of energy and quickly ceases to function properly when the blood glucose level falls much below normal.
- The major function of carbohydrate in metabolism is to serve as fuel and get oxidised to provide energy for other metabolic processes. The metabolic intermediates are used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

1. Glycolysis 2. Tricarboxylic acid cycle

GLYCOLYSIS:

- Oxidation of glucose to pyruvate is called ***glycolysis***. It was first described by ***Embden-Meyerho***f and ***Parnas***. Hence it is also called as ***Embden-Meyerh*** of pathway. Glycolysis occurs virtually in all tissues. Erythrocytes and nervous tissues derive the energy mainly from glycolysis. This pathway is unique in the sense that it can proceed in both aerobic (presence of O_2) and anaerobic (absence of O_2) conditions. All the enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.
- The overall equation for glycolysis from glucose to lactate is as follows:

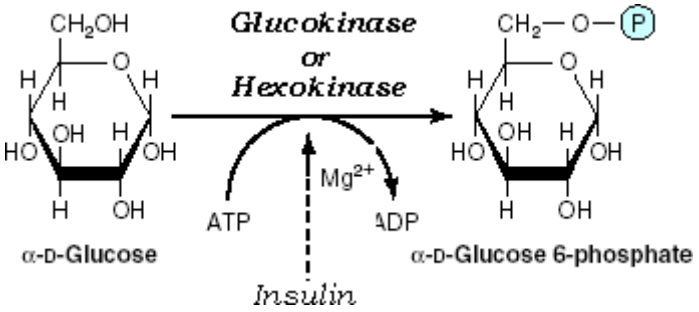
$$\text{Glucose} + 2\text{ADP} + 2\text{P}_i \rightarrow 2\text{L}(+) - \text{Lactate} + 2\text{ATP} + 2\text{H}_2\text{O}$$

Reactions of glycolytic pathway:

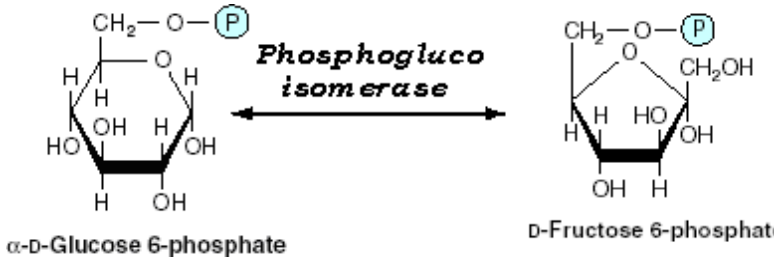
Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

***Stage I*:** This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1, 6-diphosphate by donation of two phosphate groups from ATP.

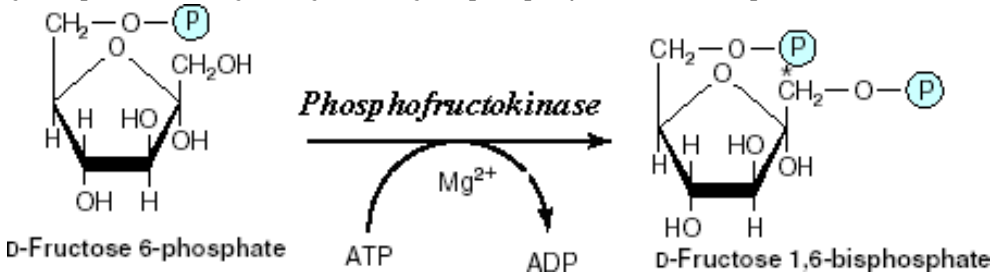
1. ***Uptake of glucose by cells and its phosphorylation:*** Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport. In other tissues ***insulin*** facilitates the uptake of glucose. Glucose is phosphorylated to form ***glucose 6-phosphate***. The enzyme involved in this reaction is *glucokinase or hexokinase*. This reaction is irreversible.



2. ***Conversion of glucose 6-phosphate to fructose 6-phosphate:*** Glucose 6-phosphate is converted to fructose 6-phosphate by the enzyme *phosphogluco isomerase*.

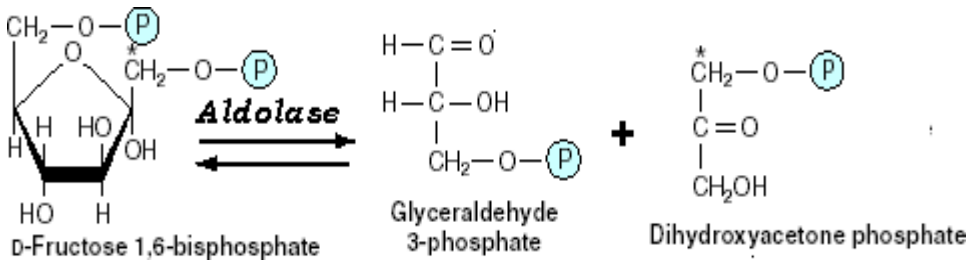


3. ***Conversion of fructose 6-phosphate to fructose 1, 6 diphosphate:*** Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme *phosphofructokinase* to produce fructose1, 6-diphosphate.

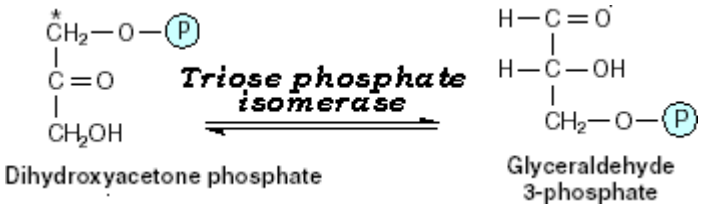


Stage II:

4. ***Actual splitting of fructose 1, 6 diphosphate:*** Fructose 1, 6 diphosphate is split by the enzyme *aldolase* into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one *ketotriose* - dihydroxy acetone phosphate. The reaction is reversible. There is neither expenditure of energy nor formation of ATP.

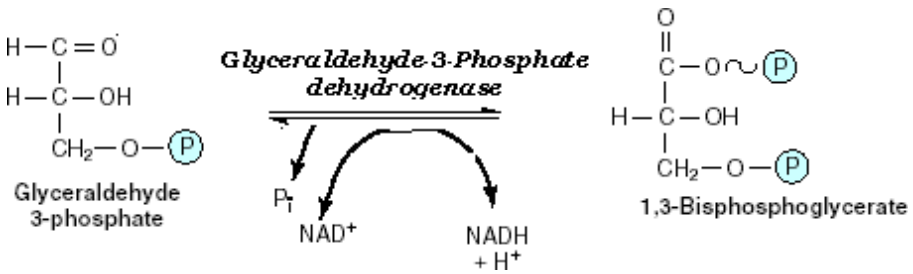


5. ***Interconversion of triose phosphates:*** Both triose phosphates are interconvertible.

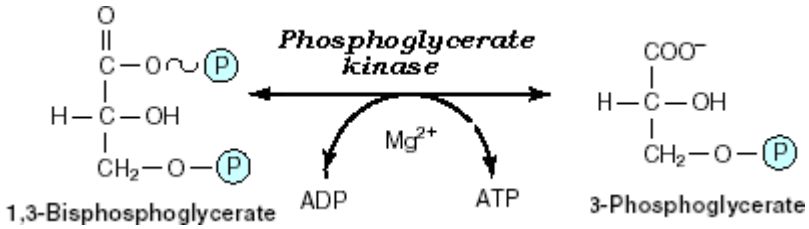


Stage III: It is the energy yielding stage. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

6. ***Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate:*** Glycolysis proceeds by the oxidation of glyceraldehyde 3-phosphate to form 1, 3−bisphosphoglycerate. The reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphate dehydrogenas*.

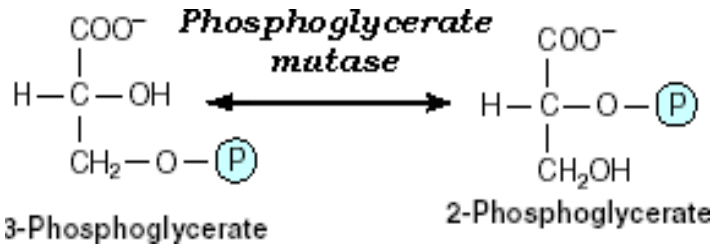


7. ***Conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate:*** The reaction is catalyzed by the enzyme *phosphoglycerate kinase*. The high energy phosphate bond at position-1 is transferred to ADP to form ATP molecule.

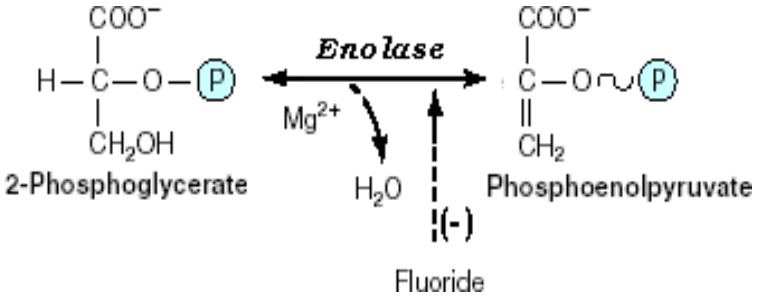


Stage IV: It is the recovery of the phosphate group from *3-phosphoglycerate*. The two molecules of *3-phosphoglycerate*, the end product of the previous stage, still retains the phosphate group, originally derived from ATP in Stage I.

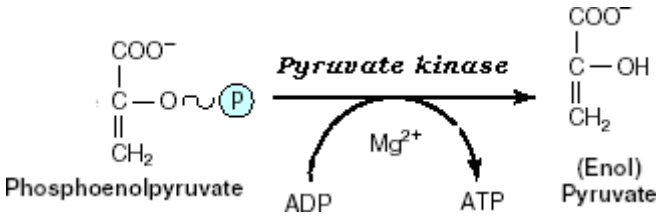
8. ***Conversion of 3-phosphoglycerate to 2-phosphoglycerate:*** *3-phosphoglycerate* formed by the above reaction is converted to 2-phosphoglycerate, catalyzed by the enzyme *phosphoglycerate mutase*.



9. ***Conversion of 2-phosphoglycerate to phosphoenol pyruvate:*** The reaction is catalyzed by the enzyme *enolase*, the enzyme requires the presence of either Mg^{2+} or Mn^{2+} ions for activity.



10. ***Conversion of phosphoenol pyruvate to pyruvate:*** *Phosphoenol pyruvate* is converted to *pyruvate*, the reaction is catalysed by the enzyme *pyruvate kinase*. The high energy phosphate group of phosphoenol pyruvate is directly transferred to ADP, producing ATP. The reaction is irreversible.

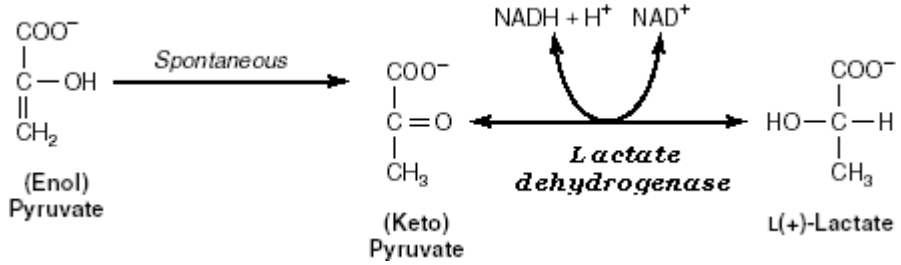


♦ *Summary of glycolysis:*

During glycolysis NAD^+ is reduced to NADH. At the same time, *glyceraldehyde 3-phosphate* is oxidized to *1, 3-bisphosphoglycerate*. To conserve the coenzyme NAD^+, NADH must be reoxidized. Under anaerobic conditions this is done when pyruvic acid is converted to lactic acid. In the presence of oxygen, NADH, can be oxidized to NAD^+ with the help of the respiratory enzymes.

♦ ***Anaerobic phase:***

- In the absence of O_2, reoxidation of NADH at glyceraldehyde 3-phosphate dehydrogenase stage cannot take place in respiratory chain. But the cells have limited coenzyme. Hence to continue the glycolysis **NADH** must be reoxidized to **NAD^+**. This is achieved by reoxidation of NADH by conversionof pyruvate to lactate (without producing ATP).



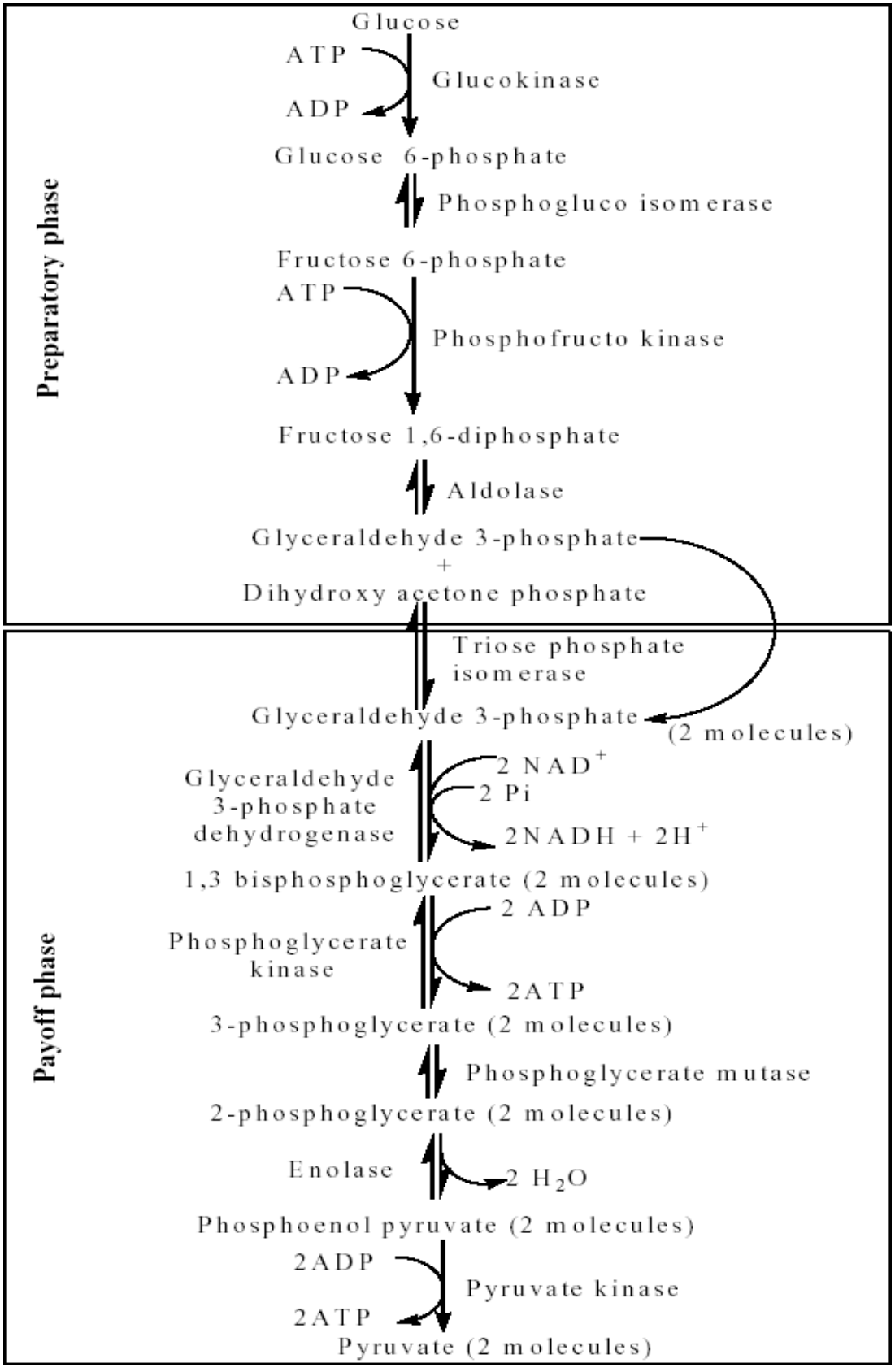
- It is to be noted that in the reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*, therefore, no ATP is produced.
- In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

Energy yield per glucose molecule oxidation:

During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

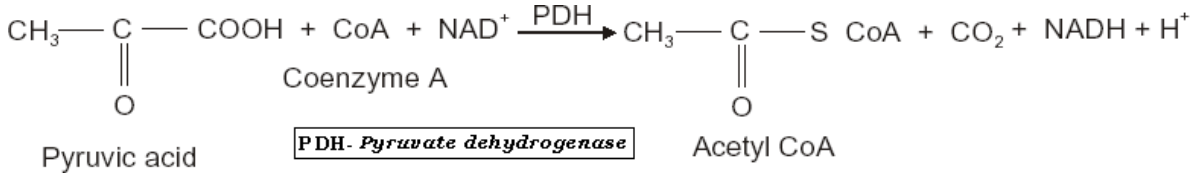
Reactions Catalyzed	***ATP used***	***ATP formed***
Stage I: 1. Glucokinase (for phosphorylation)	1	
2. Phosphofructokinase I (for phosphorylation)	1	
Stage II: 3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain)		6
4. Phosphoglycerate kinase (substrate level phosphorylation)		2
Stage IV: 5. Pyruvate kinase (substrate level phosphorylation)		2
Total	**2**	**10**
Net gain	**08**	

- **Schematic diagram of glycolytic pathway:**

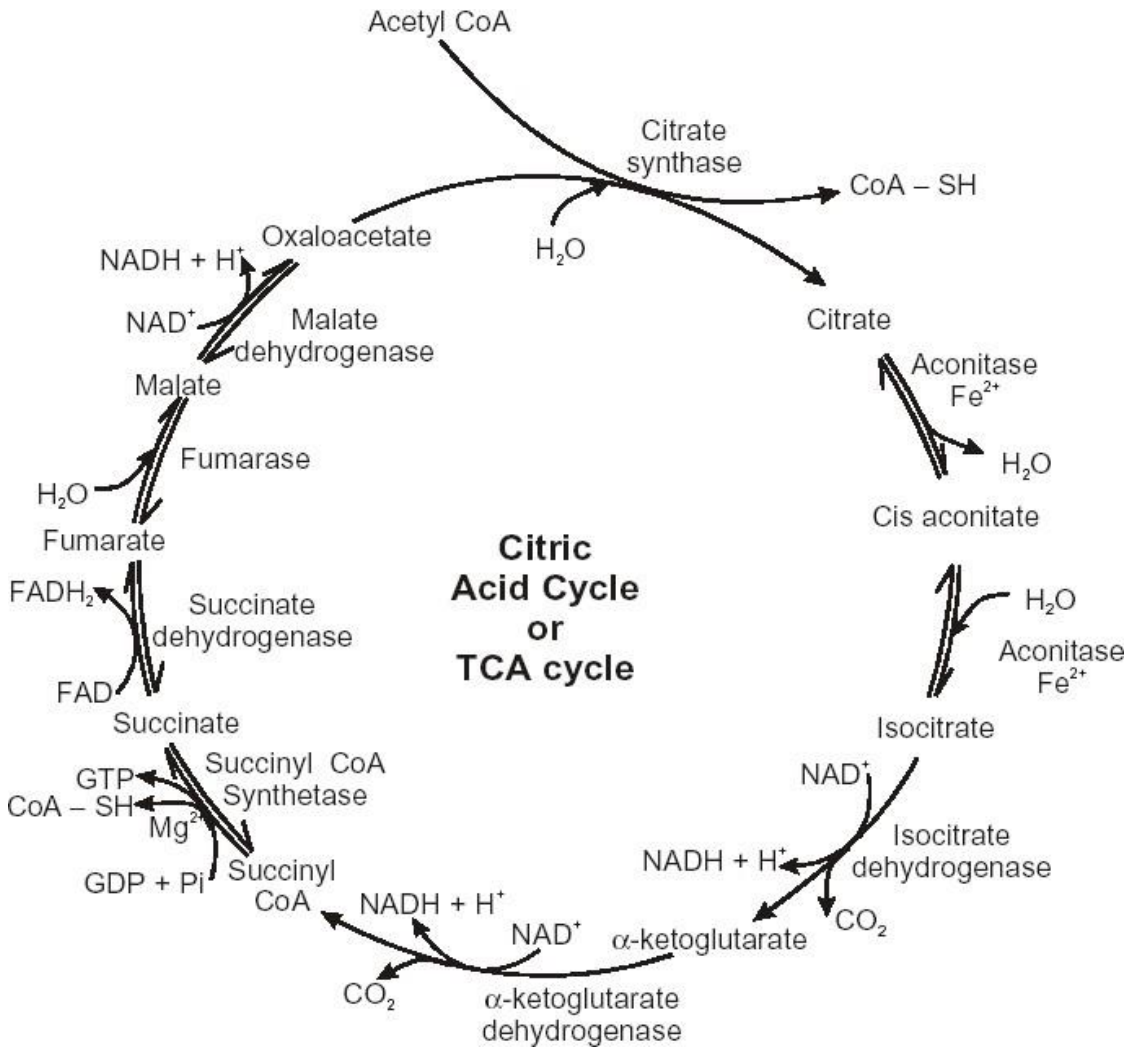


TRICARBOXYLIC ACID CYCLE (TCA CYCLE)

- This cycle is the aerobic phase of carbohydrate metabolism and follows the anaerobic pathway from the stage of pyruvate and is called as citric acid cycle or TCA cycle.
- The name citric acid cycle stems from citric acid which is formed in the first step of this cycle.
- This cycle is also named "Kerbs cycle" after H.A. Krebs, an English biochemist who worked on it.
- Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl coenzyme A (active acetate) before entering the citric acid cycle. This occurs in the mitochondrial matrix and forms a link between glycolysis and TCA cycle.
- This reaction is catalysed by the multienzyme complex known as pyruvate dehydrogenase complex.

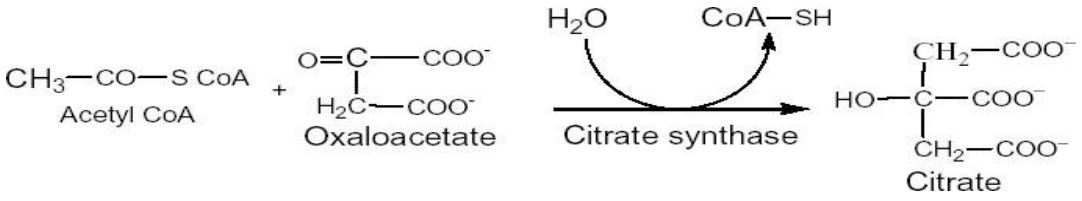


Schematic Diagram of Krebs Cycle

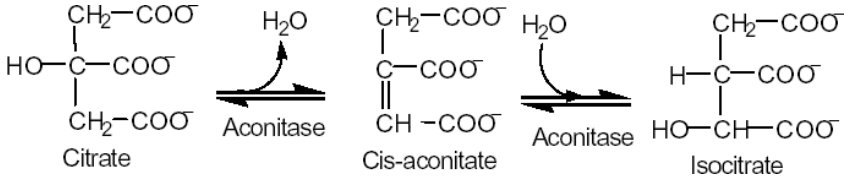


Reactions of the citric acid cycle: There are eight steps in the cycle and the reactions are as follows.

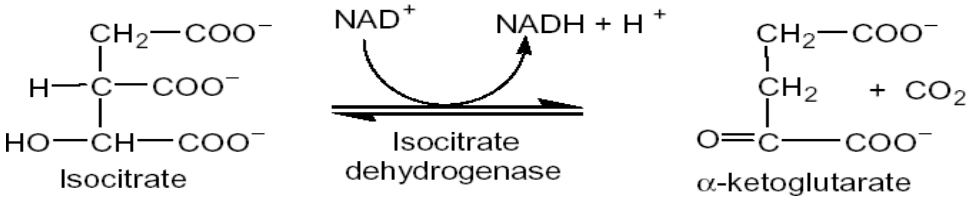
1. ***Formation of citrate:*** The first reaction of the cycle is the condensation of acetyl CoA with oxaloacetate to form citrate, catalyzed by *citrate synthase*. This is an irreversible reaction.



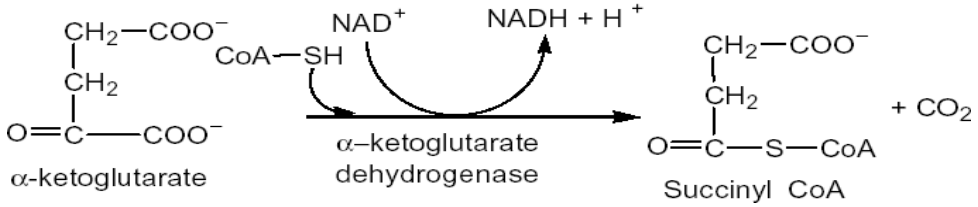
2. ***Formation of isocitrate via cis aconitate:*** The enzyme *aconitase* catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis aconitate.



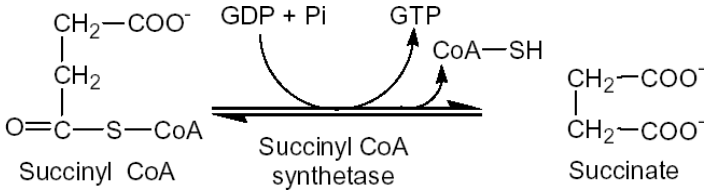
3. **Oxidation of isocitrate to α-ketoglutarate and CO_2**: In the next step, *isocitrate dehydrogenase* catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.



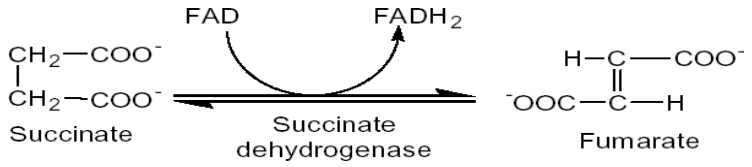
4. ***Oxidation of α-keto glutarate to succinyl CoA and CO_2:*** The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to succinyl CoA and CO2 by the action of the *α- ketoglutarate dehydrogenase* complex. The reaction is irreversible.



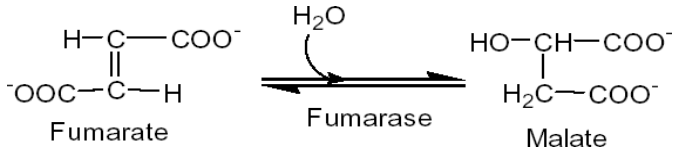
5. ***Conversion of succinyl CoA to succinate:*** The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation) and the enzyme that catalyzes this reversible reaction is called succinyl CoA synthetase or *succinic thiokinase.*



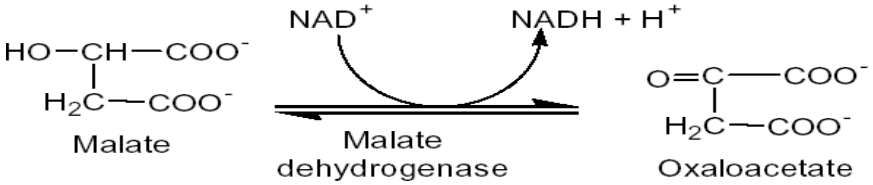
6. ***Oxidation of succinate to fumarate:*** The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme *succinate dehydrogenase*.



7. ***Hydration of fumarate to malate:*** The reversible hydration of fumarate to malate is catalyzed by *fumarase*.



8. ***Oxidation of malate to oxaloacetate:*** The last reaction of the citric acid cycle is, NAD linked *malate-dehydrogenase* which catalyses the oxidation of malate to oxaloacetate.



Energy yield from TCA cycle: If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

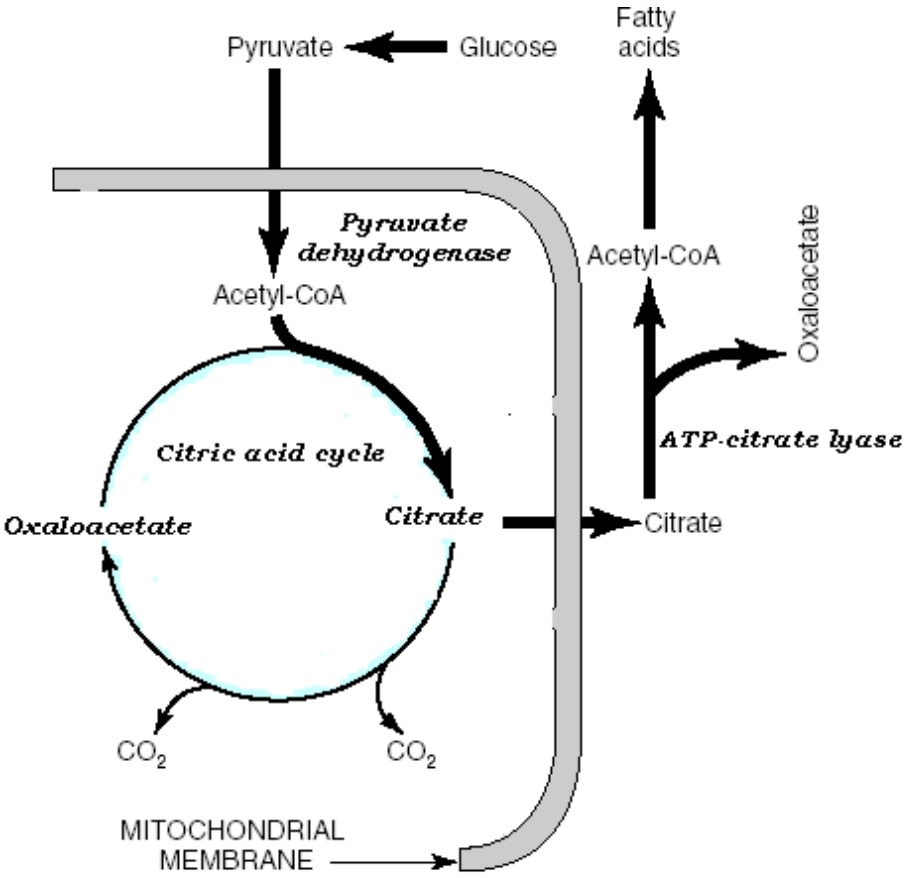
Reactions	No.of ATP formed
1. 2 isocitrate → 2 α-ketoglutarate (2 NADH + 2H+) (2 × 3)	6
2. 2 α-ketoglutarate → 2 succinyl CoA (2 NADH + 2H+) (2 × 3)	6
3. 2 succinyl CoA → 2 succinate (2 GTP = 2ATP)	2
4. 2 succinate → 2 Fumarate ($2\ FADH_2$) (2 × 2)	4
5. 2 malate → 2 oxaloacetate (2 NADH + 2H+) (2 × 3)	6
Total No.of ATP formed	24

- **Vitamins play key roles in the citric acid cycle:**

Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism:

1. ***Riboflavin***, in the form of flavin adenine dinucleotide (FAD), a cofactor in the α-ketoglutarate dehydrogenase complex and in succinate dehydrogenase.
2. ***Niacin***, in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for three dehydrogenases in the cycle — isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase.
3. ***Thiamin* (vitamin B1),** as thiamin diphosphate, the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.
4. ***Pantothenic acid,*** as part of coenzyme A, the cofactor attached to ―active‖ carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

- **The Citric Acid Cycle Takes Part in Fatty Acid Synthesis:** Acetyl-CoA, formed from pyruvate by the action of *pyruvate dehydrogenase*, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.) *Pyruvate dehydrogenase* is a mitochondrial enzyme and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl- CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by



Participation of the citric acid cycle in fatty acid synthesis from glucose

ATP-citrate lyase.

HMP shunt pathway

- ✓ Although glycolysis and citric acid cycle are the common pathways by which animal tissues oxidise glucose to CO_2 and H_2O with the liberation of energy in the form of ATP, a number of alternative pathways are also discovered. The most important one is Hexose Monophosphate Shunt Pathway (HMP shunt). The pathway occurs in the extra mitochondrial soluble portion of the cells.
- ✓ It has two major functions:
  - I. The formation of **NADPH** for synthesis of fatty acids and steroids
  - II. The synthesis of **ribose** for nucleotide and nucleic acid formation.
- ✓ The fundamental difference between NADPH and NADH (reduced nicotinamide adenine dinucleotide) is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis, for example in the biosynthesis of fatty acids and steroids.
- ✓ Glucose, fructose and galactose are the main hexoses absorbed from the gastrointestinal tract, derived principally from dietary starch, sucrose and lactose respectively. Fructose and galactose are converted to glucose, mainly in the liver.
- ✓ The overall equation of the hexose phosphate pathway is

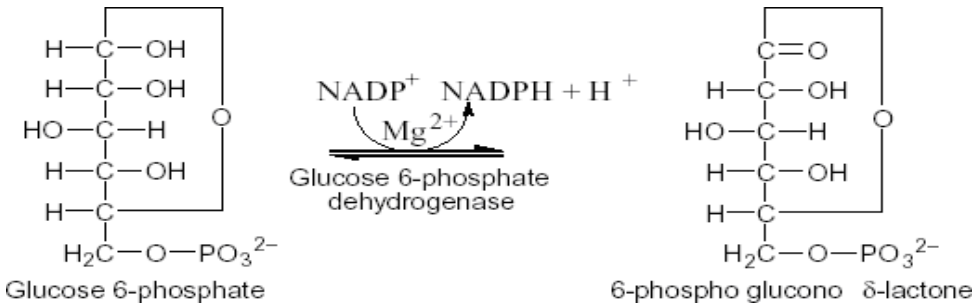
$$\text{Glucose 6-phosphate} + 2NADP^+ + H_2O \longrightarrow \text{Ribose 5-phosphate} + CO_2 + 2NADPH + 2H^+$$

and the net result is the production of NADPH, a reductant for biosynthetic reactions and ribose 5-phosphate, a precursor for nucleotide synthesis.

- ✓ *Oxidative reactions of the hexose mono-phosphate pathway:*

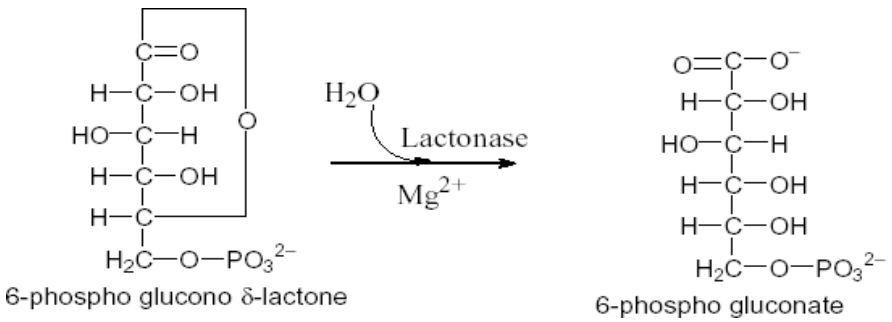
Step 1:

Glucose 6-phosphate in the presence of $NADP^+$ and the enzyme *glucose 6-phosphate dehydrogenase*, forms *6-phospho glucono-□δ-lactone*. The first molecule of $NADPH^+$ is produced in this step.



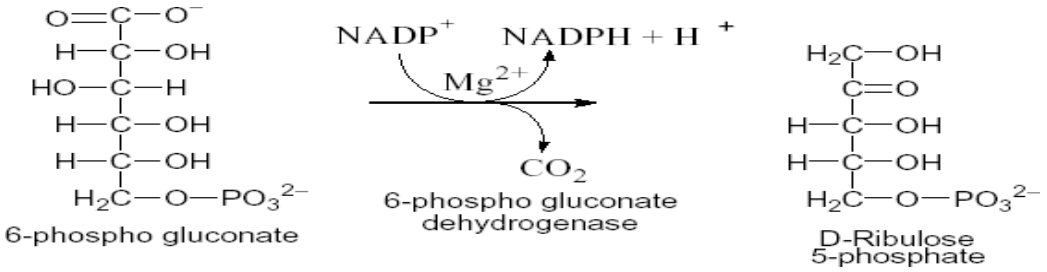
Step 2:

The *6-phospho glucono- δ-lāctone* is unstable and the ester spontaneously hydrolyses to 6- phosphogluconate.



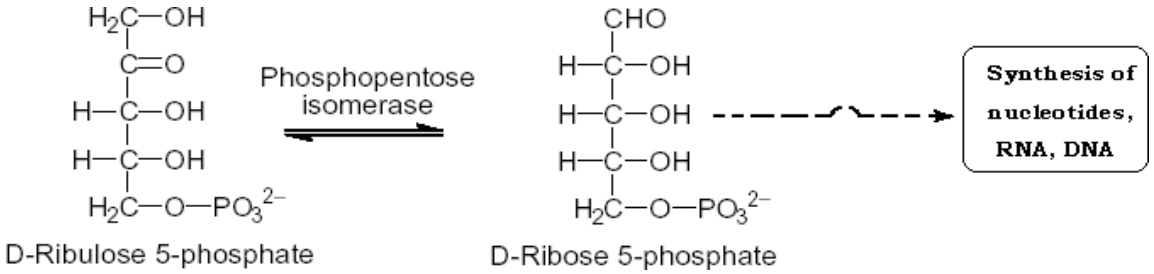
Step 3:

6-phospho gluconate further undergoes dehydrogenation and decarboxylation by *6-phosphogluconate dehydrogenase* to form the ketopentose, D-ribulose 5-phosphate. This reaction generates the second molecule of NADPH.



Step 4:

The enzyme *phosphopentose isomerase* converts ribulose 5-phosphate to its aldose isomer, D-ribose 5-phosphate.



Note By

Genetic deficiency of *glucose 6-phosphate dehydrogenase*, the first enzyme of the pentose phosphate pathway, is a major cause of hemolysis of red blood cells, resulting in **hemolytic anemia** and Glucuronic acid is synthesized from glucose via the **uronic acid pathway,** of major significance for the excretion of metabolites and foreign chemicals (xenobiotics) as **glucuronides.**

GLUCONEOGENESIS

- ✓ The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis.
- ✓ The major site of gluconeogenesis is liver.
- ✓ It usually occurs when the carbohydrate in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet and at the time of starvation, when tissue proteins are broken down to amino acids.

♦ ***Substrates for Gluconeogenesis:***

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include the intermediates of glycolysis and the citric acid cycle. Glycerol, lactate, and the α-keto acids obtained from the deamination of glucogenic amino acids are the most important gluconeogenic precursors.

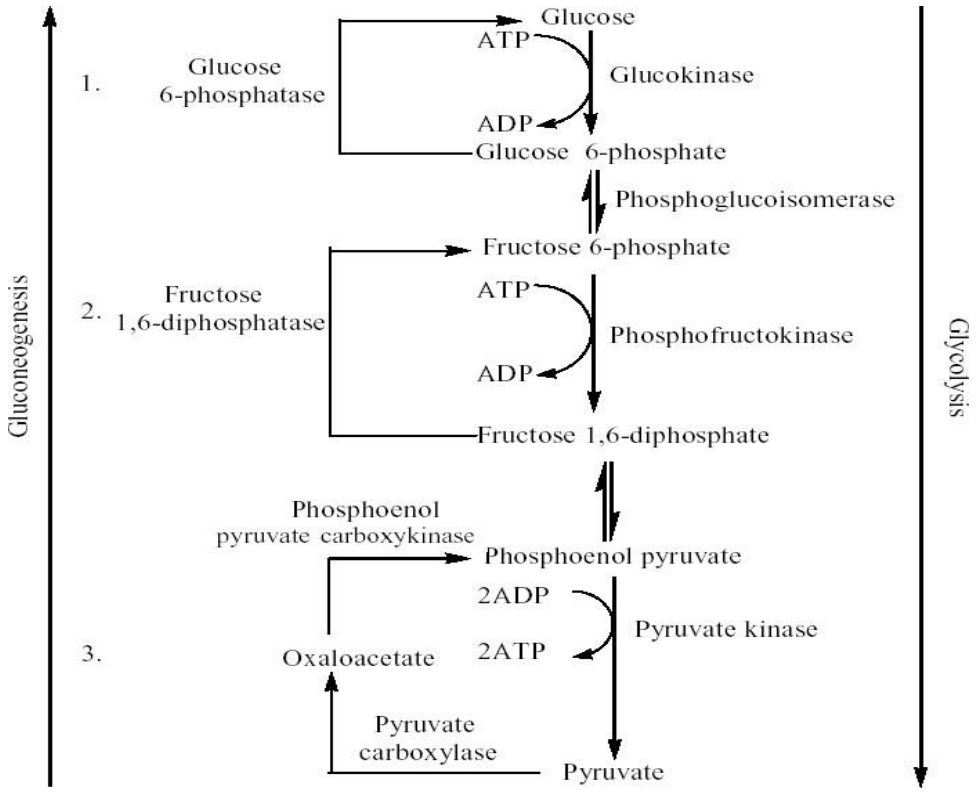
1. *Glycerol:* Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue and in the liver. Glycerol is phosphorylated by *glycerol kinase* to glycerol phosphate, which is oxidized by *glycerol phosphate dehydrogenase* to dihydroxyacetone phosphate as an intermediate of glycolysis.
2. *Lactate:* It is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells. In the Cori cycle, glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which diffuse into the circulation.
3. *Amino acids:* Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during fasting.

♦ ***Gluconeogenesis and glycolysis:***

- ✓ Gluconeogenesis and glycolysis are opposing metabolic pathways and share a number of enzymes. In glycolysis, *glucose* is converted to *pyruvate* and in gluconeogenesis *pyruvate* is converted to *glucose*. However gluconeogenesis is not exact reversal of glycolysis.
- ♦ There are three essentially irreversible steps in glycolysis which are

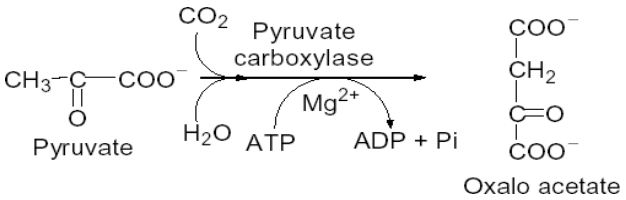
1. Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ Glucose 6-phosphate + ADP
2. Fructose 6-phosphate + ATP $\xrightarrow{\text{Phosphofructo kinase}}$ Fructose 1,6-diphosphate + ADP
3. Phosphoenol pyruvate + ADP $\xrightarrow{\text{Pyruvate kinase}}$ Pyruvate + ATP

- In gluconeogenesis these three reactions are bypassed or substituted by the following news ones.

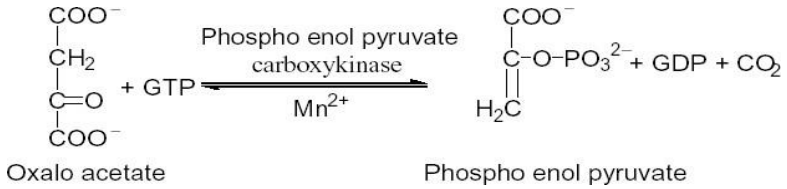


Reactions of gluconeogenesis:

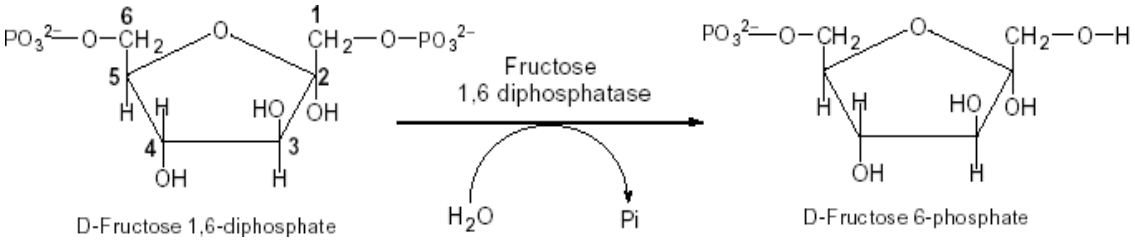
1. The formation of ***phosphoenol pyruvate*** begins with the carboxylation of ***pyruvate*** at the expense of ATP to form ***Oxalo acetate***.



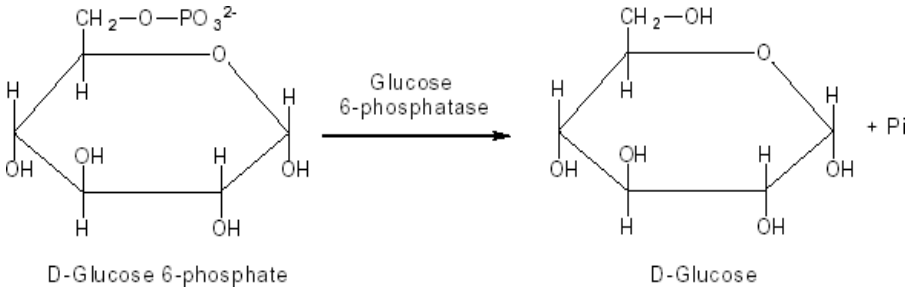
Oxalo acetate is converted to ***phosphoenolpyruvate*** by phosphorylation with GTP, accompanied by a simultaneous *decarboxylation*.



2. ***Fructose 6-phosphate*** is formed from ***fructose 1, 6-diphosphate*** by hydrolysis and the enzyme *fructose 1, 6 diphosphatase* catalyses this reaction.



3. ***Glucose*** is formed by hydrolysis of ***glucose 6-phosphate*** catalysed by *glucose 6-phosphatase*.



- ***Gluconeogenesis of amino acids:***

Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenic amino acids are converted to the intermediates of citric acid cycle either by transamination or deamination.

- ***Gluconeogenesis of Propionate:***

Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion to succinyl CoA.

Gluconeogenesis of Glycerol:

At the time of starvation glycerol can also undergo gluconeogenesis. When the triglycerides are hydrolysed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it. Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol 3-phosphate} + \text{ADP}$$

This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD+ and glycerol 3-phosphate dehydrogenase.

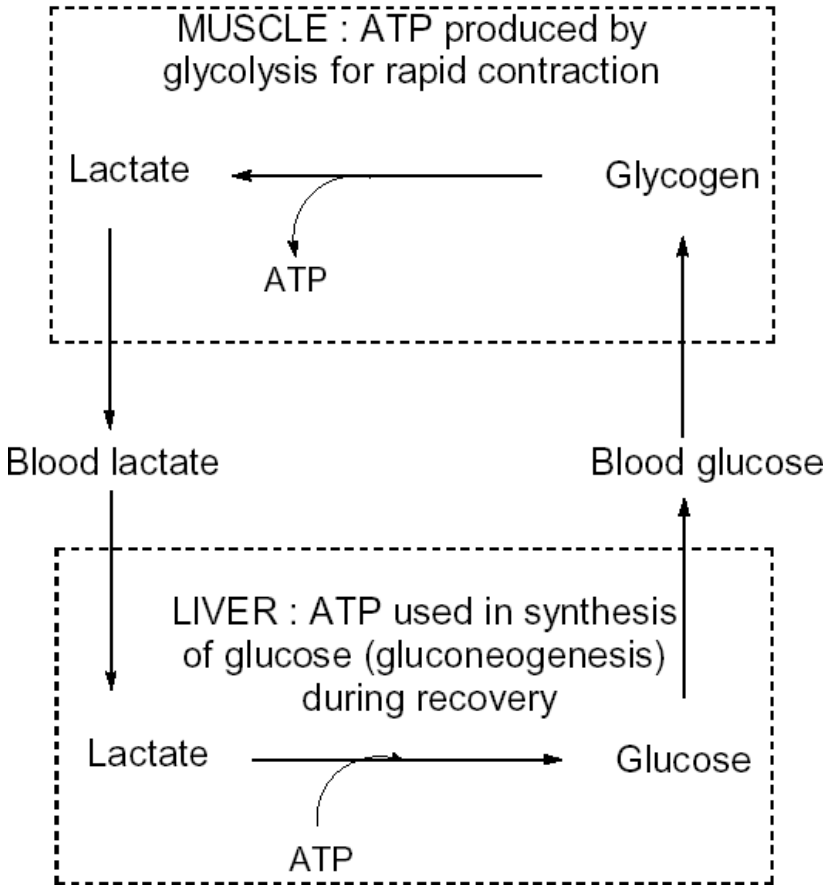
$$\text{Glycerol 3-phosphate} + \text{NAD}^+ \xrightarrow{\text{Glycerol 3-phosphate dehydrogenase}} \text{Dihydroxy acetone phosphate} + \text{NADH} + \text{H}^+$$

This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose. Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

Gluconeogenesis of lactic acid (Cori cycle):

- ✓ The liver and skeletal muscles exhibit a special metabolic cooperation as far as carbohydrates are concerned by the way of a cycle of conversions known as Cori cycle.

Cori cycle



- ✓ In this cycle liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactate produced by the active skeletal muscles.
- ✓ At the time of heavy muscular work or strenuous exercise, O_2 supply is inadequate in active muscles but the muscles keep contracting to the maximum. Hence, glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus lactate gets accumulated in the muscle. Muscle tissue lacks the enzyme *glucose 6-phosphatase* hence it is incapable of synthesizing glucose from lactic acid and the conversion take place only in the liver.
- ✓ Lactate diffuses out of the muscle and enters the liver through blood. In the liver lactate is oxidised to pyruvate which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose.
- ✓ The glycogen may be once again converted to glucose (glycogenolysis) and may be recycled to the muscle through the blood. The process of gluconeogenesis completes the cycle by converting glucose once again to muscle glycogen.

DIABETES MELLITUS

- ✓ Diabetes mellitus is an important disorder of carbohydrate metabolism. However, fat and protein metabolism are also affected in diabetic condition. Diabetes means excretion of excessive volume of urine and mellitus means sweet. So the word diabetes mellitus refers to chronic excretion of large volume of urine containing glucose.
- ✓ Diabetes mellitus, caused by a deficiency in the secretion or action of insulin, is a relatively common disease. Insulin is an endocrine hormone which is secreted by β-cells of *islets of Langerhans* of pancreas. The abnormality in glucose metabolism is indicative of diabetes or a tendency towards the condition. Diabetes mellitus is really a group of diseases in which the regulatory activity of insulin is defective.
- ✓ There are two major clinical classes of the disease:

  1. Type-I or insulin dependent diabetes mellitus (IDDM), this disease begins early in the life and quickly becomes severe.

  2. Type - II or non-insulin dependent diabetes mellitus (NIDDM), this disease is slow to develop, milder and often goes unrecognized.
- ✓ Type one requires insulin therapy and careful, life long control of the balance between glucose intake and insulin dose. The decreased or defective production of insulin is characterised by the following symptoms.

I. Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.

II. **Polyuria:** This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).

III. **Polydipsia:** The excessive thirst which leads to increased consumption of water. This condition is known as polydipsia. This is to replace the volume of water excreted due to polyuria.

IV. **Polyphagia**: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating, they lose weight and become lean and emaciated.

V. As glucose is not enough for energy production, increased mobilisation of fat from adipose tissue occurs. But the metabolism of fat is incomplete resulting in the production of large amounts of the intermediary products of fat metabolism namely ketone bodies (e.g. Acetoacetate and β-hydroxybutarate). This condition is known as 'ketosis' and excess ketone bodies cause severe acidosis, ultimately resulting in 'coma'.

VI. Deposition of lipids in the walls of the blood vessels resulting "atherosclerosis".

- Biochemical measurements on the blood and urine are essential in the diagnosis and treatment of diabetes, which causes profound changes in metabolism. A sensitive diagnostic criterion is provided by the *glucose tolerance test* (GTT).

✓ ***Classification of antidiabetic drugs:*** Antidiabetic drugs can be classified into two categories:

1. *Insulin injections*: Mostly used on serious cases of diabetes.
2. *Oral hypoglycaemic agents*: These agents are the group of drugs that may be taken singly or in combination to lower the blood glucose in type 2 diabetes. Type 2 diabetes can be due to increased peripheral resistance to insulin or to reduced secretion of insulin. Oral hypoglycaemic should be used together with changes in diet and lifestyle to achieve good glycaemic control and it is customary to monitor such changes for three months before considering medication.

   Oral hypoglycaemic agents are not usually used in type 1 diabetes, but *metformin* may be of use in overweight type 1 diabetics.

   The following groups of oral hypoglycaemics are currently available:

   Biguanides derivatives: Metformin; *Sulphonylureas derivatives:* glimepiride;

   Postprandial glucose regulators: Repaglinide and Nateglinide; *Thiazolidinediones derivatives*: Pioglitazone and Rosiglitazone and *Acarbose*: *which* acts by inhibiting intestinal alpha glucosidases, which delays the absorption and digestion of sucrose and starch.

Glucose Tolerance Test (GTT) or Oral Glucose Tolerance Test (OGTT)

A Glucose Tolerance Test in medical practice is the administration of glucose to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia. The glucose is most often given orally so technically is terms as an oral glucose tolerance test (OGTT).

REGULATION OF GLUCONEOGENESIS

The regulation of gluconeogenesis is determined primarily by the circulating level of ***glucagon***, and by the availability of gluconeogenic substrates.

A. Glucagon

This pancreatic islet hormone stimulates gluconeogenesis by three mechanisms.

- ✓ **Changes in allosteric effectors:** Glucagon lowers the level of fructose 2, 6-bisphosphate, resulting in activation of *fructose 1 , 6- bisphosphatase* and inhibition of *phosphofructokinase* , thus favoring gluconeogenesis over glycolysis.
- ✓ **Covalent modification of enzyme activity:** Glucagon, via an elevation in cyclic AMP (cAMP) level and *c AMP- dependent protein kinase* activity, stimulates the conversion of *pyruvate kinase* to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose.
- ✓ **Induction of enzyme synthesis:** Glucagon increases the transcription of the *PEP- carboxykinase* gene, thereby increasing the availability of this enzyme's activity as levels of its substrate rise during fasting.

[***Note by:*** Insulin causes decreased transcription of the mRNA for this enzyme.]

B. Substrate availability

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis.

C. Allosteric activation by acetyl CoA

Allosteric activation of hepatic *pyruvate carboxylase* by acetyl CoA occurs during fasting. As a result of excessive lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to CO_2 and H_2O. As a result, acetyl CoA accumulates and leads to activation of *pyruvate carboxylase* .

[**Note:** Acetyl CoA inhibits *pyruvate dehydrogenase* . Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle]

D. Allosteric inhibition by AMP

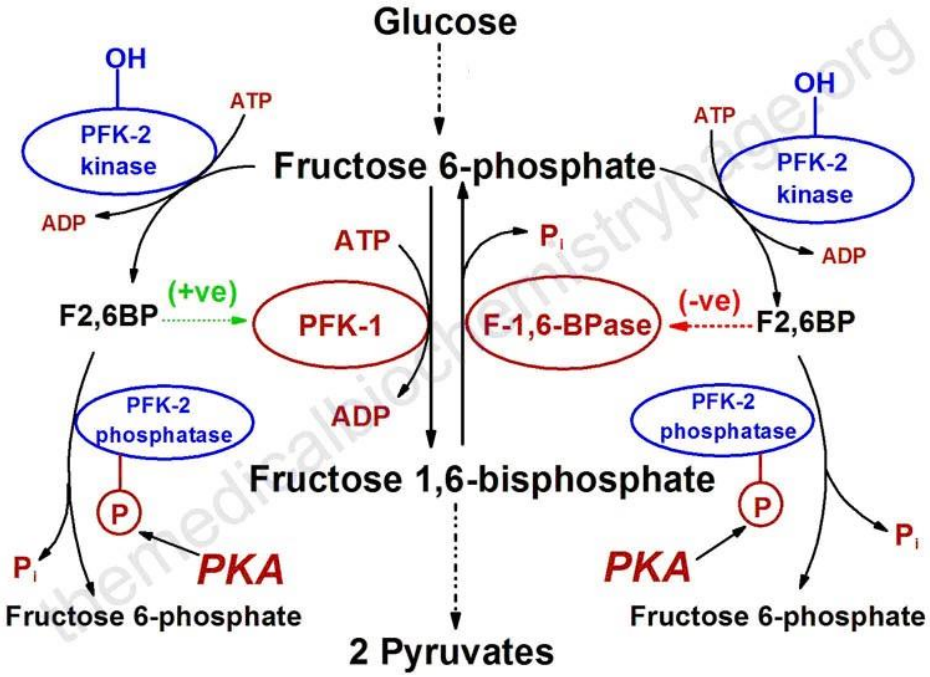
Fructose 1, 6 - bisphosphatase is inhibited by AMP—a compound that activates *phosphofructokinase* . Elevated AMP thus stimulates pathways that oxidize nutrients to provide energy for the cell.

[**Note:** ATP and NADH, produced in large quantities during fasts by catabolic pathways, such as fatty acid oxidation, are required for gluconeogenesis. Fatty acid oxidation also provides the acetyl CoA that allosterically activates *pyruvate* carboxylase]

The regulation of gluconeogenesis will be in direct contrast to the regulation of glycolysis. In general, negative effectors of glycolysis are positive effectors of gluconeogenesis. Regulation of the activity of PFK-1 and *Fructose 1, 6- bisphosphatase* is the most significant site for controlling the flux toward glucose

oxidation or glucose synthesis. As described in control of glycolysis, this is predominantly controlled by fructose-2, 6-bisphosphate, *Fructose 2, 6- bisphosphatase* which is a powerful negative allosteric effector of *Fructose 1, 6 - bisphosphatase* activity.

The level of *Fructose 2, 6 - bisphosphatase* will decline in hepatocytes in response to glucagon stimulation as well as stimulation by catecholamines. Each of these signals is elicited through activation of cAMP-dependent protein kinase (PKA). One substrate for PKA is PFK-2, the bifunctional enzyme responsible for the synthesis and hydrolysis of F2, 6BP. When PFK-2 is phosphorylated by PKA it acts as a phosphatase leading to the dephosphorylation of *Fructose 2, 6 - bisphosphatase* with a concomitant increase in *Fructose 1, 6 - bisphosphatase* activity and a decrease in PFK-1 activity. Secondarily, *Fructose 1, 6 - bisphosphatase* activity is regulated by the ATP/ADP ratio. When this is high, gluconeogenesis can proceed maximally.



Regulation of glycolysis and gluconeogenesis by fructose 2, 6-bisphosphate (F2, 6BP). The major sites for regulation of glycolysis and gluconeogenesis are the phosphofructokinase-1 (PFK-1) and fructose-1, 6-bisphosphatase (F-1, 6-BPase) catalyzed reactions. PFK-2 is the kinase activity and F-2, 6-BPase is the phosphatase activity of the bi-functional regulatory enzyme, phosphofructokinase-2/fructose-2, 6-bisphosphatase. PKA is cAMP-dependent protein kinase which phosphorylates PFK-2/F-2, 6-BPase turning on the phosphatase activity. (+ ve) and (- ve) refer to positive and negative activities, respectively.

GLYCOGEN METABOLISM

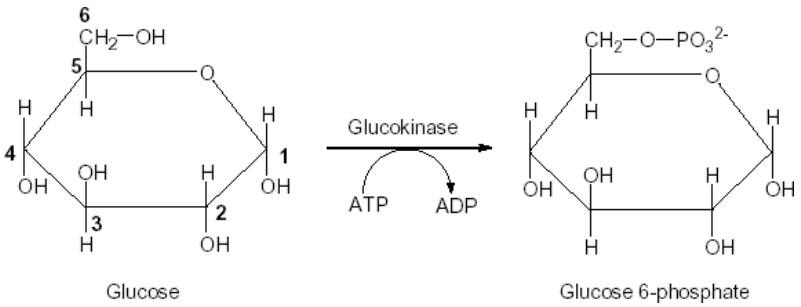
- ✓ Glycogen is a branched polymer of **α-D-glucose**.
- ✓ The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use.
- ✓ The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.
- ✓ Approximately 400 g of glycogen make up one to two percent of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to ten percent of the fresh weight of a well-fed adult liver.
- ✓ ***Structure of glycogen:*** Glycogen is a branched-chain homopolysaccharide made exclusively from α-D-glucose. The primary glycosidic bond is an **α(1→4)** linkage. After an average of eight–ten glucosyl residues, there is a branch containing an **α(1→6)** linkage. A single molecule of glycogen can have a molecular mass of up to 10^8 daltons.
- ✓ Liver glycogen stores increase during the well-fed state, and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks).

Glycogen biosynthesis:

- ✓ The process of biosynthesis of glycogen from glucose is known as glycogenesis.
- ✓ The process occurs in the cytosol, and requires energy supplied by ATP for the phosphorylation of glucose and uridine triphosphate (UTP).
- ✓ Glycogenesis is a very essential process since the excess of glucose is converted and stored up as glycogen which could be utilised at the time of requirement. In the absence of this process the tissues are exposed to excess of glucose immediately after a meal and they are starved of it at other times.
- ✓ The following are the various reactions of glycogenesis are as follows:

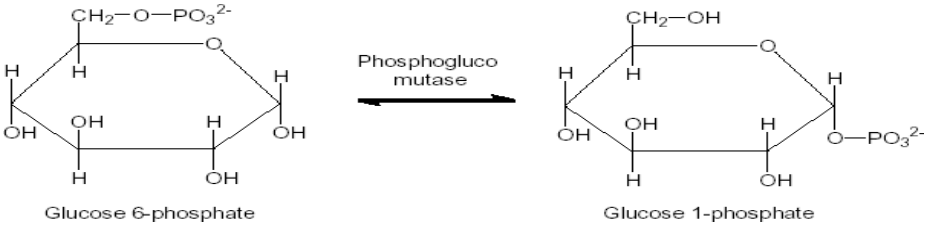
Step 1

Glucose is phosphorylated to **glucose 6-phosphate**, a reaction that is common to the first reaction in the pathway of glycolysis from glucose. This reaction is catalysed by ***hexokinase*** in muscle and ***glucokinase*** in liver in the presence of ATP.



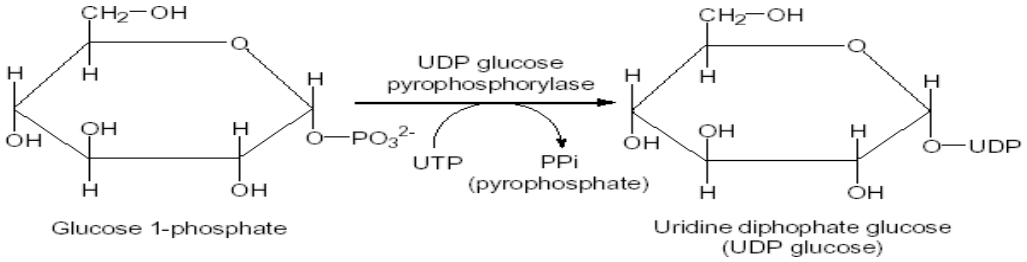
Step 2

Glucose 6-phosphate is then reversibly converted to **glucose 1-phosphate** in a reaction catalysed by enzyme ***phosphogluco mutase***. This process requires Mg^{2+} and a small amount of **glucose 1, 6-diphosphate** as coenzyme.



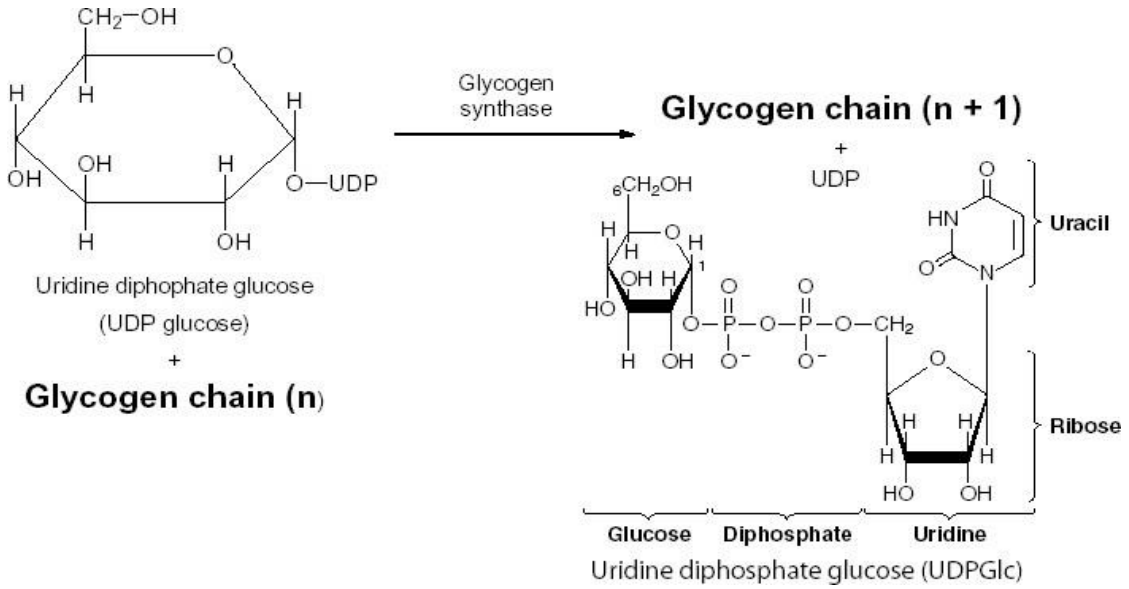
Step 3

The **glucose 1-phosphate** is then activated by the energy produced by the hydrolysis of **uridine triphosphate** (UTP) in the presence of ***uridine diphosphate glucose pyrophophosrylase***. This is a key reaction in glycogen biosynthesis.



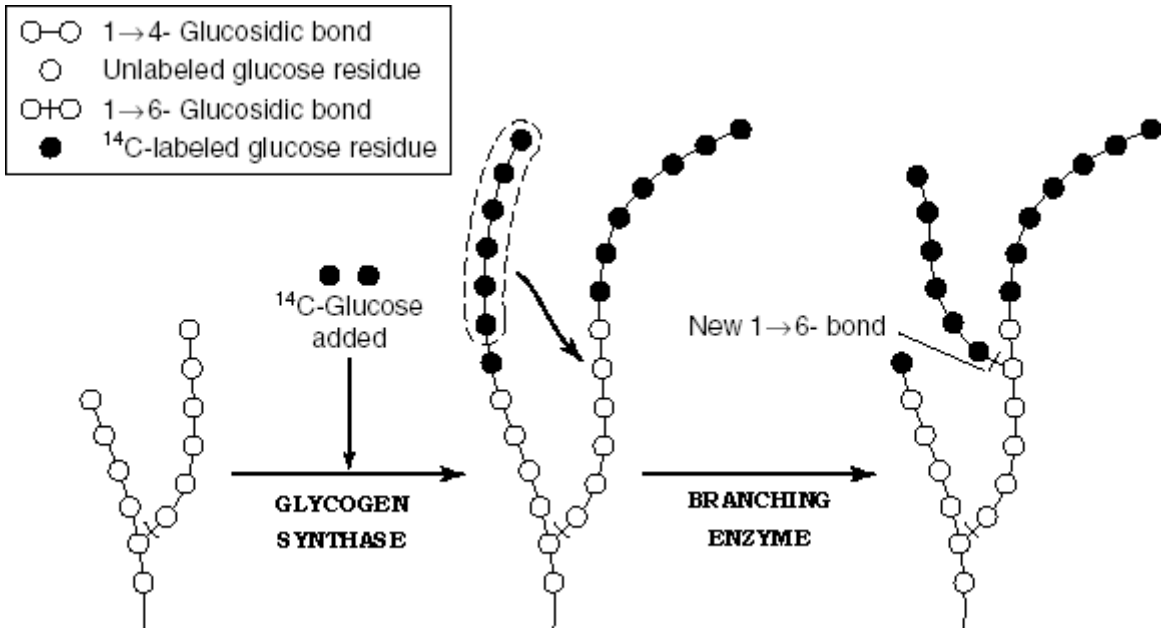
Step 4

UDP-glucose is the immediate donor of glucose residues in the reaction catalyzed by ***glycogen synthase***, which promotes the transfer of the glucose residue from UDP-glucose to a nonreducing end of a branched glycogen chain.



Step 5

When the chain has become long with more than 8 glucose units, a second enzyme, namely branching enzyme ***amylo 1-4 to 1-6 transglycosylase*** acts on the glycogen and helps in joining of 1, 4 glycogen chains with a similar neighbouring chain to form 1-6 linkage, thus forming a branching point in the molecule. Glycogen thus formed may be stored in liver, muscles and tissues.



If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) molecule of glucosyl residues attached by α(1→4) linkages. Such a compound is found in plant tissues, and is called **amylose**. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resulting in a highly branched, tree-like structure that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added.

DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

- ✓ When the blood sugar level falls (Hypoglycemia), glycogen stored in the tissues specially glycogen of liver and muscles may be broken down and this process of breakdown of glycogen is called glycogenolysis.
- ✓ When glycogen is degraded, the primary product is **glucose 1-phosphate**, obtained by breaking **α (1→4)** glycosidic bond. In addition, **free glucose** is released from each **α (1→6)**-linked glucosyl residue.

A. Shortening of chains

- Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis until four glucosyl units remain on each chain before a branch point. The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further.

B. Removal of branches

- Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme. First, ***oligo-α (1→4)→α(1→4)-glucan transferase*** removes the outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an **α(1→4)** bond is made, and the enzyme functions as a ***4:4 transferase***.
- Then the remaining single glucose residue attached in an **α(1→6**) linkage is removed hydrolytically by ***amylo-α(1→6)-glucosidase*** activity, releasing **free glucose**.
- The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units from the next branch are reached.

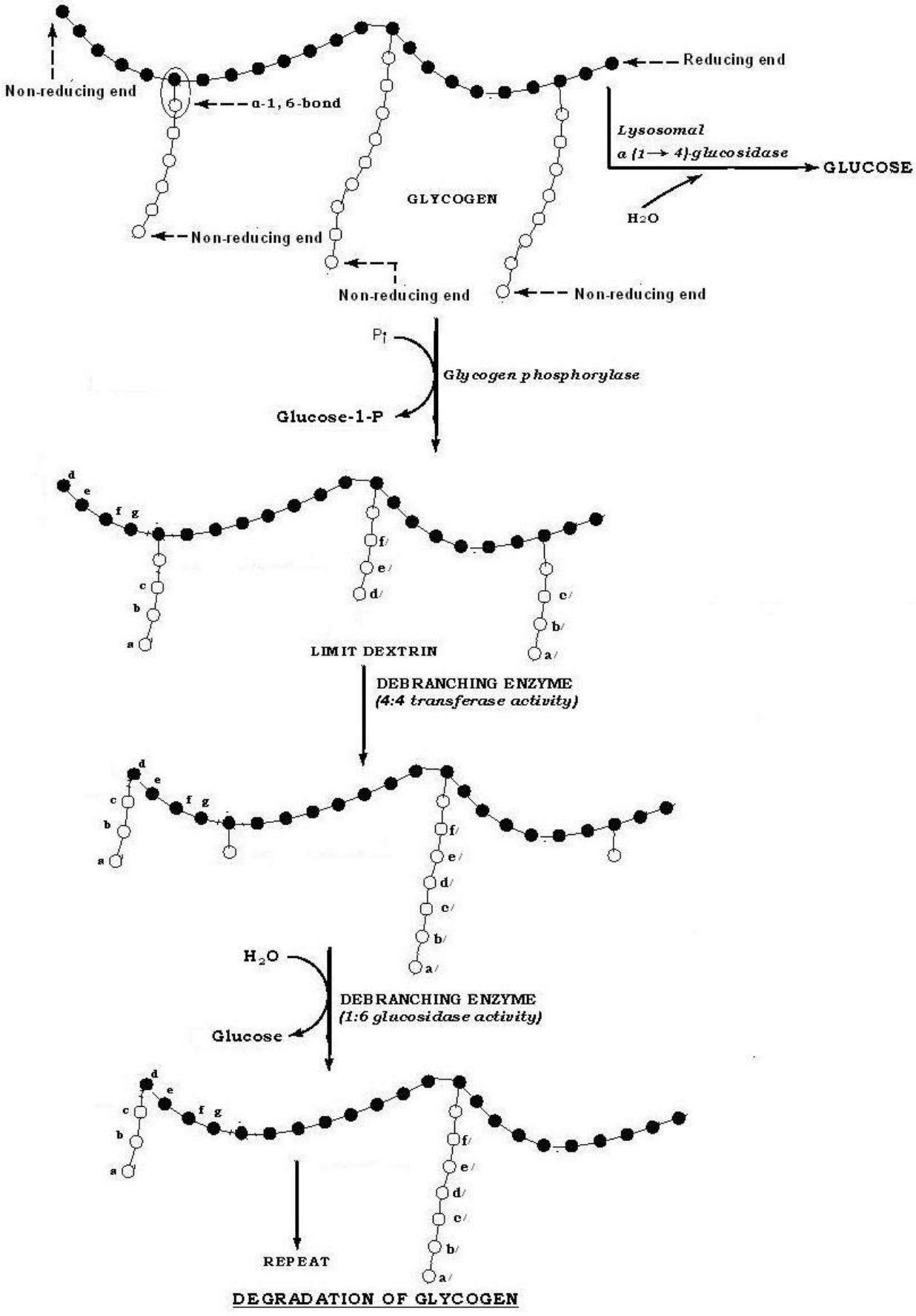
C. Conversion of glucose 1-phosphate to glucose 6-phosphate

- Glucose 1-phosphate, produced by ***glycogen phosphorylase***, is converted in the cytosol to glucose 6-phosphate by ***phosphoglucomutase***.
- In the liver, glucose 6-phosphate is translocated into the endoplasmic reticulum (ER) by ***glucose 6-phosphate translocase***. There it is converted to glucose by glucose 6-phosphatase—the same enzyme used in the last step of gluconeogenesis.
- In the muscle, glucose 6-phosphate cannot be dephosphorylated because of a lack of ***glucose 6-phosphatase***. Instead, it enters glycolysis, providing energy needed for muscle contraction.

D. Lysosomal degradation of glycogen

- A small amount (one–three percent) of glycogen is continuously degraded by the lysosomal enzyme, ***α(1→4)-glucosidase*** (***acid maltase***). The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease type II (**Pompe disease**).

Note By: Glycogen storage diseases are genetic disorders characterized by the accumulation of abnormal amounts of carbohydrates or lipids primarily due to decreased degradation.



DEGRADATION OF GLYCOGEN

GLYCOGEN STORAGE DISEASES (GSD)

- ✓ Glycogen storage disease (GSD, also glycogenosis and dextrinosis) Glycogen storage disease (GSD, also glycogenosis and dextrinosis) is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types
- ✓ Glycogen is a major source of energy for the body. It is stored in the form of glycogen in both the liver and muscles and later released with the help of enzymes. Persons affected by GSD have an inherited defect in one of the enzymes responsible for forming or releasing glycogen as it is needed by the body during exercise and/or between meals.
- ✓ *Types of Glycogen Storage Disease*

1. *Type 0 - glycogen synthase deficiency*:

- The enzyme *glycogen synthase* is needed for the body to make glycogen. A deficiency results in very low amounts of glycogen stored in the liver. A person between meals can develop very low blood sugar levels, known as hypoglycemia.

2. *Type I - Von Gierke Disease*:

- It is also known as *glucose-6-phosphatase* deficiency, in which the body cannot break down glycogen for energy.
- Gycogen is stored in the liver and muscles and is normally broken down into glucose when you do not eat. It occurs when the body lacks the protein (enzyme) that releases glucose from glycogen. This causes abnormal amounts of glycogen to build up in certain tissues. When glycogen is not broken down properly, it leads to low blood sugar.
- *Von Gierke* disease is inherited, which means it is passed down through families. If both parents carry the defective gene related to this condition, each of their children has a 25% chance of developing the disease.

3. *Glycogen Storage Disease Type II*:

- It is also known as Pompe disease or acid maltase deficiency.
- It is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body's cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally.
- Three types of Pompe disease,

i. The classic form of infantile-onset Pompe disease begins within a few months of birth. Infants with this disorder typically experience muscle weakness (myopathy), poor muscle tone (hypotonia), an enlarged liver (hepatomegaly), and heart defects. Affected infants may also fail to gain weight and grow at the expected rate (failure to thrive) and have breathing problems. If untreated, this form of Pompe disease leads to death from heart failure in the first year of life.

ii. The non-classic form of infantile-onset Pompe disease usually appears by age 1. It is characterized by delayed motor skills (such as rolling over and sitting) and progressive muscle weakness. The heart may be abnormally large (cardiomegaly), but affected individuals usually do not experience heart failure. The

muscle weakness in this disorder leads to serious breathing problems, and most children with non-classic infantile-onset Pompe disease live only into early childhood.

iii. The late-onset type of Pompe disease may not become apparent until later in childhood, adolescence, or adulthood. Late-onset Pompe disease is usually milder than the infantile-onset forms of this disorder and is less likely to involve the heart. As the disorder progresses, breathing problems can lead to respiratory failure take place.

4. *Glycogen Storage Disease Type IV*:

- It is also known as *Andersen disease* or *brancher enzyme* deficiency.
- Deficient activity of the glycogen-branching enzyme is the cause of GSD Type IV. It results in accumulation of abnormal glycogen in the liver, muscle and other tissues.

5. *Glycogen Storage Disease Type V*:

- It is also known as *McArdle Disease.*
- It cause due to *myophosphorylase* deficiency.
- It is a rare metabolic disorder which causes muscle pain in everyday activities and exercise. If activity is prolonged despite the pain then muscle damage ensues with the risk of muscle breakdown and kidney failure.
- **Note by**: *Myophosphorylase* is the muscle isoform of the enzyme *glycogen phosphorylase*. This enzyme helps break down glycogen (a form of stored carbohydrate) into glucose-1-phosphate, (not glucose) so that it can be utilized within the muscle cell.

6. *Glycogen Storage Disease Type VI*:

- It is also known as *Hers* disease.
- It cause due to *liver phosphorylase* deficiency.
- **Note by**: *liver phosphorylase* is an enzyme that catalyzes the breakdown of liver glycogen to glucose-1-phosphate.

7. *Glycogen Storage Disease Type VII*:

- It is also known as *Tarui* disease.
- It cause due to *muscle phosphofructokinase* deficiency.
- The phosphofructokinase enzyme which is needed to facilitate the breakdown of glycogen into energy in muscle. This results in reduced amount of energy available to muscles during exercise.
- The body breaks down muscle when trying to attain energy, which causes symptoms such as muscle pain, cramping, fatigue and tenderness. With the breakdown of muscle and the release of the red protein myoglobin, red-brown urine may be seen.
- The enzyme deficiency is due to abnormalities in the muscle phosphofructokinase gene. GSD VII is inherited as an autosomal recessive genetic disorder.

8. *Glycogen Storage Disease Type IX*:

- It cause due to *liver glycogen phosphorylase kinase* deficiency**.**
- In most individuals apart from liver enlargement there are few other problems. There is usually no tendency to low blood sugar, the liver becomes smaller with age and children grow normally.

Summary of Glycogen storage disease (GSD, also glycogenosis and dextrinosis)

Glycogenosis	*Name*	*Cause of Disorder*	*Characteristics*
Type I	Von Gierke's disease	Deficiency of glucose-6-phosphatase	Liver cells and renal tubule cells loaded with glycogen. Hypoglycemia, lactic-acidemia, ketosis, hyperlipemia.
Type II	Pompe's disease	Deficiency of lysosomal α-1→4- and 1→6-glucosidase (acid maltase)	Fatal, accumulation of glycogen in lysosomes, heart failure.
Type III	Limit dextrinosis, Forbes' or Cori's disease	Absence of debranching enzyme	Accumulation of a characteristic branched polysaccharide.
Type IV	Amylopectinosis, Andersen's disease	Absence of branching enzyme	Accumulation of a polysaccharide having few branch points. Death due to cardiac or liver failure in first year of life.
Type V	Myophosphorylase deficiency, McArdle's syndrome	Absence of muscle phosphorylase	Diminished exercise tolerance; muscles have abnormally high glycogen content (2.5–4.1%). Little or no lactate in blood after exercise.
Type VI	Hers' disease	Deficiency of liver phosphorylase	High glycogen content in liver, tendency toward hypoglycemia.
Type VII	Tarui's disease	Deficiency of phosphofructokinase in muscle and erythrocytes	As for type V but also possibility of hemolytic anemia.
Type VIII		Deficiency of liver phosphorylase kinase	As for type VI.

HORMONE CONTROL OF CARBOHYDRATE METABOLISM

Introduction:

The metabolism of carbohydrates is regulated by a variety of hormones and other molecules. Some of these have already been mentioned in previous sections. The proper functions of the body are dependent on precise control of the glucose concentration in the blood. The normal fasting level of glucose in the blood is 70-90 mg/100 ml.

If the concentration of glucose in blood is too high (above 120 mg/100 mL) a condition known as **hyperglycemia** results. Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates.

If the concentration of glucose is too low (below 70 mg/100 ml) a condition of hypoglycemia exists. Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse perspiration, rapid heart beat, and possible loss of consciousness.

INSULIN:

Insulin, a polypeptide, is secreted from the pancreas in response to a hyperglycemia condition which usually results shortly after ingesting a meal.

The major effect of insulin is to promote the transport of sugar across the cell membrane of fat and muscle cells. In addition, insulin promotes anabolic processes such as increasing the rate of synthesis for glycogen (glycogenesis), fatty acids, and proteins. Insulin inhibits the catabolic processes such as the breakdown of glycogen and fat.

A deficiency of insulin (hypoinsulinism) results in a permanent hyperglycemic condition known as diabetes mellitus. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood. Fatty acid metabolism is also upset. For this reason, a detailed study of diabetes mellitus must wait until the next chapter.

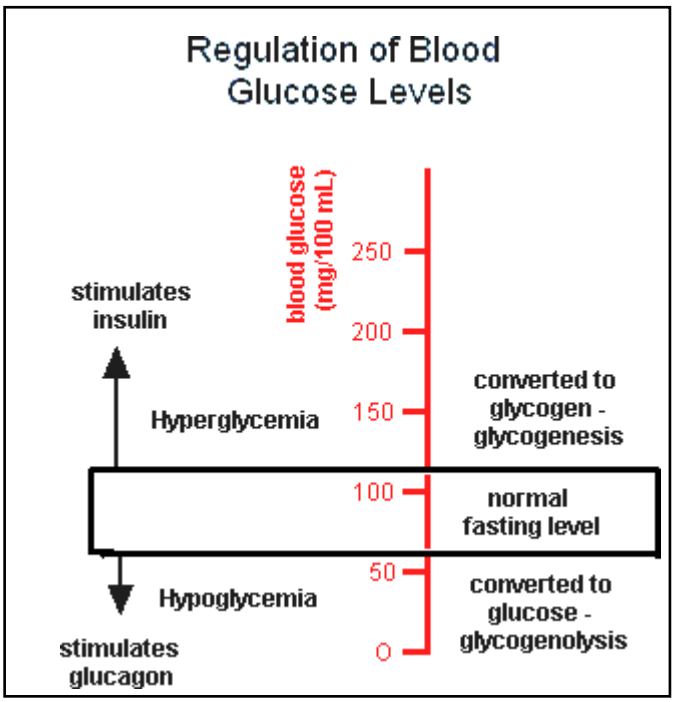
Hyperinsulinism (too much insulin) leads to the hypoglycemic condition. Excessive amounts of glucose are removed from the blood. Severe hypoglycemia may result when a diabetic injects too much insulin. A severe insulin shock may result in a coma since glucose does not reach the brain. A diabetic usually carries a glucose rich food, such as candy, to provide a quick supply of glucose to replenish depleted glucose levels caused by too much insulin.

A functional type of hypoglycemia results in some individuals from an over stimulation of insulin. The causes of hypoglycemia are not completely understood, but it occurs in some people after eating heavily sugared food such as heavily sugared cereal and/or coffee and sweet rolls. The initial high glucose levels over stimulates the pancreas to produce too much insulin. The excess insulin causes blood sugar levels to drop below normal after 2-3 hours which may cause the person to feel sleepy, irritable, and generally tired. The condition is only exacerbated by a "quick fix" of more sweetened coffee, pastry, or candy since more insulin is produced again. A protein rich breakfast would correct the condition by allowing glucose to enter the blood stream more slowly.

GLUCAGON:

If one hormone, insulin, controls the excess of glucose in the blood by stimulating synthesis of glycogen, then other hormones must respond to low levels of glucose. The liver is more responsive to **glucagon**, a peptide also secreted by the pancreas.

Glucagon increases glucose levels in the blood by stimulating the breakdown of glycogen (glycogenolysis) in the liver into glucose which leaves the liver cells and enters the blood stream. The method of hormone stimulation is a complex cascade effect. The exact sequence has been worked out in the most detail for epinephrine (adrenalin) although glucagon works in a similar fashion.



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#Special thanks to Dr Suman M. & Medical Biochemistry Page.