Chapter

6

Metabolism of Carbohydrates (Part I)

Competency achievement: The student after reading the chapter should be able to:

BI3.4: Define and differentiate the pathways of carbohydrate metabolism, (glycolysis, gluconeogenesis, glycogen metabolism, HMP shunt).

BI3.5: Describe and discuss the regulation, functions and integration of carbohydrate along with associated diseases/disorders.

BI3.6: Describe and discuss the concept of TCA cycle as a amphibolic pathway and its regulation.

BI3.7: Describe the common poisons that inhibit crucial enzymes of carbohydrate metabolism (e.g. fluoride, arsenate).

BI3.8: Discuss and interpret laboratory results of analytes associated with metabolism of carbohydrates.

DIETARY CARBOHYDRATES

Most of the carbohydrates ingested in a natural diet consists of *starch,* the plant storage polysaccharide that is similar in structure to glycogen. Cane sugar or sucrose—a disaccharide of glucose with another hexose, fructose—is abundant in **'civilized'** diet, and lactose is a significant component in a high milk diet, such as that of infants. Small amounts of free hexoses (glucose and fructose) are found in **fruits** and **honey**.

There is no specific carbohydrate requirement for the human, but in a balanced diet, about 50% of the energy requirement of the body should be provided from this source. The only carbohydrate derivative required in the diet is ascorbic acid, or vitamin C. Virtually, all members of the animal kingdom are capable of synthesizing this six-carbon compound by three enzymatic conversions of glucose. Man, however, in common with other primates and the guinea pig, lacks one of the enzymes required for this metabolic sequence. This represents a type of inborn error of metabolism that arose by a mutation during primate evolution. Other important carbohydrates, such as galactose and the pentoses, may be synthesized in the body and are not required for human nutrition.

A few persons possess an inherited inability to utilize fructose from the diet. Apart from an abnormal excretion of fructose in the urine (which could be misinterpreted in the diagnosis of diabetes mellitus by undifferentiated sugar tests); such **fructosuria** is benign and unimportant clinically. More serious is the importance of galactose in the diet, experienced by infants with the inborn error known as galactosemia, which may lead to mental retardation and cataract; these may be prevented by the avoidance of milk and other sources of galactose in the diet. In terms of oral health, large amount of free sugars in the diet should be avoided because these sugars are excellent substrates for bacteria in the mouth and hence contribute to dental caries.

Assimilation

Assimilation is the combination of two processes to supply nutrients to the cells. The first is the process of absorption of vitamins, minerals, and other chemicals from food within the gastrointestinal tract. In humans, this is always done with a chemical breakdown (enzymes and acids) and physical breakdown (oral mastication and stomach churning).

The second process is the chemical alteration of substances in the bloodstream by the liver or cellular secretions. By this process; the absorbed food reaches the cells via the liver.

Assimilation in simple words takes place in the human body in the small intestine where the villi absorb and transport simpler substances/molecules.

Examples of biological assimilation

- **Photosynthesis**, a process whereby CO_2 and H_2O are transformed into a number of organic molecules in plant cells.
- **Nitrogen fixation** from the soil into organic molecules by **symbiotic bacteria** which live in the roots of certain plants, such as those of Leguminosae family.
- The **absorption of nutrients** into the body after digestion in the intestine and its transformation in biological tissues and fluids.

Assimilation of carbohydrates, lipids and proteins inside the cell and their storage

Carbohydrates

Food, which we have taken, is digested in the mouth, where enzyme in saliva, called salivary amylase (**ptyalin**), starts breaking complex molecules into simpler sugars and other enzyme found in the mouth, known as **lingual lipase** starts the digestion of lipids/fats. Chewed-up food passes through the esophagus and stomach with little digestion in the intestine, where it is broken down into the simplest sugar molecules which are then absorbed through the small intestine wall into the bloodstream which is later on utilized by our body.

$$\text{Starch} \xrightarrow{\text{Salivary amylase}} \text{Maltose} + \text{Dextrin}$$

The excess of monosaccharides—the glucose, fructose and galactose—is usually stored in the liver and muscle cells in the form of glycogen (**glycogenesis**). Whenever there is a deficiency of glucose in the blood, the glycogen is converted into glucose (**glycogenolysis**).

Muscle glycogen is utilized during muscle contraction. Glucose is utilized in the production of energy for various body activities. **A considerable amount of glucose is converted into fat and stored as such.**

Lipids

Fats and oils are not digested easily. **Bile**, produced by the liver and stored in the gallbladder, can attach to molecules of both water and fat. **Lipase**, a digestive enzyme, can break it down into simpler molecules. The broken down fat particles—fatty acids and cholesterol—are absorbed through the intestinal walls into the bloodstream, where they accumulate in the chest veins and are then carried to fat-deposit areas throughout the body to be stored and used for fuel, when necessary.

The fat is stored in the fat depots of the body, such as mesenteries, subcutaneous layers, etc. The fat stored is a readily available source of fuel for the cells. Fat has important insulating properties in connection with the conservation of heat and maintenance of body temperature.

Fat also plays a protective role as a filling around packing material and between organs. In the liver, phospholipids are formed which are returned to the blood to be used by all the cells. In the liver cells, the fats are converted into amino acids and carbohydrates.

Proteins

Protein molecules are quite large. **Churning** helps break proteins down into smaller particles for digestion; later a number of enzymes, including from the substances from the pancreas and even amino acids digest the proteins.

Amino acids are not stored but are taken up by the cells in connection with the synthesis of proteins, which are then used for growth, repair, etc.

Excess amino acids can be converted into glucose and then to fat and are thus stored. This is an irreversible reaction. Amino acids can also be converted to glucose and used as fuel for the cell. During their conversion to glucose, the amino acids are deaminated (removal of amino groups $-NH_2$).

The liver is the chief site for deamination, i.e. a process by which the amino group is removed from the amino acids resulting in the production of **ammonia**. The ammonia is soon converted into urea, which is filtered from the blood in the kidney.

Egestion (Defecation)

The elimination of faeces from the alimentary canal is called egestion or defecation. The faeces is a waste matter discharged from the alimentary canal.

Mechanism of egestion

Peristalsis gradually pushes the indigestible materials of the small intestine into the large intestine or colon. Normally, 1500 ml of chyme passes into the large intestine per day. The colon absorbs most of the water. **It also absorbs electrolytes, including sodium and chloride from the chyme.**

The epithelial cells of the colon also excrete certain salts such as iron and calcium from the blood. *Escherichia coli* (bacterium) lives in the colon which feeds on undigested matter. This bacterium, in turn, produces vitamin B_{12} (cobalamin), vitamin K, vitamin B_1 (thiamine) and vitamin B_2 (riboflavin) which are absorbed by the wall of colon. Consequently, the chyme is converted into semisolid faeces.

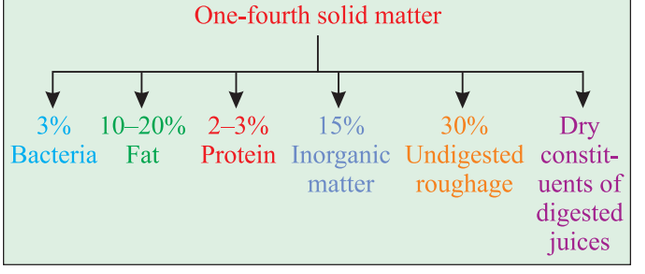
As the pellets of faeces enter the rectum, distension in the rectal wall induces the feeling of defecation due to a '**defecation reflex**'. This reflex initiates peristalsis in the last part of the colon (sigmoid colon) and the rectum, forcing the faeces towards anus. As the faeces reaches the anus, involuntary relaxation of the internal sphincter and voluntary relaxation of external sphincter cause defecation.

Voluntary contraction of the diaphragm and abdominal muscles forces the sphincter open, the faeces is expelled through the anus (contraction of the abdominal muscles and lowering of the diaphragm increases the intra-abdominal pressure which aids in the process of defecation). In infants, the defecation occurs by reflex action without the voluntary control of the external anal sphincter.

Contributents of faeces

The faeces consists of the following two parts (Flowchart 6.1).

Flowchart 6.1: Constituents of solid matter of faeces



1. About three-fourths water
2. One-fourth solid matter

Dead mucosal cells, mucus and cholesterol molecules are also found in the faeces. Its brown colour is due to the brown pigments, stercobilinogen and stercobilin, which are derivatives of bilirubin.

The liver and the carbohydrates

Carbohydrates are essential metabolites for a wide range of processes in the body. Free sugars, particularly glucose, are important energy fuels in the nervous system, muscles and many other tissues. In combined form the pentose sugars contribute to structures of nucleotide coenzymes and polynucleotides of DNA and RNA. Sugars may also be combined with lipids forming ***glycolipids,*** which are of particular significance in myelin and other membranes of nerves and in the erythrocytes where they determine the blood group reactions. Combined with proteins as ***glycoproteins***, the sugars play a role in secretion and the external recognition properties of cell membranes. Finally, the sugars may polymerize among themselves to produce the storage energy substance, *glycogen,* or they may form heteropolymers, such as the glycosaminoglycans of the connective tissue ground substance. Anomalies in carbohydrate metabolism include the common medical problem of diabetes mellitus, as well as several less frequent **genetic disorders such as the glycogen storage diseases**.

The hepatic cells of the liver serve as a major retailoring site and exert a regulatory influence upon the dietary carbohydrates. Once absorbed, the sugars are taken from the intestinal lining cells into the blood capillaries that drain into the portal vein. The latter vessel then conveys the monosaccharides derived from the diet directly to the liver. Here, the sugars are rapidly taken up by the hepatic cells, where glucose may be formed from the fructose and galactose.

As in other tissues of the body, the energy needs of the liver itself may be met by the intracellular catabolism of incoming glucose. Two distinct processes are involved in glucose catabolism: (1) the **glycolytic pathway**, which converts one mole of glucose to two moles of lactate and also yields two moles of ATP, and (2) the **pentose phosphate pathway,** which converts one mole of glucose to six moles of CO_2 and also yields 12 moles of NADPH. Both processes are anaerobic, both enzyme systems are contained in the soluble cytoplasm and both pathways require prior phosphorylation of glucose to glucose-6-phosphate by the ATP requiring enzyme, glucokinase. Here, however, the similarities end. Whereas, glycolysis provides the energy for cellular phosphorylations, protein synthesis and other processes that require ATP. The pentose-phosphate pathway is a primary source of reducing energy for biosynthetic reactions such as the steps in fatty-acid and steroid syntheses that require NADPH. The pentose-phosphate pathway breaks down the hexose molecule completely to CO_2 under anaerobic conditions but glycolysis affects only the partial catabolism of glucose to three-carbon fragments; for complete oxidation of the latter, the presence of oxygen and mitochondria is required. Under aerobic conditions, the combined actions of glycolysis in the cytoplasm and of the citric-acid cycle and oxidative phosphorylation in the mitochondria will result in a maximum yield of 38 moles of ATP per mole of glucose.

The two pathways differ also in the intermediates formed: the pentose-phosphate pathway involves the intermediate production of pentose sugars, which may be tapped off to provide the cell's requirements for ribose in the formation of nucleotides and nucleic acids. The glycolytic pathway can be diverted to produce α-glycerophosphate for fat synthesis by the reduction of triose intermediates. It also produces pyruvate, which may be used in the net synthesis of alanine aspartate and various other substances through oxaloacetate formation, as well as of the myriad cellular constituents formed from acetyl-CoA. Although, reactions within the pentose phosphate pathway may be used to form glucose in photosynthetic organisms, in mammalian tissues, the process is essentially irreversible in the direction of glucose breakdown. In contrast, the reactions of glycolysis may be reversed to allow the synthesis of glucose by **gluconeogenesis**. In the liver, approximately one-third of the glucose oxidized follows the pentose phosphate pathway, the remaining two-thirds being metabolized by the glycolytic reactions.

Glucose utilization in the erythrocytes

Glucose enters the erythrocytes from the surrounding plasma by means of an energy-requiring, facilitated transport system that is not influenced by insulin. Under normal circumstances, over 90% of the glucose utilization by erythrocytes is by means of glycolytic pathway, which provides the ATP required for energy-mediated transport reactions across the cell membrane. In addition to providing for glucose uptake, these transport mechanisms, which are coupled to **ATP** hydrolysis, promote the movement of $\mathbf{Na^+}$ outward and $\mathbf{K^+}$ inward to maintain the internal composition (high $\mathbf{K^+}$, low $\mathbf{Na^+}$) against the concentration gradients of blood plasma (low $\mathbf{K^+}$, high $\mathbf{Na^+}$). The triose-phosphate dehydrogenase reaction of glycolysis also generates NADH, which is necessary to reduce any excess of *methemoglobin,* (an oxidized form of hemoglobin that does not combine with O_2) that may be formed in the erythrocyte by oxidizing agents.

A byproduct of glycolytic pathway that is found in uniquely high concentration in erythrocytes is **2,3-diphosphoglycerate**. The latter serves several functions: Firstly, it is the major phosphate containing compound in the cell and serves as an important anion to balance the intracellular cations as well as acts as a buffering agent; secondly, it may serve as an emergency energy reserve for a cell that lacks phosphocreatine and glycogen stores; and thirdly, it binds to hemoglobin to lower its affinity for O_2, and, thus, facilitates the unloading of oxygen to tissues. The pentose-phosphate pathway normally accounts for only a small percentage of total glucose catabolism, but under conditions where the NADPH requirements are increased, the activity of this 'shunt' may accelerate many times. The major need for NADPH in the erythrocyte is to maintain the important intracellular reductant, *glutathione,* in its reduced –SH form; hence, the presence of agents that promote the oxidation of glutathione to the –S–S– form will activate the pentose phosphate pathway to provide compensatory reducing equivalents in the form of NADPH.

HEMOLYTIC ANAEMIAS

The importance of carbohydrate and energy metabolism for the survival of the erythrocyte is emphasized by several inborn errors of metabolism that lead to **hemolysis** and **anaemia.** In type VII glycogen-storage disease, e.g. **phosphofructokinase enzyme** of the erythrocytes is partly deficient, which results in reduced efficiency of ATP production and **mild hemolytic symptoms.**

A more severe disorder is associated with another recessively inherited condition, ***pyruvate-kinase deficiency,*** in which the enzyme in the erythrocytes is specifically decreased. Since glycolysis is blocked before the last energy-producing step, there can be no net ATP generation from glucose catabolism. The affected cells also accumulate increased amounts of 2,3-diphosphoglycerate, but they cannot use the latter for ATP production because of the kinase deficit. The resulting lack of cellular energy sources causes an impaired maintenance of internal composition and **disruption of membrane integrity**. Deformation and other alterations in surface properties lead to the recognition by the spleen of such cells as abnormal, and they are consequently removed and destroyed by the reticuloendothelial system. Other similarly severe hemolytic anaemias have been described with inherited lesions of the erythrocytic enzymes of the glycolytic pathway (triosephosphate isomerase, glucosephosphate isomerase, phosphoglycerate kinase and hexokinase), but these conditions seem to be very rare.

The significance of pentose phosphate pathway for the ability of the erythrocyte to withstand external stresses is demonstrated by a group of common, drug-induced, **hemolytic anaemias that are characterized by glucose-6-phosphate dehydrogenase deficiency.** The diseases are transmitted as sex-linked recessive traits and are found most frequently among black people who are inhabitants of the Mediterranean area.

Carbohydrate metabolism

The major **anabolic route** open for carbohydrates in the human body is *glycogenesis* in which genesis of glycogen (biosynthesis of the polysaccharide glycogen) takes place whereas the catabolic route is **glycogenolysis.** The main aim of catabolic pathway is to liberate energy which is used for various purposes in the human body. Side-by-side, certain non-carbohydrate substances lead to the biosynthesis of glucose and glycogen; this process is referred to as *gluconeogenesis* or neoglucogenesis (another anabolic pathway).

Amphibolic pathways

The term **amphibolic** is used to describe a biochemical pathway that involves both **catabolism** and **anabolism**. Catabolism is a degenerative phase of metabolism in which large molecules are converted into smaller and simpler molecules, which involves two types of reactions. The term 'amphibolic' was proposed by B. Davis in 1961 to emphasize the dual metabolic role of such pathway. These pathways are considered to be central metabolic pathways.

All the reactions associated with the synthesis of biomolecule converge into the following pathway, viz., glycolysis, Krebs cycle and electron transport chain, exist as amphibolic pathway meaning that they can function anabolically as well as catabolically.

Important amphibolic pathways are:

1. Citric acid cycle
2. Embden-Meyerhof pathway
3. Pentose phosphate pathway
4. Entner-Doudoroff pathway (alternate pathway in microbes)

The Embden-Meyerhof pathway along with Krebs cycle are the centre of metabolism in nearly all bacteria and eukaryotes (humans); they not only provide energy but also provide precursors for the biosysnthesis of macromolecules that make-up living system.

Citric acid cycle

Citric acid cycle (Krebs cycle) is a good example of an amphibolic pathway because it functions in both the degradative (carbohydrate, protein and fatty acid) and biosynthetic processes. The cycle occurs in the cytosol of bacteria and within the mitochondria of eukaryotic cells. The TCA cycle provides electrons to the ETC which is used to drive the production of ATP in oxidative phosphorylation. Intermediates of the TCA cycle, such as oxaloacetate, are used to synthesize macromolecule constituents such as amino acids, e.g. glutamate and aspartate.

The first reaction of the cycle, in which OAA (a 4-carbon compound) condenses with acetate (a 2-carbon compound) to form citrate (a 6-carbon compound) is typically anabolic. The next few reactions, which are intramolecular rearrangements, produce isocitrate. The conversion of D-isocitrate to α-ketoglutarate followed by its conversion to succinyl-CoA are typically catabolic. COO is lost in each step and succinate (a 4-carbon compound) is produced.

There is an interesting and critical difference in the coenzymes used in catabolic and anabolic pathways. In catabolism, NAD^+ serves as an oxidizing agent when it is reduced to NADH, whereas in anabolism, the coenzyme NADPH serves as the reducing agent and is converted to its oxidized form $NADP^+$.

Citric acid has two modes that play two roles. The first role is **energy production** that is produced by oxidative mode, as the acetyl group of acetyl-CoA is fully oxidized to CO_2. This mode produces most of the ATP in the metabolism of aerobic heterotrophic metabolism, as this energy conversion in the membrane structure (cytoplasmic membrane in bacteria and mitochondria in eukaryotes) by oxidative phosphorylation by moving electron from donor (NADH and $FADH_2$) to the ecceptor O_2. Every cycle gives 3 NADH, 1 $FADH_2$, CO_2 and GTP.

The second role is biosynthetic, as TCA cycle regenerates OAA when cycle intermediates are removed for biosysnthesis.

Pentose phosphate pathway (PPP)

The PPP gets its name because it involves several intermediates that are phosphorylated 5-carbon sugars (pentoses). The PPP provides monomers for many metabolic pathways by transforming glucose into 4-carbon sugar (erythrose) and 5-carbon sugar (ribose), that are important monomers in many matabolic pathways. Many of the reactants in the PPP are those similar to glycolysis, also both occur in cytosol. The ribose-5-PO_4 can be transported to nucleic acid metabolism, producing the basis of DNA and RNA monomers, the nucleotides.

GLYCOGENESIS

Glycogenesis (genesis, i.e. synthesis of glycogen, Fig. 6.1) is an anabolic process which requires energy expenditure both from ATP and uridine triphosphate (UTP). This process may be referred to as the genesis (synthesis) of glycogen from glucose or other sugars. Glycogen is synthesized in practically all the tissues of the body but the major sites are:

(a) Liver, and (b) Muscles

Glycogenesis starts with glucose. In the very first reaction glucose gets phosphorylated to form glucose-6-phosphate in the presence of ATP; this reaction is catalyzed by glucokinase. Thus, it may be seen that glucose-6-phosphate so formed occupies a key position as a common intermediate in carbohydrate metabolic pathways: It may be converted reversibly to pyruvate by glycolysis or gluconeogenesis; it may be converted to pentoses or to CO_2 irreversibly by the pentose phosphate pathway; and it may be converted reversibly to glycogen also.

In the next step, mutation of the phosphate group of glucose-6-phosphate from position 6 to position 1 takes place in the presence of a cofactor glucose-1,6-diphosphate; this reaction is catalysed by the enzyme phosphoglucomutase. In the next reaction, glucose-1-phosphate reacts with uridine triphosphate (UTP) to form active nucleotide, i.e. uridine diphosphate glucose in the presence of an enzyme UDPG pyrophosphorylase; in this process, two terminal phosphates are removed from UTP as inorganic pyrophosphate, while the remaining UMP portion gets joined by a pyrophosphate bridge to glucose-1-phosphate to form UDP-glucose.

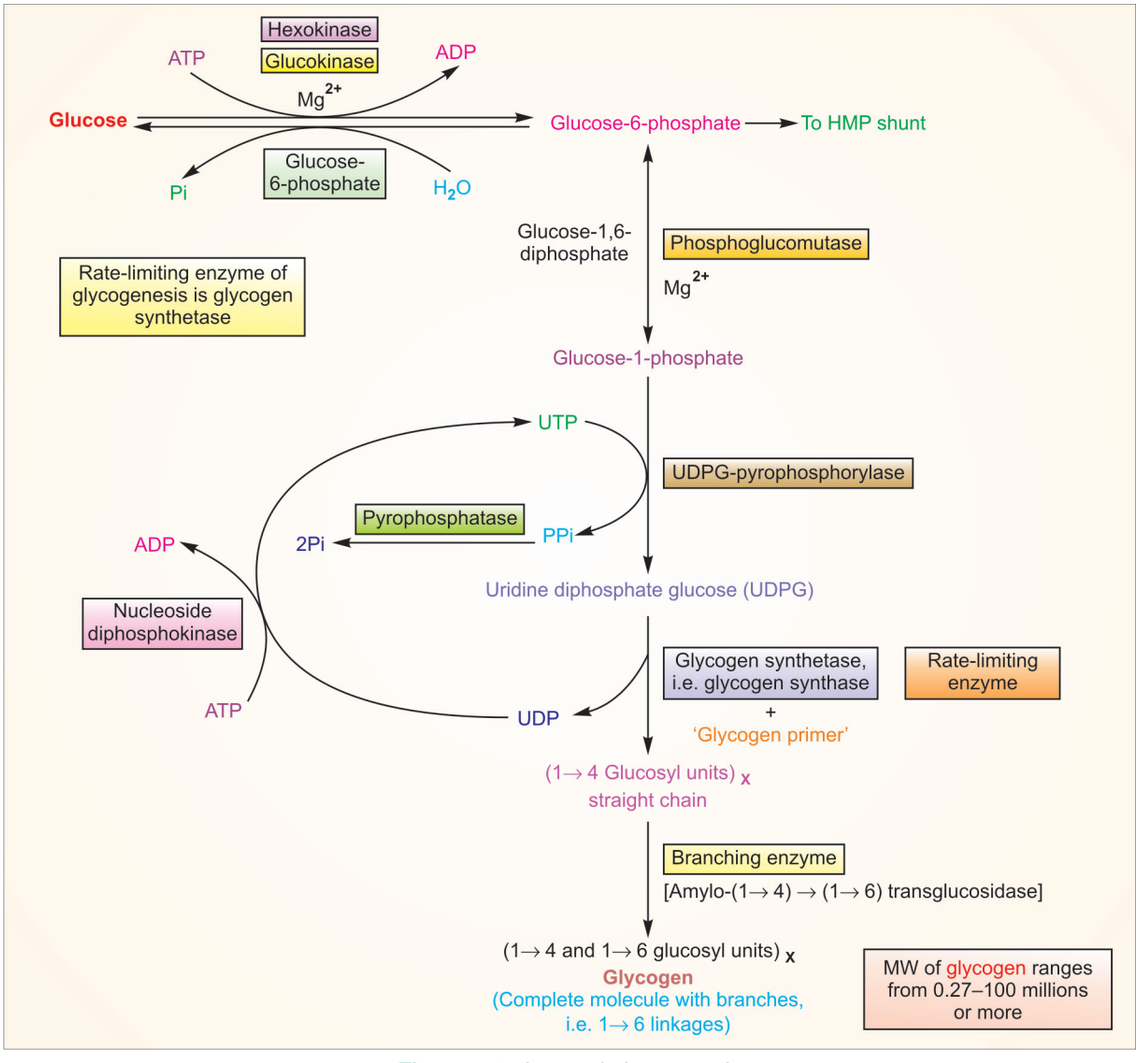


Fig. 6.1: Pathway of glycogenesis

The next reaction is catalyzed by the enzyme glycogen synthetase, in this reaction, the C_1 of the activated glucose of UDPG forms a glycosidic bond with the C_4 of a terminal glucose residue of glycogen, liberating uridine diphosphate (UDP) with the formation of unnatural glycogen, i.e. straight chain; a pre-existing glycogen or 'primer' must be present to initiate this reaction (Fig. 6.1).

Mechanism of branching

The addition of a glucose residue to the pre-existing glycogen chain or 'primer' occurs at the non-reducing outer end of the molecule, so that branches of the glycogen become elongated as successive 1→4 linkages occur, **as a result of which a 'tree' like molecule of glycogen gets synthesized.** When the chain acquires a length of minimum of 11 glucose residues, a second enzyme called a branching enzyme (amylo-[l→4]→[1→6]-transglucosidase) transfers a part of the 1→4 chain (minimum length of 6 glucose residues) to a neighbouring chain to form α-1→6 linkage, thus, a branch point is established in the molecule. The branches grow by further additions of 1→4-glucosyl units and further branching. This highly branched structure serves two major purposes in the cell:

(a) **Firstly**, it provides numerous ends for glucose molecules to be attached or removed rapidly;
(b) **Secondly**, it forms a dense and compact storage particle in the cell.

In this way, natural molecule of glycogen with α-1,6 linkages, i.e. branches is synthesized.

Significance of the pathway

Significance of this pathway lies in the fact that it acts as a source of energy in the form of sugar, both for the blood that nourishes other tissues and for the needs of the liver itself especially when one is starved or sick. **Glycogen**, in fact, is a **reservoir** of energy. After a heavy intake of carbohydrates, as much as one-tenth of the liver mass may consist of this storage form of glucose, whereas in starvation or during illness when there is loss of appetite, the glycogen reserve may be almost depleted in order to meet out the energy requirements of the body.

GLYCOGENOLYSIS

This process (Fig. 6.2) may be referred to as the breakdown of glycogen in the tissues. It may be broken down to glucose as in liver and kidneys or glucose-6-phosphate as in muscles. The process gets enhanced by hypoglycemia or under the influence of certain hyperglycaemic hormones. Liver glycogen is metabolically more easily available for this pathway as compared to muscle glycogen.

In the very first reaction of this pathway (Fig. 6.2), enzyme phosphorylase breaks the linkages between glucose monomers of the glycogen chains by adding inorganic phosphate. This enzyme cleaves off the glucose units one at a time from the tips of the glycogen tree, rather than by random attack. Phosphorylase can act only upon the linear portions of the polymer down to the branch points; a separate *debranching enzyme,* i.e. amylo-1,6-glucosidase hydrolyzes the 1→6 linkage, yielding free glucose. Since, the enzyme phosphorylase adds phosphate at the linear 1→4 linkages, the products of phosphorylase action will be glucose-1-phosphate molecules, which outnumber the free glucose molecules from the debranching process by a ratio of roughly 10:1.

In the next reaction, in the liver/kidney tissues, glucose-1-phosphate molecule is converted to glucose-6-phosphate by the action of enzyme phosphoglucomutase in the presence of coenzyme glucose-1,6-diphosphate; this reaction being reversible. Now, in the third and last reaction of this pathway, glucose-6-phosphate is acted upon by the enzyme glucose-

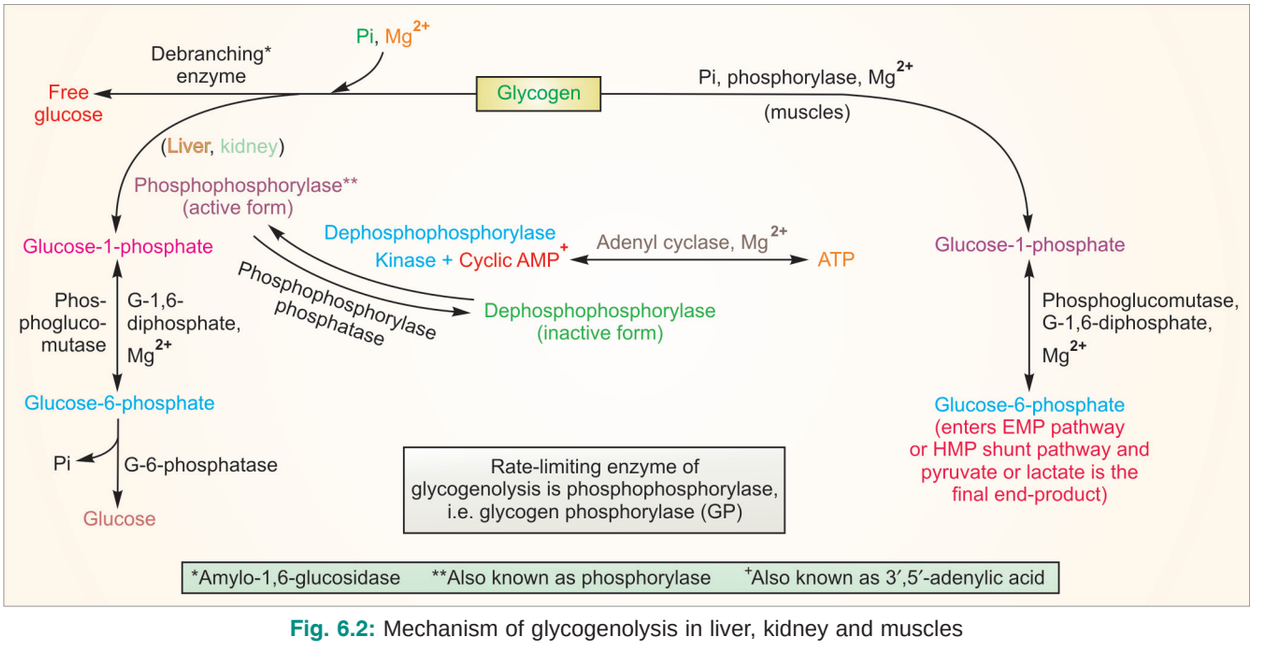


Fig. 6.2: Mechanism of glycogenolysis in liver, kidney and muscles

6-phosphatase, as a result of which glucose is formed along with the liberation of inorganic phosphate. Glucose, so obtained is freely diffusible through the bloodstream.

Be it known, that muscles lack the enzyme glucose-6-phosphatase, therefore, in these tissues, the end-product of muscular glycogenolysis is always glucose-6-phosphate and not glucose as in the case of hepatic and renal tissues.

However, glucose-6-phosphate formed in hepatic, renal and muscular tissues may be utilized directly for energy purposes either through glycolytic or pentose-phosphate pathways.

Regulatory factors of glycogenolysis

There are several regulatory factors which play vital role in the regulation of this pathway, namely:

(a) **Active phosphorylase or phosphophosphorylase** is the *rate-limiting* enzyme of glycogenolysis: Inactive dephosphophosphorylase is converted to its active form i.e; phosphophosphorylase by the action of enzyme dephosphophosphorylase kinase (Fig. 6.2).

(b) **There is yet another enzyme phosphophosphorylase phosphatase** which may convert phosphophosphorylase into dephosphophosphorylase. In this way, the above named phosphatase enzyme has a regulatory effect. If the rate of this pathway is to be decreased, then the activity of this phosphatase becomes more and vice-versa.

(c) Role of **cyclic AMP,** the presence of which is must for the activity of enzyme dephosphophosphorylase kinase.

(d) Role of enzyme **adenyl cyclase**, the main function of which is to convert ATP into cyclic AMP, and vice-versa in the presence of magnesium ions.

(e) Role of certain **hormones:** Hepatic and muscular glycogenolysis get accelerated by the action of adrenaline, which, in turn, converts more ATP into cyclic AMP; likewise hepatic glycogenolysis is also influenced in positive direction by the action of glucagon; more to say, in adrenal cortex, hormone ACTH plays the same role.

Insulin appears to suppress glucose-6-phosphatase activity, thereby reducing glycogenolysis. Contrarily, starvation, less carbohydrate consumption, glucocorticoids, thyroid hormones and glucagon may stimulate glycogenolysis by enhancing the activity of enzyme glucose-6-phosphatase.

Significance

It lies in the fact that this pathway is very important and essential as well from the point of view of giving energy to various tissues of the body, especially in starvation, sickness, carbohydrate deprivation and other similar conditions.

GLYCOLYSIS OR EMBDEN-MEYERHOF-PARNAS PATHWAY (EMP pathway)

There is a minimum requirement of glucose in all the tissues of the body but some tissues *like brain and erythrocytes require it in a good concentration.* It is the major pathway for the utilization of glucose which operates in all the cells. It is a unique pathway because it can operate under aerobic and as well as anaerobic conditions.

GLYCOLYTIC PATHWAY

This is a very important pathway (Fig. 6.3) from several points and may be referred to as the breakdown of glucose or glycogen to produce pyruvate or lactate. This operates in almost all the tissues of the body but liver and muscles are the main sites. Various enzymes involved in this pathway are found in the extramitochondrial soluble fraction of the cell, the cytosol.

Glucose units derived from dietary carbohydrates or from glucose synthesized in the liver, enter cells as free glucose. In the very first reaction of this pathway glucose molecule gets phosphorylated with ATP by an enzyme called hexokinase to yield glucose-6-phosphate (Fig. 6.3) in the presence of Mg^{2+} ions. Several hereditary disorders of hexokinase deficiency are known today, the reason being that it exists in different isoenzymic forms. In each case, the main symptom of enzymic deficiency is anaemia. Another glucose phosphorylating enzyme exists in liver which is called glucokinase; this has got greater specificity for glucose than the hexokinase.

The very first reaction, i.e. the conversion of glucose to G-6-P, is irreversible but can be reversed by another enzyme glucose-6-phosphatase, which remains present in liver and kidney tissues. Muscles do not contain glucose-6-phosphatase, hence the formation of glucose from glucose-6-phosphate is not possible in such tissues. Side-by-side, glucosyl units obtained from glycogen in the presence of inorganic phosphate may also be converted to glucose-1-phosphate by the action of phosphorylase; these units do not require ATP for activation. G-1-P may then be converted to G-6-P by the action of phosphoglucomutase in the presence of cofactor glucose-1,6-diphosphate and Mg^{2+} ions.

Glucose-6-phosphate forms the active substrate of glycolysis, which is now isomerized to form fructose-6-P in the presence of enzyme phosphohexose-isomerase. At this juncture fructose can also enter into glycolytic pathway by forming fructose-6-P; this reaction being catalysed by an unspecific hexokinase in the presence of ATP and Mg^{2+} ions. In the next reaction, fructose-6-P is phosphorylated at C-1 position forming a sugar diphosphate, i.e. fructose-1,6-diphosphate under the influence of enzyme phosphofructokinase (PFK), ATP and Mg^{2+} ions; *this reaction is also irreversible.* The reaction can, however, be reversed by another enzyme, i.e. fructose-1,6-diphosphatase (Fig. 6.3). F-l,6-diP is now cleaved under the influence of an enzyme termed aldolase as a result of which dihydroxyacetone phosphate and glyceraldehyde-3-phosphate are formed. The two triose phosphates formed are convertible into each other under the influence of an enzyme called triose phosphate isomerase and these two triose phosphates can further be condensed to form fructose-1,6-diP by the reversal of aldolase activity. Glyceraldehyde-3-P can now enter the subsequent reactions of glycolysis. The equilibrium of the reactions is such that dihydroxyacetone phosphate (DHAP) also gets changed into glyceraldehyde-3-P and enters into subsequent reactions of glycolysis. At this juncture, glycerol obtained from fat degradation can also enter into the forward or

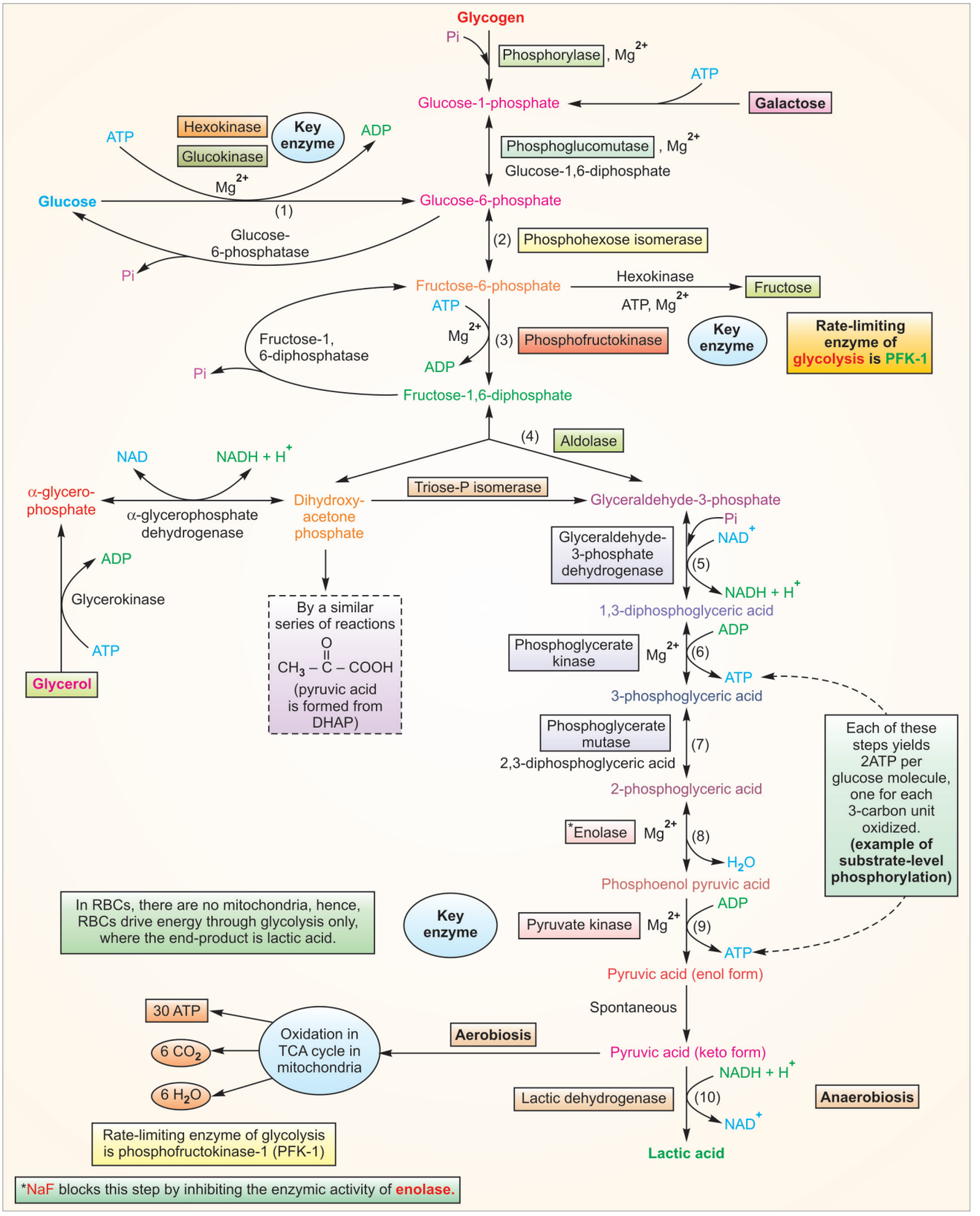


Fig. 6.3: Glycolytic pathway (glycolysis)

backward reactions of glycolysis by first getting converted into α-glycerophosphate and then into DHAP.

Now, glyceraldehyde-3-P is oxidized to form 1,3-diphosphoglyceric acid by the action of enzyme glyceraldehyde-3-P-dehydrogenase in the presence of nicotinamide adenine dinucleotide (NAD) as hydrogen acceptor and inorganic phosphate. The phosphate group gets attached at position-1 of glycerol by a high-energy bond which is formed by intramolecular rearrangement of energy during dehydrogenation. In the next reaction, high energy phosphate group at C_1 is transferred to ADP forming ATP and 3-phosphoglyceric acid (Fig. 6.3) under the influence of enzyme phosphoglycerate kinase and Mg^{2+} ions. Now, 3-PGA is changed into 2-phosphoglyceric acid by the enzyme phosphoglycerate mutase in the presence of cofactor 2,3-diphosphoglyceric acid. In the next reaction, 2-PGA loses a molecule of water which is associated with a simultaneous intramolecular rearrangement of energy forming a high energy bond compound, i.e. phosphoenolpyruvic acid under the influence of enzyme enolase and Mg^{2+} ions.

The high energy phosphate group in phosphoenolpyruvic acid molecule is transferred to ADP in the presence of enzyme pyruvate kinase and Mg^{2+} ions with the formation of ATP and enolpyruvic acid. Enol form of pyruvic acid gets changed into keto form of pyruvic acid spontaneously under the influence of same enzyme, i.e. pyruvate kinase. **The overall reaction of conversion of phosphoenolpyruvic acid into pyruvic acid is irreversible.**

Pyruvic acid forms the main end product of glycolysis in those tissues which are supplied oxygen in sufficient quantity, but in those tissues where oxygen supply is not met fully, e.g. skeletal muscles, lactic acid forms the usual end product of glycolysis. In such tissues, pyruvic acid is reduced to lactic acid under the influence of enzyme lactic dehydrogenase in the presence of reduced NAD, which is supplied by the triose-phosphate dehydrogenase reaction. Conversion of pyruvic acid into lactic acid allows glycolysis to run smoothly under anaerobic conditions.

Biomedical importance of glycolysis

1. **It is the main pathway for the oxidation of glucose.**
2. It is also the main pathway for the metabolism of fructose and galactose, which one consumes daily in moderate quantity via diet.
3. It provides ATP.
4. A small number of diseases occur in which enzymes of glycolysis (e.g. pyruvate kinase) are deficient in activity; such conditions are mainly manifested as hemolytic anaemias.
5. In fast growing cancerous cells, this pathway proceeds at a much higher rate resulting into more production of pyruvate. This, in turn, results in excessive production of lactate, which makes the internal environment of the tumour acidic, a situation that may have implications for certain types of cancer therapy.
6. Deficiency of pyruvate dehydrogenase enzyme causes lactic acidosis.

Role of arsenate and fluoride in glycolysis

Glyceraldehyde-3-P is converted to 1,3-diphosphoglyceric acid by the enzyme **glyceraldehyde-3-P-dehydrogenase** in the presence of NAD^+ and inorganic phosphate. If ***arsenate*** is present, it will compete with inorganic phosphate (Pi) in the above reaction to give 1-arseno-3-phosphoglyceric acid, which hydrolyzes spontaneously to give 3-phosphoglyceric acid along with heat, without generating ATP. This is an important example of the ability of arsenate to accomplish uncoupling of oxidation and phosphorylation.

2-PGA is converted to phosphoenolpyruvic acid, reaction being catalysed by enolase in the presence of Mg^{2+} ions. **Enolase** is inhibited by **fluoride**, a property that can be made use of when it is required to prevent glycolysis prior to the estimation of blood glucose.

Regulation of the glycolytic pathway

The irreversible reactions of glycolysis (Fig. 6.4) are catalysed by:

(a) Hexokinase,
(b) Phosphofructokinase (PFK), and
(c) Pyruvate kinase

These are regulated by a number of allosteric modifiers as shown in Fig. 6.4. Regulation of these enzymes is important in controlling the rate of glycolysis.

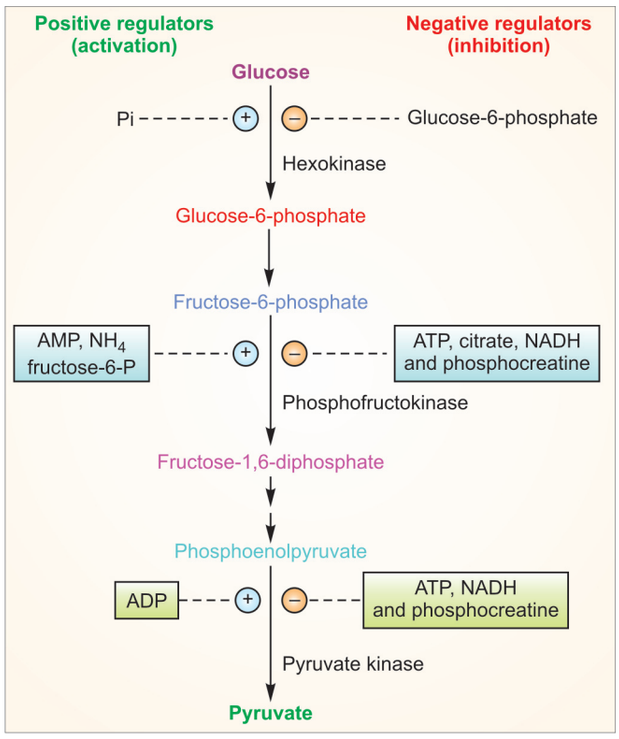


Fig. 6.4: Allosteric modifiers; positive and negative regulators

Shuttle systems

There are some reactions that take place in the cytosol which produce NADH which have to be oxidized through the electron transport chain situated in the inner mitochondrial

membrane. NADH is not permeable to the mitochondrial membrane; therefore, shuttle systems operate for its transport.

There are three types of shuttle system (Fig. 6.5):

1. **Glycerophosphate shuttle,**
2. **Malate-aspartate shuttle, and**
3. **Isocitrate shuttle**

1. Glycerophosphate (Glycerol-phosphate) shuttle

This shuttle starts with the transfer of a pair of electrons (reducing equivalents) from cytosolic NADH to dihydroxyacetone phosphate (an intermediate of glycolysis) converting it into glycerol-3-phosphate by a cytosolic dehydrogenase. Glycerol-3-phosphate enters into mitochondria and is oxidized to DHAP by a mitochondrial dehydrogenase. The electron pair of glycerol-3-phosphate is transferred to the coenzyme FAD to form FADH which finally gets oxidized through ETC yielding 1.5 molecules of ATP. This shuttle occurs in muscles and other tissues.

2. Malate-aspartate shuttle

In this cycle, reducing equivalents are transferred from NADH in the cytosol to oxaloacetate, forming malate, which then enters into mitochondria and oxidized by malate dehydrogenase (a Krebs cycle enzyme). In this reaction, NADH and oxaloacetate are generated. NADH gets oxidized by ETC yielding **2.5 molecules of ATP** instead of **1.5 ATP** as in glycerol-phosphate shuttle.

The OAA, thus, regenerated cannot cross the inner mitochondria membrane and, therefore, undergoes transamination with glutamate to form aspartate and α-ketoglutarate. The aspartate then enters into cytosol and transaminates with α-KG to form oxaloacetate and glutamate and the cycle then gets restarted.

This shuttle is mostly operative in heart and liver, whereas, glycerol-phosphate shuttle takes place in muscles and other tissues.

3. Isocitrate shuttle

Isocitrate dehydrogenase (IDH) is an enzyme that catalyses the oxidative decarboxylation of isocitrate, producing α-ketoglutarate and CO_2. This is a two-step process which involves oxidation of isocitrate (a secondary alcohol) to oxalosuccinate (a ketone) followed by the decarboxylation of the carboxyl group beta to the ketone, forming α-ketoglutarate.

ENERGETICS OF GLYCOLYSIS

Glycolysis as a whole is an energy liberating process. Energy obtained from this pathway under aerobic and anaerobic

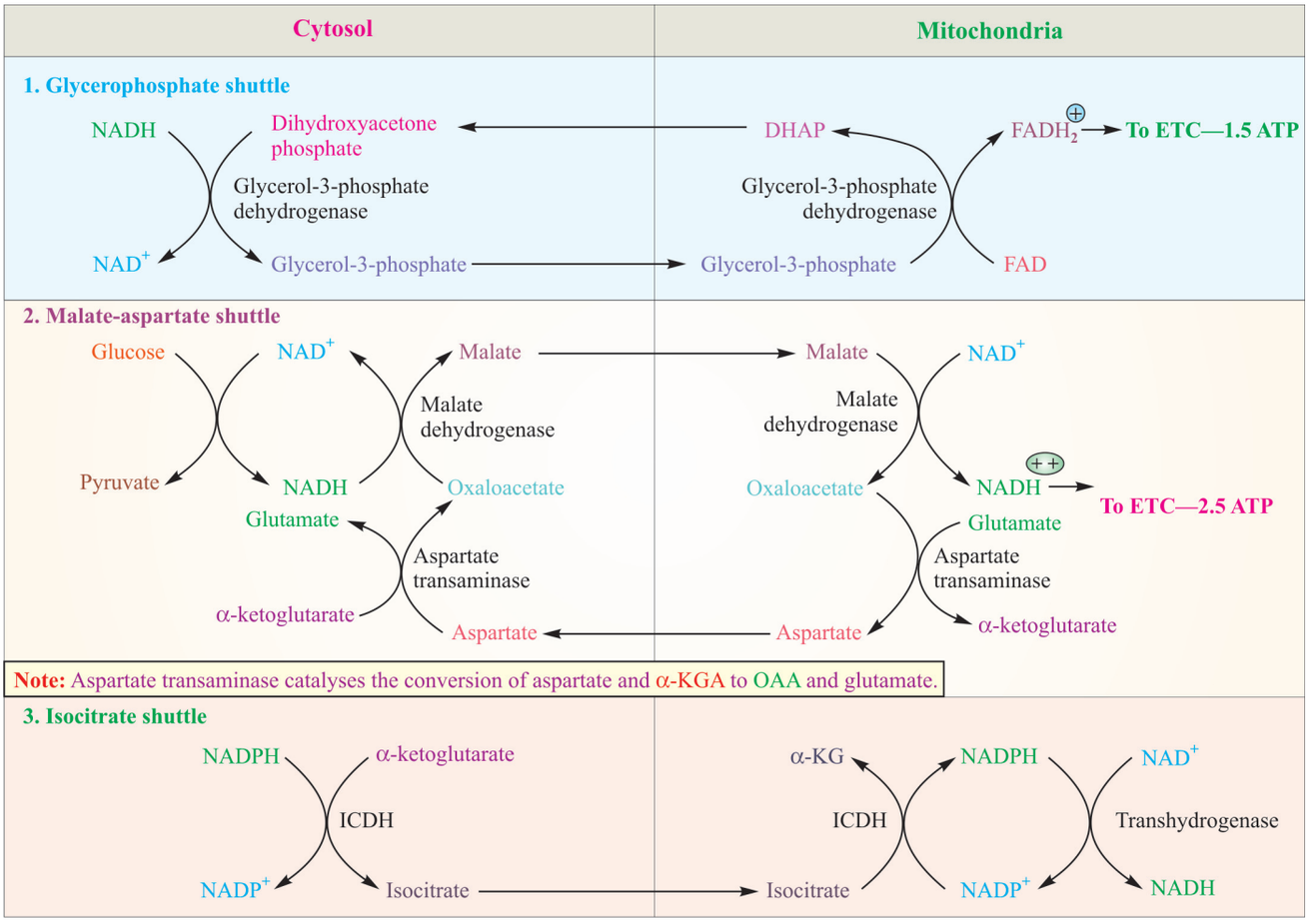


Fig. 6.5: Glycerophosphate, malate-aspartate and isocitrate shuttles

conditions is different. It is also dependent upon whether it is the free glucose or the glucosyl unit of glycogen which is entering into glycolysis.

In general, the sites of energy production in glycolysis are—(1) during conversion of 1,3-diphosphoglyceric acid (energy rich) into 3-PGA (low energy) and (2) during conversion of phosphoenol pyruvic acid (energy rich) into enolpyruvic acid (low energy). If ADP is present in these reaction systems, energy of energy-rich compounds is captured with the formation of ATP. One mole of ATP at each of the above two steps is synthesized per mole of 1,3-diphosphoglyceric acid or phosphoenolpyruvic acid utilized for forward reaction. DHAP formed by the action of aldolase also undergoes similar series of reactions as glyceraldehyde-3-P, mean to say, in all two moles of ATP are synthesized during this process. *Thus, four moles of ATP are formed in all per mole of hexose used up under anaerobic conditions.* Besides these, there exists one more site of energy production which comes into play under *aerobic* conditions, this is during conversion of glyceraldehyde-3-phosphate into 1,3-diphosphoglyceric acid when 1 mole of reduced NAD is formed.

The reduced NAD may be oxidized by respiratory chain under *aerobic* conditions forming 2.5 moles of ATP per mole of NADH. Since, during breakdown of 1 mole of glucose, or glucosyl unit of glycogen, two moles of triose phosphates are formed and both may enter the subsequent glycolytic reactions, hence, two moles of NADH may be produced which on oxidation would give rise to 5 moles of ATP under aerobic conditions, therefore, 9, i.e. (4 + 5) moles of ATP may be produced per mole of glucose being oxidized via glycolysis.

There are also sites in glycolysis where ATP is consumed for activation purposes. First of all, one mole of ATP is consumed in the very first reaction, i.e. in the conversion of glucose to glucose-6-phosphate. However, if the starting substance is the glucosyl unit of glycogen, this step is bypassed. The second site of ATP consumption is when F-1, 6-diP is formed from F-6-P, here too 1 mole of ATP is consumed.

In order to evaluate the net gain of energy, the energy consumption has also got to be taken into consideration and must be subtracted from total energy production. Under *anaerobic conditions;* therefore, there occurs a net gain of 3 ATP moles per glucosyl unit of glycogen and 2 ATP moles per free glucose mole broken down and oxidized to lactic acid (Table 6.1). Under *aerobic conditions* there occurs a net gain of 8 ATP moles per glucosyl unit and 7 ATP moles per free glucose molecule broken down to pyruvic acid (Table 6.2).

Table 6.1: Energy yield (i.e. number of ATP molecules generated) per molecule of glucose oxidized in glycolysis under anaerobic conditions, i.e. in the absence/deficiency of oxygen

Step	Reaction catalysed by enzyme (in glycolysis)	Source	Number of ATPs generated/consumed
1	Hexokinase/glucokinase	—	–1 (consumed)
3	PFK	—	–1 (consumed)
6	Phosphoglycerate kinase	ATP	1 × 2 = 2 (generated)
9	Pyruvate kinase	ATP	1 × 2 = 2 (generated)
		Total	4 – 2 = 2 ATP

Note: If glucosyl unit of glycogen participates in glycolysis, then there is yield of 3 ATP per mole of glucose oxidized under anaerobic conditions as the first step of glycolysis is bypassed saving 1 mole of ATP.

Table 6.2: Energy yield (i.e. number of ATP molecules generated) per molecule of glucose oxidized in glycolysis under aerobic conditions, i.e. when oxygen is available/sufficient

Step	Reaction catalysed by enzyme (in glycolysis)	Source	Number of ATPs generated/consumed
1	Hexokinase/glucokinase	—	–1 (consumed)
3	Phosphofructokinase	—	–1 (consumed)
5	Glyceraldehyde-3-phosphate dehydrogenase	NADH	2.5 × 2 = 5 (generated)
6	Phosphoglycerate kinase	ATP	1 × 2 = 2 (generated)
9	Pyruvate kinase	ATP	1 × 2 = 2 (generated)
		Total	9 – 2 = 7 ATP

Note: If glucosyl unit of glycogen involved in aerobic glycolysis, then a total of 8 moles of ATP are generated; as there is saving of 1 mole of ATP because of the bypassing of 1st step of glycolysis.

Hexokinases/glucokinase

There are four types of important mammalian hexokinase isoenzymes, namely hexokinases I, II, III and IV or A, B, C and D, respectively. Hexokinase IV (D) is often called glucokinase and differs from other hexokinases in kinetics and functions.

A hexokinase is an enzyme that phosphorylates hexoses (six-carbon sugars), forming hexose phosphate. In most organisms, glucose is the most important substrate of hexokinases, and glucose-6-phosphate is the most important product. Hexokinase has the capability to transfer an inorganic phosphate group (Pi) from ATP to a substrate.

Hexokinases should not be confused with glucokinase, which is a specific isoform of hexokinase. While other hexokinases are capable of phosphorylating several hexoses, glucokinase acts with a 50-fold lower substrate affinity and its only hexose substrate is glucose.

Glucokinase

Most of the glucokinase in a mammal is found in the liver which provides approximately 95% of the hexokinase activity in hepatocytes. Phosphorylation of glucose to glucose-6-phosphate (G-6-P) by glucokinase is the first step of both glycolysis and glycogen synthesis in the liver. This enzyme plays an important regulatory role in the metabolism of carbohydrates. In the β cells of the pancreatic islets, it serves as a glucose sensor to control insulin release, and similarly, controls glucagon release in the α cells. In hepatocytes of the liver, glucokinase responds to changes of ambient

(surrounding) glucose levels by increasing or decreasing the synthesis of glycogen (**glycogenesis**).

Differences between hexokinase and glucokinase

These are the enzymes that convert glucose to glucose-6-phosphate which is the first step in the two very important pathways of carbohydrate metabolism, i.e. glycolysis and glycogenesis. The differences between the two are given in Table 6.3.

TRICARBOXYLIC ACID CYCLE (TCA CYCLE) OR KREBS CYCLE OR CITRIC ACID CYCLE

Both the names, i.e. Krebs cycle and TCA cycle are popular. The name 'Krebs cycle' has been given to this cycle after the discoverer's name, i.e. Hans Krebs. For this brilliant discovery **Dr. Hans Adolf Krebs** was given the title of **'Sir'**. He was also later on awarded **Nobel Prize in 1953** for his brilliant research work in physiology and medicine. As the first product of this cycle, i.e. citric acid, contains three carboxyl groups, hence the name **'Tricarboxylic Acid Cycle'** has been given to this cyclic process.

This cycle may be referred to as a mechanism whereby acetyl coenzyme A may be completely oxidized to CO_2 and H_2O. This process is **aerobic**, requiring oxygen as the final oxidant of the reduced coenzymes. The enzymes of the citric acid cycle are located in the mitochondrial matrix, either free or attached to the **inner mitochondrial membrane** (Fig. 6.6) and the crista membrane, where the enzymes and isoenzymes of the respiratory chain (**ETC**) are also found.

Pyruvic acid, formed from carbohydrates is an important source, but not the only source of acetyl coenzyme A. Under anaerobic conditions pyruvic acid is converted to lactic acid as the chief end product of glycolysis; but under aerobic conditions, a different series of reactions occurs, which is made possible by the availability of an adequate supply of the oxidized form of NAD. In the presence of magnesium ions, NAD, FAD, thiamine pyrophosphate, lipoic acid and coenzyme A, an ***oxidative decarboxylation*** of pyruvic acid takes place. The products of the reaction are acetyl coenzyme A, CO_2 and reduced nicotinamide adenine dinucleotide

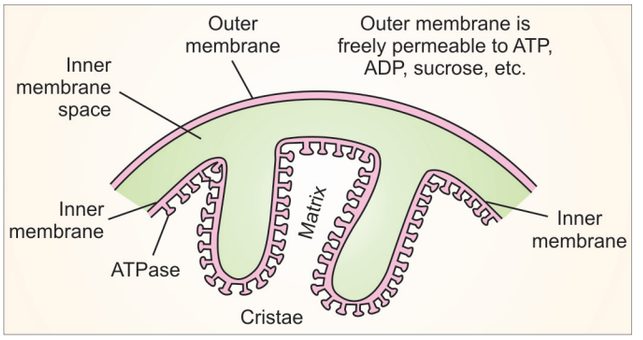


Fig. 6.6: A portion of mitochondria

($NADH_2$). The series of the reactions is quite complex but the overall process has been summarized in Fig. 6.7.

In the course of the reaction, cofactors other than coenzyme A and NAD (nicotinamide adenine dinucleotide) are regenerated and reutilized. The most important thing of the reaction is its irreversibility.

Carbohydrates are the important sources of pyruvic acid but certain amino acids may also be converted to pyruvic acid as shown in the reaction (Fig. 6.7); besides these, fatty acids, other lipid materials and proteins may eventually give rise to acetyl coenzyme A. Therefore, TCA cycle serves as the principal pathway for the complete oxidation of lipids and amino acids as well besides the carbohydrates, mean to say, this cycle should not be considered to be a feature of carbohydrate metabolism alone.

The principal reactions of the cycle are shown in Fig. 6.8. The very first reaction of the cycle which occurs in the presence of an enzyme called the condensing enzyme (citrate synthetase) is a condensation between acetyl-CoA and oxaloacetic acid to form citric acid and coenzyme A. This reaction may be considered to be irreversible. The next reaction, i.e. conversion of citric acid to *cis*-aconitic acid is catalysed by aconitase in the presence of Fe^{2+} whereby a molecule of water is lost. Then, a molecule of water is added forming isocitric acid, reaction being catalysed by the previous enzyme, i.e. aconitase in the presence of Fe^{2+}. Both the reactions catalysed by aconitase are reversible. Thus, we

Table 6.3: Differences between hexokinase and glucokinase

	Hexokinase	Glucokinase
1. Occurrence	Ubiquitous, i.e. found in all tissues	Found mainly in liver
2. Affinity to substrate	High affinity	Low affinity
3. K_m value	Low K_m (about 0.01 mM) for substrates	High K_m (about 10 mM) for glucose
4. V_{max}	Low	High
5. Feedback inhibition	Feedback inhibited by glucose-6-phosphate	No direct feedback inhibition
6. Induction by insulin	Not induced by insulin	Induced by insulin
7. Specificity	Acts on all hexoses, i.e. glucose, fructose and mannose (catalyses the phosphorylation of all hexoses)	Acts on glucose only (catalyses the phosphorylation of glucose only)
8. Capacity	Low (about 0.01 mM) for substrates	High (10 mM) for substrate, i.e. glucose
9. Function, i.e. phosphorylation	Acts even when blood sugar level is low, glucose is phosphorylated, vis-a-vis utilised by the cells of the body	Acts only when blood sugar level is high (more than 100 mg/dl), then, glucose is taken up for phosphorylation by the cells of the liver for the synthesis of glycogen (glycogenesis)

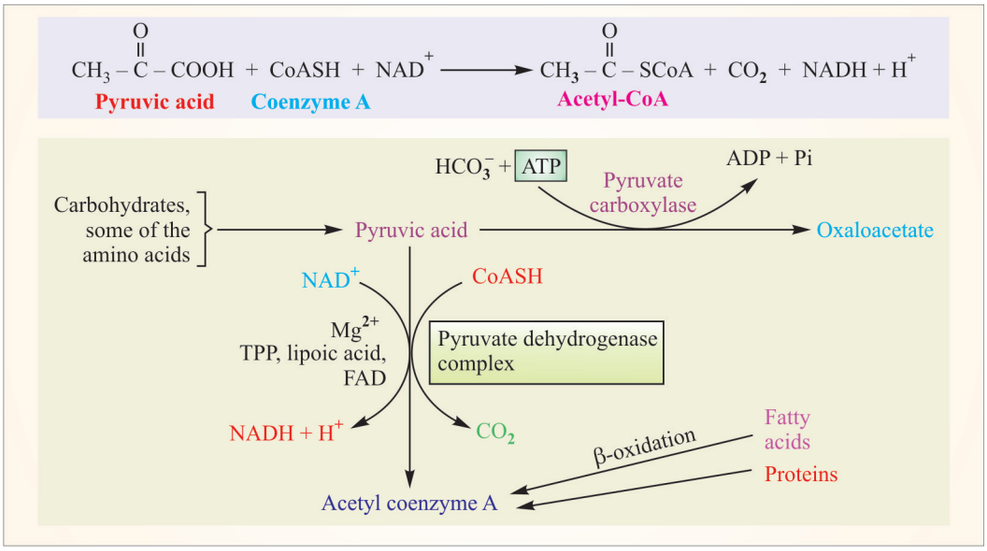


Fig. 6.7: Conversion of pyruvic acid to acetyl coenzyme A

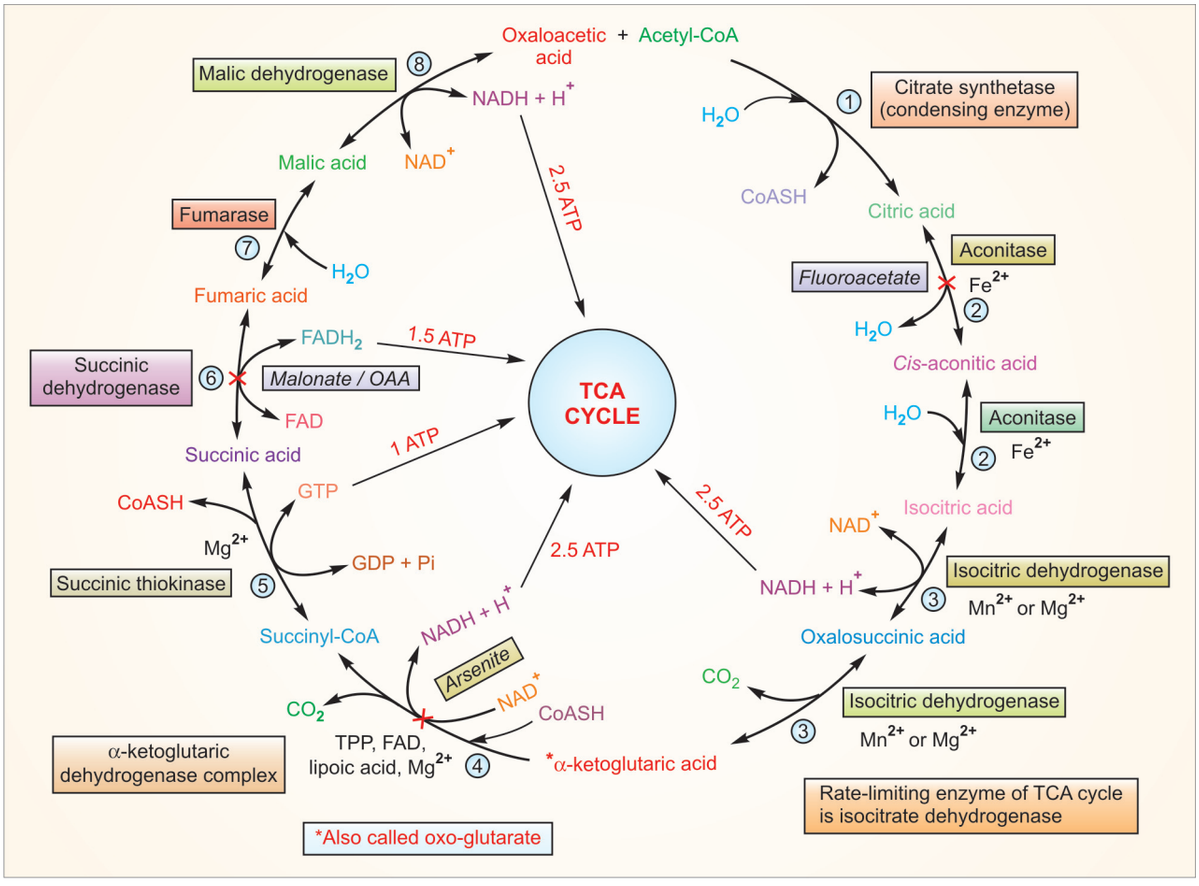


Fig. 6.8: The citric acid (Krebs) cycle. Oxidation of NADH and FADH in the respiratory chain leads to the generation of ATP via oxidative phosphorylation

see that aconitase enzyme is a very important enzyme which brings about an equilibrium between citric acid, *cis*-aconitic acid and isocitric acid by the removal and addition of water.

An enzyme, isocitric dehydrogenase, brings about the oxidation of isocitric acid, to oxalosuccinic acid, with the reduction of NAD^+. Oxalosuccinic acid, while remaining still bound to the enzyme surface, is decarboxylated to form α-ketoglutaric acid; the reaction being catalysed by the same enzyme, i.e. isocitric dehydrogenase in the presence of Mn^{2+} or Mg^{2+} ions. The α-keto acid, so formed, now undergoes an

oxidative decarboxylation forming succinyl coenzyme A in the presence of thiamine pyrophosphate, NAD^+, FAD, CoA, lipoic acid and Mg^{2+}, in a manner exactly analogous to the oxidative decarboxylation of pyruvic acid, as shown in Fig. 6.8, which is also an α-keto acid; reaction being catalysed by α-ketoglutaric dehydrogenase complex. Succinyl-CoA, being a thioester, contains a high-energy bond. The equilibrium of this reaction is so much in favour of succinyl-CoA formation that the reaction must be physiologically considered as unidirectional.

Now, succinyl-CoA is converted to succinic acid by the enzyme succinic thiokinase. The reaction requires GDP or IDP, which is converted in the presence of inorganic phosphate to either GTP or ITP. This is the only example in the whole of TCA cycle where *generation of a high-energy phosphate takes place at the substrate level.* By means of a phosphokinase, ATP may be formed from either GTP or ITP.

$$GTP + ADP \xleftrightarrow{\text{Phosphokinase}} GDP + ATP$$

Succinic acid is now dehydrogenated forming fumaric acid and reduced FAD in the presence of FAD, reaction being catalysed by succinic dehydrogenase. Enzyme succinic dehydrogenase remains bound to the inner surface of the inner mitochondrial membrane. It is the only dehydrogenation in the TCA cycle which involves the *direct transfer of hydrogen from the substrate to a flavoprotein without the participation of NAD^+.*

A molecule of water is added to fumaric acid to form malic acid in the presence of enzyme fumarase, and finally, malic acid is oxidized by malic dehydrogenase in the presence of NAD^+, resulting in the formation of reduced NAD and oxaloacetic acid. The cycle can then be repeated by the entrance of another molecule of acetyl-CoA.

This process, which occurs in the mitochondria, results in the reduction of five molecules of NAD^+ or FAD (starting from pyruvate). These reduced cofactors are reoxidized by a process called the hydrogen transport system, or the electron transport system. The hydrogen, with the intermediation of a variety of cytochromes and other factors, is finally oxidized to water by combination with 'activated' molecular oxygen. The reoxidized cofactors can then be reutilized in various reactions of the TCA cycle. Simultaneously, with the oxidation of hydrogen to water, there occurs phosphorylation of ADP by inorganic phosphate to form ATP. The two processes, which are coupled by an unknown mechanism, are referred to as *oxidative phosphorylation.* ATP, which is the principal compound supplying energy for many reactions, such as muscle contraction, is generated in greater amount by oxidative phosphorylation than by any other means. The continued operation of this process is essential for life in aerobic organisms (cyanide, e.g. is toxic because it reacts with the cytochromes, preventing their normal functioning). Since, the hydrogen transport system serves as the primary mean for the reoxidation of cofactors such as NADH, the TCA cycle is said to be an *aerobic process*. Oxygen is necessary in the transport system to reoxidize NAD, FAD, etc. The oxidized factors are necessary for the TCA cycle to operate.

Inhibitors of TCA cycle

- **Fluoroacetate** inhibits the conversion of citric acid to *cis*-aconitic acid, causing citric acid to accumulate. It is the first blockade point in the TCA cycle.
- **Arsenite** inhibits the conversion of α-KGA to succinyl-CoA, causing α-KGA to accumulate. It is the second blockade point.
- **Malonate or oxaloacetate (OAA)** blocks the conversion of succinic acid to fumaric acid by inhibiting the enzyme succinic dehydrogenase. It is the third and last blockade point. ***Competitive inhibition*** takes place here. This step results in the accumulation of succinic acid.

Importance of TCA cycle

The importance of this pathway lies in the fact that it is one of the pathways that generates the major part of the ATP and NADH in the cell.

TCA cycle is amphibolic in nature as it plays role in anabolism and catabolism both but, by and large, its role in catabolism is much as it liberates lot of energy.

Anabolic in the sense that it is responsible for the biosynthesis of various substances as well, which include:

1. Carbohydrates,
2. Amino acids, and
3. Fats

Catabolic in the sense that it is the common pathway for the oxidation of carbohydrates, fats and proteins, as a result of which much energy gets liberated.

NADPH (which is required for the synthesis of fatty acids, steroids and various other substances) can be formed from $NADP^+$ and NADH by a mitochondrial transhydrogenase, linked to the electron transport system and essentially irreversible.

$$NADH + NADP^+ \rightarrow NADPH + NAD^+$$

ATP, thus, produced is utilized by the cell for its functions which are numerous and NADPH for the biosynthesis of fatty acids, steroids and other important substances of the human body.

Generation of ATP in TCA cycle

Three NADH molecules on oxidation by electron transport chain (ETC) will yield $3 \times 2.5 = 7.5$ high energy phosphates, i.e. ATPs. The $FADH_2$ will yield 1.5 molecules of ATP. In addition, one molecule of GTP (equivalent to one molecule of ATP) is formed by substrate level phosphorylation. Hence, per turn of the cycle, 10 high energy phosphates are generated. These steps are marked in Fig. 6.8 and Table 6.4.

Energetics of the catabolism (complete oxidation) of one mole of glucose via glycolysis and TCA cycle

Complete oxidation of one molecule of glucose via glycolysis and TCA cycle has been explained in detail in Table 6.4. The net result is the formation of CO_2 and H_2O from pyruvate, along with the generation of 32 moles of ATP.

Table 6.4: Energy yield (number of ATPs generated) per molecule of glucose when it is completely oxidized through glycolytic pathway (glycolysis) and TCA cycle under aerobic conditions

Pathway	Step	Reaction catalysed by enzyme	Source	Method of ATP generation	Number of ATPs generated per molecule of glucose	
Glycolysis	1	Hexokinase	—		–1	
Glycolysis	3	PFK	—		–1	
Glycolysis	5	Glyceraldehyde-3-P-dehydrogenase	NADH	Respiratory chain oxidation (ETC) of 2 moles of NADH	$2 \times 2.5 = 5$	
Glycolysis	6	Phosphoglycerate kinase	ATP	Substrate level phosphorylation	$1 \times 2 = 2$	
Glycolysis	9	Pyruvate kinase	ATP	Substrate level phosphorylation	$1 \times 2 = 2$	
Conversion of pyruvate to acetyl-CoA	—	Pyruvate dehydrogenase	NADH	Respiratory chain oxidation (ETC) of 2 moles of NADH	$2 \times 2.5 = 5$	
TCA cycle	3	Isocitrate dehydrogenase	NADH	-do-	$2 \times 2.5 = 5$	20 moles of ATP
-do-	4	α-ketoglutarate dehydrogenase	NADH	-do-	$2 \times 2.5 = 5$	
-do-	5	Succinate thiokinase	GTP	Substrate level phosphorylation	$2 \times 1 = 2$	
-do-	6	Succinic dehydrogenase	$FADH_2$	Respiratory chain oxidation (ETC) of 2 moles of $FADH_2$	$2 \times 1.5 = 3$	
-do-	8	Malic dehydrogenase	NADH	Respiratory chain oxidation (ETC) of 2 moles of NADH	$2 \times 2.5 = 5$	
				Total	**34 – 2 = 32 ATP**	

SUMMARY

Net generation of ATP via glycolytic pathway (aerobic conditions)	9 – 2 = 7	12 moles of ATP
Generation of ATP via pyruvate dehydrogenase reaction (junction point)	$2 \times 2.5 = 5$	
Generation of ATP via TCA cycle	= 20	

Therefore, net generation of ATP from one mole of glucose, if oxidised completely via glycolysis and TCA cycle under aerobic conditions: 12 + 20 = 32

Note : (a) In the ETC, upon oxidation, one mole of NADH generates 2.5 moles of ATP, and (b) in the ETC, upon oxidation, one mole of FADH generates 1.5 moles of ATP.

Role of vitamins in the TCA cycle

There are five vitamins of the B complex group which play vital role in the functioning of TCA cycle, namely:

1. ***Riboflavin*** in the form of flavin adenine dinucleotide (FAD), a cofactor in the α-KGA dehydrogenase complex and in succinic dehydrogenase.
2. ***Niacin*** in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for 3 dehydrogenases in the cycle, i.e. isocitrate dehydrogenase, α-KGA dehydrogenase, and malic dehydrogenase.
3. ***Thiamine*** (B_1) in the form of *thiamine pyrophosphate,* the coenzyme for decarboxylation in the α-KGA dehydrogenase reaction.
4. ***Pantothenic acid*** as part of *coenzyme-A,* the cofactor attached to 'active' acyl residues such as acetyl-CoA and succinyl-CoA.
5. **Lipoic acid** in the reactions catalysed by pyruvate dehydrogenase complex and α-ketoglutarate dehydrogenase complex.

Regulation of the TCA cycle

TCA cycle is regulated by the following three enzymes, namely: (a) Citrate synthetase, (b) isocitric dehydrogenase, and (c) α-ketoglutaric dehydrogenase as shown in Fig. 6.8.

Both isocitrate dehydrogenase and α-ketoglutaric dehydrogenase get activated by Ca^{2+}, which may be of importance in muscle in relation to contraction stimulated by Ca^{2+}.

The above named three enzymes are in non-equilibrium which means that the equilibrium of the 3 reactions catalysed by them is neither in the forward direction and nor in the backward.

Acetyl coenzyme A (acetyl-CoA)

It consists of a two-carbon activated acetyl unit attached to coenzyme A in thioester linkage. Acetyl-CoA is central to energy generation from the degradative pathways of oxidative fuel metabolism and to a number of biosynthetic pathways that utilize the activated two-carbon acetyl unit.

In aerobic cells, it is the product of all the major catabolic pathways of fuel metabolism, including β-oxidation of fatty acids, ketone body degradation, glycolysis and pyruvate oxidation, ethanol oxidation, and the oxidative degradation of many amino acids. The two-carbon acetyl unit of acetyl-CoA formed from these pathways can be completely oxidised to CO_2 in the tricarboxylic acid cycle (TCA cycle), thus providing aerobic cells with energy from the complete oxidation of fuels. The acetyl unit of acetyl-CoA is also the basic building block of fatty acids, cholesterol, and other

compounds, and it can be transferred to other molecules in acetylation reactions (e.g. synthesis of *N*-acetylated sugars).

The ability of acetyl-CoA to participate in these diverse metabolic pathways is derived from the thioester bond formed between the acyl carbon of the acetyl unit and the sulfhydryl group of coenzyme A (CoASH).

Pathway of the formation of acetyl-CoA

It can be easily understood from Fig. 6.9.

Acetyl-CoA (acetyl coenzyme A) is a molecule that participates in many biochemical reactions in protein, carbohydrates and lipid metabolism, the main function of which is to deliver the acetyl group to the citric acid cycle (Krebs cycle) to be oxidized for energy production. Coenzyme A (CoASH or CoA) consists of a β-mercapto-ethylamine group linked to the vitamin pantothenic acid through an amide linkage and 3′-phosphorylated ADP. The acetyl group of acetyl-CoA is linked to the sulfhydryl substituent of the β-mercaptoethylamine group. This thioester linkage is a '**high energy**' bond which is particularly active. Hydrolysis of the thioester bond is exergonic (–31.5 kJ/mol).

CoA is acetylated to acetyl-CoA by the breakdown of carbohydrates through glycolysis and by the breakdown of fatty acids through β-oxidation. Acetyl-CoA then enters the TCA cycle, where the acetyl group is oxidized to CO_2 and H_2O, and the energy released and captured in the form of ATP and one GTP per acetyl group.

Konard Bloch and Feodor Lynen were awarded the Nobel Prize in Physiology and Medicine for their fascinating discoveries linking acetyl-CoA and fatty acid metabolism in the year 1964. Fritz Lipmann won the Nobel Prize in 1953 for his discovery of the cofactor coenzyme A.

The acetylation of CoA is determined by the carbon sources.

As evidenced from Fig. 6.9, acetyl-CoA is formed from many substances (sources), viz.

(a) Fatty acids
(b) Glucose and other sugars
(c) Ethanol
(d) Ketone bodies
(e) Various amino acids
(f) Malate

Thus, acetyl-CoA plays an important role in the integration of metabolism of carbohydrate, lipid, protein and ethanol—a unique and unprecedented role.

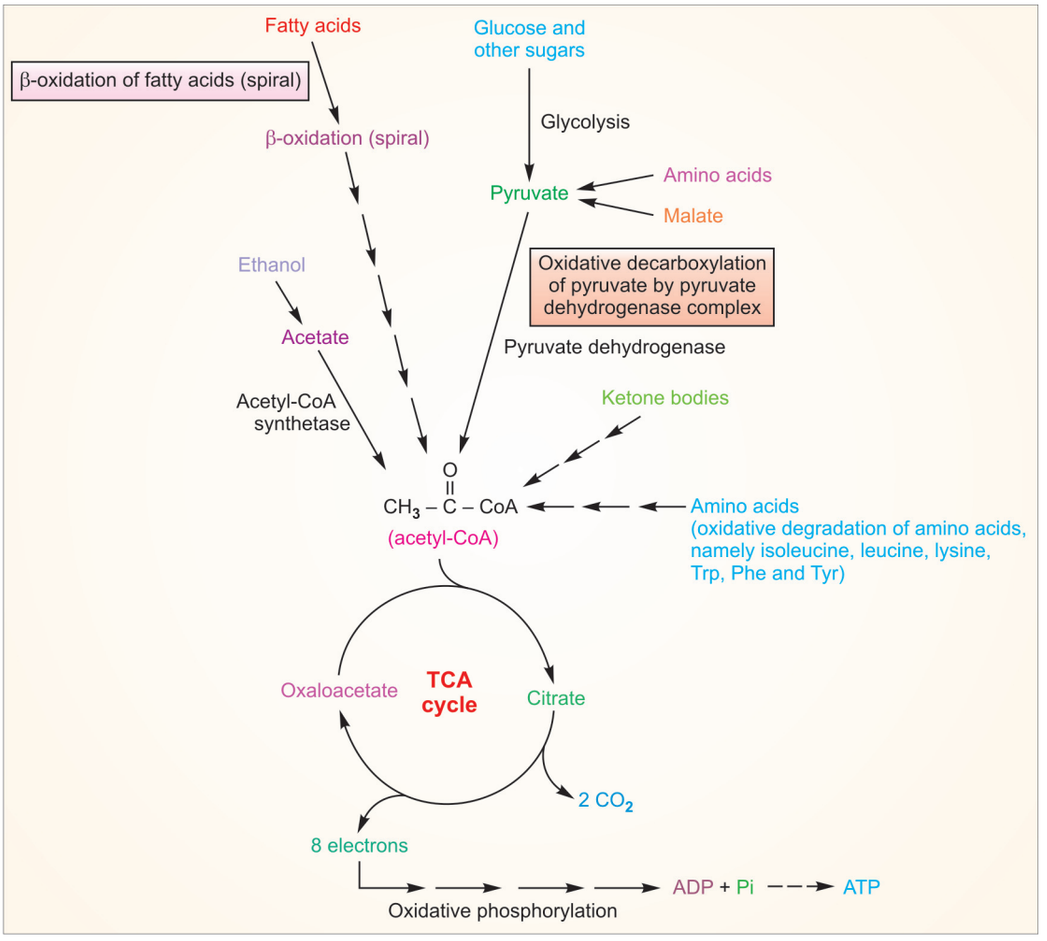


Fig. 6.9: Formation of acetyl coenzyme A which plays role in oxidative fuel metabolism. Cycle shows wonderful integration of metabolism of carbohydrate, lipid, protein and ethanol.

HEXOSE MONOPHOSPHATE SHUNT (HMP SHUNT) OR PENTOSE PHOSPHATE PATHWAY (PPP)

This is an alternative pathway for the oxidation of glucose. *This pathway does not generate any ATP.* This oxidative pathway is also known as **Warburg-Dickens-Lipmann phosphogluconate pathway** (Figs 6.10 a and b).

Significance of HMP shunt

- **Alternative route for oxidation of glucose.** Instead of glucose going through the glycolytic pathway; it is shunted through this pathway, so, it is known as the **'shunt pathway'**. In the glycolysis, there are a few bisphosphate intermediates; but in this pathway, there are **mono-phosphates** only, hence this is called hexose mono-phosphate (HMP) pathway.
- **Main sites** are adrenal cortex, testis, liver, adipose tissue, lactating mammary glands, leucocytes and erythrocytes.
- **Its enzymes** are found in the **extra-mitochondrial soluble fraction of the cell, the cytosol.**
- The enzymes of this pathway are important in providing NADPH for the biosynthesis of fatty acids, steroids and other important substances.
- This pathway also helps in the synthesis of nucleotides and nucleic acids by providing ribose sugar.
- **Two dehydrogenases** are involved in the production of NADPH:

  (a) Glucose-6-phosphate dehydrogenase.
  (b) 6-Phosphogluconate dehydrogenase.

It is quantitatively of no significance in highly glycolytic tissues such as skeletal muscles and non-lactating mammary glands.

It is a multicyclic process in which three molecules of glucose-6-phosphate give rise to three molecules of CO_2 and three-5-carbon residues namely ribulose 5-phosphate, ribose-5-phosphate and xylulose-5-phosphate. The latter are arranged to regenerate two molecules of glucose-6-phosphate and one molecule of the glycolytic intermediate, i.e. glyceraldehyde-3-phosphate. Since two molecules of glycer-

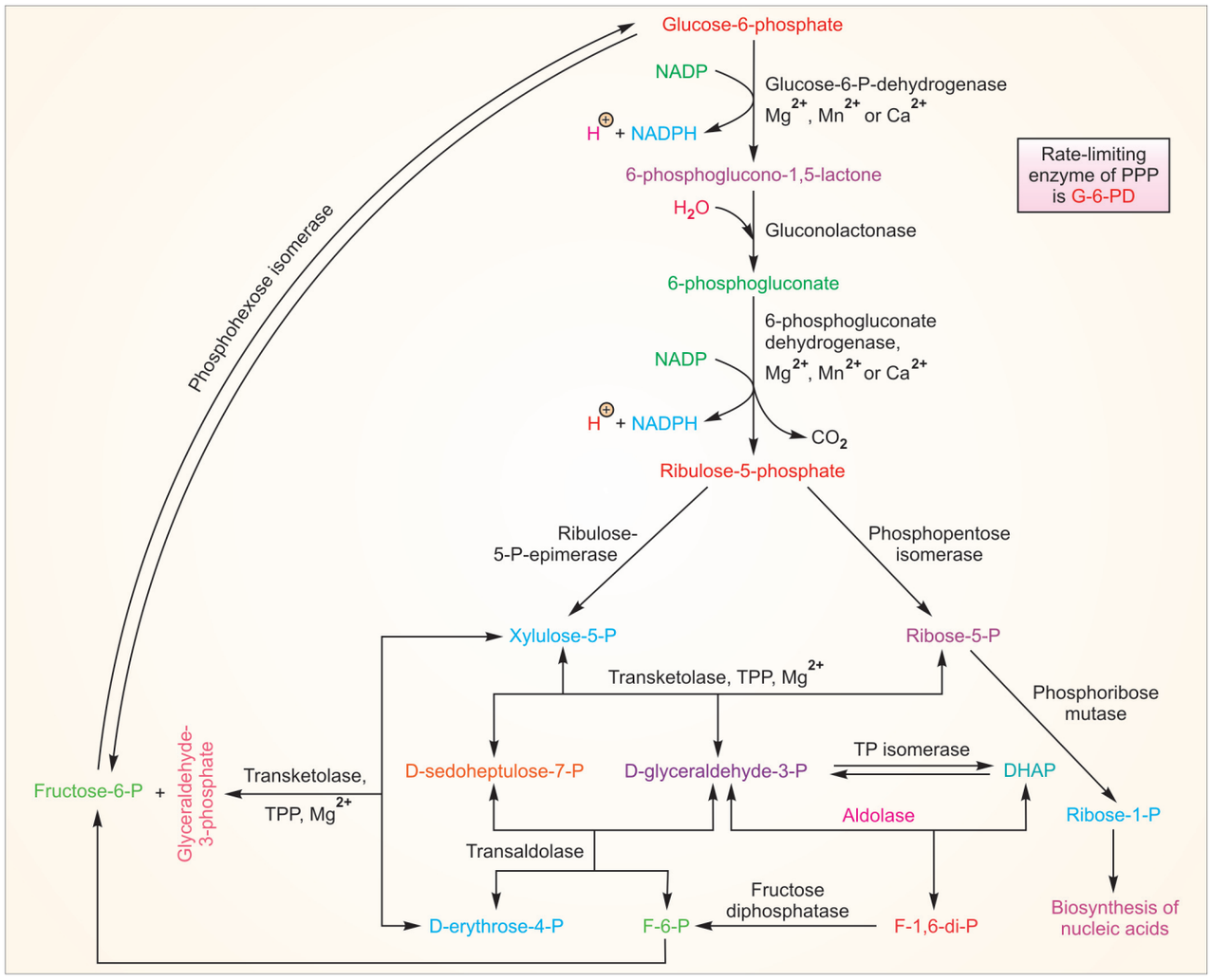


Fig. 6.10 a: Pentose phosphate pathway

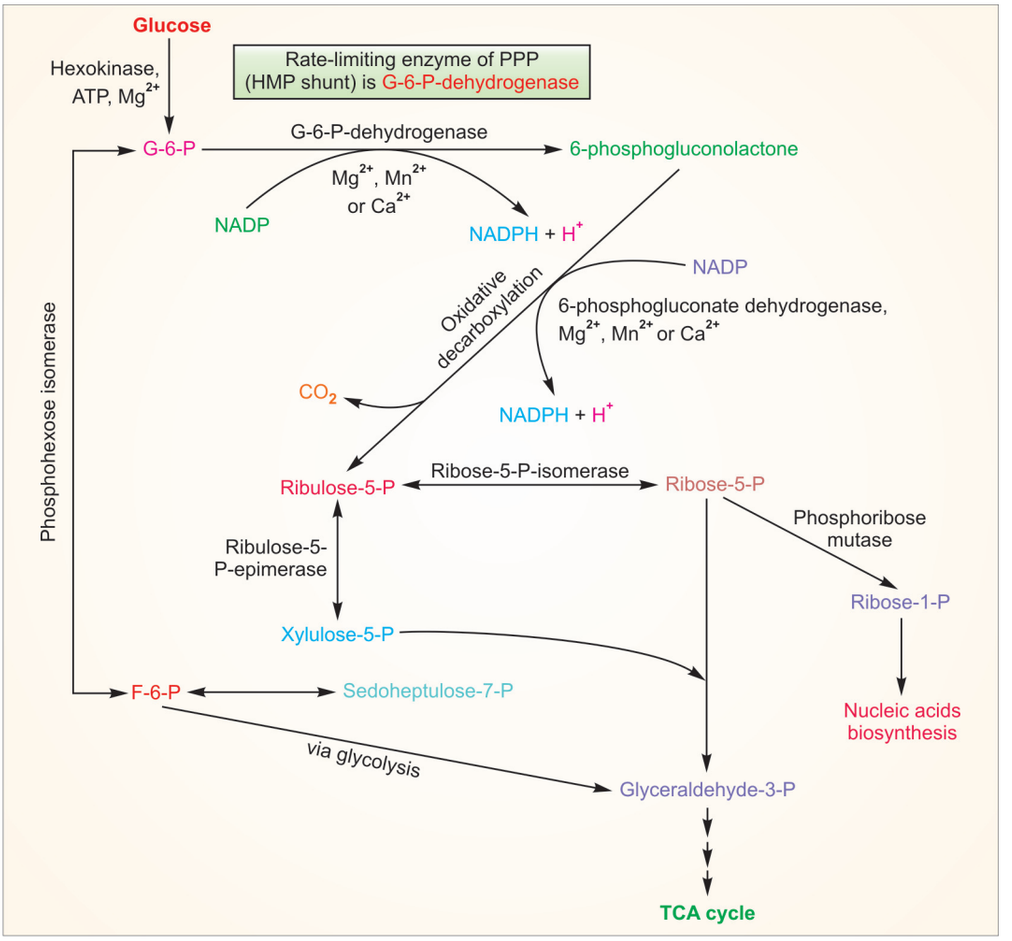


Fig. 6.10 b: Pentose phosphate pathway (simplified presentation)

aldehyde-3-phosphate can regenerate glucose-6-phosphate; glucose may be completely oxidized by this pathway. In nutshell, equation may be summarized as in Fig. 6.11.

3 glucose-6-phosphate + 6 $NADP^+ \rightarrow$ 3 CO_2 + 2 glucose-6-phosphate + glyceraldehyde 3-phosphate + 6 NADPH + 6 H^+

Fig. 6.11: Reaction to show summary of PPP pathway

Mechanism of HMP shunt

In the very first reaction of this pathway, oxidation of glucose-6-P to 6-phosphogluconolactone takes place; this reaction is catalyzed by glucose-6-phosphate dehydrogenase in the presence of NADP as a result of which NADPH is formed.

In the next reaction, 6-phosphogluconolactone is rapidly hydrolyzed to 6-phosphogluconate by the enzyme gluconolactonase.

The equilibrium of the reaction lies far to the right and it appears to play an important role in generating TPNH (NADPH) for reductive processes in the synthesis of fatty acids and cholesterol.

In the next reaction, 6-phosphogluconate is oxidized to ribulose-5-phosphate by the enzyme 6-phosphogluconate dehydrogenase in the presence of coenzyme NADP forming NADPH; this enzyme is activated by Mg^{2+}, Mn^{2+} or Ca^{2+} ions. NADPH so formed may serve as a reducing agent in other reactions coupled with it, such as those of fatty acid and the synthesis of cholesterol. In this reaction, one mole of CO_2 is split out. **This is the only step where CO_2 is formed in whole PPP.**

Now, ribulose-5-phosphate is subjected to the action of two enzymes namely phosphopentose isomerase which forms ribose-5-P and ribulose-5-phosphate epimerase which forms xylulose-5-P. Ribose-5-P so formed may be converted to ribose-l-P by the action of phosphoribosemutase. Ribose-1-P so produced is responsible for the synthesis of nucleic acids.

In the next reaction, enzyme transketolase is involved, which is responsible for the transfer of a ketol group from xylulose-5-phosphate to an aldehyde acceptor. This enzyme requires the presence of cofactor TPP and Mg^{2+}.

A number of aldehydes, such as ribose-5-phosphate, glyceraldehyde-3-P, glyceraldehyde, and glycolaldehyde can serve as acceptor. Thus, products formed by the action of transketolase are sedoheptulose-7-P and glyceraldehyde-3-P (Fig. 6.10 a), which are then subjected to the action of transaldolase forming erythrose-4-phosphate and fructose-6-phosphate. The function of transaldolase is to transfer a

dihydroxyacetone unit from sedoheptulose-7-phosphate (donor) to glyceraldehyde 3-P, D-erythrose-4-P, or ribose-5-P (acceptors). The enzyme appears to require no cofactors.

Now, erythrose-4-phosphate and fructose-6-P are again subjected to the action of a transketolase forming fructose-6-P and glyceraldehyde-3-P.

Glyceraldehyde-3-P formed previously by the action of a transketolase (Fig. 6.10 a) may be converted to dihydroxyacetone phosphate by the action of a triose-P isomerase. Now, dihydroxyacetone phosphate (DHAP) and the glyceraldehyde-3-phosphate formed in the last reaction catalysed by a transketolase are subjected to the action of the enzyme aldolase forming fructose-6-P.

It is evident from PPP that two transketolase reactions and a transaldolase reaction are involved in this pathway.

Fructose-6-P formed in the end is converted to glucose-6-P by phosphohexose isomerase for recycling through the pentose phosphate pathway.

Genetic error

Genetic deficiency of glucose-6-P-dehydrogenase enzyme, supposed to be a key enzyme of PPP may enhance erythrocyte fragility as a result of which several disorders like hemolysis of RBCs, anaemia and jaundice, particularly after the administration of vitamin K, sulpha drugs, fava beans and quinine may take place. This rare genetic disorder is more common in some races like **Punjabis, Parsis** and **Sindhis** (i.e. **P, P and S**) of our country. The disease may be due to a failure of the body to provide **NADPH for protecting hemoglobin against oxidation**.

This enzyme, i.e. G-6-P-dehydrogenase is very important and it has been estimated that as many as **100 million** people in the world may have genetically determined low levels of this enzyme, owing to variant forms which may be distinguished from each other by electrophoresis and various other techniques.

Significance of the pentose phosphate pathway

1. A considerable proportion of glucose metabolism in various important tissues like liver, lactating mammary glands, adipose tissue, leukocytes, testis, and adrenal cortex appears to take place through pentose phosphate pathway (PPP).
2. It gives rise to the formation of pentoses vis-a-vis nucleotides and nucleic acids biosynthesis.
3. This pathway helps in the interconversion of pentoses and hexoses.
4. This pathway requires only half the ATP per glucose molecule metabolized than the glycolysis-TCA cycle combined route, and is independent of the TCA cycle components.
5. One of the most important functions of the PPP is to provide TPNH (NADPH), which serves as a reducing agent **in the synthesis of fatty acids, steroids, and various other substances.** This pathway operates especially in tissues where such synthesis takes place.
6. About 10% of glucose molecules per day are entering via this pathway. The liver and RBCs metabolise about 30% glucose via this pathway.
7. In this process glucose or glycogen is synthesized from non-carbohydrate sources like amino acids, glycerol and lactic acid.
8. It is a useful pathway especially when an individual is consuming less carbohydrates.
9. It helps in the **maintenance of blood sugar level** in its normal range.
10. **It brings about proper disposal of lactic acid and glycerol.**

Lactic acid

Lactic acid is an organic compound with the formula $CH_3CH(OH)COOH$.

Be it known that, somehow, if there is some defect in the disposal (metabolism) of lactic acid then it goes on accumulating in blood causing **lactic acidosis vis-a-vis change in the pH of blood—a very bad sign.**

Lactic acid is a weak acid, the accumulation of which is responsible to shift the pH of blood (7.4) towards acidic side from alkaline one.

GLUCONEOGENESIS (*DE NOVO* FORMATION OF GLUCOSE)

This process (Fig. 6.12) may be referred to as the synthesis of glucose or glycogen from non-carbohydrate sources like α-amino acids, α-keto acids, lactate, etc. The mechanisms involved in this pathway are essentially the reversal of TCA cycle and glycolytic pathway (Fig. 6.12). Since, some of the reactions are not reversible, such reactions do take place by alternative routes. Any substance which can form any of the intermediates of the TCA cycle or glycolysis can, therefore, easily give rise to glucose or glycogen. Such substances which are responsible for this phenomenon, are termed as gluconeogenic substances which ordinarily include several amino acids like alanine, serine, aspartic acid, valine, isoleucine, threonine, glutamic acid, histidine, arginine, proline, etc., lactate and glycerol (formed from the catabolism of fats). **Main sites of this pathway are:**

(a) **Liver**
(b) **Kidneys**

Out of the above two sites, **liver is the main site**.

Glycolytic pathway is made reversible with the help of following enzymes; such enzymes are named as **gluconeogenic enzymes:**

(a) **Glucose-6-phosphatase** (glucose-6-phosphate → glucose) is a hydrolytic enzyme in the membrane of the endoplasmic reticulum.
(b) **Fructose-1,6-diphosphatase** (fructose-1,6-diphosphate → fructose-6-phosphate) is a hydrolytic enzyme found in the cytosol.
(c) **Pyruvate carboxylase** (pyruvate + CO_2 → oxaloacetate). This is the same enzyme which provides oxaloacetate for citric acid cycle activity. It is located within the mitochondrion.

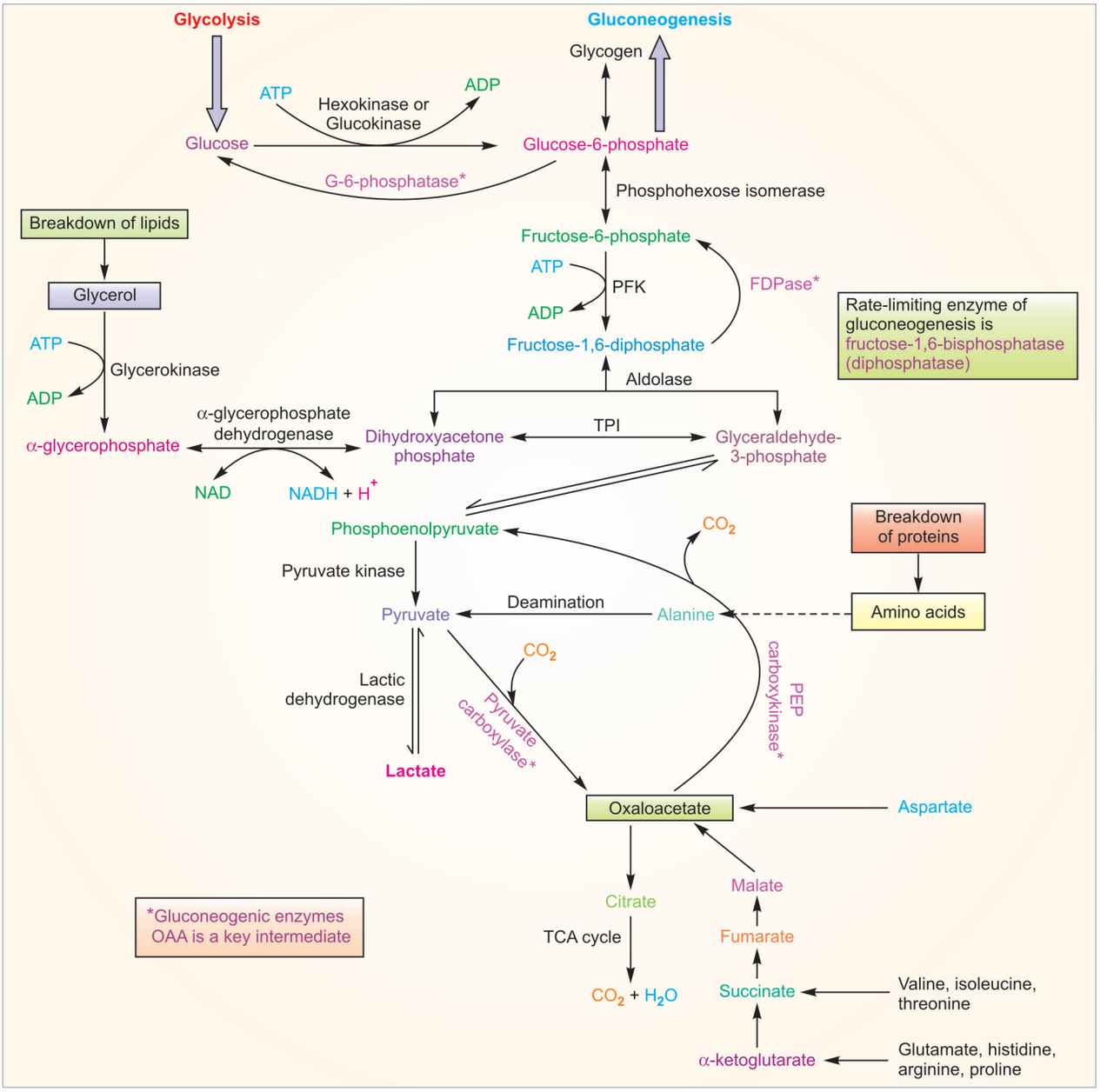


Fig. 6.12: Pathway of gluconeogenesis showing the synthesis of glucose from (a) lactate, (b) gluconeogenic amino acids, and (c) glycerol. Major substrates are shown in boxes.

(d) **Phosphoenolpyruvate (PEP) carboxykinase** (Oxaloacetate → phosphoenolpyruvate + CO_2). It is partially located in the mitochondrion and partially in the cytosol.

Only alanine directly provides pyruvate after removal of the amino group whereas carbon skeletons of other amino acids must undergo a series of metabolic conversions to yield pyruvate (Fig. 6.12).

Lactate provides pyruvate directly after oxidation by lactate dehydrogenase. Glycerol can be converted to glucose if it is first phosphorylated (by glycerol kinase) to glycerol phosphate, which can be oxidized to dihydroxyacetone phosphate by glycerol phosphate dehydrogenase (Fig. 6.12).

Regulators of gluconeogenesis

Regulatory system must ensure that the catabolic reactions of glycolysis are **'off'** while gluconeogenesis is taking place; otherwise the cell might engage in a futile energy-dissipating cycle:

$$\text{Glucose} \rightarrow 2\ \text{Lactate} + 2\ \text{ATP}$$

$$2\ \text{Lactate} + 6\ \text{ATP} \rightarrow \text{Glucose}$$

There are several regulators, some of them are intracellular whereas some are extracellular as given below.

Intracellular regulators

Glucose-6-phosphatase, FDPase, pyruvate carboxylase and PEP carboxykinase are the key regulatory enzymes of this pathway.

The amino acid, alanine plays an important role, since it furnishes pyruvate upon deamination and acts as a negative allosteric modifier of pyruvate kinase.

Citrate acts as a **negative feedback agent** by inhibiting PFK to block glycolysis at an early stage.

Extracellular regulators

These include several endocrine agents like **insulin, glucocorticoids, glucagon, etc.**

Significance

This pathway plays various important roles in the body, namely:

1. This process helps in the regulation of blood sugar level especially when an individual is taking less carbohydrates via diet. Eventually, this protects delicate organs, e.g. brain against harmful effects that might take place due to hypoglycaemia.
2. It brings about proper disposal of lactic acid, produced by the muscles during and after exercise. In vigorous exertion, the muscles can produce **50 or even 100 g lactate in a few minutes.** Some of this lactate is taken up by other tissues such as the heart and perhaps by resting skeletal muscles and oxidized to CO_2 and H_2O; the rest diverted towards gluconeogenesis.
3. It brings about proper disposal of glycerol, produced in the adipose tissue due to turnover of the fats and, thus, prevents its wastage.

The linking sites of various gluconeogenic substances in glycolysis and TCA cycle are reversible; hence at the time of emergency, biosynthesis of lipids and proteins from carbohydrates or vice versa is possible through these sites. We mean to say that a dynamic equilibrium is, thus, maintained among carbohydrates, lipids and proteins through this pathway, i.e. gluconeogenesis.

Regulation of glycolysis and gluconeogenesis

The balance between the catabolism and the anabolism of glucose in liver cells is subject to an elaborate series of regulatory influences, including the concentrations of metabolites and nucleotides as well as the effects of hormones. It should be noted that the liver cells and, to a lesser extent, those of kidney and intestinal mucosa are unique in that they contain full complement of enzymes to allow the reversal of glycolysis. Other tissues have controlling factors that are solely involved with turning the glycolytic process on or off in parallel with their own cellular energy demands. Hepatic cells, on the other hand, have large responsibilities to the rest of the body; **they must sense the requirement for glucose elsewhere and switch on the gluconeogenic reactions accordingly.** Moreover, the regulatory system must also ensure that the catabolic reactions of glycolysis are **'off'** while gluconeogenesis is taking place; otherwise, the cell might engage in a futile, energy-dissipating cycle:

$$\text{Glucose} \rightarrow 2\ \text{Lactate} + 2\ \text{ATP}$$

$$2\ \text{Lactate} + 6\ \text{ATP} \rightarrow \text{Glucose}$$

Regulators of glycolysis and gluconeogenesis have been discussed earlier.

Glucose–alanine cycle

Alanine is the major amino acid involved in gluconeogenesis. The output from the muscle exceeds that of all other amino acids. Alanine, thus, serves as the donor of the amino groups and carbon substrate from the muscle to the liver for the conversion to urea and glucose, respectively and would be resynthesized in the muscle by the transamination of pyruvate derived from glucose or other amino acids (Fig. 6.13).

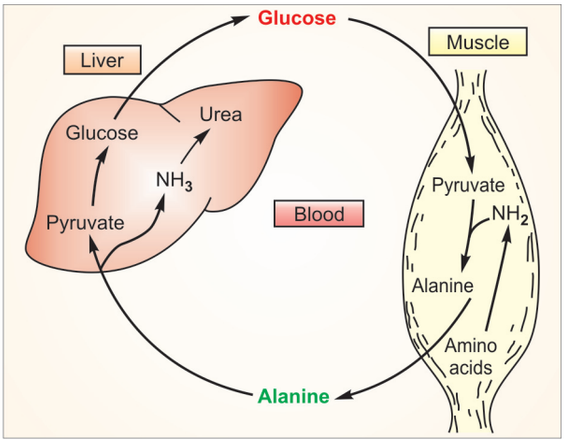


Fig. 6.13: Glucose–alanine cycle

Thus, the operation of this cycle permits the catabolism of muscle proteins without the release of ammonia. It would not, however, lead to net glucose production unless pyruvate is formed from another source in the muscle or liver. Since much of the branched chain amino acids are catabolised in the muscles, **alanine** provides a non-toxic alternative to ammonia in the transfer of $-NH_2$ groups from the periphery to the liver.

Significance: Alanine output and the uptake by the liver are important factor in the reduction of hepatic gluconeogenesis and protein catabolism during prolonged fasting.

Uronic acid pathway

Besides the major pathways of glucose metabolism, this is an alternative route/pathway for the oxidation of glucose as a result of which glucose is converted to important substances as in Fig. 6.14.

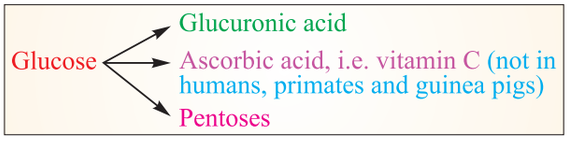


Fig. 6.14: Conversion of glucose to important substances

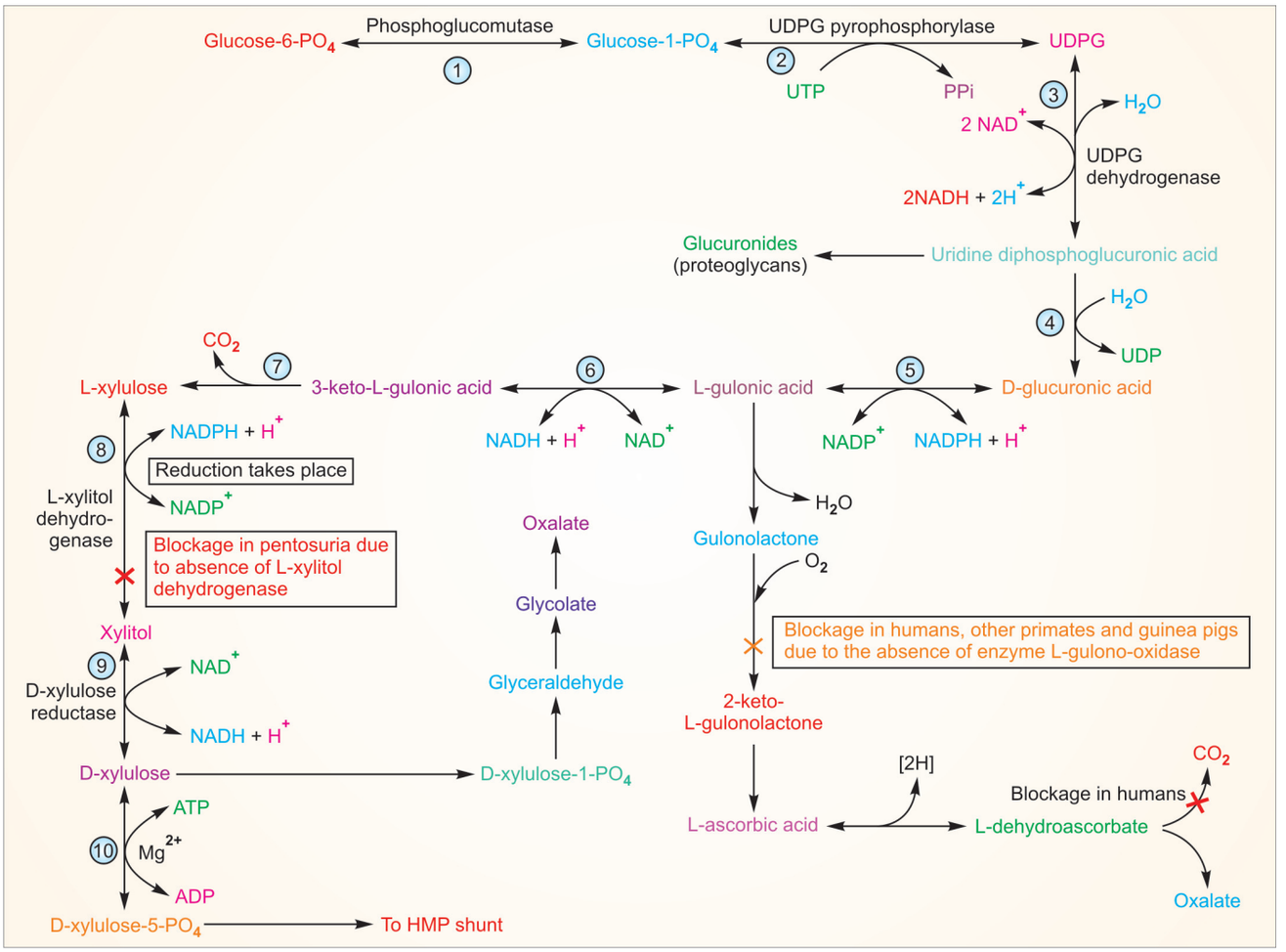


Fig. 6.15: Uronic acid pathway

Box 6.1: Oxalosis

Parenteral administration of xylitol may lead to **oxalosis** causing calcium oxalate deposition in brain and kidney tissues. This results from the conversion of D-xylulose to oxalate via xylulose-1-PO_4, glycolaldehyde, and glycolate formation (Fig. 6.15).

Other sources of **oxalosis** may be large intake of **vitamin C** and **glycine**.

- In humans and other primates as well as in guinea pigs, ascorbic acid is not synthesized and gulonic acid is the final product which also is finally converted to **L-xylulose.**
- But like the pentose phosphate pathway, this pathway also does not yield any ATP.
- Its schematic diagram has been shown in Fig. 6.15.

Metabolic reactions

First of all glucose is converted to glu-6-PO_4 as in glycolytic pathway.

1. Now, glucose-6-PO_4 is converted to glucose-1-PO_4, the reaction being catalysed by the enzyme phospho-glucomutase. Reaction is reversible.
2. Now, glucose-1-PO_4 thus formed reacts with UTP to form the active nucleotide, i.e. uridine diphosphate glucose (UDPG), reaction being catalysed by UDPG pyro-phosphorylase.
3. In the next reaction, UDPG is oxidized to uridine diphosphoglucuronic acid by the enzyme UDPG dehydro-genase in the presence of NAD^+. UDP-glucuronic acid is the **'active form'** of glucuronic acid for the reaction involving incorporation of glucuronic acid into **proteo-glycans**.
4. Now UDP-glucuronic acid is converted to D-glucuronic acid in the presence of a water molecule forming D-glucuronic acid liberating UDP (Fig. 6.15).
5. Glucuronic acid is reduced to L-gulonic acid in the presence of reduced NADP. L-gulonic acid, thus formed is the direct precursor of **ascorbic acid (vitamin C)** in these animals capable of synthesizing this vitamin, but in humans, other primates and as well as guinea pigs, this vitamin is not synthesized due to the absence of **enzyme L-gulono-oxidase** in them.
6, 7 and 8. In man, other primates and as well as guinea pigs, L-gulonic acid is oxidized to 3-keto-L-gulonic acid which is then decarboxylated to form L-xylulose which is then converted to xylitol in the presence of reduced

NADP; reaction being catalysed by enzyme L-xylitol dehydrogenase. This step gets blocked in a rare hereditary disorder called **essential pentosuria** (Fig. 6.15), as a result of which one finds large quantities of L-xylulose appearing in the urine of such patients. **Essential pentosuria is a harmless condition/disorder.**

9 and 10. Now, xylitol in the presence of NAD^+ is converted to D-xylulose in the presence of enzyme D-xylulose reductase. D-xylulose, being converted to D-xylulose-5-PO_4 in the presence of ATP which then enters in HMP shunt.

Importance of glucuronic acid

1. Bilirubin, benzoic acid, chloramphenicol, phenol, etc. get conjugated with D-glucuronic acid forming mono and di-glucuronides, chloramphenicol glucuronide and phenol glucuronide respectively (detoxication by conjugation mechanism).
2. Drugs and other xenobiotics are first hydroxylated by mono-oxygenase cytochrome P-450 system and then get conjugated with D-glucuronic acid.
3. D-glucuronic acid is incorporated to chondroitin sulphates, hyaluronic acid and heparin.
4. UDP-glucuronic acid, which is the active form, acts as a donor in liver.
5. UDP-glucuronic acid gets decarboxylated in cornea, and cartilage by a specific enzyme and NAD^+ to yield UDP-xylose, which is used for the synthesis of muco-proteins.
6. UDP-glucuronic acid is changed to UDP-L-iduronic acid with the help of UDP-glucuronic acid-5-epimerase. L-iduronic acid is then incorporated in the formation of dermatan sulphate (chondroitin sulphate B) in the skin.

Luebering-Rapoport pathway/shunt (Rapoport-Luebering cycle)

This pathway is also known as the Luebering-Rapoport shunt which occurs in mature erythrocytes involving the formation of 2,3-bis-phosphoglycerate (2,3-BPG). The function of this pathway is to regulate oxygen release from hemoglobin and deliver it to tissues. 2,3-BPG, the reaction product of the Luebering-Rapoport pathway was first described and isolated in 1925 by the Austrian biochemist Samuel Mitja Rapoport and his Technical Assistant Janet Luebering (Fig. 6.16).

Through the Luebering-Rapoport pathway bisphospho-glycerate mutase catalyzes the transfer of a phosphoryl group from C1 to C2 of 1,3-BPG, giving 2,3-BPG. Now, 2,3-bis-phosphoglycerate, the most concentrated organophosphate in the RBCs, forms 3-PG by the action of bisphosphoglycerate phosphatase. The concentration of 2,3-BPG varies proportionately with the pH, since it is inhibitory to catalytic action of bisphosphoglyceromutase.

Significance

1. In hypoxic conditions, 2,3-BPG enhances the supply of oxygen to the tissues.

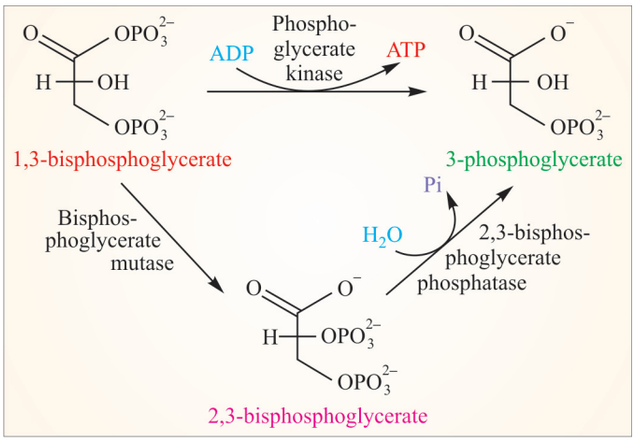


Fig. 6.16: Luebering-Rapoport pathway

2. At high altitudes, oxygen level in blood is low (hypoxia), then the level of BPG gets increased to compensate the low oxygen level in blood.
3. **In obstructive pulmonary disease, BPG level gets increased to reduce tissue hypoxia.**
4. The level of BPG is also increased in severe anaemia. This is a compensatory mechanism to take care of oxygen delivery to the tissues of the low Hb level.

CORI CYCLE (LACTIC ACID CYCLE)

The Cori cycle (also known as the lactic acid cycle), named after the discoverers, Carl Ferdinand Cori and Gerty Cori, refers to the metabolic pathway in which lactate produced from the oxidation of glucose (anaerobic glycolysis) in the muscles moves to the liver via blood where it is again converted to pyruvate and then glucose by a pathway known as gluconeogenesis (**reversal of glycolysis**), which then again returns to the muscles and is again metabolized to **lactate** (Figs 6.17 a and b).

This recycling of lactic acid is referred to as the Cori cycle, as a result of which muscles never feel fatigued and continuously receive energy.

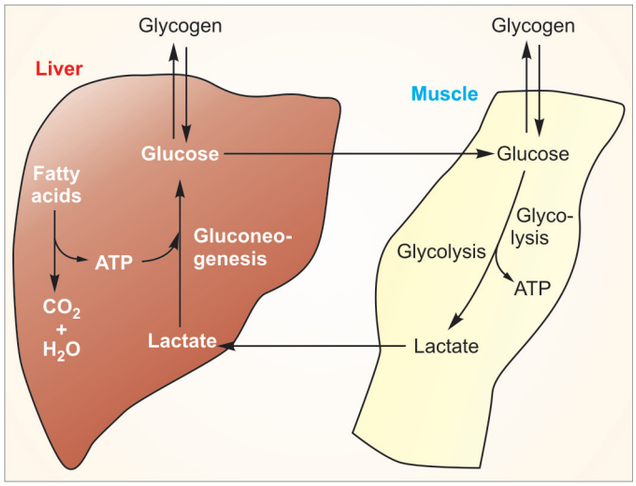


Fig. 6.17 a: Cori cycle (lactic acid cycle)

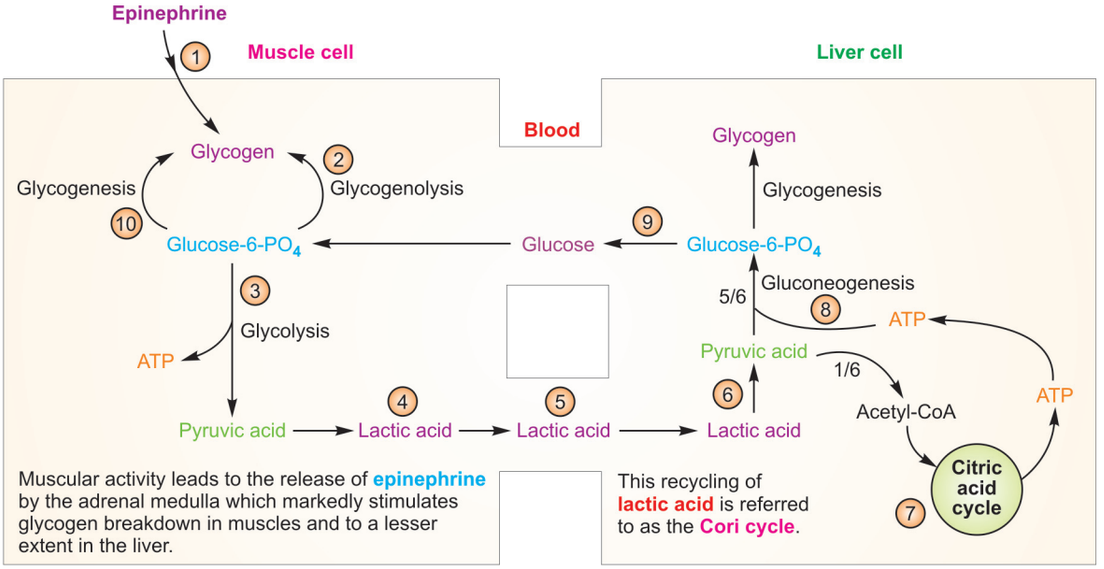


Fig. 6.17 b: Cori cycle or lactic acid cycle (in detail)

In nutshell, the series of events by which glucose is converted into lactate and back again to glucose is called the Cori cycle (Figs 6.17 a and b).

Muscular activity requires ATP, which is provided by the breakdown of glycogen in the skeletal muscles. The breakdown of glycogen, a process known as glycogenolysis, releases glucose in the form of glucose-1-phosphate (G-1-P), which is then converted to G-6-P by the action of enzyme phosphoglucomutase. G-6-P is readily fed into glycolysis (or can go into the pentose phosphate pathway if G-6-P concentration is high, a process that provides ATP to the muscle cells as energy source. During muscular activity, the store of ATP needs to be constantly replenished (filled again). When the supply of oxygen is sufficient, this energy comes from feeding pyruvate, one product of glycolysis, into the Krebs cycle.

When the oxygen supply is insufficient, typically during intense muscular activity, energy must be released through anaerobic metabolism. Lactic acid fermentation converts pyruvate to lactate by the action of **lactate dehydrogenase**. Most importantly, fermentation regenerates NAD^+, maintaining the NAD^+ concentration so that additional glycolysis reactions can occur. The fermentation step oxidizes the NADH produced by glycolysis back to NAD^+, transferring two electrons from NADH to reduce pyruvate into lactate.

Instead of accumulating inside the muscle cells, lactate produced by anaerobic fermentation is taken up by the liver. This initiates the other half of the Cori cycle. In the liver, gluconeogenesis occurs. From an intuition, gluconeogenesis reverses both glycolysis and fermentation by converting lactate first into pyruvate, and finally back to glucose. The glucose is then supplied to the muscles through the blood stream. It is ready to be fed into further glycolysis reactions. If muscle activity has stopped, the glucose is used to replenish (fill) the supplies of glycogen through glycogenesis.

How is Cori cycle important to metabolism?

1. It involves two organs, the contracting muscle and the liver.
2. It functions in anaerobic conditions when the muscles are contracting under reduced oxygen.
3. The contracting muscles produce lactate (instead of pyruvate proceeding to acetyl-CoA to TCA cycle) which is supplied to the liver.
4. In the liver, gluconeogenesis converts lactate to pyruvate.
5. Glucose is then metabolized by contracting muscle via glycolysis, to pyruvate and acetyl-CoA under aerobic condition (sufficient oxygen), and acetyl-CoA enters TCA cycle, otherwise the glucose goes through anaerobic glycolysis and the Cori cycle goes on till O_2 is sufficient.

Overall, the glycolysis part of the cycle produces 2 ATP molecules at a cost of 6 ATP consumed in the gluconeogenesis part. Each repetition of the cycle must be maintained by a net consumption of 4 ATP molecules. As a result, the cycle cannot be sustained indefinitely. The intensive consumption of ATP molecules indicates that the Cori cycle shifts the metabolic burden from the muscles to the liver.

Significance of Cori cycle

1. The cycle's importance lies in the fact that it prevents hazards of lactic acidosis in the muscles under anaerobic conditions. However, normally, before this happens the lactic acid is moved out of the muscles and into the liver.
2. The cycle is also important in producing ATP, an energy source, during muscle activity.
3. The **Cori cycle** is a much more important source of substrate for gluconeogenesis than food. The contribution of Cori cycle lactate to overall glucose production increases with fasting duration. Specifically, after 12, 20 and 40 hours of fasting by human volunteers, the contribution of Cori cycle lactate to gluconeogenesis is 41%, 71% and 92%, respectively.

4. The drug metformin (antidiabetic drug) can cause lactic acidosis in patients with renal failure because metformin inhibits the hepatic gluconeogenesis of the Cori cycle, particularly the mitochondrial respiratory chain complex.

Normally, the excess lactate would be cleared by the kidneys, but in patients with renal failure, the kidneys cannot handle the excess lactic acid.

- Lactic acid (lactate) is the major end product of glycolysis under anaerobic conditions in the muscles. Muscle tissue is incapable of resynthesizing glucose from lactate. The conversion entirely takes place in the liver.
- Muscle lactate is transported to the liver by the blood where it is converted to glucose and glycogen by reversal of glycolysis, i.e. gluconeogenesis.
- Liver glycogen is converted to glucose which is carried back to the muscle by blood.
- The conversion of muscle lactate to glucose in liver and its re-entry into muscles is called Cori cycle.
- Thus, lactic acid (lactate) formed by the oxidation of glucose in the skeletal muscles and erythrocytes, is transported to the liver and kidney where it re-forms glucose, which again becomes available via the circulation for oxidation in the tissues. This process is known as Cori cycle or lactic acid cycle.
- The significance of this cycle is that it helps in the regulation of blood sugar level. Via this cycle, muscle metabolism can attribute a lot to blood glucose indirectly.

CONVERSION OF GLUCOSE

Glucose, the very important sugar (carbohydrate) of the blood and other tissues may be easily converted to other very important substances like **glycoproteins, glycolipids, proteoglycans, etc.** by a series of biochemical reactions as shown in Fig. 6.18.

- **Glycoproteins:** They play several very important roles for the body, such as helping the immune, digestive and reproductive systems.
- **Glycolipids:** Their role is to maintain the stability of the cell membrane and to facilitate cellular recognition which is crucial to the immune response.
- **Proteoglycans:** Significant component of extracellular matrix acts as '**filler substance**'. Some proteoglycans play a role in the regulation of certain biological processes like coagulation, wound repair, host defenses and cellular packaging.

METABOLISM OF OTHER MONOSACCHARIDES

Metabolism of galactose

Metabolism of galactose is more complicated (Fig. 6.19), despite the fact that it differs from glucose only in the position of the hydroxyl group at C-4. It enters the bloodstream from the intestine, being formed in the digestion of lactose. It is derived chiefly from lactose of the diet.

In the very first reaction, galactose is firstly converted to galactose-1-phosphate in the presence of ATP by the enzyme

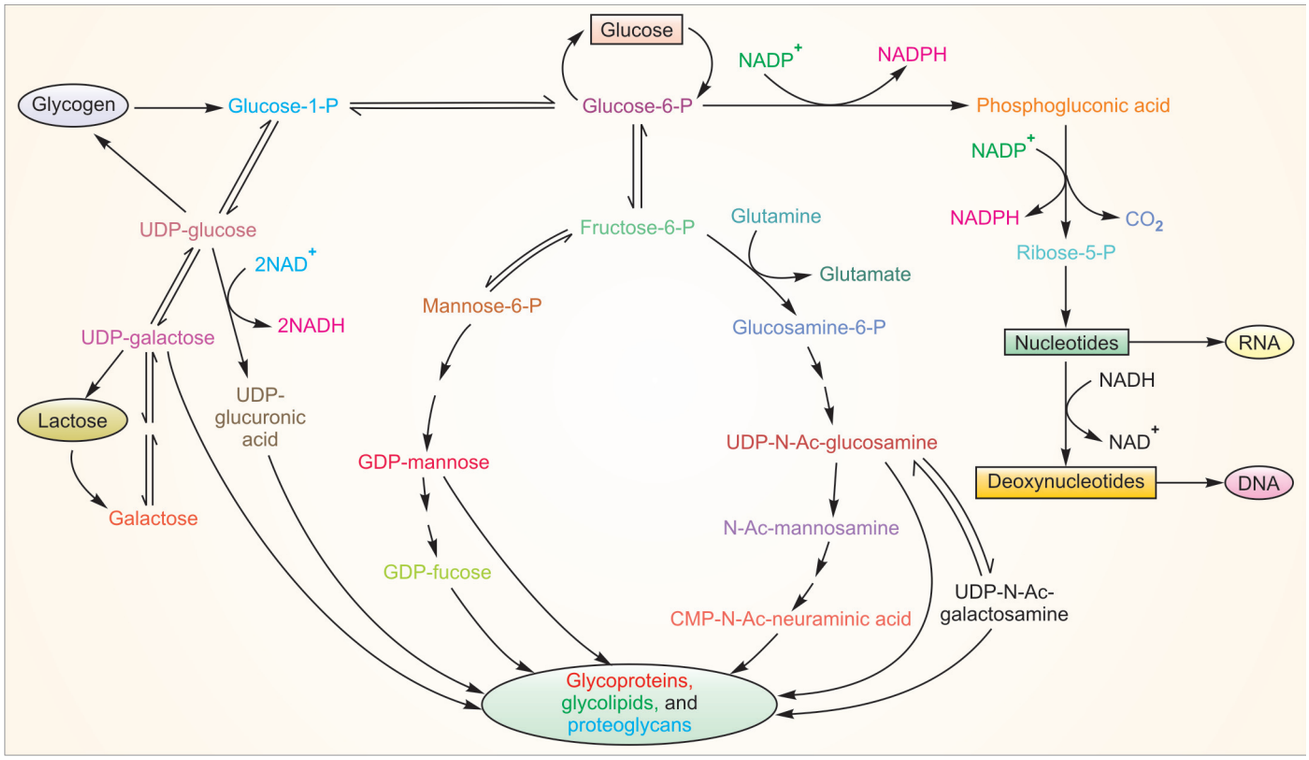


Fig. 6.18: Conversion of glucose to other sugars and important substances like glycoproteins, glycolipids and proteoglycans

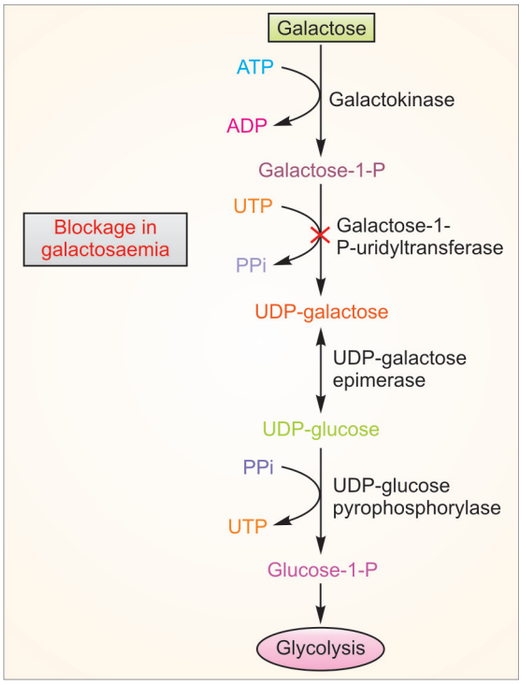


Fig. 6.19: Metabolism of galactose

galactokinase. Now, galactose-1-P is metabolized to a nucleotide derivative, i.e. UDP galactose in the presence of UTP with the formation of inorganic pyrophosphate; this reaction being catalysed by a very important enzyme of this pathway, **i.e. galactose-1-phosphate uridyl transferase.** Then UDP-galactose gets converted to UDP-glucose (uridine diphosphate glucose) with the help of enzyme UDP-galactose epimerase. In the last stage, UDP-glucose gets converted to glucose-1-P in the presence of inorganic pyrophosphate with the formation of uridine triphosphate; this reaction is catalysed by UDP-glucose pyrophosphorylase.

Glucose-1-P so formed now can easily enter into glycolytic pathway for further oxidation.

In a hereditary disorder known as **galactosaemia,** the second enzyme, i.e. **galactose-1-P-uridyl transferase is found to be missing as a result of which galactose-1-P is not further broken down to form UDP-galactose;** meaning to say that further metabolism of galactose is blocked at this very point. The inability to metabolize galactose results in abnormally high concentrations of galactose and galactose-1-P in blood and tissues. If this disease is not detected in early infancy, then it is very fatal and may lead to severe consequences. Such infants are unable to digest milk. Treatment consists in giving them galactose- (lactose-) free diet, i.e. no milk should be given to such infants/children.

Galactose-1-phosphate accumulates in erythrocytes and other tissues causing damage particularly to the liver, brain and optic lens.

Significance

Galactose is important in the body for the formation of various important substances, like certain glycolipids, glycoproteins, and of course for the formation of lactose during lactation.

The incidence of ***galactosaemia*** is not much; it may occur as frequently as **once every 18,000** births. If such infants are fed milk, then they may develop high blood sugar chiefly due to galactose-1-phosphate, galactosuria and sometimes aminoaciduria and ketonuria. **There is usually hepatomegaly, cataract and mental retardation in galactosaemic children.**

In cataract of ***diabetic patients,*** fructose and sorbitol are accumulated in the lens and glucose metabolism is also inhibited in the lens. Lens ATP declines as does lens protein synthesis, but the exact reason for cataract is not clear.

Whereas, in cases of galactosaemia, **dulcitol** [reduction of galactose gives dulcitol (a sugar alcohol)] accumulates in the lens in sufficient quantity to increase osmolarity, with consequent disruption of the lens fibre structure. Accumulation of galactose-1-P inhibits glycolysis in the lens. **Scientist *Kalckar*** has done tremendous work on galactosaemia and has noticed **large accumulation of galactose-1-P in erythrocytes, liver, brain and optic lens.**

Removal of milk from the diet of the infants results in the decrease of blood sugar concentration and the disappearance of galactosaemia. However, soya-milk (an artificial preparation) may be safely given to such infants.

Metabolism of fructose

There appear to be **two ways** by which fructose may enter the metabolic stream. Fructose may be phosphorylated by a hexokinase in the presence of ATP and Mg^{2+} ions as a result of which fructose-6-phosphate is formed along with ADP as in Fig. 6.20.

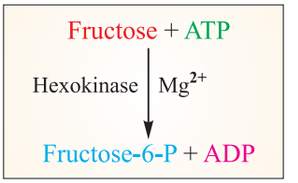


Fig. 6.20: Metabolism of fructose

The product formed, i.e. F-6-P, is an intermediate of glycolytic pathway which joins the metabolic pool.

A second fructokinase which is specific and found in liver and muscles causes the formation of fructose-1-phosphate (Fig. 6.21), which is cleaved by aldolase B to glyceraldehyde and dihydroxyacetone phosphate which are the intermediate compounds of glycolytic pathway. In this way, DHAP enters the glycolytic pathway whereas glyceraldehyde is reduced to form glycerol.

In a hereditary disorder known as *fructose intolerance,* the symptoms of which are lack of glucose in the bloodstream and **a failure to gain weight which can result into death**, ***aldolase B enzyme*** **is found to be missing in such disorder. Once diagnosed, the treatment is simple: replacement of the sucrose in the diet by lactose or glucose.**

Significance

Fructose administered intravenously is utilized better by the diabetic patients than glucose. D-fructose remains present in

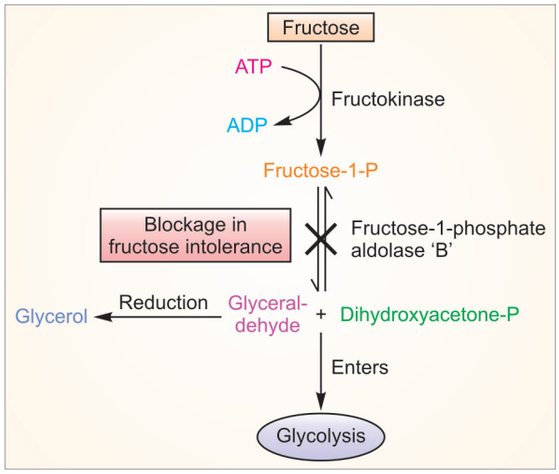


Fig. 6.21: Metabolism of fructose

significant amount in seminal fluid and is synthesized in the prostrate gland by the following pathway (Fig. 6.22).

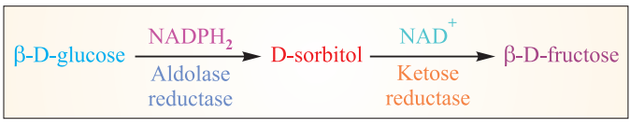


Fig. 6.22: Synthesis of fructose from glucose

In cases of fructose intolerance, one may find the appearance of appreciable amount of fructose sugar in the urine (fructosuria). There is an interesting recent claim that **children with Tay-Sachs disease (a disease of lipid metabolism) have a deficiency of F-1-P aldolase.**

Metabolism of mannose

The metabolism of mannose (Fig. 6.23) is initiated by phosphorylation in the presence of hexokinase and Mg^{2+} ions as a result of which mannose-6-phosphate is formed as shown below. Now, M-6-P is converted to F-6-P, reaction being catalysed by an isomerase. Once F-6-P is formed, now being an intermediate of glycolysis, it can easily enter into glycolytic pathway.

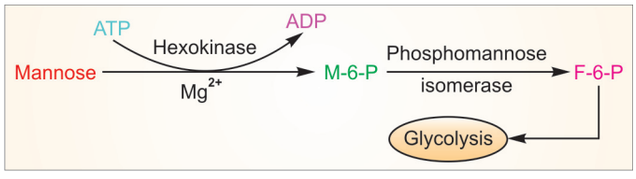


Fig. 6.23: Metabolism of mannose

Metabolism of pyruvate

Complete oxidation of the pyruvate produced in glycolysis is by the final common pathway shared by carbohydrates, fats and proteins, namely, the citric acid cycle. Before entering the cycle, pyruvate first must be carried from the cytoplasm into the mitochondria; there the pyruvate dehydrogenase enzyme system acts by exactly analogous mechanisms to those described earlier for the oxidative decarboxylation of α-ketoglutarate within the cycle (Fig. 6.24). Thus, the complex multienzyme system first uses thiamine pyrophosphate to decarboxylate pyruvate to acetaldehyde; the latter while still bound to the enzyme, is oxidized to an acetyl group; the acetyl group is transferred to lipoic acid and the hydrogens from oxidation are transferred to NAD^+ through FAD; and finally, this ester retained, is transferred to a free molecule of coenzyme A. The overall reaction is as in Fig. 6.25.

The liver, as all tissues in the human body, has no mechanism to convert the energy of the thioester of acetyl-CoA to ATP that is parallel to the mechanism for succinyl-CoA; rather the energy is used for condensation reactions, such as the citrate synthetase reaction, or **for converting the acetyl groups to fatty acids, sterols, and other important constituents of the liver.**

The oxidative decarboxylation of pyruvate is essentially non-reversible, and the pyruvate dehydrogenase reaction will lead to depletion of the body pool of glucose because there is no process in humans that can convert acetyl-CoA into glucose. Thus, the control of pyruvate oxidation is important for the conversion of glucose in liver and other tissues. Pyruvate dehydrogenase is subject to feedback regulation at two levels by the end products of its reaction: (1) acetyl-CoA and NADH will inhibit the reaction by competing with CoASH and NAD^+, respectively; (2) ATP will favour the phosphorylation. When there is abundant acetyl-CoA for citric acid cycle oxidations (e.g. from fat catabolism), and when there is ample cellular energy as indicated by high NADH and ATP levels, pyruvate dehydrogenase will be turned off, and glucose will be conserved for other requirements.

Pyruvate dehydrogenase is also a key control point of lipogenesis in adipose tissue. Insulin will favour dephosphorylation of the enzyme, hence, activating it to produce more acetyl-CoA from glucose for fatty acid biosynthesis.

Moreover, the action of malic enzyme involves pyruvate:

$$\text{Malate} + NADP^+ \rightleftharpoons \text{Pyruvate} + CO_2 + NADPH + H^+$$

This reaction may constitute an important source of reducing power for these cellular anabolic paths, such as **fatty-acid and sterol biosynthesis,** that require a source of reduced NADP. Thus, pyruvate in a true sense represents crossroads where the manifold processes of carbohydrate, fat, and protein metabolism converge and interlock with one another.

Significance

Pyruvate, in fact, occupies a crucial position in carbohydrate metabolism; it may generate lactate to sustain glycolysis in the absence of O_2; it may be fully oxidized by way of the citric acid cycle in the presence of O_2 to yield enormous amount of energy; and it may be carboxylated to keep the cycle operational or to reverse glycolysis. In addition to these important functions relating primarily to carbohydrate metabolism, there are numerous ways in which interconversions involving pyruvate strike upon other metabolic pathways. Either by direct transamination to alanine or through other keto acids (through which it is a precursor to aspartate, glutamate and others), pyruvate contributes and is

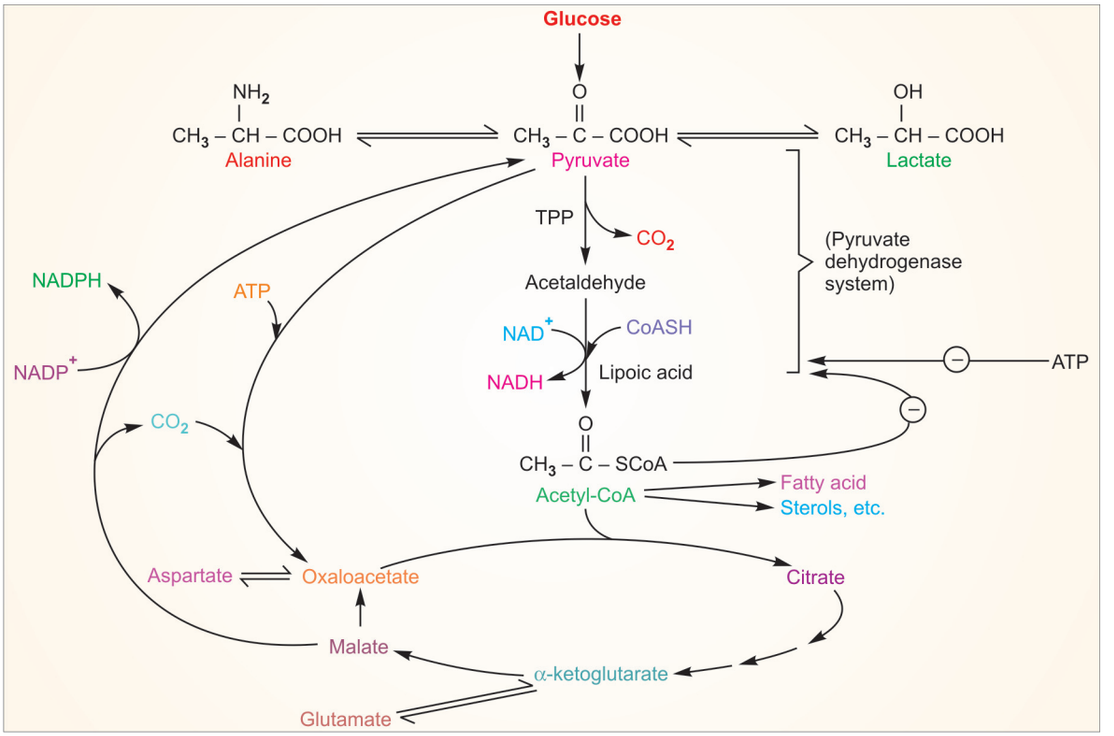


Fig. 6.24: Metabolism of pyruvate (TPP = thiamine pyrophosphate)

$$\text{Pyruvate} + \text{CoASH} + \text{NAD}^+ \rightarrow \text{Acetyl-SCoA} + \text{NADH} + \text{CO}_2$$

Fig. 6.25: Conversion of pyruvate to acetyl coenzyme A

itself regenerated through amino acid metabolism. **In the form of acetyl-CoA, pyruvate contributes to the anabolism of a variety of lipids and other important compounds such as acetylcholine.**

GLYCOGEN STORAGE DISEASES (GLYCOGENOSES)

These diseases (Table 6.5) are referred to as the diseases caused by the storage (accumulation) of polysaccharide glycogen. Tissues involved are generally liver and muscles. Such diseases are genetically transmitted from one generation to another, which means these are hereditary.

Today, seven types of such diseases are known. All these diseases are examples of **inborn errors of metabolism.** In each case, only a single enzyme appears to be missing or inactivated which suggests that the part of the chromosome responsible for producing that particular enzyme has been damaged in some way without affecting any other part of that chromosome or any other chromosome; that is why phosphorylases of muscles and liver are separately genetically controlled despite their similarity.

In every case, major finding is an increased storage of glycogen in one or the other tissue. Excessive hepatic glycogen storage (glycogenosis) in some patients will result in such massive enlargement of the liver tissue that it is readily palpable and may grossly distend the abdomen. Such kind of **hepatomegaly** is a characteristic feature of such disorders.

Table 6.5: Glycogen storage diseases (GSDs)

Type	Name	Defective enzyme	Organ affected
I	von Gierke	Glucose-6-phosphatase	Liver and kidney
II	Pompe's	α-1,4-glucosidase, i.e. lysosomal storage disease (defect in lysosomal metabolism)	All organs
III	Cori	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver
IV	Andersen's	α-1,4→α-1,6 (branching enzyme)	Liver and spleen
V	McArdle's	Phosphorylase	Muscle
VI	Hers	Phosphorylase	Liver
VII	Tarui	Phosphofructokinase	Muscle
VIII		Phosphorylase kinase	Liver

Note: Types I through VII are inherited as autosomal recessives, whereas type VIII is sex-linked.

Tissues involved generally are liver and muscles. Type I disease is more common than others. **Glycogen storage diseases are very rare.**

Following are the diseases (Table 6.5):

1. Type I glycogenosis (von Gierke disease)

The first described and most common of the hepatic glycogen-storage diseases is referred to as type I or, after its discoverer, von Gierke disease. In this disease, there is found to be an **absence of glucose-6-phosphatase in liver and kidney** as a result of which free glucose can not be formed from glucose-6-phosphate. Since G-6-P is unable to escape easily across the cell membranes, which means that the glucose in the form

of its 6-phosphate will be trapped in the liver and kidney and eventually will not be able to enter the blood. In addition, the high concentration of G-6-P inside the cells results in permanent stimulation of enzyme glycogen synthetase, which is responsible for the synthesis of glycogen. In this way, large stores of normal glycogen are found to be accumulated in liver and kidney tissues.

Since, in this disorder, glycogen metabolism is affected, therefore, such patients even on fasting for only a short period suffer from severe hypoglycaemia, mild ketosis and mild lactic acidosis. This hypoglycaemia results in convulsions in acute conditions and in growth retardation due to chronic suppression of insulin production.

2. Type II glycogenosis (Pompe disease)

Stores of normal glycogen are found in almost every organ of the body, including the heart. The disease is congenital and the infants often die from cardiac failure. In this disease one finds the **absence of lysosomal enzyme α-1→4- and 1→6-glucosidase (acid maltase),** whose function is to degrade glycogen, which otherwise accumulates in the lysosomes. **This disease is fatal owing to deposits of glycogen in heart.**

3. Type III glycogenosis (limit dextrinosis; Forbes or Cori disease)

This disease is characterized by increased deposits of glycogen with very short outer branches in the liver and other tissues. In this disease, there is found to be an **absence of debranching enzyme**, i.e. amylo-1,6-glucosidase. In this disease, phosphorylase acts on α-l,4 bonds until an α-l,6 bond is reached. The resulting molecule is called a limit dextrin.

4. Type IV glycogenosis (amylopectinosis; Andersen disease)

This disease is caused due to the **deficiency of the branching enzyme**, i.e. amylo-(1→4)→(1→6)-transglucosidase which results in the accumulation of glycogen with abnormally long chains. This disease invariably leads to liver cirrhosis and death in infancy. Death due to cardiac or liver failure usually occurs in the first year of life.

5. Type V glycogenosis [myophosphorylase (muscle phosphorylase) deficiency glycogenosis; McArdle disease]

In this disease, patients exhibit markedly diminished tolerance to exercise as a result of which they feel muscular pain, weakness and stiffness after only mild exercise. Their skeletal muscles are found to have an abnormally high content of glycogen (2.5–4.1%) but little or no lactic acid is detectable in their blood after exercise. In this disease, there is **found deficiency of muscle phosphorylase**.

6. Type VI glycogenosis (Hers disease)

In this disease, there is found an **absence of liver phosphorylase** as a result of which glycogen is not further broken down, instead, glycogen (with normal structure) accumulates in the liver. Symptoms are similar to but milder than those of type I disease.

7. Type VII glycogenosis (Tarui disease)

In this disease, there is found absence of enzyme phosphofructokinase (PFK) in the muscles and erythrocytes. Net result is pain and cramps in muscles.

Apart from the type I form, these glycogen storage diseases appear to be quite rare.

8. GSD VIII glycogenosis

One of the mildest forms of glycogenoses, characterized by hepatomegaly, growth retardation, hyperketosis, hypercholesterolemia, hypertriglyceridemia, etc.

Important Questions

Short Answer Type Questions

Write a short note on the following.

1. Dietary carbohydrates
2. Hemolytic anaemias
3. Amphibolic pathways
4. Glucose utilization in the RBCs
5. Hexokinases/glucokinase
6. Glucose-6-phosphatase dehydrogenase deficiency

Essay Type Questions

1. Describe glycogenesis in detail.
2. Describe glycogenolysis in detail.
3. Describe glycolysis in detail giving its energetics and its biomedical importance.
4. Describe in detail shuttle systems of carbohydrate metabolism.
5. Describe TCA cycle in detail, giving its energetics, role of vitamins, etc.
6. Describe formation of acetyl-CoA and its role in metabolism.
7. Describe in detail pentose phosphate pathway along with its significance.
8. Describe gluconeogenesis giving its schematic diagram and its regulation.
9. Describe in detail glucose-alanine cycle and its significance.
10. Describe uronic acid pathway in detail.
11. Describe Luebering-Rapoport pathway in detail.
12. Describe in detail Cori cycle giving its schematic diagram and significance.
13. Write down in detail about metabolism of galactose, fructose and mannose.
14. Describe pyruvate metabolism in detail and its significance.
15. Describe glycogen storage diseases at length.

Multiple Choice Questions

Read the following questions carefully and put a tick (✓) mark in the box against the correct option.

1. Formation of galactose-1-P from galactose and ATP is catalysed by:
   (a) Hexokinase ☐
   (b) Galactokinase ☐
   (c) Galactose-1-P uridyltransferase ☐
   (d) Glucokinase ☐
2. Galactosemia is an inborn error of metabolism which is characterised by:
   (a) Non-accumulation of high concentration of galactose-1-P in the RBCs ☐
   (b) Accumulation of high concentration of galactose-1-P in the RBCs ☐
   (c) Accumulation of high concentration of glucose-1-P in the RBCs ☐
   (d) Accumulation of mannose-1-P in the RBCs ☐
3. Glycogen storage disease includes:
   (a) McArdle disease ☐
   (b) Wilson disease ☐
   (c) Alkaptonuria ☐
   (d) Phenylketonuria ☐
4. Symptom(s) of diabetes mellitus is/are:
   (a) Gastrointestinal disorders ☐
   (b) Polydipsia ☐
   (c) Sweating ☐
   (d) Blackening near eyes ☐
5. Symptom(s) of diabetes insipidus is/are:
   (a) Sweating ☐
   (b) Watery mouth ☐
   (c) Edema in the limbs ☐
   (d) Large quantity of urine (more than 4 litres per day) ☐
6. Glycogenolysis is a process by which glycogen in muscles is finally broken down to produce:
   (a) Glucose ☐
   (b) Pyruvic acid ☐
   (c) Lactic acid ☐
   (d) Glucose-6-phosphate ☐
7. Glycogenesis is a process by which glycogen is synthesized from:
   (a) Pyruvic acid ☐
   (b) Lactic acid ☐
   (c) Certain keto acids ☐
   (d) Glucose ☐
8. Gluconeogenesis is a process by which glucose is synthesized from:
   (a) Glycogen ☐
   (b) Alanine ☐
   (c) RNA ☐
   (d) Starch ☐
9. Glycolysis is a process by which glycogen or glucose is broken down to produce finally (under aerobic conditions):
   (a) Lactic acid ☐
   (b) Dextrins ☐
   (c) Pyruvic acid ☐
   (d) Amino acids ☐
10. HMP shunt is a process by which one of the following is synthesized:
    (a) Ribose-5-phosphate ☐
    (b) Maltose ☐
    (c) Insulin ☐
    (d) Glycogen ☐
11. Main site of gluconeogenesis is:
    (a) Brain ☐
    (b) Lungs ☐
    (c) Pancreas ☐
    (d) Liver ☐
12. Main site of glycolysis is:
    (a) Brain ☐
    (b) Spleen ☐
    (c) Muscles ☐
    (d) Pancreas ☐
13. Main sites of glycogenesis are:
    (a) Pancreas ☐
    (b) Kidney ☐
    (c) Liver ☐
    (d) Thymus ☐
14. Enzymes of TCA cycle are found in the:
    (a) Nucleus ☐
    (b) Nucleolus ☐
    (c) Golgi bodies ☐
    (d) Mitochondria ☐
15. TCA cycle is a process by which pyruvic acid is completely oxidized to produce:
    (a) Ketone bodies ☐
    (b) Lipids ☐
    (c) Carbon dioxide ☐
    (d) Glucose ☐
16. How many molecules of ATP are produced according to new concept when one molecule of glucose gets completely oxidized?
    (a) 30 ☐
    (b) 32 ☐
    (c) 36 ☐
    (d) 38 ☐
17. How many molecules of ATP are generated in glycolysis per molecule of glucose oxidized under anaerobic conditions?
    (a) 2 ☐
    (b) 4 ☐
    (c) 6 ☐
    (d) 8 ☐
18. How many molecules of ATP are generated per molecule of glucose oxidized in glycolysis under aerobic conditions?
    (a) 6 ☐
    (b) 7 ☐

(c) 8 ☐
(d) 9 ☐

19. In erythrocytes, the most abundantly found phosphate ester is:
(a) Glucose-6-P ☐
(b) Fructose-6-P ☐
(c) Fructose-1,6-diphosphate ☐
(d) 2,3-diphosphoglycerate ☐

20. In muscle cells, a substance is found which acts as an immediate source of useful energy in the first few seconds of muscular contraction, before the whole process of glucose (as fatty acids) catabolism can be speeded up to produce the extra energy required.
(a) Creatine phosphate ☐
(b) Adenosine triphosphate ☐
(c) β-glycerophosphate ☐
(d) Fructose-1,6-diphosphate ☐

21. Crystalline insulin was isolated from pancreas in 1926 by:
(a) Abel ☐
(b) Fischer ☐
(c) Benedict ☐
(d) Sanger ☐

22. Amino acid sequence of insulin was established in 1953 by:
(a) Smith ☐
(b) Sanger ☐
(c) Davidson ☐
(d) Jacob ☐

23. Traces of which metal are required for the crystallization of insulin?
(a) Cobalt ☐
(b) Aluminium ☐
(c) Zinc ☐
(d) Iron ☐

24. Insulin hormone consists of how many amino acids in its structure?
(a) 49 ☐
(b) 50 ☐
(c) 51 ☐
(d) 52 ☐

25. Insulin hormone consists of how many polypeptide chains in its structure?
(a) Two ☐
(b) Three ☐
(c) Four ☐
(d) Five ☐

26. A and B polypeptide chains of insulin hormone consist of how many amino acids in them?
(a) 20 and 31 ☐
(b) 21 and 30 ☐
(c) 22 and 29 ☐
(d) 23 and 28 ☐

27. The two polypeptide chains (A and B) of insulin hormone remain linked to each other with the help of disulfide bridges at positions:
(a) 7–5 and 20–7 ☐
(b) 7–3 and 20–8 ☐
(c) 7–7 and 20–19 ☐
(d) 7–8 and 20–20 ☐

28. An intradisulfide bridge between two amino acids also occurs in the A polypeptide chain of insulin hormone:
(a) Between 6 and 10th ☐
(b) Between 6 and 11th ☐
(c) Between 6 and 12th ☐
(d) Between 6 and 13th ☐

29. Insulin hormone has a molecular weight of:
(a) 5,000 daltons ☐
(b) 5,700 daltons ☐
(c) 5,734 daltons ☐
(d) 5,808 daltons ☐

30. Normal requirement of insulin per day by a normal man is about:
(a) 50 units ☐
(b) 100 units ☐
(c) 150 units ☐
(d) 200 units ☐

31. Normal pancreas can store insulin which is nearly:
(a) 150 units ☐
(b) 200 units ☐
(c) 250 units ☐
(d) 300 units ☐

32. Insulin secretion is reduced by:
(a) Laparotomy ☐
(b) Vagotomy (branches of vagus nerve cut) ☐
(c) Nephrectomy ☐
(d) Vasectomy ☐

33. Glucagon hormone circulates in plasma in free form, its half-life in plasma is:
(a) ~5 minutes ☐
(b) ~10 minutes ☐
(c) ~15 minutes ☐
(d) ~20 minutes ☐

34. By consuming 100 ml beer one gets how many kilo-calories?
(a) 38 ☐
(b) 48 ☐
(c) 58 ☐
(d) More than 58 ☐

Answers

1. b **2.** b **3.** a **4.** b **5.** d **6.** d **7.** d **8.** b **9.** c **10.** a **11.** d **12.** c **13.** c **14.** d **15.** c **16.** b **17.** a **18.** b **19.** d **20.** a **21.** a **22.** b **23.** c **24.** c **25.** a **26.** b **27.** c **28.** b **29.** d **30.** a **31.** c **32.** b **33.** a **34.** a