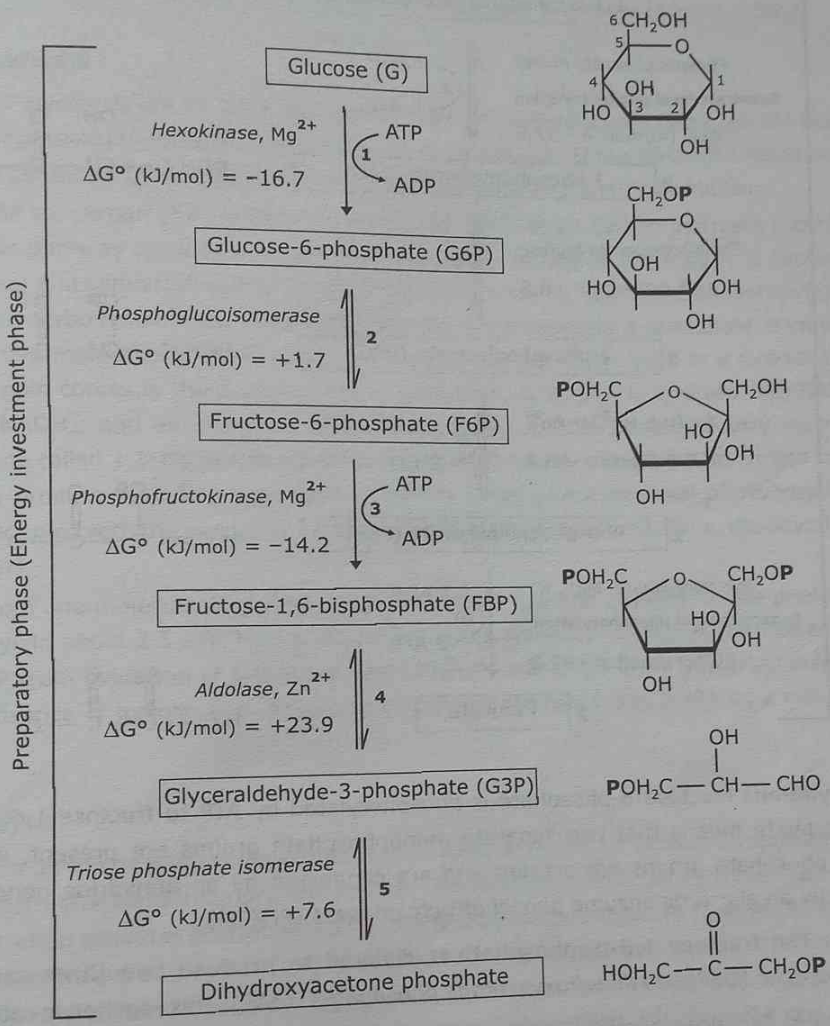
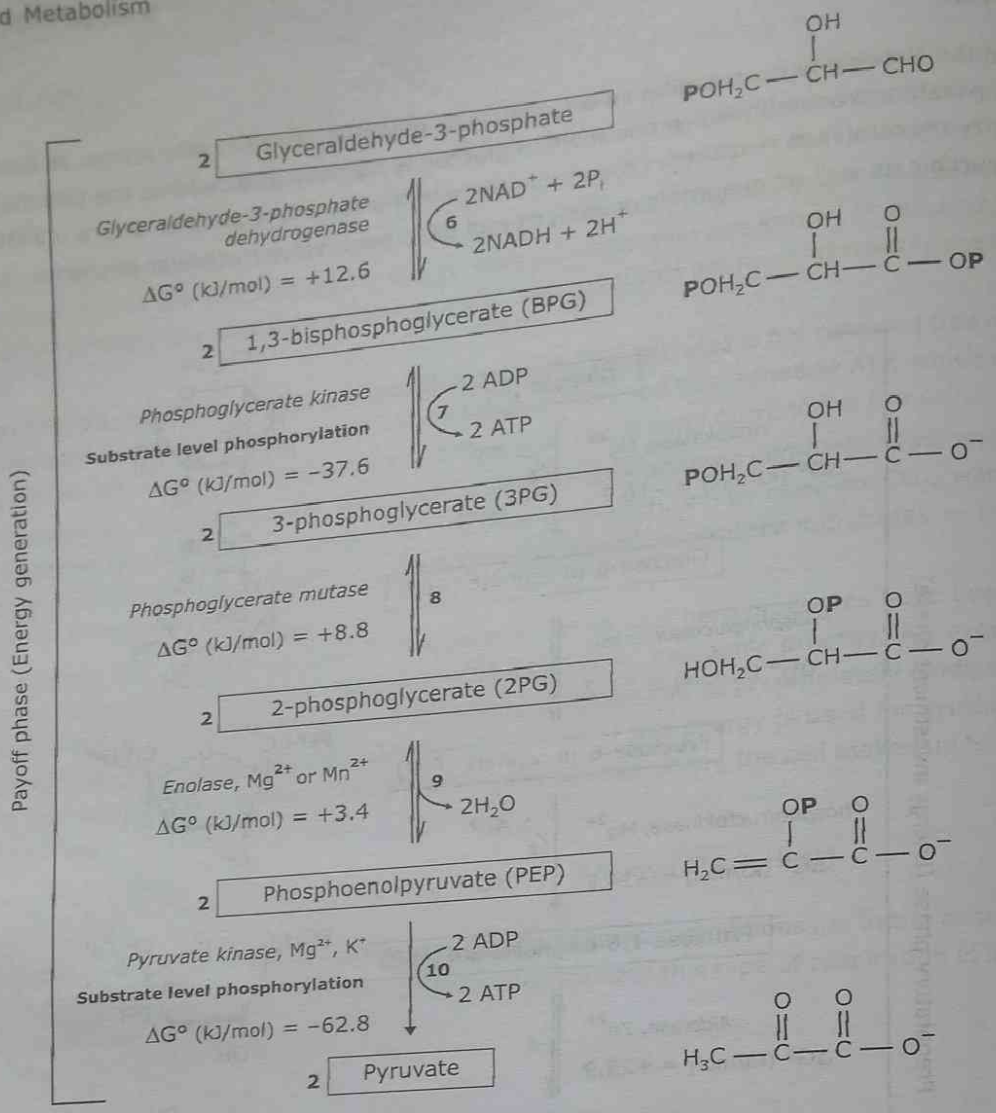
## 2.3.2 Glycolysis

*Glycolysis* (from the Greek *glykys*, meaning *sweet*, and *lysis*, meaning *splitting*) also known as *Embden-Meyerhof pathway*, is an oxidative process in which one mole of glucose is partially oxidized into the two moles of pyruvate in a series of enzyme-catalyzed reactions. Glycolysis occurs in the cytosol of all cells. It is a unique pathway that occurs in both aerobic as well as anaerobic conditions and does not involve molecular oxygen.




**Step 1 :** (**Phosphorylation**) Glucose is phosphorylated by ATP to form a glucose 6-phosphate. The negative charge of the phosphate prevents the passage of the glucose 6-phosphate through the plasma membrane, trapping glucose inside the cell. This *irreversible* reaction is catalyzed by *hexokinase*. Hexokinase is present in all cells of all organisms. Hexokinase requires divalent metal ions such as $Mg^{2+}$ or $Mn^{2+}$ for activity. Hepatocytes and β-cells of the pancreas also contain a form of hexokinase called **glucokinase** (hexokinase D). Hexokinase and glucokinase are isozymes. Glucokinase is present in liver and beta-cells of the pancreas and has a high $K_m$ and $V_{max}$ as compared to hexokinase.

**Step 2 :** (**Isomerization**) A readily reversible rearrangement of the chemical structure (isomerization) moves the carbonyl oxygen from carbon 1 to carbon 2, forming a ketose from an aldose sugar. Thus, the isomerization of glucose 6-phosphate to fructose 6-phosphate is a conversion of an aldose into a ketose.




**Step 3 :** (**Phosphorylation**) Fructose 6-phosphate is phosphorylated by ATP to fructose 1,6-bisphosphate. The prefix *bis-* in bisphosphate means that two separate monophosphate groups are present, whereas the prefix *di-* means that two phosphate groups are present and are connected by an anhydride bond. This *irreversible* reaction is catalyzed by an allosteric enzyme *phosphofructokinase-1* (PFK-1).

**Step 4 :** (**Cleavage**) The fructose 1,6-bisphosphate is cleaved to produce two three-carbon molecules - *glyceraldehyde 3-phosphate* (G3P) and *dihydroxyacetone phosphate* (DHAP). This reaction is catalyzed by *aldolase.*

**Step 5 :** (**Isomerization**) Dihydroxyacetone phosphate is isomerized to form glyceraldehyde 3-phosphate. The isomerization of these three-carbon phosphorylated sugars is catalyzed by *triose phosphate isomerase.*

**Step 6 :** The two molecules of glyceraldehyde 3-phosphate are oxidized. Enzyme *glyceraldehyde 3-phosphate dehydrogenase* catalyzes the conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate (1,3-BPG). The reaction occurs in two steps: first the *oxidation* of the aldehyde to a carboxylic acid by $NAD^+$ and second the *joining* of the carboxylic acid and orthophosphate to form the acyl-phosphate product. **Iodoacetate** is a potent inhibitor of *glyceraldehyde-3 phosphate dehydrogenase* because it forms a covalent derivative of the essential -SH group of the enzyme active site, rendering it inactive.

**Step 7 :** In this step high-energy phosphate group is transferred from 1,3-bisphosphoglycerate to ADP. The formation of ATP is referred to as *substrate-level phosphorylation* because the phosphate donor, 1,3-bisphosphoglycerate, is a substrate with high phosphoryl-transfer potential. This step is catalyzed by enzyme *phosphoglycerate kinase.*

**Step 8 :** The remaining phosphate ester linkage in 3-phosphoglycerate, which has a relatively low free energy of hydrolysis, is moved from carbon 3 to carbon 2 to form 2-phosphoglycerate.

**Step 9 :** The removal of water from 2-phosphoglycerate creates a high-energy enol phosphate linkage. The enzyme catalyzing this step, *enolase*, is inhibited by *fluoride*.

**Step 10 :** The transfer of the high-energy phosphate group that was generated in step 9 to ADP forms ATP. This last step in glycolysis is the irreversible transfer of the phosphoryl group from phosphoenolpyruvate to ADP is catalyzed by *pyruvate kinase*. Pyruvate kinase requires either $K^+$ or $Mg^{2+}$.

*Net reaction* : Glucose + $2NAD^+$ + 2ADP + $2H_2PO_4^-$ $\longrightarrow$ 2Pyruvate + 2NADH + 2ATP + $2H_2O$

**Glycolysis : An overview**

All of the enzymes of glycolysis are found in the cytosol. Certain trypanosomes carry out the first seven reactions of glycolysis in an organized cytoplasmic organelles called *glycosome*. Three glycolytic reactions are irreversible. These reactions are catalyzed by hexokinase, phosphofructokinase and pyruvate kinase.

The breakdown of the six-carbon glucose into two molecules of the three-carbon pyruvate occurs in ten steps. The first five steps of this pathway constitute the *preparatory phase*. In this phase, energy is consumed as glucose is phosphorylated twice, and converted to FBP. For both phosphorylations, ATP is the phosphoryl group donor. FBP is split to yield two three-carbon molecules. One of the products, dihydroxyacetone phosphate, is immediately converted to G3P. This yields two molecules of G3P, which are then converted to pyruvate in a five-step process of payoff phase. The energy gain comes in the *payoff phase* of glycolysis. First, G3P is oxidized with $NAD^+$ as the electron acceptor (to form NADH), and an inorganic phosphate (not by ATP) is simultaneously incorporated to give a high-energy molecule called 1,3-bisphosphoglycerate. The high-energy phosphate on carbon one is subsequently donated to ADP to produce ATP. This synthesis of ATP is called *substrate-level phosphorylation* because ADP phosphorylation is coupled with the exergonic breakdown of a high-energy bond. Thus, the payoff phase of glycolysis yields ATP and NADH.

The energy yield from one molecule of glucose in glycolysis is 2 ATP and 2 NADH. In the presence of oxygen, one NADH reoxidize to yield about 2.5 ATP. Hence, glycolysis in the presence of oxygen (termed as aerobic glycolysis) yields 7 ATP (5 ATP from oxidation of two molecules of NADH and 2 ATP from substrate level phosphorylation). Glycolysis in the absence of oxygen (i.e. anaerobic glycolysis) produces only 2 ATP as a result of substrate-level phosphorylation.

### 2.3.4 Krebs cycle

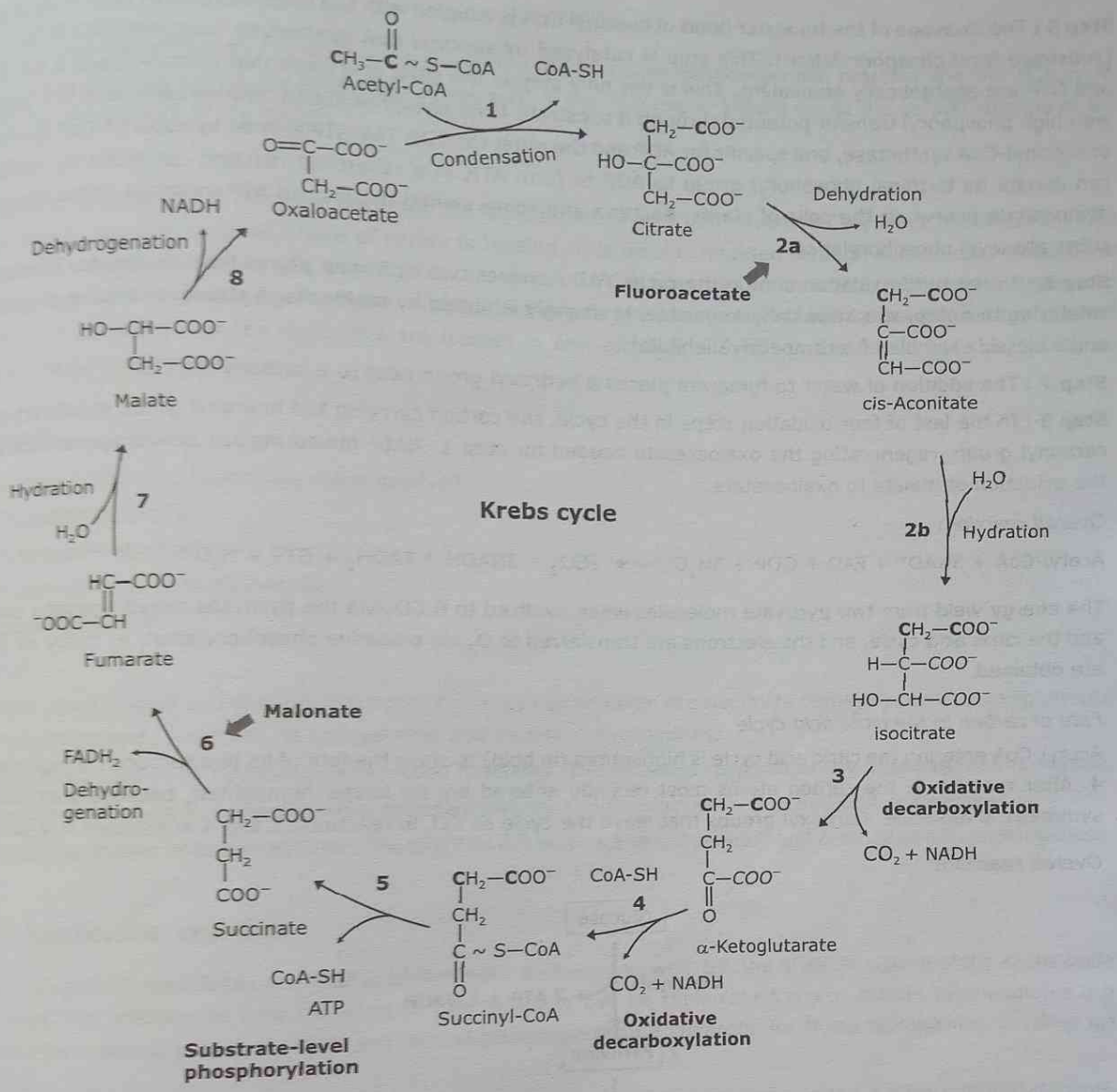
Krebs cycle (also known as the citric acid cycle or tricarboxylic acid cycle) was discovered by H. A. Kreb, a German born British Biochemist, who received the Nobel prize in 1953. This cycle occurs in the matrix of mitochondria (cytosol in prokaryotes). The whole cycle is explained in the following figure. The net result of Kreb cycle is that for each acetyl group entering the cycle as acetyl-CoA, two molecules of $CO_2$ are produced.

**Step 1 :** The Krebs cycle begins with the condensation of an oxaloacetate (four carbon unit), and the acetyl group of acetyl-CoA (two-carbon unit). Oxaloacetate reacts with acetyl-CoA and $H_2O$ to yield citrate and coenzyme A. This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by *citrate synthase.* Citrate has no chiral center but has the potential to react asymmetrically if an enzyme with which it interacts has an active site that is asymmetric. Such molecule is called *prochiral molecule*.

**Step 2a and 2b :** An isomerization reaction, in which water is first removed and then added back, moves the hydroxyl group from one carbon atom to its neighbour. The enzyme catalyzing this step, *aconitase* (nonheme iron protein), is the target site for the toxic compound **fluoroacetate** (used as a pesticide). Fluoroacetate blocks the citric acid cycle by its metabolic conversion of *fluorocitrate*, which is a potent inhibitor of aconitase.

**Step 3 :** Isocitrate is oxidized and decarboxylated to α-ketoglutarate (also called oxoglutarate). In the first of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group. The immediate product is unstable, losing $CO_2$ while still bound to the enzyme. The oxidative decarboxylation of isocitrate is catalyzed by *isocitrate dehydrogenase.*

**Step 4 :** A second oxidative decarboxylation reaction results in the formation of succinyl-CoA from α-ketoglutarate. *α-ketoglutarate dehydrogenase* catalyzes this oxidative step and produces NADH, $CO_2$, and a high-energy thioester bond to coenzyme A.




1. Citrate synthase
2a. Aconitase
2b. Aconitase
3. Isocitrate dehydrogenase
4. α-Ketoglutarate dehydrogenase
5. Succinate thiokinase
6. Succinate dehydrogenase
7. Fumarase
8. Malate dehydrogenase

**Figure 2.7** Krebs cycle. Acetyl-CoA is the fuel for the Krebs cycle. A four-carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid (citrate). An isomer of citrate is then oxidatively decarboxylated. The resulting five-carbon compound (α-ketoglutarate) also is oxidatively decarboxylated to yield a four carbon compound (succinate). Oxaloacetate is then regenerated from succinate. During oxidative decarboxylation, two carbon atoms come out as $CO_2$ from the oxidation of isocitrate and α-ketoglutarate. The energy released during this cycle is used in the reduction of three $NAD^+$ and one FAD and the production of one ATP or GTP. Note that the two carbon atoms appearing as $CO_2$ are not the same two carbons that entered in the form of the acetyl group; additional turns around the cycle are required to release these carbons as $CO_2$.

**Step 5 :** The cleavage of the thioester bond of succinyl CoA is coupled with the phosphorylation of an ADP or a GDP (*substrate level phosphorylation*). This step is catalyzed by *succinyl CoA synthetase* (succinate thiokinase). ATP and GTP are energetically equivalent. This is the only step in the citric acid cycle that directly yields a compound with high phosphoryl transfer potential through a substrate-level phosphorylation. Animal cells have two isozymes of succinyl-CoA synthetase, one specific for ADP and the other for GDP. The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by *nucleoside diphosphate kinase*. In the cells of plants, bacteria and some animal tissues, an ATP molecule forms directly by substrate-level phosphorylation.

**Step 6 :** In the third oxidation step in the cycle, FAD removes two hydrogen atoms from succinate. The enzyme catalyzing this step, succinate dehydrogenase, is strongly inhibited by **malonate**, a structural analog of succinate and a classic example of a competitive inhibitor.

**Step 7 :** The addition of water to fumarate places a hydroxyl group next to a carbonyl carbon.

**Step 8 :** In the last of four oxidation steps in the cycle, the carbon carrying the hydroxyl group is converted to a carbonyl group, regenerating the oxaloacetate needed for step 1. $NAD^+$ linked *malate dehydrogenase* catalyzes the oxidation of malate to oxaloacetate.

*Overall reaction*:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + 3H_2O \longrightarrow 2CO_2 + 3NADH + FADH_2 + GTP + H_2O$$

The energy yield from two pyruvate molecules when oxidized to 6 $CO_2$ via the pyruvate dehydrogenase complex and the citric acid cycle, and the electrons are transferred to $O_2$ via oxidative phosphorylation, as many as 25 ATP are obtained.

*Fate of carbon in the citric acid cycle*

Acetyl-CoA entering the citric acid cycle is highlighted (in bold) to show the fate of its two carbons through reaction 4. After reaction 5, the carbon atoms most recently entered are no longer highlighted, because succinate is a symmetrical molecule. Carboxyl groups that leave the cycle as $CO_2$ in reactions 3 and 4 are shown in *italic* letter.

*Overall reaction*:

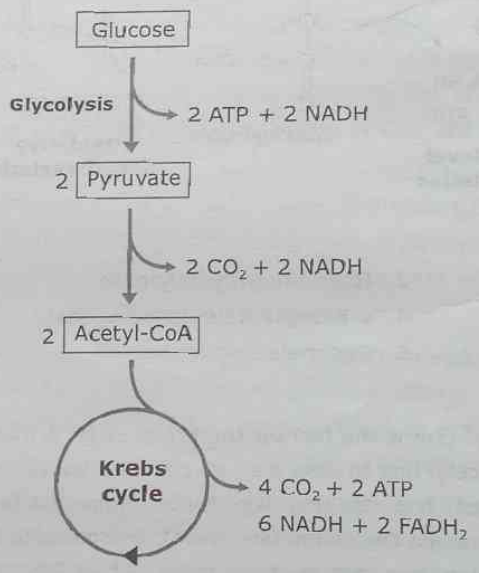



**Figure 2.8** Glycolysis and the Krebs cycle, produce only 4 ATP molecules per glucose molecule, all by substrate level phosphorylation: 2 net ATP from glycolysis and 2 ATP from the Krebs cycle. At this point, molecules of NADH (and $FADH_2$) account for most of the energy extracted from the glucose. These electron escorts link glycolysis and the Krebs cycle to the machinery of oxidative phosphorylation, which uses energy released by the electron transport chain to power ATP synthesis.

### 2.9.1 Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors. Gluconeogenesis is a universal pathway, found in all animals, plants, fungi and microorganisms.

*Site of gluconeogenesis* : In higher animals, gluconeogenesis occurs in the liver and, to a smaller extent, in the kidney cortex. Under normal circumstances, the liver is responsible for 85% to 95% of the glucose that is made. During starvation or during metabolic acidosis, the kidney is capable of making glucose and then may contribute up to 50% of the glucose formed, since, in these conditions, the amount contributed by the liver decreases considerably.

*Precursors of gluconeogenesis* : Gluconeogenetic precursors include:

1. Glycolytic products like lactate, pyruvate, glycerol
2. Citric acid cycle intermediates and
3. Some amino acids (termed glucogenic amino acids). Lysine and leucine are the only amino acids that are not substrate for gluconeogenesis. These amino acids produce only acetyl-CoA upon degradation.

Animal cells can carry out gluconeogenesis from three- and four- carbon precursors, but not from the two acetyl carbons of acetyl-CoA. Animal cells have no way to convert acetyl-CoA to pyruvate or oxaloacetate. Thus, fatty acids are not substrates for gluconeogenesis in animals, because most fatty acids yield only acetyl-CoA upon degradation. Unlike animals, plants and some microorganisms can convert acetyl-CoA derived from fatty acid oxidation to glucose.

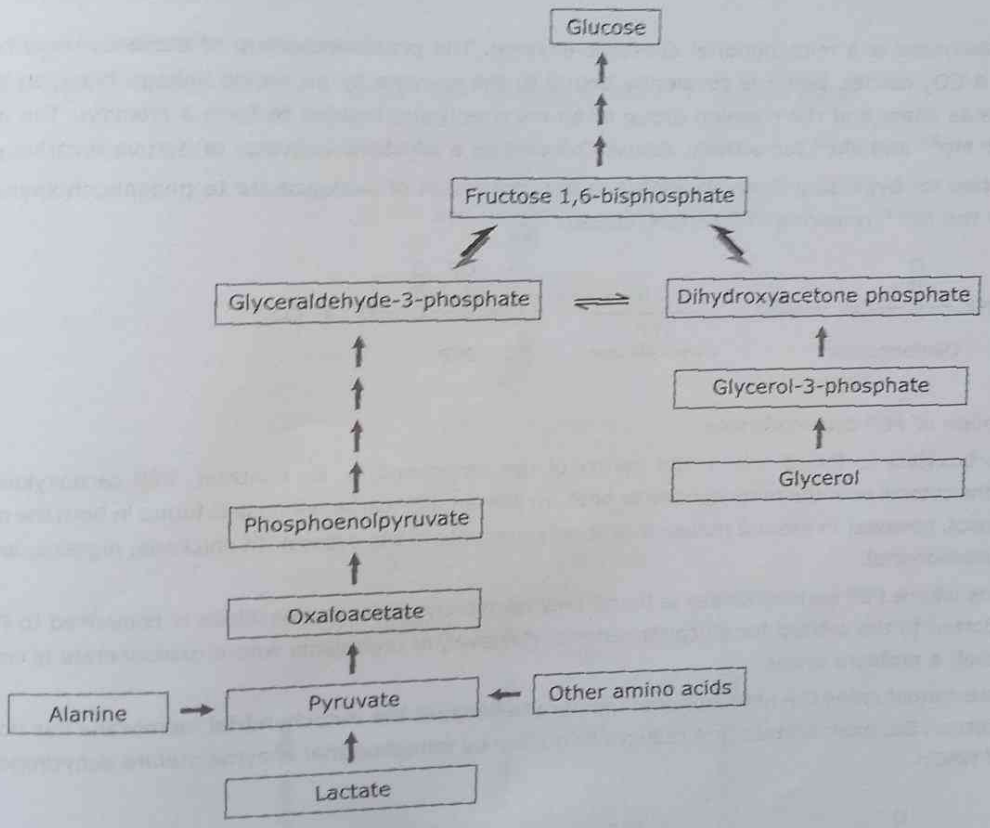



**Figure 2.64** Major gluconeogenic precursors.

**Conversion of PEP to glucose**

This pathway is opposite of glycolysis. However,

One step in glycolytic pathway where PFK-1 is involved is irreversible so during gluconeogenesis enzyme fructose 1,6 bisphosphatase acts without using ATP and converts fructose-1,6-bisphosphate to fructose-6-phosphate. Fructose-1,6-bisphosphatase is an allosterically regulated enzyme. Citrate stimulates bisphosphatase activity, but fructose-2,6-bisphosphate is a potent allosteric inhibitor. AMP also inhibits the bisphosphatase.

Another step where glucose converted into glucose-6-phosphate during glycolysis is catalyzed by hexokinase and requires ATP. This reaction is also irreversible. During gluconeogenesis, conversion of glucose-6-phosphate to glucose requires glucose-6-phosphatase and no ATP is required. This enzyme is present in the membranes of the endoplasmic reticulum of liver and kidney cells, but is absent in muscle and brain. For this reason, gluconeogenesis is not carried out in muscle and brain.

*Energetics of gluconeogenetic pathway*

$$2\ \text{Pyruvate} + 2\text{NADH} + 4\text{ATP} + 2\text{GTP} + 6\text{H}_2\text{O} \longrightarrow \text{Glucose} + 2\text{NAD}^+ + 2\text{GDP} + 4\text{ADP}$$

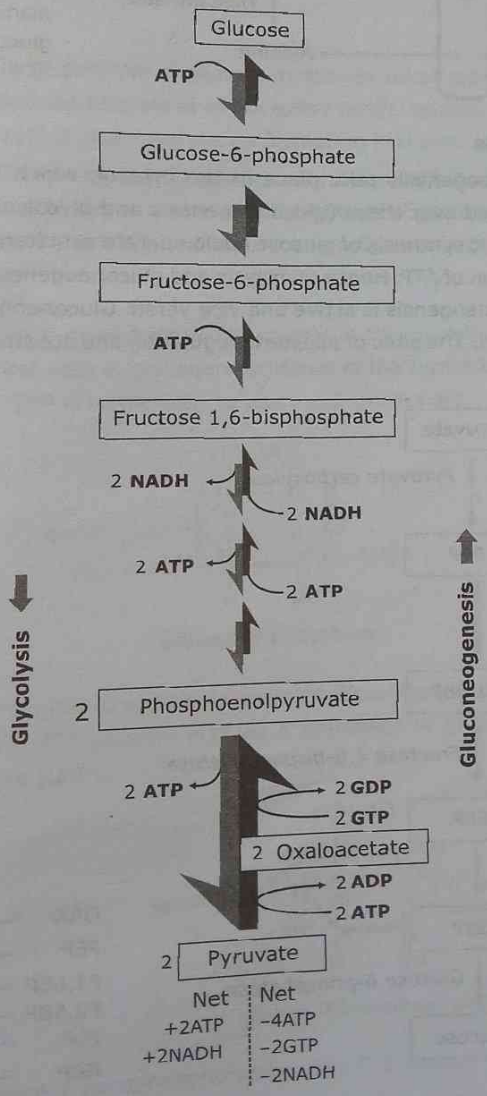



**Figure 2.65** Reactions of glycolysis and gluconeogenesis.