Tricarboxylic acid cycle (Keres cycle)

The complete cycle was proposed by Sir Hans Krebs in 1937 (Nobel prize, 1953). The cycle is therefore named after him. Please note that the name is Krebs cycle (there is no apostrophe). Functions of the Citric Acid Cycle

1. It is the final common oxidative pathway that oxidizes acetyl CoA to CO2 2. It is the source of reduced co-enzymes that provide the substrate for the respiratory chain.

3. It acts as a link between catabolic and anabolic pathways (amphibolic role).

4. It provides precursors for synthesis of amino acids and nucleotides.

5. Components of the cycle have a direct or indirect controlling effects on key enzymes of other pathways. Reactions of the Cycle Preparatory Steps Acetyl CoA enters the cycle, and is completely oxidized. During this process, energy is trapped. The sources of acetyl CoA. Pyruvate derived from glycolysis is oxidatively decarboxylated to acetyl CoA by the pyruvate

dehydrogenase. This is the link between the TCA cycle and glycolysis. All the enzymes of citric acid cycle are located inside the mitochondria.

1st Step: Formation of Citric Acid

The 4 carbon, oxaloacetate condensed with 2 carbon, acetyl CoA to form 6 carbon compound, the citrate. The enzyme is citrate synthase. The sources and utilization of acetyl CoA are shown .

2nd Step: Formation of Isocitrate

Citrate is isomerized to isocitrate by aconitase. This reaction is a two-step process. At first, one water molecule is removed from citrate forming cis aconitate; a transient compound. Immediately, one water molecule is added to aconitate to form isocitrate.

3rd Step: Formation of Alpha Keto Glutarate

i. This reaction is a two-step process, both catalyzed by the same enzyme, isocitrate dehydrogenase. In the first part of the reaction, isocitrate is dehyro-genated to form oxalo succinate. It is an unstable compound which undergoes spontaneous decarboxylation to form alpha ketoglutarate.

ii. The NADH generated in this step is later oxidized in electron transport chain (ETC) to generate 3 ATPs.

iii. Isocitrate (6 carbons) undergoes oxidative decarboxylation to form alpha keto glutarate (5 carbons). In this reaction, one molecule of CO 2 is liberated.

4th Step: Formation of Succinyl CoA

i. Next, alphaketo glutarate is oxidatively decarboxylated to form succinyl CoA by the enzyme alpha keto glutarate dehydrogenase (Step 4, Fig. 14.2).

ii. The NADH thus generated enters into ETC to generate 3 ATPs.
iii. Another molecule of CO 2 is removed in this step.
iv. This is the only irreversible step in the whole reaction cycle.
v. The enzyme alpha keto glutarate dehydrogenase is a multi-enzyme complex having 3 enzyme proteins and 5 co-enzymes. This is similar to the pyruvate dehydrogenase reaction.

5th Step: Generation of Succinate

The next reaction involves a substrate level phosphorylation whereby a high energy phosphate is generated from the energy trapped in the thio-ester bond of succinyl CoA. The enzyme is succinate thiokinase. A molecule of GDP is phosphorylated to GTP and succinate is formed. The GTP can be converted to ATP by reacting with an ADP molecule:

$GTP + ADP \rightarrow GDP + ATP$

6th Step: Formation of Fumarate:

Succinate is dehydrogenated to fumarate, an unsaturated dicarboxylic acid, by succinate dehydrogenase. The hydrogen
atoms are accepted by FAD. The FADH 2 then enters into ETC to generate 2 ATPs. The succinate dehydrogenase is competitively inhibited by malonate.

7th Step: Formation of Malate

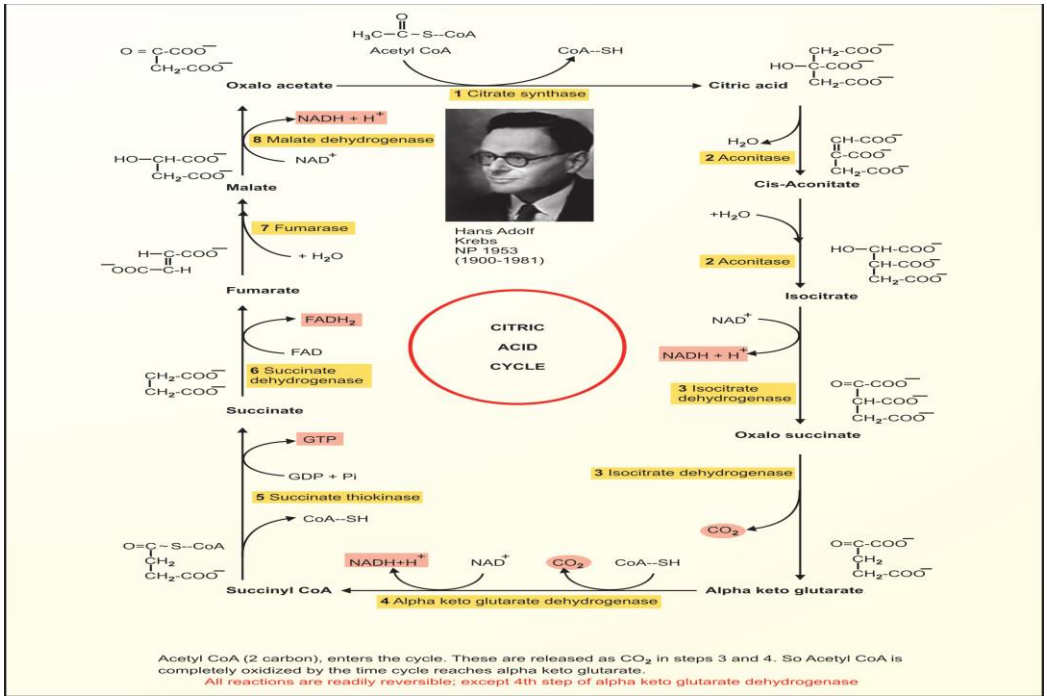
The formation of malate from fumarate is catalysed by fumarase.

8th Step: Regeneration of Oxaloacetate

Finally malate is oxidized to oxaloacetate by malate dehydrogenase (Step 8, The co-enzyme is NAD + . The NADH is generated in this step, which enters the electron transport chain, when 3 ATPs are produced. The oxaloacetate can further condense with another acetyl CoA molecule and the cycle continues. Oxalo acetate may be viewed as a catalyst, which enters into the reaction, causes complete oxidation of acetyl CoA and comes out of it without any change.

Regulation of the Citric Acid Cycle:

1. Citrate: The formation of citrate from oxalo acetate and acetyl CoA is an important part of control. ATP acts as an allosteric inhibitor of citrate synthase. Citrate allosterically inhibits PFK, the key enzyme of glycolysis.
2. Availability and cellular need of ATP: When the energy charge of the cell is low, as indicated by high level of NAD + and FAD, the cycle operates at a faster rate. The cycle is tightly coupled to the respiratory chain providing
ATP. The Krebs cycle is the largest generator of ATP among metabolic pathways.



The tricarboxylic cycle (TCA) with all enzymes and co- factors

ENERGY PRODUCED BY THE TCA CYCLE: Two carbon atoms enter the cycle as acetyl CoA and leave as CO2. The cycle does not involve net consumption or production of oxaloacetate or of any other intermediate. Four pairs of electrons are transferred during one turn of the cycle: three pairs of electrons reducing three NAD+ to NADH and one pair reducing FAD to FADH2. Oxidation of one NADH by the electron transport chain leads to formation of approximately three ATP, whereas oxidation of FADH2 yields approximately two ATP. The total yield of ATP from the oxidation of one acetyl CoA is?

3 NADH 3 NAD^+ → 2 FADH2 FAD → 1 GDP + Pi GTP Energy producing reaction Number of ATP produced 12 ATP/acetyl CoA oxidized

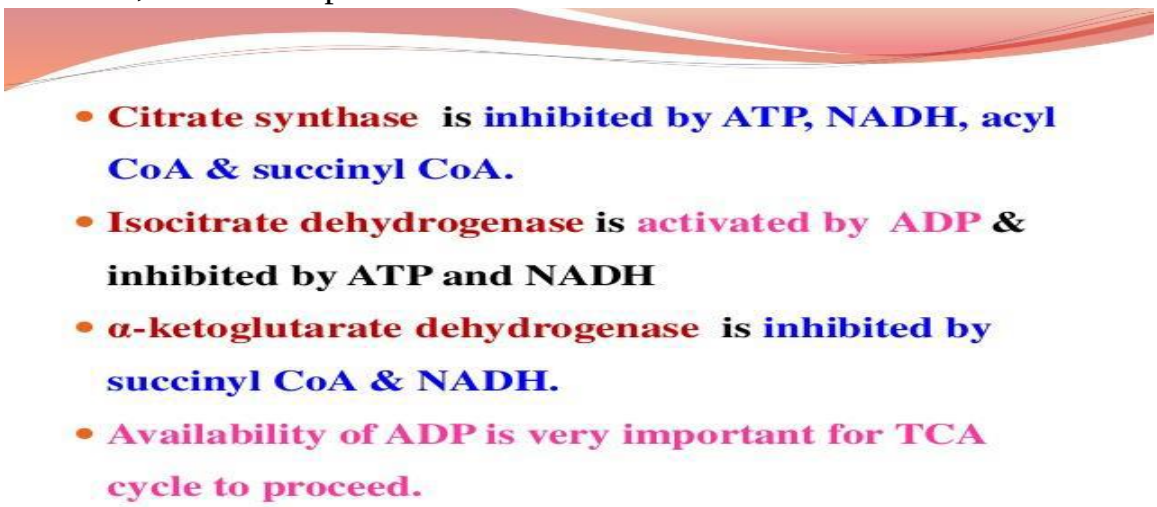
Inhibitors of TCA Cycle

The above-said mechanisms are physiological and regulatory in nature. But the following are toxic or poisonous (non-physiological) agents which inhibit the reactions.

A. Aconitase (citrate to aconitate) is inhibited by fluoro-acetate. This is non-competitive inhibition.

B. Alpha keto glutarate dehydrogenase is inhibited by Arsenite (non-competitive inhibition).

C. Succinate dehydrogenase (succinate to fumarate) is inhibited by malonate; this is competitive inhibition.



In the TCA cycle, oxaloacetate is first condensed with an acetyl group from acetyl coenzyme A (CoA), and then is regenerated as the cycle is completed. Thus, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates. [Note: Two carbons entering the cycle as acetyl CoA are balanced by two CO_2 exiting.]

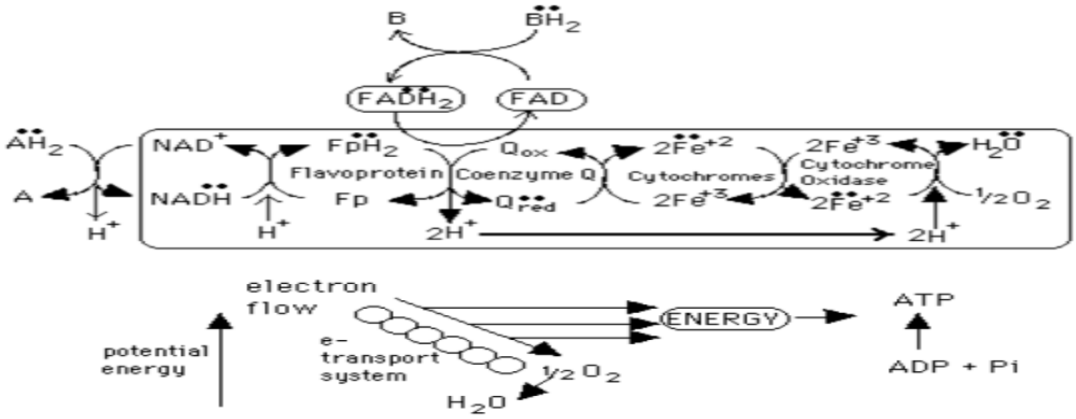
A. Oxidative decarboxylation of pyruvate

Pyruvate, the endproduct of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle. This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane. Once in the matrix, pyruvate is converted to acetyl CoA by the pyruvate dehydrogenase complex, which is a multienzyme complex. Strictly speaking, the pyruvate dehydrogenase complex is not part of the TCA cycle proper, but is a major source of acetyl CoA—the two-carbon substrate for the cycle.

Oxidative Phosphorylation & The Electron Transport Chain:

• OXIDATIVE PHOSPHORYLATION is the indirect way of generating ATP from ADP and Pi using the energy released from REDOX REACTIONS on the ELECTRON TRANSPORT (RESPIRATORY) CHAIN.

• The ELECTRON TRANSPORT CHAIN is a series of compounds on the INNER MITOCHONDRIAL MEMBRANE which transfers H_2 or electrons from one compound to another in a series of REDOX REACTIONS.



The Oxidative Phosphorylation & the Electron Transport Chain

Gluconeogenesis:

OVERVIEW Some tissues, such as the brain, red blood cells, kidney medulla, lens and cornea of the eye, testes, and exercising muscle, require a continuous supply of glucose as a metabolic fuel. Liver glycogen, an essential postprandial source of glucose, can meet these needs for only 10–18 hours in the absence of dietary intake of carbohydrate. During a prolonged fast, however, hepatic glycogen stores are depleted, and glucose is formed from precursors such as lactate, pyruvate, glycerol (derived from the backbone of triacylglycerols), and α-ketoacids (derived from the catabolism of glucogenic amino acids). The formation of glucose does not occur by a simple reversal of glycolysis, because the overall equilibrium of glycolysis strongly favors pyruvate formation. Instead, glucose is synthesized by a special pathway, gluconeogenesis, that requires both mitochondrial and cytosolic enzymes. During an overnight fast, approximately 90% of gluconeogenesis occurs in the liver, with the kidneys providing 10% of the newly synthesized glucose molecules. However, during prolonged fasting, the kidneys become major glucose-producing organs, contributing an estimated 40% of the total glucose production. This process is called gluconeogenesis and converts lactic acid, glycerol & glucogenic amino acids to glucose. - During exercise it converts LACTIC ACID back to glucose. which is sent back to the muscles to reenter glycolysis

A. Carboxylation of pyruvate The first "roadblock" to overcome in the synthesis of glucose from pyruvate is the irreversible conversion in glycolysis of PEP to pyruvate by pyruvate kinase. In gluconeogenesis, pyruvate is first carboxylated by pyruvate carboxylase to OAA, which is then converted to PEP by the action of PEP-carboxykinase

B. Transport of oxaloacetate to the cytosol

OAA must be converted to PEP for gluconeogenesis to continue. The enzyme that catalyzes this conversion is found in both the mitochondria and the cytosol in humans. The PEP that is generated in the mitochondria is transported to the cytosol by a specific transporter, whereas that generated in the cytosol requires the transport of OAA from the mitochondria to the cytosol. However, OAA is unable to directly cross the inner mitochondrial membrane; it must first be reduced to malate by mitochondrial malate dehydrogenase. Malate can be transported from the mitochondria to the cytosol, where it is reoxidized to oxaloacetate by cytosolic malate dehydrogenase as NAD+ is reduced.

C. Decarboxylation of cytosolic oxaloacetate

Oxaloacetate is decarboxylated and phosphorylated to PEP in the cytosol by PEP-carboxykinase (also referred to as PEPCK). The reaction is driven by hydrolysis of guanosine triphosphate . The combined actions of pyruvate carboxylase and PEPcarboxykinase provide an energetically favorable pathway from pyruvate to PEP. Then, PEP is acted on by the reactions of glycolysis running in the reverse direction until it becomes fructose 1,6-bisphosphate

D. Dephosphorylation of fructose 1,6-bisphosphate

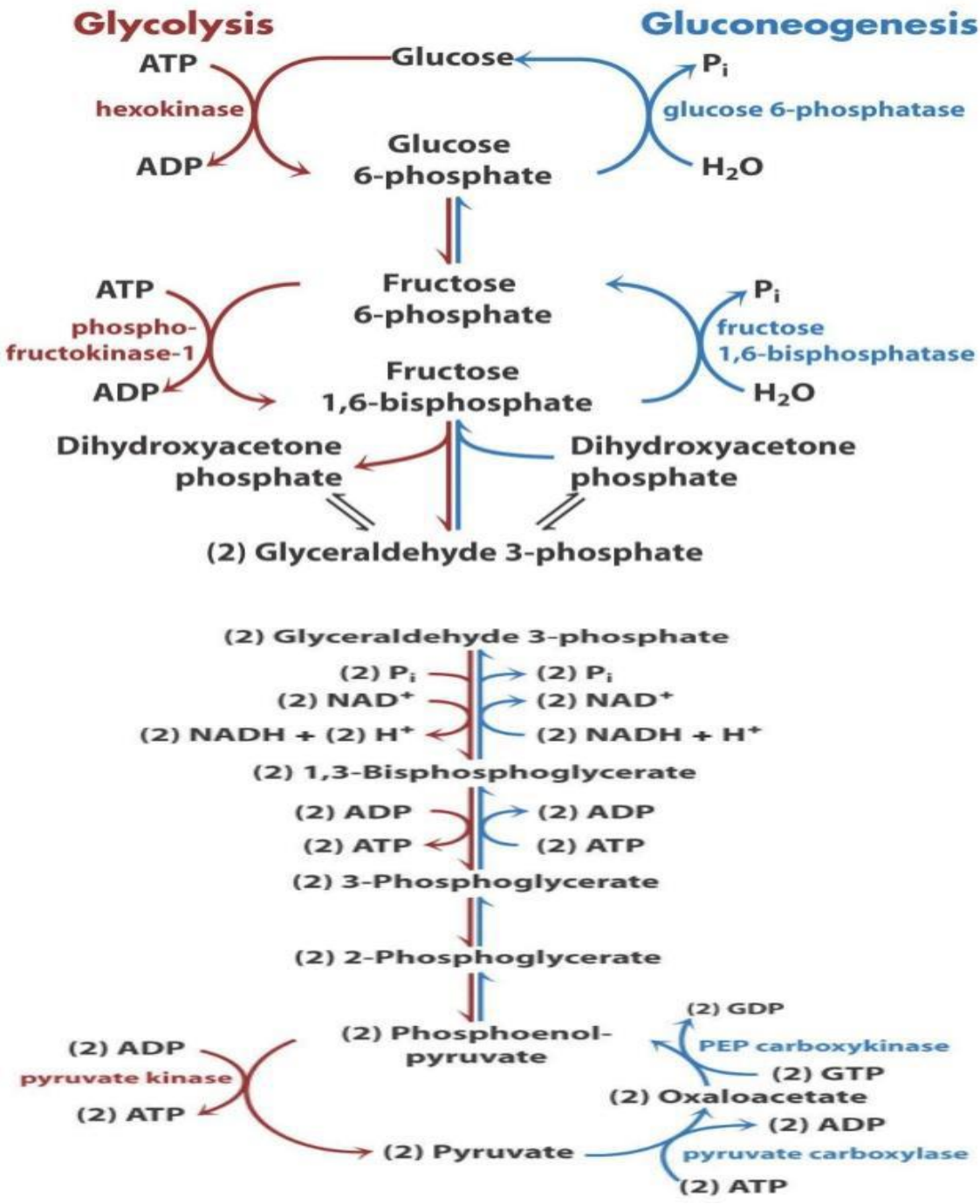
Hydrolysis of fructose 1,6-bisphosphate by fructose 1,6-bisphosphatase bypasses the irreversible phosphofructokinase-1 reaction, and provides an energetically favorable pathway for the formation of fructose 6-phosphate . This reaction is an important regulatory site of gluconeogenesis

E. Dephosphorylation of glucose 6-phosphate

Hydrolysis of glucose 6-phosphate by glucose 6-phosphatase by passes the irreversible hexokinase reaction, and provides an energetically favorable pathway for the formation of free glucose

F. Summary of the reactions of glycolysis and gluconeogenesis

Of the 11 reactions required to convert pyruvate to free glucose, seven are catalyzed by reversible glycolytic enzymes . The irreversible reactions of glycolysis catalyzed by hexokinase, phosphofructokinase-1, and pyruvate kinase are circumvented by glucose 6-phosphatase, fructose 1,6-bisphosphatase, and pyruvate carboxylase/PEP-carboxykinase. In gluconeogenesis, the equilibria of the seven reversible reactions of glycolysis are pushed in favor of glucose synthesis as a result of the essentially irreversible formation of PEP, fructose 6-phosphate, and glucose catalyzed by the gluconeogenic enzymes. [Note: The stoichiometry of gluconeogenesis from pyruvate couples the cleavage of six high-energy phosphate bonds and the oxidation of two NADH with the formation of each molecule of glucose.



Gluconeogenis pathway

The liver:

The liver is the most important single organ in ensuring a constant energy supply for other tissues, including the brain, under a wide variety of conditions. The hepatic cells are in a key position to buffer the hyperglycemic effect of a high carbohydrate meal. Liver can adapted different roles like:

- **Converted glucose to glycogen (glycogenesis)**
- **Converted glucose to fatty acid, which are ultimately stored as triglyceride in adipose tissue.**
- **During fasting, liver can converted fatty acid to glucose when there is short supply.**
- **Break down of glycogen to glucose (glycogenolysis).**

in muscles :

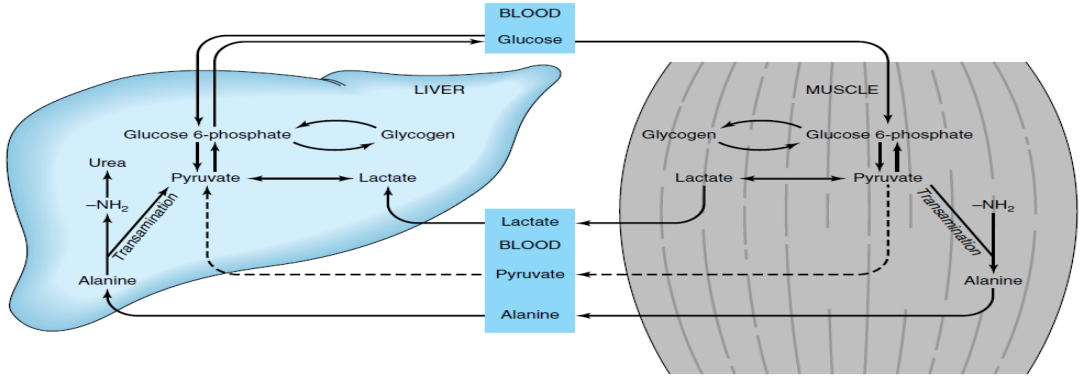
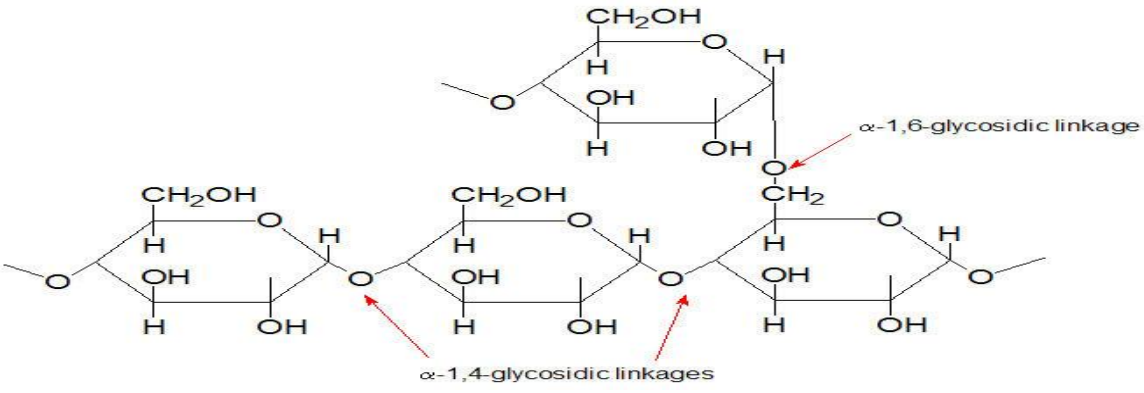


Figure 19–4. The lactic acid (Cori) cycle and glucose-alanine cycle.

GLYCOGEN

The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use. The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.

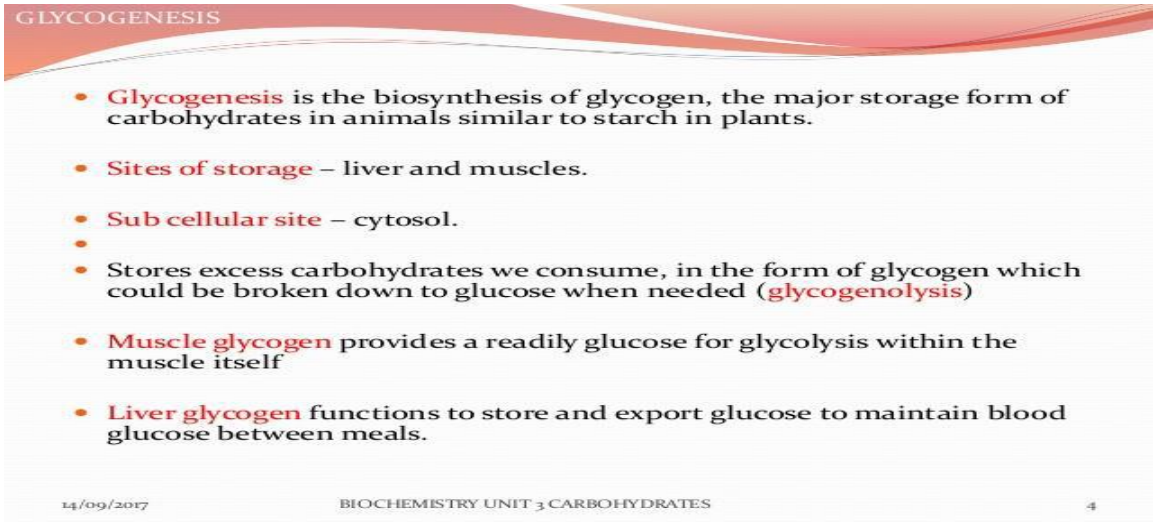
A. Amounts of liver and muscle glycogen. Approximately 400 g of glycogen make up 1–2% of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to 10% of the fresh weight of a well-fed adult liver.



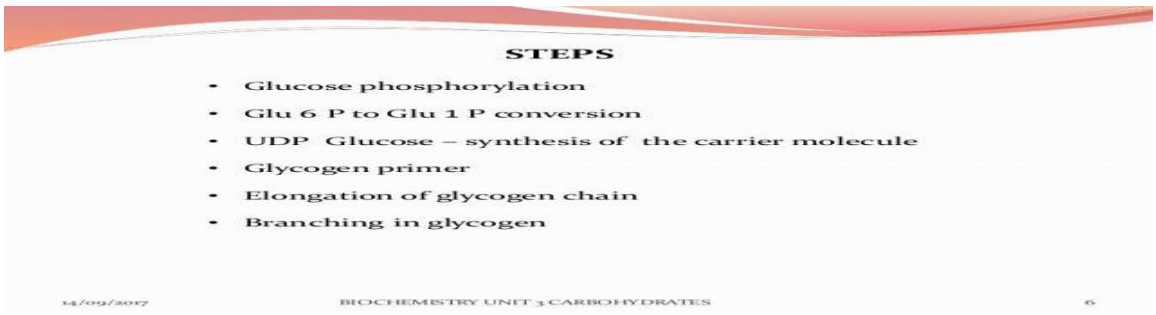
Glycogen structuer

b. Structure of glycogen

Glycogen is a branched-chain polysaccharide made exclusively fromα-D-glucose. The primary glycosidic bond is an α (1→4) linkage. After an average of eight to ten glucosyl residues, there is a branch containing an α (1→6) linkage. A single molecule of glycogen can have a molecular mass of up to 108 daltons. These molecules exist in discrete cytoplasmic granules that also contain most of the enzymes necessary for glycogen synthesis and degradation.



SYNTHESIS OF GLYCOGEN (GLYCOGENESIS):



Functions of Glycogen

1. Glycogen is the storage form of carbohydrates in the human body.
2. The major sites of storage are liver and muscle. The major function of liver glycogen is to provide glucose during starvation.
3. When blood glucose level lowers, liver glycogen is broken down and helps to maintain blood glucose level.

4. The function of muscle glycogen is to act as reserve fuel for muscle contraction.
5. After taking food, blood sugar tends to rise, which causes glycogen deposition in liver. About 5 hours after taking food, the blood sugar tends to fall. But, glycogen is lysed to glucose so that he energy needs are met.A
6. after about 18 hrs fasting, most of the liver glycogen is depleted, when depot fats are hydrolysed and energy requirement is met by fatty acid oxidation.

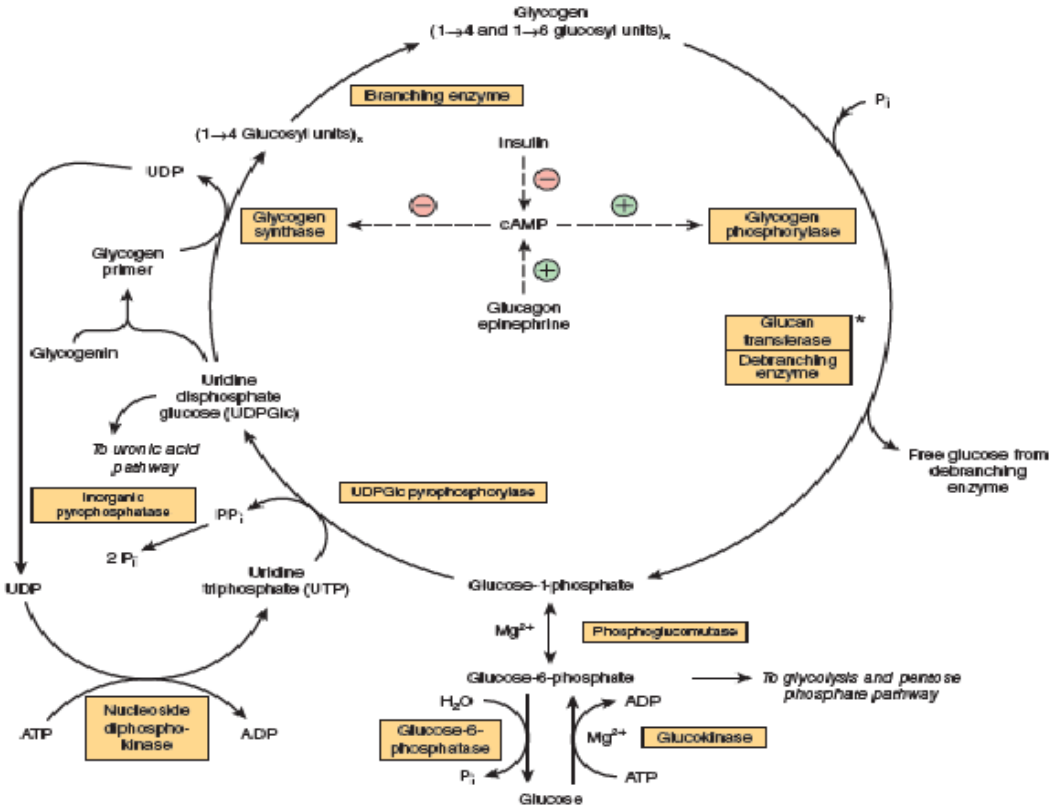


FIGURE '. **Pathways of glycogenesis and glycogenolysis in the liver.** (⊕, Stimulation; ⊖, inhibition.) Insulin decreases the level of cAMP only after it has been raised by glucagon or epinephrine; that is, it antagonizes their action. Glucagon is active in heart muscle but not in skeletal muscle. *Glucan transferase and debranching enzyme appear to be two separate activities of the same enzyme.

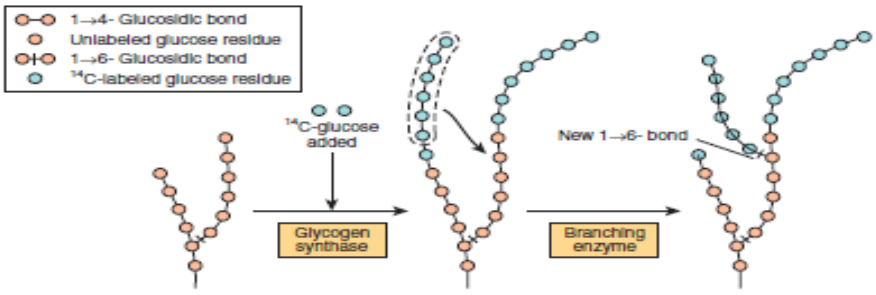
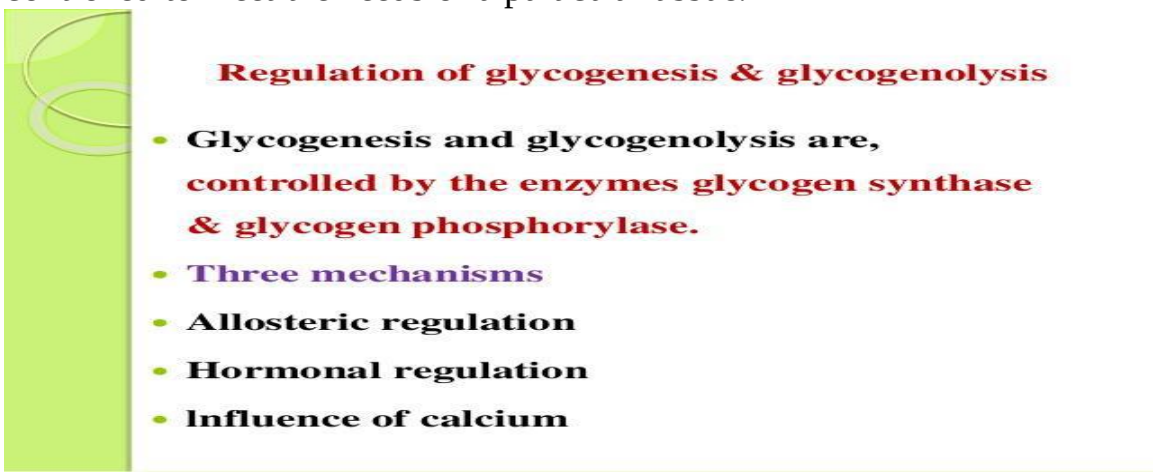


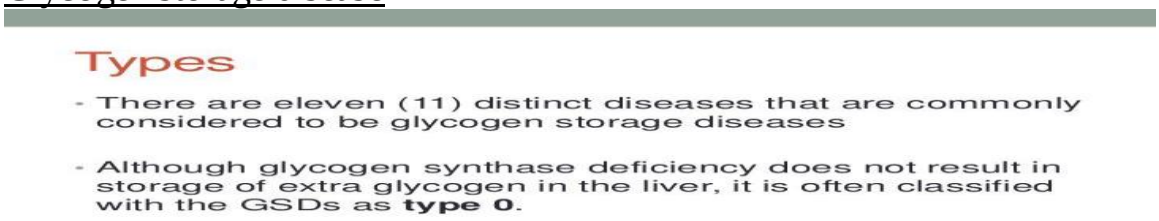
FIGURE 18–3 **The biosynthesis of glycogen.** The mechanism of branching as revealed by feeding ^{14}C-labeled glucose and examining liver glycogen at intervals.

REGULATION OF GLYCOGEN SYNTHESIS AND DEGRADATION

Because of the importance of maintaining blood glucose levels, the synthesis and degradation of its glycogen storage form are tightly regulated. In the liver, glycogenesis accelerates during periods when the body has been well fed, whereas glycogenolysis accelerates during periods of fasting. In skeletal muscle, glycogenolysis occurs during active exercise, and glycogenesis begins as soon as the muscle is again at rest. Regulation of glycogen synthesis and degradation is accomplished on two levels. First, glycogen synthase and glycogen phosphorylase are hormonally regulated to meet the needs of the body as a whole. Second, the pathways of glycogen synthesis and degradation are allosterically controlled to meet the needs of a particular tissue.



Glycogen storage disease



Type I, Von Gierke's disease

Affected enzyme: glucose-6-phosphatase

Affected tissue: Liver and kidney

Clinical features:

- Large quantities of glycogen are formed and stored in hepatocytes, renal and intestinal mucosa cells. The liver and kidneys become enlarged.

Type II, Pompe's disease

Cause:

- The deficiency of the lysosomal enzyme alpha-1,4-glucosidase (acid maltase) leads to the accumulation of glycogen in many tissues.

Clinical feature:

- The clinical spectrum is continuous and broad, with presentation in infants, children and adults.
- In the infantile form, accumulation of glycogen in cardiac muscle leads to cardiac failure.

- Accumulation may also occur in the liver, which results in hepatomegaly and elevation of hepatic enzymes.
- Glycogen accumulation in muscle and peripheral nerves causes hypotonia and weakness.
- Glycogen deposition in blood vessels may result in intracranial aneurysms.

Type III, Cori disease

- **Affected enzyme:** Glycogen debranching enzyme. Deposition of abnormal glycogen structure.
- **Affected tissues:** Liver and muscle.
- **Clinical features:**
  - About 15% affect liver only. Hypoglycaemia, poor growth, hepatomegaly, moderate progressive myopathy.
  - Symptoms can regress with age.
  - A few cases of liver cirrhosis and hepatocellular carcinoma have been reported.

Type IV, Andersen's disease, Amylopectinosis

- **Affected enzyme:** Glycogen branching enzyme. Abnormally structured glycogen forms.
- **Affected tissues:** Many, including liver. Rare variant affects peripheral nerves.
- **Clinical features:**
  - Hepatomegaly, failure to thrive, cirrhosis, splenomegaly, jaundice, hypotonia, waddling gait, lumbar lordosis.

Type 0, Lewis disease

- **Affected enzyme:** Hepatic glycogen synthase.
- **Affected tissues:** Liver.
- **Clinical features**
  - Seizures can occur.
  - Fatigue and muscle cramps after exertion.
  - Mild growth retardation in some cases.