Al-Mustaqbal University

جامعة المستقبل
AL MUSTAQBAL UNIVERSITY

College of Medical and Health Techniques

Medical Laboratories Techniques Departments

Biochemistry Lectures for 2nd Year Students

First Semester

(2 Credit Hrs. Theory + 4 Credit Hrs. Practice / Week = 4 Credit Unit

Academic Year: 2024 - 2025

Course Organizers:

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Lecture No. 10

Date: Dec., 15th , 2024

Carbohydrate Metabolism – Citric Acid Cycle

Objectives:

The reader will be able to answer questions on the following topics:

1. Citric acid cycle
2. Significance of citric acid cycle
3. Regulation of citric acid cycle

Functions of the Citric Acid Cycle

1. The final common oxidative pathway that oxidizes acetyl-CoA to CO_2.
2. The source of reduced co-enzymes that provide the substrate for the respiratory chain.
3. The link between catabolic and anabolic pathways (amphibolic role).
4. Provides precursors for synthesis of amino acids and nucleotides.
5. Components of the cycle have a direct or indirect controlling effect 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 are shown in **(Fig. 1).** Pyruvate derived from glycolysis is oxidatively decarboxylated to acetyl-CoA by the pyruvate dehydrogenase as mentioned previously.

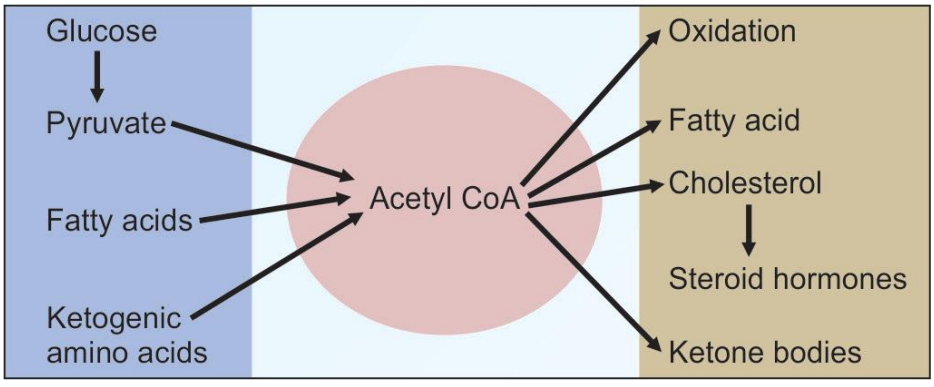

Fig.1: Sources and utilization of acetyl-CoA

This is the link between the TCA cycle and glycolysis. The pyruvate dehydrogenase reaction occurs in the mitochondria. Pyruvate with the help of a carrier can enter the mitochondria from the cytoplasm. The acetyl-CoA derived from beta oxidation of fatty acids is formed in the mitochondria itself. All the enzymes of citric acid cycle are located inside the mitochondria. Cellular respiration occurs in three major stages (Fig. 2).

1. In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl group of acetyl-coenzyme A (acetyl-CoA).
2. In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH + H^+ and FADH2.
3. In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons. The electrons are transferred across various complexes to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.

Citric acid cycle, also called the tricarboxylic acid (TCA) cycle or the Krebs cycle. In aerobic organisms, the final pathway where the oxidative catabolism of glucose and other monosaccharides, most amino acids, and fatty acids converge their carbon skeletons being converted to carbon dioxide (CO_2). Before entering the citric acid cycle, the carbon skeletons of sugars and fatty acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input. Many amino acid carbons also enter the cycle this way, although several amino acids are degraded to other cycle intermediates. The pyruvate dehydrogenase complex is the prototype for two other important enzyme complexes: α-ketoglutarate dehydrogenase, of the citric acid cycle, and the branched-chain α-keto acid dehydrogenase, of the oxidative pathways of several amino acids. The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation, an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA.

This oxidation provides energy for the production of the majority of ATP in most animals, including humans. Because the TCA cycle occurs totally in mitochondria, it is in close proximity to the respiratory chain or electron transport chain (ETC), which oxidizes the reduced coenzymes nicotinamide adenine dinucleotide (NADH + H^+) and flavin adenine dinucleotide (FADH2) produced by the cycle. The TCA cycle is an aerobic pathway, because oxygen (O_2) is required as the final electron acceptor. The TCA cycle also provides intermediates for a number of important anabolic reactions, such as glucose formation from the carbon skeletons of some amino acids and the synthesis of some amino acids and heme. Therefore, this cycle should not be viewed as a closed system but, instead, as an open one with compounds entering and leaving as required, see Figure 2 and 3.

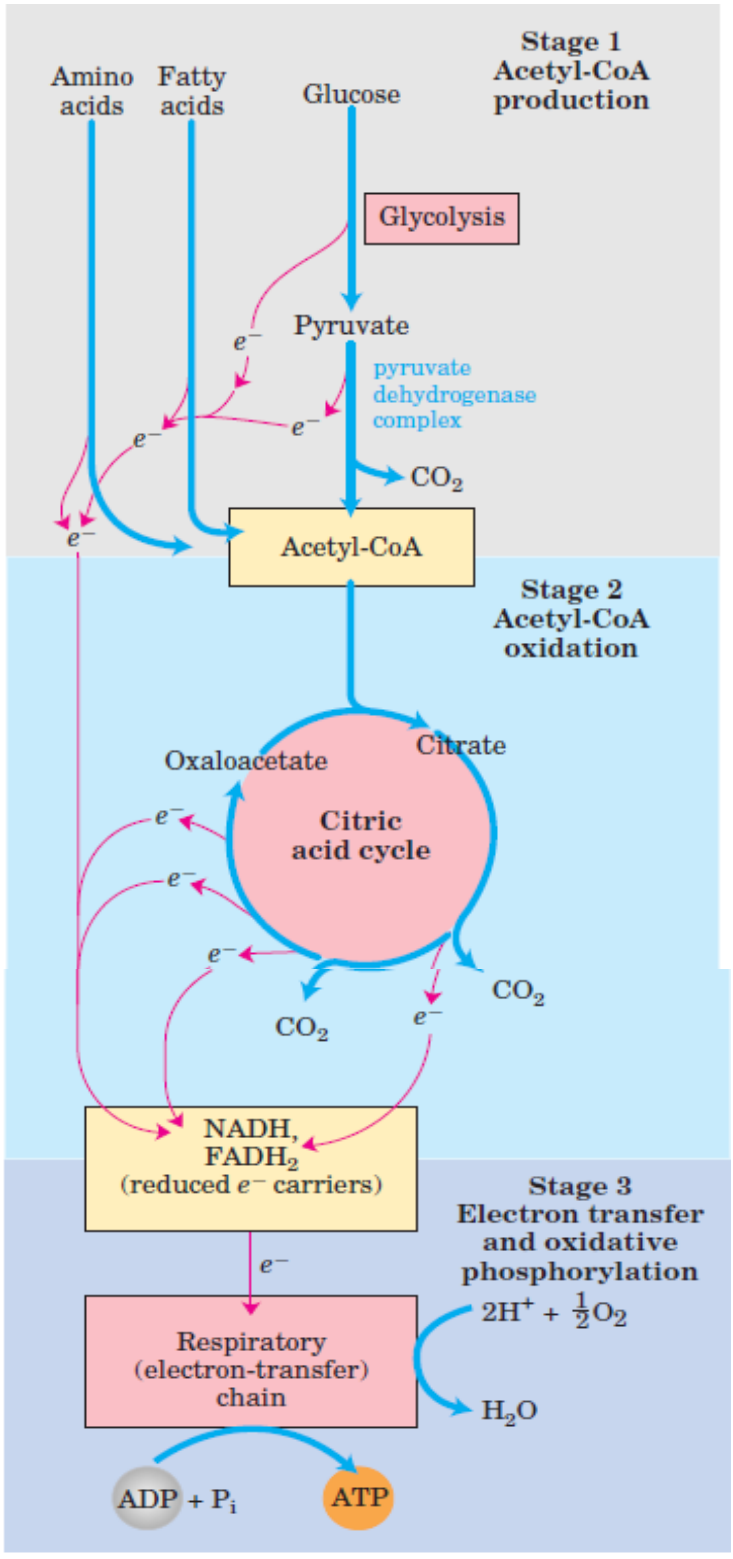

Fig.2. Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration.

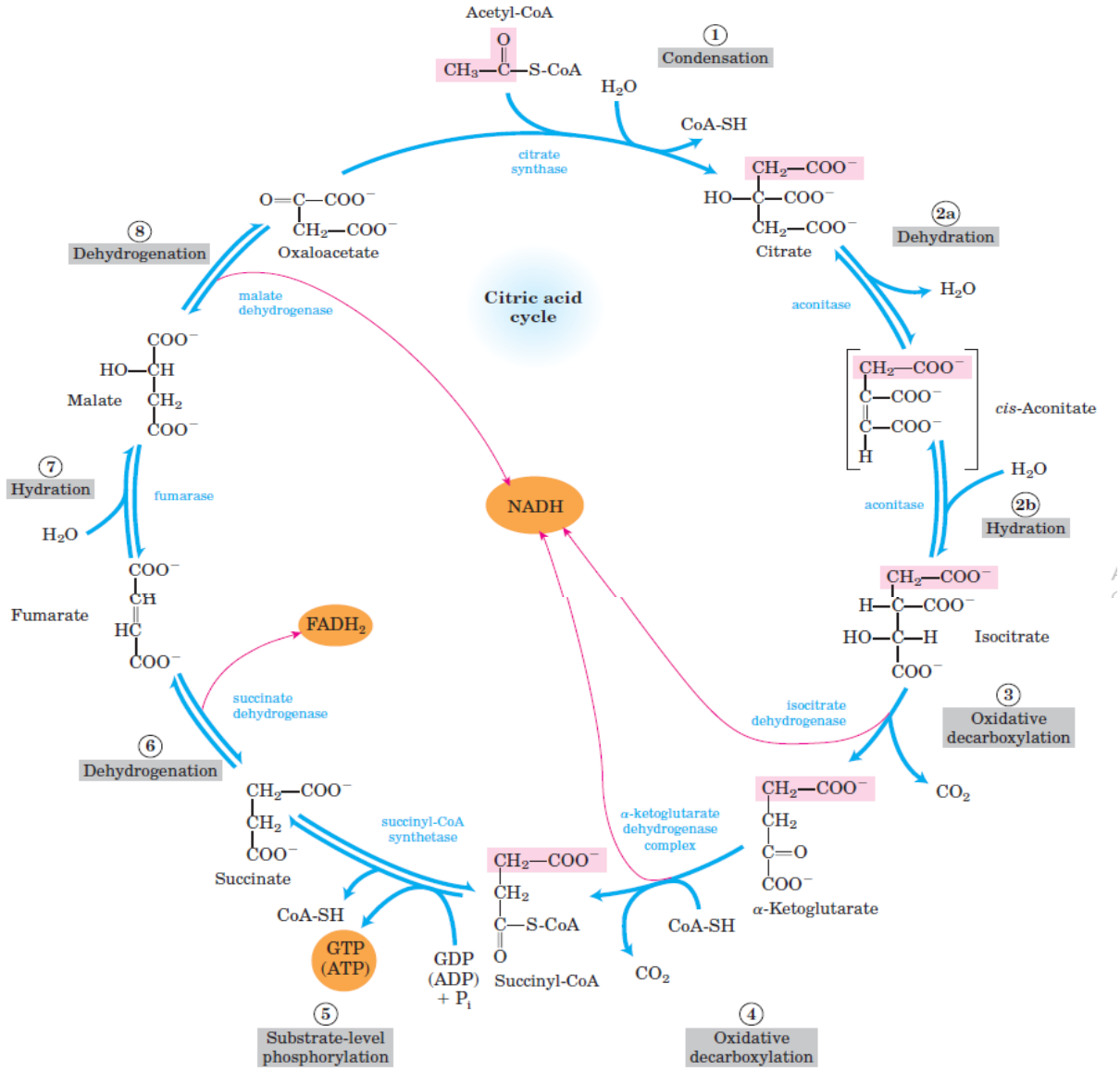

Fig. 3. Reactions of the citric acid cycle.

The enzyme α-ketoglutarate dehydrogenase (step 4 Fig. 3) is a multienzyme complex having 3 enzyme proteins and 5 co-enzymes. This is similar to the pyruvate dehydrogenase reaction. The first two enzyme activities are similar to the corresponding components of pyruvate dehydrogenase complex and the 3rd enzyme is the same in both complexes. The reactions of citric acid cycle is summarized in (Fig. 3).

Reaction (step 5 Fig. 3) involves a substrate level phosphorylation whereby a high energy phosphate is generated from the energy trapped in the thioester 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 ---------► GDP + ATP

Oxaloacetate as a Junction Point

Oxaloacetate 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. Oxaloacetate is an important junction point in metabolisms. Significance of citric acid cycle is shown in Box 1.

Box 1. Significance of TCA cycle

1. Complete oxidation of acetyl-CoA.
2. ATP generation
3. Final common oxidative pathway
4. Integration of major metabolic pathways
5. Fat is burned on the wick of carbohydrates
6. Excess carbohydrates are converted as neutral fat
7. No net synthesis of carbohydrates from fat
8. Carbon skeletons of amino acids finally enter the citric acid cycle

Figure 4 indicate a summary of citric acid cycle with their regulatory steps and the enzymes are numbered. The two carbons of acetate are removed or eliminated in reactions number 3 and 4 are carbon dioxide. Physiological regulatory steps are: Step No.1(citrate synthase) is physiologically inhibited by ATP. Step No.3 (ICDH) is inhibited by NADH and activated by ADP. Steps where energy is trapped are marked with the co-enzyme and the number of ATP generated during that reaction. Recent work shows that in the electron transport chain, NADH may produce only 2 ½ ATPs and FADH only 1 ½ ATPs. Acetyl-CoA contains 2 carbon atoms. Net result is that acetyl-CoA is completely oxidized during one turn of cycle.

ATP Generating Steps in TCA Cycle

There are 3 NADH molecules generated during one cycle, each of them will give rise to 2 ½ ATPs on oxidation by electron transport chain (ETC); so altogether they will give 3 × 2 ½ = 7 ½ (7.5) high energy phosphates as ATP. The FADH2 will generate 1½ 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 as ATP are produced. These steps are marked in (Fig. 4) and in Table 1.

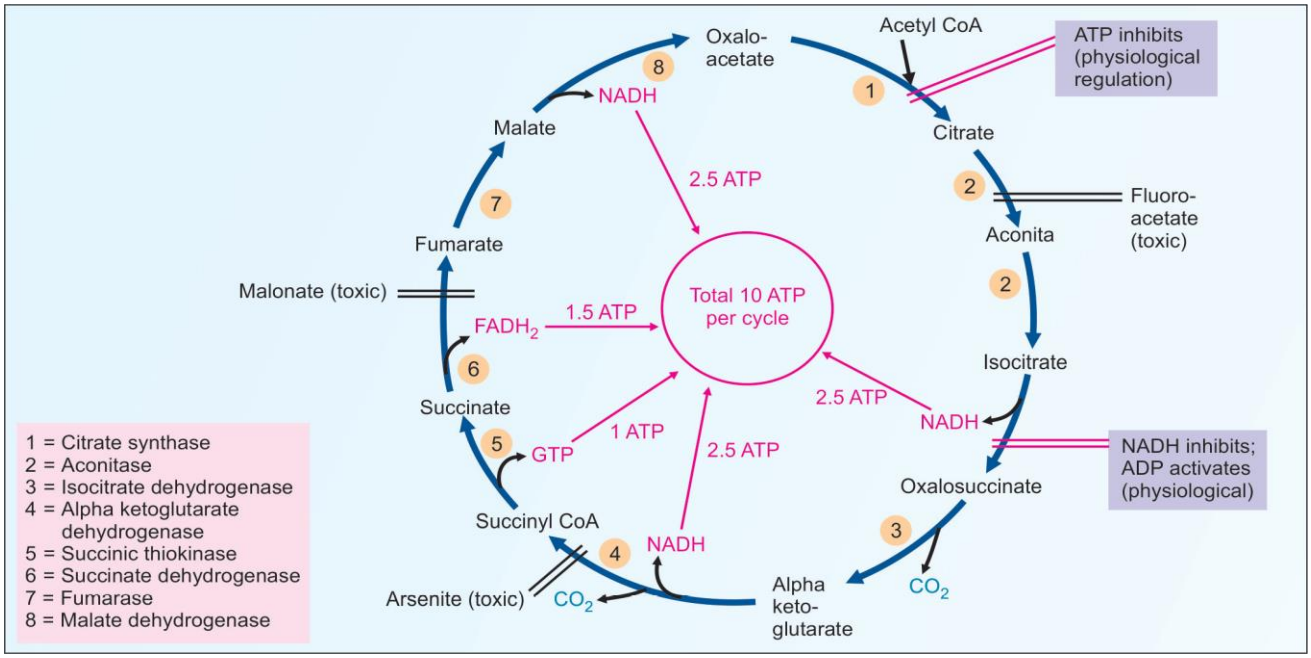

Fig. 4. Summary of Krebs citric acid cycle. Enzymes are numbered.

Table 1. ATP generation steps

Step No	*Reactions*	*Co-enzyme*	*ATPs (old-calculation)*	*ATPs (new calculation)*
3	Isocitrate → alpha keto glutarate	NADH	3	2.5
4	Alpha keto glutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA→Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxalo acetate	NADH	3	2.5
		Total	12	10

Alpha ketoglutarate dehydrogenase reaction is the only one irreversible step in the cycle. The free energy changes of the reactions of the cycle are such that the cycle will operate spontaneously in the clockwise direction. Only about 33% of energy liberated is trapped as ATP. The rest is used to keep the body temperature at a higher level than the environment.

Figure 5 indicate the communication of citric acid cycle with other metabolic pathways. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways. Shown in red are four anaplerotic reactions that replenish depleted cycle intermediates.

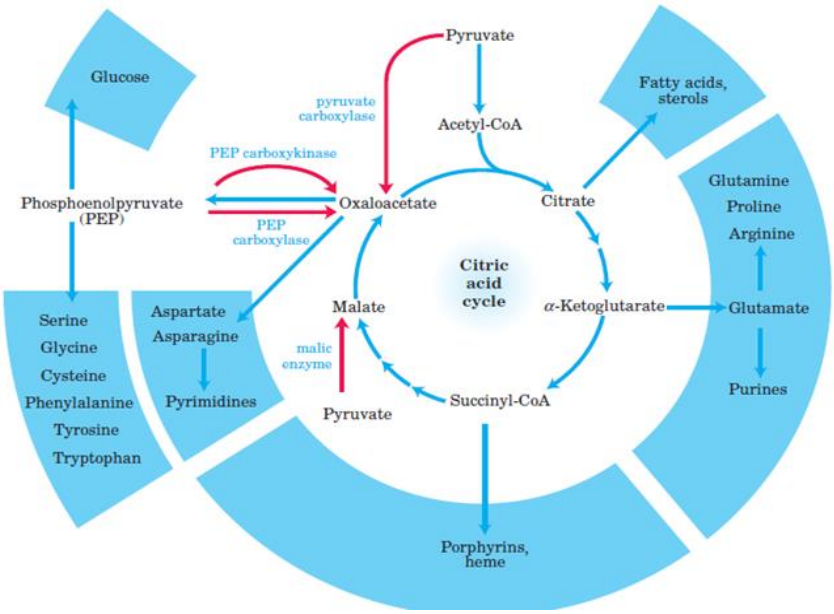

Fig. 5. Role of the citric acid cycle in anabolism.

Integration of Major Metabolic Pathways

1. Carbohydrates are metabolized through glycolytic pathway to pyruvate, then converted to acetyl-CoA, which enters the citric acid cycle.
2. Fatty acids through beta oxidation, are broken down to acetyl-CoA and then enters this cycle.
3. Glucogenic amino acids after transamination enter at some or other points in this cycle (Fig. 6). Ketogenic amino acids are converted into acetyl-CoA.
4. The integration of metabolism is achieved at junction points by key metabolites (Fig. 6). Several pathways can converge at this point with the result that carbon atoms from one source can be used for synthesis of another. Important intermediates are pyruvate, acetyl-CoA and oxaloacetate.

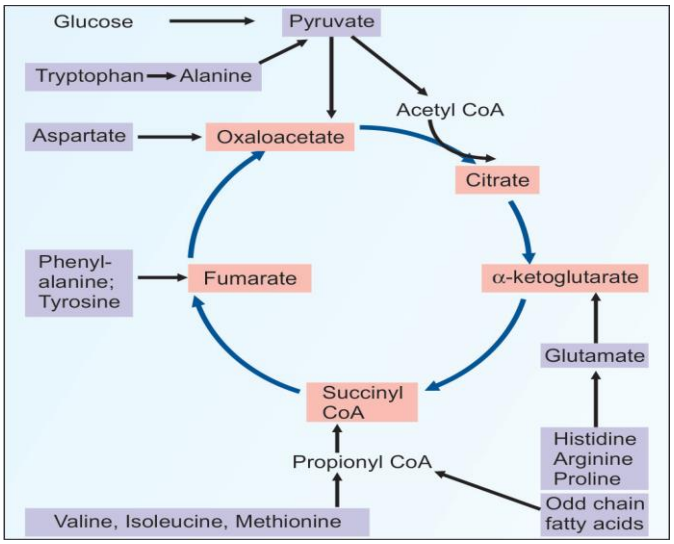

Fig. 6. Influx of TCA cycle intermediates

Excess Carbohydrates are Converted as Neutral Fat

Excess calories are deposited as fat in adipose tissue. The pathway is glucose to pyruvate to acetyl-CoA to fatty acid. However, fat cannot be converted to glucose because pyruvate dehydrogenase reaction (pyruvate to acetyl-CoA) is an absolutely irreversible step. Acetyl-CoA entering in the cycle is completely oxidized to CO2 by the time the cycle reaches succinyl-CoA. So, acetyl-CoA is completely broken down in the cycle. Thus acetyl-CoA cannot be used for gluconeogenesis. Therefore, there is no net synthesis of carbohydrates from fat.

Anaplerotic Role of TCA Cycle

The citric acid cycle acts as a source of precursors of biosynthetic pathways, e.g. heme is synthesized from succinyl-CoA and aspartate from oxaloacetate. To counter-balance such losses, and to keep the concentrations of the 4-carbon units in the cell, anaplerotic reactions are essential. This is called anaplerotic role of TCA cycle. Anaplerotic reactions are “filling up” reactions or “influx” reactions or “replenishing” reactions which supply 4-carbon units to the TCA cycle. The important anaplerotic reactions are:

A. Pyruvate to oxaloacetate by pyruvate carboxylase enzyme. It needs ATP.
B. Glutamate is transaminated to alpha keto glutarate; and aspartate to oxaloacetate.
C. Pyruvate can be carboxylated to malate by $NADP^+$ dependent malic enzyme.

Summary of the Regulation of Citric Acid Cycle

1. The overall rate of the citric acid cycle is controlled by the rate of conversion of pyruvate to acetyl-CoA and by the flux through citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. These fluxes are largely determined by the concentrations of substrates and products: the end products ATP and NADH are inhibitory, and the substrates NAD^+ and ADP are stimulatory.
2. The production of acetyl-CoA for the citric acid cycle by the PDH complex is inhibited allosterically by metabolites that signal a sufficiency of metabolic energy (ATP, acetyl-CoA, NADH, and fatty acids) and stimulated by metabolites that indicate a reduced energy supply (AMP, NAD^+, CoA).