

The Citric Acid Cycle: The Catabolism of Acetyl-CoA

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BIOMEDICAL IMPORTANCE

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) is a series of reactions in mitochondria that oxidize acetyl residues (as acetyl-CoA) and reduce coenzymes that upon reoxidation are linked to the formation of ATP.

The citric acid cycle is the final common pathway for the aerobic oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle. It also has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids. Many of these processes occur in most tissues, but the liver is the only tissue in which all occur to a significant extent. The repercussions are therefore profound when, for example, large numbers of hepatic cells are damaged as in acute **hepatitis** or replaced by connective tissue (as in **cirrhosis**). Very few, if any, genetic abnormalities of citric acid cycle enzymes have been reported; such abnormalities would be incompatible with life or normal development.

THE CITRIC ACID CYCLE PROVIDES SUBSTRATE FOR THE RESPIRATORY CHAIN

The cycle starts with reaction between the acetyl moiety of acetyl-CoA and the four-carbon dicarboxylic acid oxaloacetate, forming a six-carbon tricarboxylic acid, citrate. In the subsequent reactions, two molecules of CO₂ are released and oxaloacetate is regenerated (Figure 16–1). Only a small quantity of oxaloacetate is needed for the oxidation of a large quantity of acetyl-CoA; oxaloacetate may be considered to play a **catalytic role**.

The citric acid cycle is an integral part of the process by which much of the free energy liberated during the oxidation of fuels is made available. During oxidation of acetyl-CoA, coenzymes are reduced and subsequently reoxidized in the respiratory chain, linked to the formation of ATP (oxidative phosphorylation; see Figure 16–2 and also Chapter 12). This process is **aerobic**, requiring oxygen as the final oxidant of the reduced coenzymes. The enzymes of the citric acid cycle are lo-

cated in the **mitochondrial matrix**, either free or attached to the inner mitochondrial membrane, where the enzymes of the respiratory chain are also found.

REACTIONS OF THE CITRIC ACID CYCLE LIBERATE REDUCING EQUIVALENTS & CO₂ (Figure 16–3)*

The initial reaction between acetyl-CoA and oxaloacetate to form citrate is catalyzed by **citrate synthase** which forms a carbon-carbon bond between the methyl carbon of acetyl-CoA and the carbonyl carbon of oxaloacetate. The thioester bond of the resultant citryl-CoA is hydrolyzed, releasing citrate and CoASH—an exergonic reaction.

Citrate is isomerized to isocitrate by the enzyme **aconitase** (aconitate hydratase); the reaction occurs in two steps: dehydration to *cis*-aconitate, some of which remains bound to the enzyme; and rehydration to isocitrate. Although citrate is a symmetric molecule, aconitase reacts with citrate asymmetrically, so that the two carbon atoms that are lost in subsequent reactions of the cycle are not those that were added from acetyl-CoA. This asymmetric behavior is due to **channeling**—transfer of the product of citrate synthase directly onto the active site of aconitase without entering free solution. This provides integration of citric acid cycle activity and the provision of citrate in the cytosol as a source of acetyl-CoA for fatty acid synthesis. The poison **fluoroacetate** is toxic because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits aconitase, causing citrate to accumulate.

Isocitrate undergoes dehydrogenation catalyzed by **isocitrate dehydrogenase** to form, initially, oxalosuccinate, which remains enzyme-bound and undergoes decarboxylation to α -ketoglutarate. The decarboxylation

*From Circular No. 200 of the Committee of Editors of Biochemical Journals Recommendations (1975): “According to standard biochemical convention, the ending *ate* in, eg, palmitate, denotes any mixture of free acid and the ionized form(s) (according to pH) in which the cations are not specified.” The same convention is adopted in this text for all carboxylic acids.

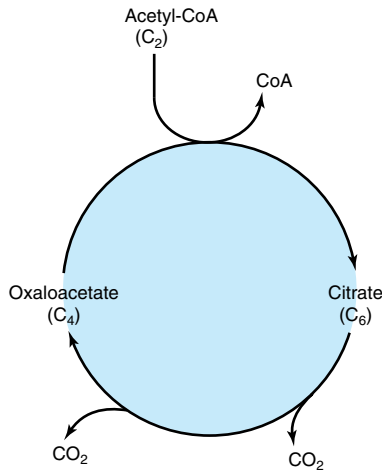


Figure 16-1. Citric acid cycle, illustrating the catalytic role of oxaloacetate.

requires Mg^{2+} or Mn^{2+} ions. There are three isoenzymes of isocitrate dehydrogenase. One, which uses NAD^+ , is found only in mitochondria. The other two use NADP^+ and are found in mitochondria and the cytosol. Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+ -dependent enzyme.

α -Ketoglutarate undergoes **oxidative decarboxylation** in a reaction catalyzed by a multi-enzyme complex similar to that involved in the oxidative decarboxylation of pyruvate (Figure 17-5). The **α -ketoglutarate dehydrogenase complex** requires the same cofactors as the pyruvate dehydrogenase complex—thiamin diphosphate, lipoate, NAD^+ , FAD, and CoA—and results in the formation of succinyl-CoA. The equilibrium of this reaction is so much in favor of succinyl-CoA formation that it must be considered physiologically unidirectional. As in the case of pyruvate oxidation (Chapter 17), arsenite inhibits the reaction, causing the substrate, **α -ketoglutarate**, to accumulate.

Succinyl-CoA is converted to succinate by the enzyme **succinate thiokinase (succinyl-CoA synthetase)**. This is the only example in the citric acid cycle of substrate-level phosphorylation. Tissues in which gluconeogenesis occurs (the liver and kidney) contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for ADP. The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis and provides a regulatory link between citric acid cycle activity and the withdrawal of oxaloacetate for gluconeogenesis. Nongluconeogenic tissues have only the isoenzyme that uses ADP.

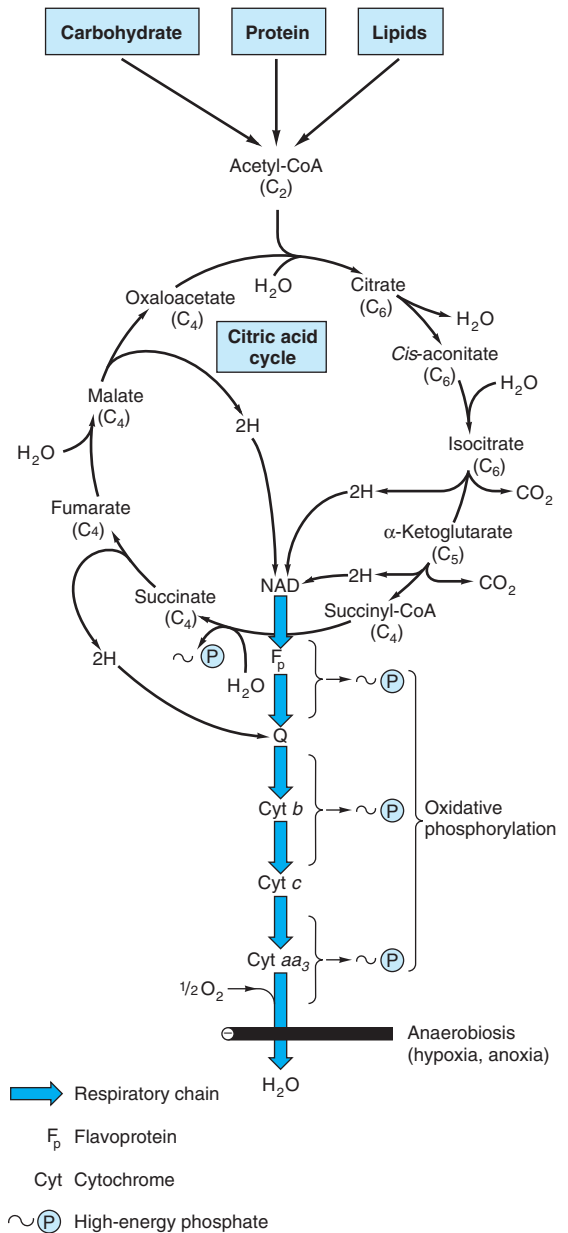


Figure 16-2. The citric acid cycle: the major catabolic pathway for acetyl-CoA in aerobic organisms. Acetyl-CoA, the product of carbohydrate, protein, and lipid catabolism, is taken into the cycle, together with H_2O , and oxidized to CO_2 with the release of reducing equivalents (2H). Subsequent oxidation of 2H in the respiratory chain leads to coupled phosphorylation of ADP to ATP. For one turn of the cycle, 11 ~P are generated via oxidative phosphorylation and one ~P arises at substrate level from the conversion of succinyl-CoA to succinate.

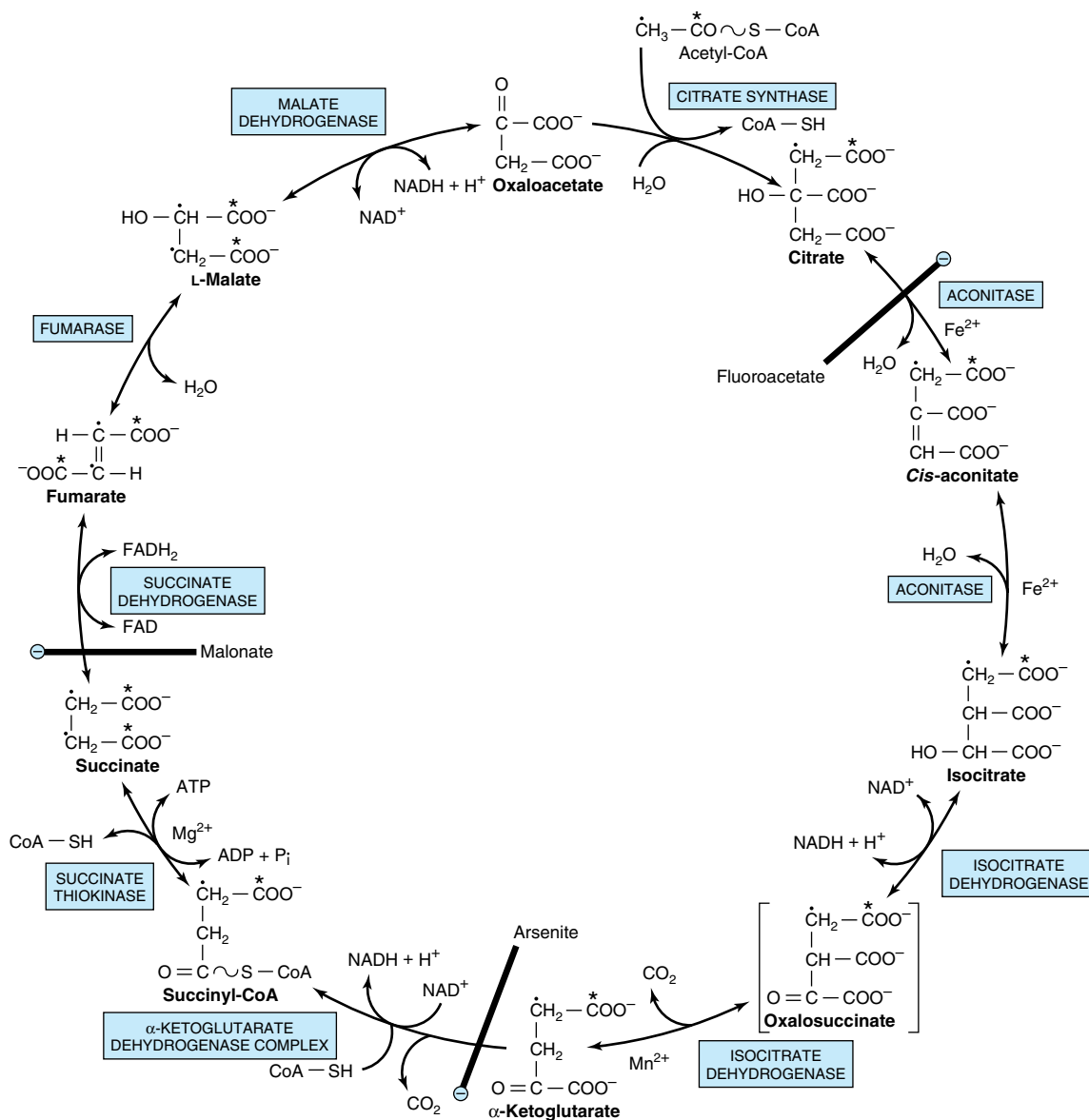


Figure 16-3. Reactions of the citric acid (Krebs) cycle. Oxidation of NADH and FADH_2 in the respiratory chain leads to the generation of ATP via oxidative phosphorylation. In order to follow the passage of acetyl-CoA through the cycle, the two carbon atoms of the acetyl radical are shown labeled on the carboxyl carbon (designated by asterisk) and on the methyl carbon (using the designation *). Although two carbon atoms are lost as CO_2 in one revolution of the cycle, these atoms are not derived from the acetyl-CoA that has immediately entered the cycle but from that portion of the citrate molecule that was derived from oxaloacetate. However, on completion of a single turn of the cycle, the oxaloacetate that is regenerated is now labeled, which leads to labeled CO_2 being evolved during the second turn of the cycle. Because succinate is a symmetric compound and because succinate dehydrogenase does not differentiate between its two carboxyl groups, "randomization" of label occurs at this step such that all four carbon atoms of oxaloacetate appear to be labeled after one turn of the cycle. During gluconeogenesis, some of the label in oxaloacetate is incorporated into glucose and glycogen (Figure 19-1). For a discussion of the stereochemical aspects of the citric acid cycle, see Greville (1968). The sites of inhibition (\ominus) by fluoroacetate, malonate, and arsenite are indicated.

When ketone bodies are being metabolized in extrahepatic tissues there is an alternative reaction catalyzed by **succinyl-CoA–acetoacetate-CoA transferase (thio-phorase)**—involving transfer of CoA from succinyl-CoA to acetoacetate, forming acetoacetyl-CoA (Chapter 22).

The onward metabolism of succinate, leading to the regeneration of oxaloacetate, is the same sequence of chemical reactions as occurs in the β -oxidation of fatty acids: dehydrogenation to form a carbon-carbon double bond, addition of water to form a hydroxyl group, and a further dehydrogenation to yield the oxo- group of oxaloacetate.

The first dehydrogenation reaction, forming fumarate, is catalyzed by **succinate dehydrogenase**, which is bound to the inner surface of the inner mitochondrial membrane. The enzyme contains FAD and iron-sulfur (Fe:S) protein and directly reduces ubiquinone in the respiratory chain. **Fumarase (fumarate hydratase)** catalyzes the addition of water across the double bond of fumarate, yielding malate. Malate is converted to oxaloacetate by **malate dehydrogenase**, a reaction requiring NAD^+ . Although the equilibrium of this reaction strongly favors malate, the net flux is toward the direction of oxaloacetate because of the continual removal of oxaloacetate (either to form citrate, as a substrate for gluconeogenesis, or to undergo transamination to aspartate) and also because of the continual reoxidation of NADH.

TWELVE ATP ARE FORMED PER TURN OF THE CITRIC ACID CYCLE

As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of FADH_2 are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle. These reducing equivalents are transferred to the respiratory chain (Figure 16–2), where reoxidation of each NADH results in formation of 3 ATP and reoxidation of FADH_2 in formation of 2 ATP. In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.

VITAMINS PLAY KEY ROLES IN THE CITRIC ACID CYCLE

Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism: (1) **riboflavin**, in the form of flavin adenine dinucleotide (FAD), a cofactor in the α -ketoglutarate dehydrogenase complex and in succinate dehydrogenase; (2) **niacin**, in the form of nicotinamide adenine dinucleotide (NAD),

the coenzyme for three dehydrogenases in the cycle—**isocitrate dehydrogenase**, **α -ketoglutarate dehydrogenase**, and **malate dehydrogenase**; (3) **thiamin (vitamin B₁)**, as thiamin diphosphate, the coenzyme for decarboxylation in the α -ketoglutarate dehydrogenase reaction; and (4) **pantothenic acid**, as part of coenzyme A, the cofactor attached to “active” carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

THE CITRIC ACID CYCLE PLAYS A PIVOTAL ROLE IN METABOLISM

The citric acid cycle is not only a pathway for oxidation of two-carbon units—it is also a major pathway for interconversion of metabolites arising from **transamination** and **deamination** of amino acids. It also provides the substrates for **amino acid synthesis** by transamination, as well as for **gluconeogenesis** and **fatty acid synthesis**. Because it functions in both oxidative and synthetic processes, it is **amphibolic** (Figure 16–4).

The Citric Acid Cycle Takes Part in Gluconeogenesis, Transamination, & Deamination

All the intermediates of the cycle are potentially gluconic, since they can give rise to oxaloacetate and thus net production of glucose (in the liver and kidney, the organs that carry out gluconeogenesis; see Chapter 19). The key enzyme that catalyzes net transfer out of the cycle into gluconeogenesis is **phosphoenolpyruvate carboxykinase**, which decarboxylates oxaloacetate to phosphoenolpyruvate, with GTP acting as the donor phosphate (Figure 16–4).

Net transfer into the cycle occurs as a result of several different reactions. Among the most important of such **anaplerotic reactions** is the formation of oxaloacetate by the carboxylation of pyruvate, catalyzed by **pyruvate carboxylase**. This reaction is important in maintaining an adequate concentration of oxaloacetate for the condensation reaction with acetyl-CoA. If acetyl-CoA accumulates, it acts both as an allosteric activator of pyruvate carboxylase and as an inhibitor of pyruvate dehydrogenase, thereby ensuring a supply of oxaloacetate. Lactate, an important substrate for gluconeogenesis, enters the cycle via oxidation to pyruvate and then carboxylation to oxaloacetate.

Aminotransferase (transaminase) reactions form pyruvate from alanine, oxaloacetate from aspartate, and α -ketoglutarate from glutamate. Because these reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of these amino acids. Other amino acids contribute to gluconeogenesis because their carbon skeletons give rise to citric acid cycle

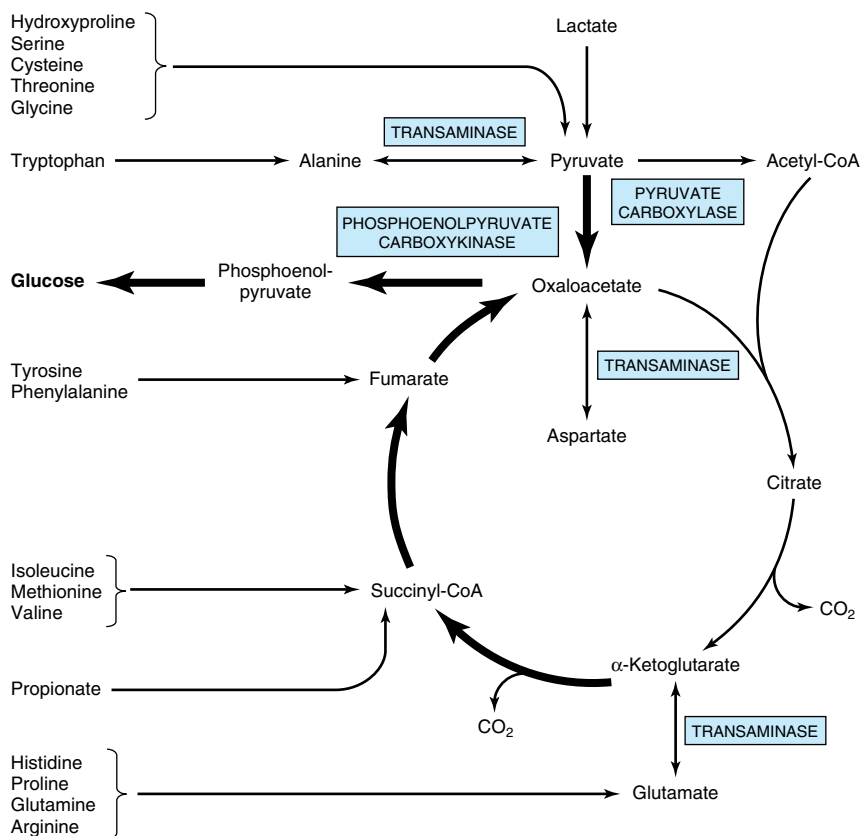


Figure 16-4. Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis.

intermediates. Alanine, cysteine, glycine, hydroxyproline, serine, threonine, and tryptophan yield pyruvate; arginine, histidine, glutamine, and proline yield α -ketoglutarate; isoleucine, methionine, and valine yield succinyl-CoA; and tyrosine and phenylalanine yield fumarate (Figure 16-4).

In ruminants, whose main metabolic fuel is short-chain fatty acids formed by bacterial fermentation, the conversion of propionate, the major glucogenic product of rumen fermentation, to succinyl-CoA via the methylmalonyl-CoA pathway (Figure 19-2) is especially important.

The Citric Acid Cycle Takes Part in Fatty Acid Synthesis (Figure 16-5)

Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.)

Pyruvate dehydrogenase is a mitochondrial enzyme, and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl-CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by **ATP-citrate lyase**.

Regulation of the Citric Acid Cycle Depends Primarily on a Supply of Oxidized Cofactors

In most tissues, where the primary role of the citric acid cycle is in energy-yielding metabolism, **respiratory control** via the respiratory chain and oxidative phosphorylation regulates citric acid cycle activity (Chapter 14). Thus, activity is immediately dependent on the supply of NAD^+ , which in turn, because of the tight coupling between oxidation and phosphorylation, is dependent on the availability of ADP and hence, ulti-

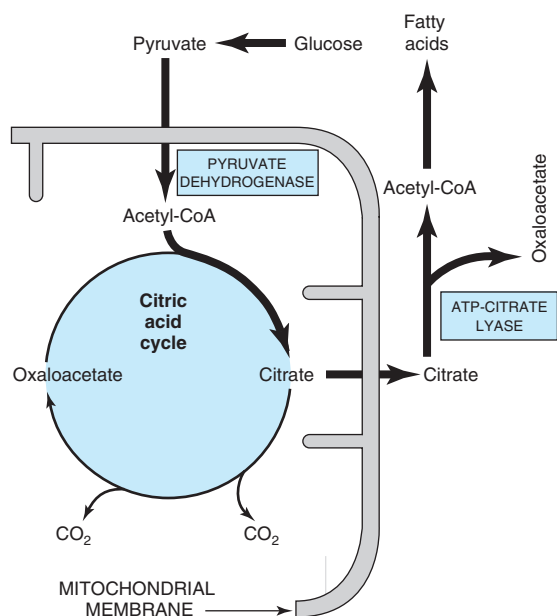


Figure 16–5. Participation of the citric acid cycle in fatty acid synthesis from glucose. See also Figure 21–5.

mately, on the rate of utilization of ATP in chemical and physical work. In addition, individual enzymes of the cycle are regulated. The most likely sites for regulation are the nonequilibrium reactions catalyzed by pyruvate dehydrogenase, citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase. The dehydrogenases are activated by Ca^{2+} , which increases in concentration during muscular contraction and secretion, when there is increased energy demand. In a tissue such as brain, which is largely dependent on carbohydrate to supply acetyl-CoA, control of the citric acid cycle may occur at pyruvate dehydrogenase. Several enzymes are responsive to the energy status, as shown by the $[\text{ATP}]/[\text{ADP}]$ and $[\text{NADH}]/[\text{NAD}^+]$ ratios. Thus, there is allosteric inhibition of citrate synthase by ATP and long-chain fatty acyl-CoA. Allosteric activation of mitochondrial NAD-dependent isocitrate dehydrogenase by ADP is counteracted by ATP and NADH. The α -ketoglutarate dehydrogenase complex is

regulated in the same way as is pyruvate dehydrogenase (Figure 17–6). Succinate dehydrogenase is inhibited by oxaloacetate, and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the $[\text{NADH}]/[\text{NAD}^+]$ ratio. Since the K_m for oxaloacetate of citrate synthase is of the same order of magnitude as the intramitochondrial concentration, it is likely that the concentration of oxaloacetate controls the rate of citrate formation. Which of these mechanisms are important in vivo has still to be resolved.

SUMMARY

- The citric acid cycle is the final pathway for the oxidation of carbohydrate, lipid, and protein whose common end-metabolite, acetyl-CoA, reacts with oxaloacetate to form citrate. By a series of dehydrogenations and decarboxylations, citrate is degraded, releasing reduced coenzymes and 2CO_2 and regenerating oxaloacetate.
- The reduced coenzymes are oxidized by the respiratory chain linked to formation of ATP. Thus, the cycle is the major route for the generation of ATP and is located in the matrix of mitochondria adjacent to the enzymes of the respiratory chain and oxidative phosphorylation.
- The citric acid cycle is amphibolic, since in addition to oxidation it is important in the provision of carbon skeletons for gluconeogenesis, fatty acid synthesis, and interconversion of amino acids.

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