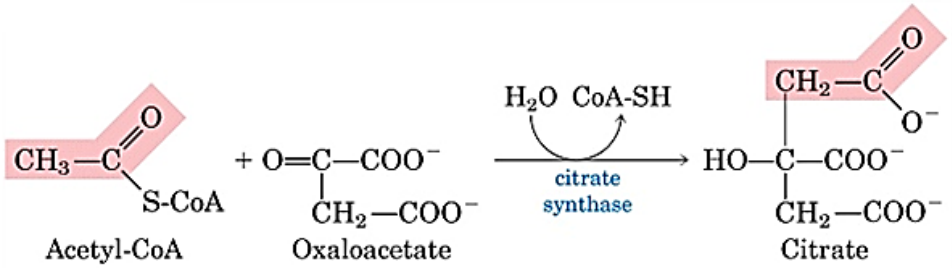
THE CITRIC ACID CYCLE

The Central Pathway of Carbohydrate, Lipid & Amino Acid Metabolism

The citric acid cycle (the Krebs or tricarboxylic acid cycle) is a sequence of reactions ***in mitochondria***. It is the final common pathway for the oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle. The citric acid cycle also has a central role in gluconeogenesis, lipogenesis, and interconversion of amino acids.

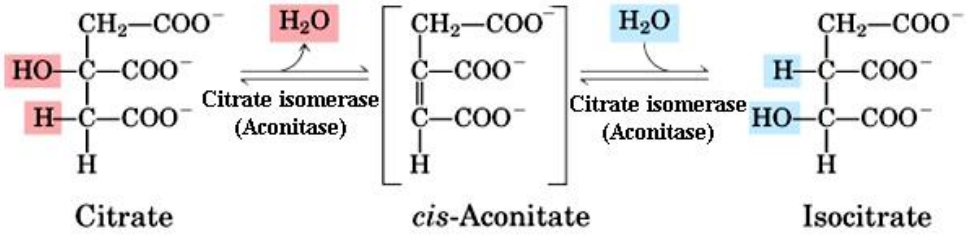
Reactions of The Citric Acid Cycle

1. Acetyl-CoA and oxaloacetate are condensed to form citrate, catalyzed by ***citrate synthase***.



First, a carbon-carbon bond is formed between the methyl carbon of acetyl-CoA and the carbonyl carbon of oxaloacetate. Then, the thioester bond of the resultant citryl-CoA is hydrolyzed, releasing citrate and CoASH. This reaction is irreversible.

2. Citrate is isomerized to isocitrate by the enzyme ***aconitase*** (citrate isomerase).

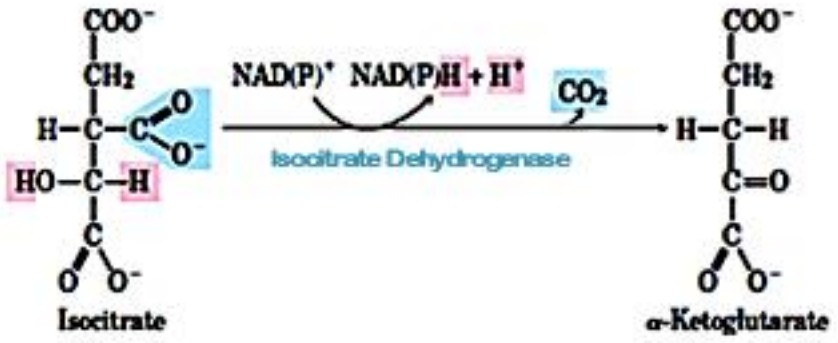


The reaction occurs in two steps: dehydration to *cis*-aconitate and rehydration to isocitrate. Although citrate is a symmetric molecule, aconitase reacts with citrate asymmetrically (atoms originated from acetyl-CoA is not used). This asymmetric behavior is the result of channeling or the transfer of the product of citrate synthase

directly onto the active site of aconitase, without entering free solution. This channeling provides integration of citric acid cycle activity and providing citrate in the cytosol as a source of acetyl-CoA for fatty acid synthesis. Citrate is only available in free solution to be transported from the mitochondria to the cytosol for fatty acid synthesis when aconitase is inhibited by accumulation of its product, isocitrate.

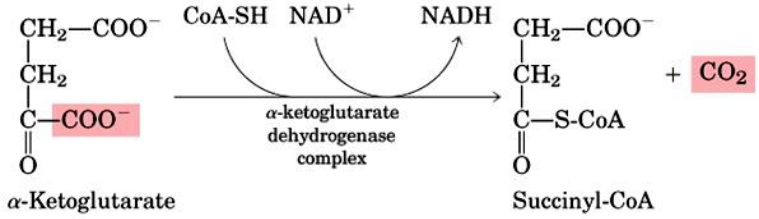
The poison fluoracetate is found in some of plants, and their consumption can be fatal to animals. Some fluorinated compounds used as anticancer agents and industrial chemicals (including pesticides) are metabolized to fluoroacetate. It is toxic because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits aconitase, causing citrate to accumulate.

3. Isocitrate undergoes dehydrogenation (forming oxalosuccinate) then decarboxylation to form α –ketoglutarate; the reaction is catalyzed by ***isocitrate dehydrogenase***.



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.

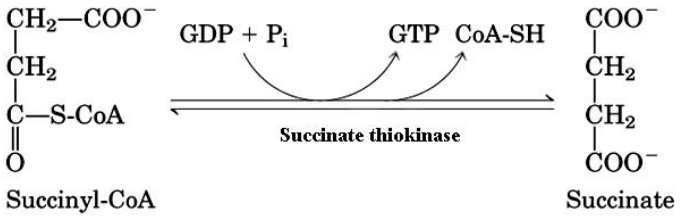
4. α -Ketoglutarate undergoes oxidative decarboxylation reaction catalyzed by α -ketoglutarate dehydrogenase complex forming succinyl-CoA.



α -ketoglutarate dehydrogenase complex is a multienzyme complex similar to that involved in the oxidative decarboxylation of pyruvate. It requires the same

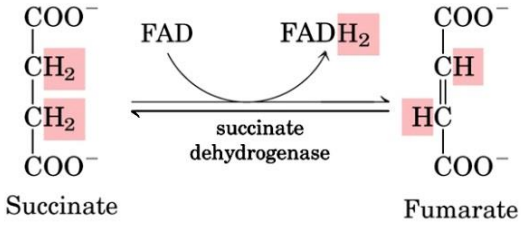
cofactors as the pyruvate dehydrogenase complex (thiamin diphosphate, lipoate, NAD^+, FAD, and CoA). As in the case of pyruvate dehydrogenase, arsenite inhibits the enzyme, causing the substrate, α -ketoglutarate, to accumulate. High concentrations of ammonia inhibit α -ketoglutarate dehydrogenase.

5. Succinyl-CoA is converted to succinate by the enzyme ***succinate thiokinase*** (succinyl-CoA synthetase).

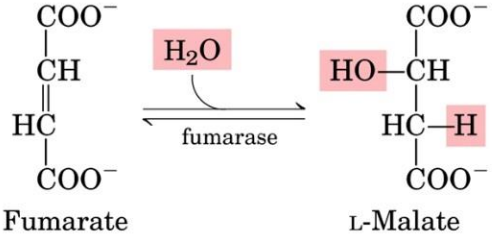


This is the only example of substrate level phosphorylation in the citric acid cycle. Tissues in which gluconeogenesis occurs (the liver and kidney) contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for ADP. The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis. Nongluconeogenic tissues have only the isoenzyme that phosphorylates ADP.

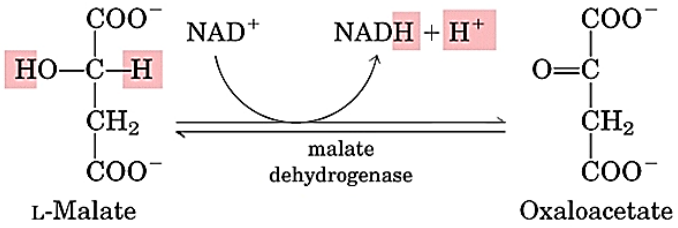
6. Dehydrogenation of succinate forming fumarate, catalyzed by ***succinate dehydrogenase***. Malonate competitively inhibits succinate dehydrogenase.



7. Fumarate is hydrated to **L-malate** by ***fumarase***.



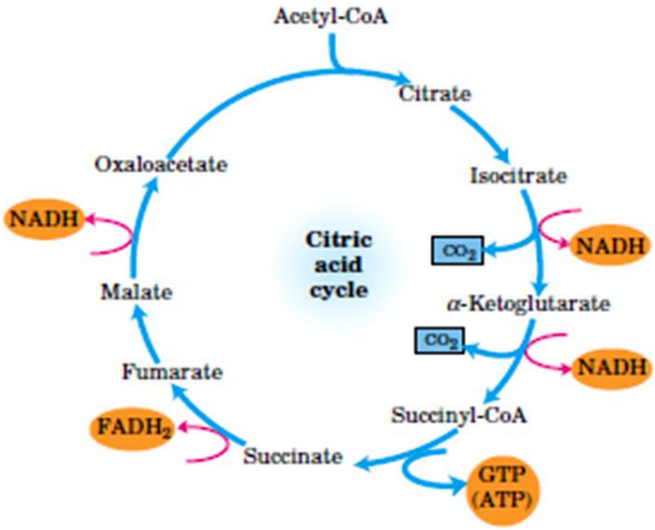
8. Malate is dehydrogenated by ***malate dehydrogenase*** to yield **oxaloacetate.**

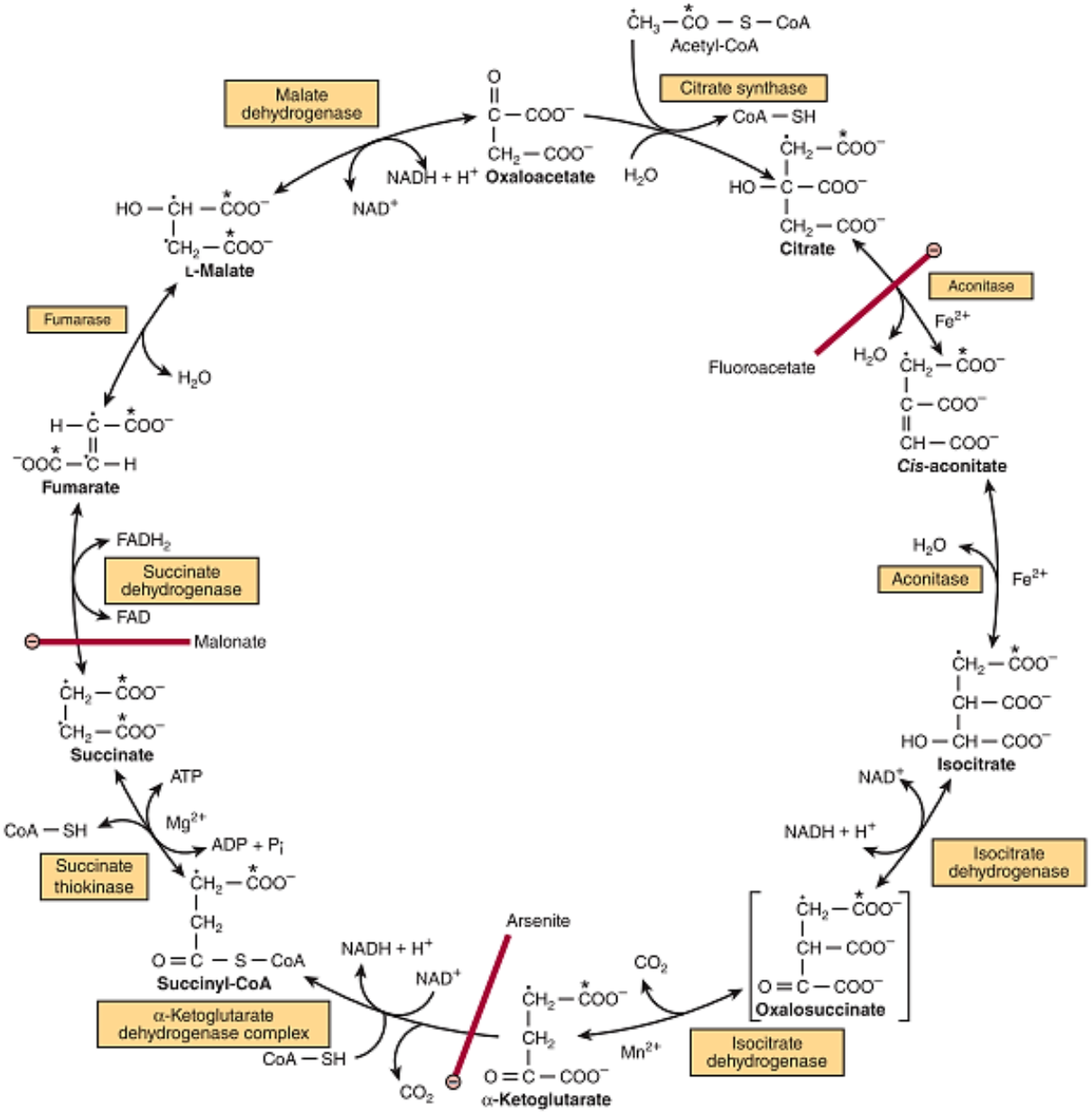


There is no direct participation of oxygen in the citric acid cycle. However, the cycle operates only under **aerobic conditions.** This is due to the fact that NAD^+ and FAD (from NADH and $FADH_2$, respectively) required for the operation of the cycle can be regenerated in the electron transport chain only in the presence of O_2. Therefore, citric acid cycle is ***strictly aerobic*** in contrast to glycolysis which operates in both aerobic and anaerobic conditions.

Energetics 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, where reoxidation of each NADH results in formation of ~2.5 ATP, and of $FADH_2$ ~1.5 ATP. In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.





The overall reactions of the citric acid cycle, showing sites of action of inhibitors

Roles of the B vitamins in the Citric Acid Cycle

Four of the B vitamins are essential in the citric acid cycle. The name, form and the reaction in which they participate are shown in the following table.

Vitamin	Form	Reaction catalyzed by:
Thiamin (B_1)	Thiamin diphosphate	α -ketoglutarate dehydrogenase (4)
Riboflavin (B_2)	FAD (flavin adenine dinucleotide)	Succinate dehydrogenase (6)
Niacin (B_3)	NAD^+ (nicotinamide adenine dinucleotide)	Isocitrate dehydrogenase (3) α -ketoglutarate dehydrogenase (4) Malate dehydrogenase (8)
Pantothenic acid (B_5)	Coenzyme A	α -ketoglutarate dehydrogenase (4)

Anaplerotic reactions

The citric acid cycle is an ***amphibolic*** pathway, and is not only a pathway for oxidation of the two carbon units of acetyl-CoA; but, it is also a major pathway for interconversion of metabolites arising from amino acids (by tansamination and deamination), and providing the substrates for amino acid synthesis, as well as for gluconeogenesis, and fatty acid synthesis. Thus, the citric acid cycle intermediate may be used in the synthesis of many biological molecules; and in order to maintain efficient metabolic pathway these intermediate need to be replenished. Different ***anaplerotic*** reactions do so. Anaplerosis is the act of replenishing the citric acid cycle intermediates that have been extracted for biosynthesis.

Regulation of the Citric Acid Cycle

Regulation occurs through regulation of acetyl-CoA formation by pyruvate dehydrogenase, or regulation of the reactions of the cycle itself. The main sites for regulation of the cycles' reaction are the nonequilibrium reactions catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The energy status as shown by the **[ATP]**/[ADP] and **[NADH]**/[NAD^+] ratios regulate the activity of these enzymes. Allosteric inhibition of isocitrate dehydrogenase by ATP results in isocitrate accumulation and thus citrate accumulation, which transport to the cytoplasm where it inhibits phosphofructokinase, an important regulatory enzyme of glycolysis.

The dehydrogenases are activated by **Ca^{2+}**, which increases in concentration during contraction of muscle and during secretion by other tissues, when there is increased energy demand. The **concentration of oxaloacetate**, limits the rate of the citrate synthase reaction.