CITRIC ACID CYCLE

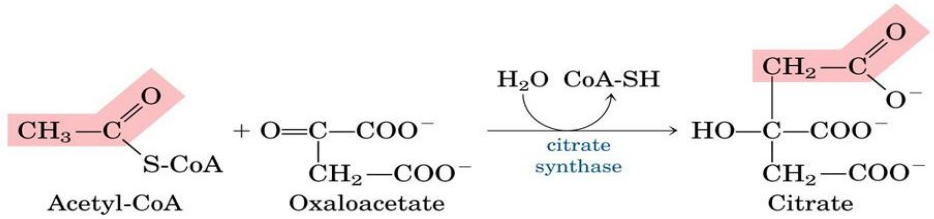
Also called as Tricarboxylic acid (TCA) Cycle because the precursor is an acid having three carboxyl groups and Krebs Cycle because Hans A. Krebs postulated the cycle in 1973.

To begin a turn of the cycle, acetyl-CoA donates its acetyl group to the four-carbon compound oxaloacetate to form the six-carbon citrate. Citrate is then transformed into isocitrate, also a six-carbon molecule, which is dehydrogenated with loss of CO_2 to yield the five-carbon compound aketoglutarate. The latter undergoes loss of CO_2 and ultimately yields the four-carbon compound succinate and a second molecule of CO_2. Succinate is then enzymatically converted in three steps into the fourcarbon oxaloacetate, with which the cycle began; thus, oxaloacetate is ready to react with another molecule of acetyl-CoA to start a second turn. In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave. In each turn, one molecule of oxaloacetate is used to form citrate but-after a series of reactions the oxaloacetate is regenerated. Therefore no net removal of oxaloacetate occurs; one molecule of oxaloacetate can theoretically suffice to bring about oxidation of an infinite number of acetyl groups. Four of the eight steps in this process are oxidations, in which the energy of oxidation is conserved, with high efficiency, in the formation of reduced cofactors (NADH and $FADH_2$).

THE CITRIC ACID CYCLE HAS EIGHT STEPS

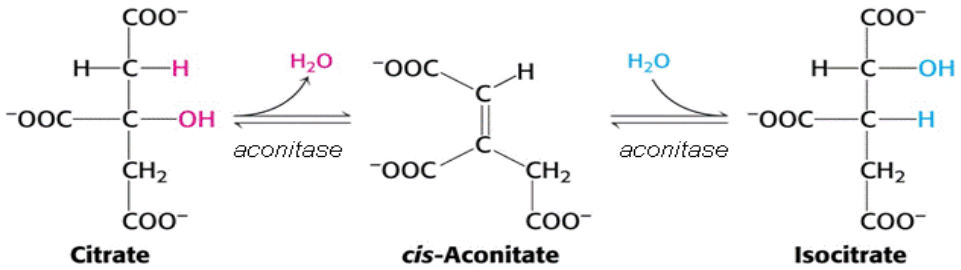
(l) ***Formation of Citrate (Condensation)***

The first reaction of the cycle is the **condensation** of acetyl-CoA with **oxaloacetate** to form citrate, catalyzed by **citrate synthase.**



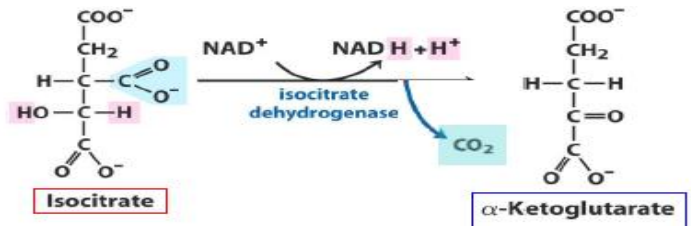
(2) ***Formation of Isocitrate via cis-Aconitate (Isomerization via dehydration and rehydration)***

The enzyme **aconitase** (more formally, **aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**, through the intermediary formation of the tricarboxylic acid **cis-aconitate**, which normally does not dissociate from the active site. Aconitase can promote the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate in two different ways, one leading to citrate and the other to isocitrate.



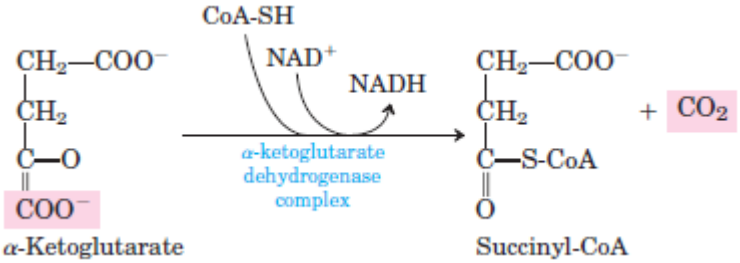
(3) Oxidation of Isocitrate to α-Ketoglutarate and CO_2 (Decarboxylation)

In the next step **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form **α-ketoglutarate.**



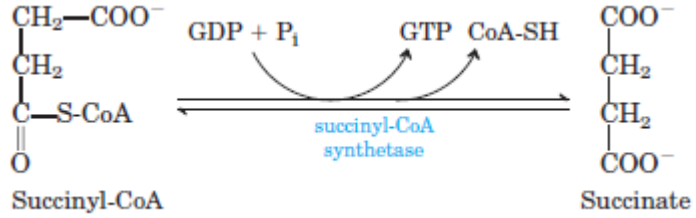
(4) Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2 (Decarboxylation)

The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to **succinyl-CoA** and O_2 by the action of the **a-ketoglutarate dehydrogenase complex**; NAD^+ serves as electron acceptor.



(5) Conversion of Succinyl-CoA to Succinate (Substrate-level Phosphorylation)

Succinyl-CoA, like acetyl-CoA, has a strongly negative free energy of hydrolysis of its thioester bond ($\Delta G^{\circ\prime}$ = -36 kJ/mol). In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, and **succinate** is also formed in the process. The enzyme that catalyzes this reversible reaction is called either **succinyl-CoA synthetase** or **succinic thiokinase.**

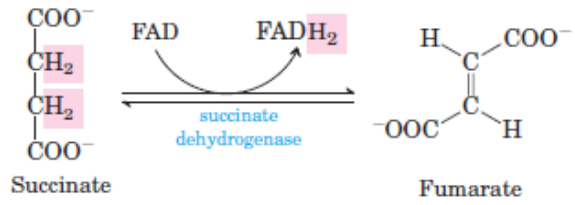


The GTP formed by succinyl-CoA synthetase may donate its terminal phosphate group to ADP to form ATP, by the reversible action of **nucleoside diphosphate kinase**. ATP and GTP are energetically equivalent.

$$GTP + ADP \longrightarrow GDP + ATP \qquad \Delta G'^{\circ} = 0 \text{ kJ/mol}$$

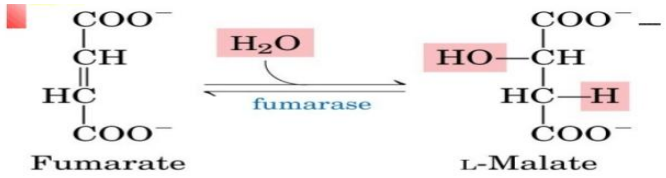
(6) Oxidation of Succinate to Fumarate (Dehydrogenation)

The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**. In eukaryotes, succinate dehydrogenase is tightly bound to the inner mitochondrial membrane (in prokaryotes, to the plasma membrane); it is the only enzyme of the citric acid cycle that is membrane-bound. Electron flow from succinate through these carriers to the final electron acceptor, O_2, is coupled to the synthesis of two ATP molecules per pair of electrons (respiration-linked phosphorylation).



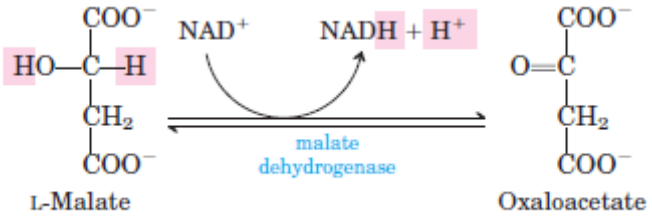
(7) Hydration of Fumarate to Produce Malate (Hydration)

The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase (fumarate hydratase)**

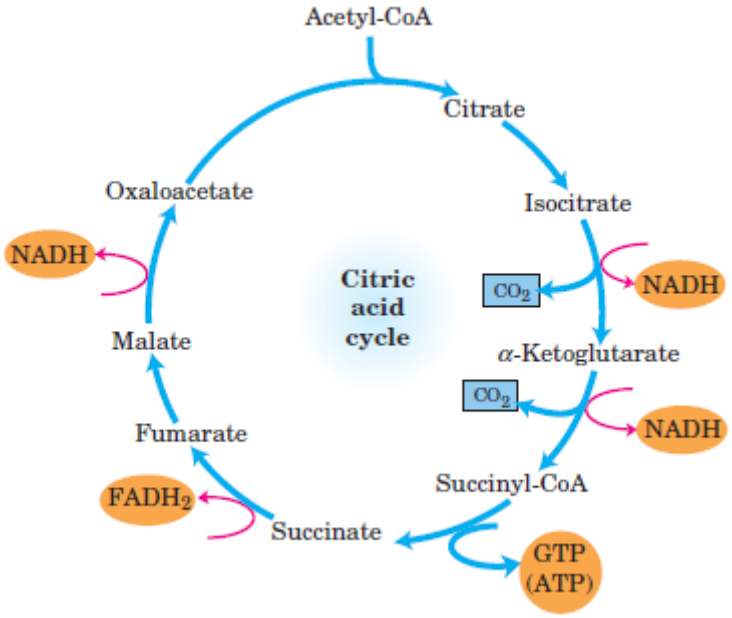


(8) Oxidation of Malate to Oxaloacetate (Dehydrogenation)

In the last reaction of the citric acid cycle, NAD-linked L-malate dehydrogenase catalyzes the oxidation of L-malate to oxaloacetate.



Products of one turn of the citric acid cycle: At each turn of the cycle, three NADH, one $FADH_2$, one GTP (or ATP), and two CO2 are released.



(b) Overall reaction: $\text{acetyl CoA} + 3\ NAD^+ + FAD + ADP + P_i + 3\ H_2O \longrightarrow 2\ CO_2 + 3\ NADH + 3\ H^+ + FADH_2 + ATP + CoA$

OVERVIEW OF CITRIC ACID CYCLE

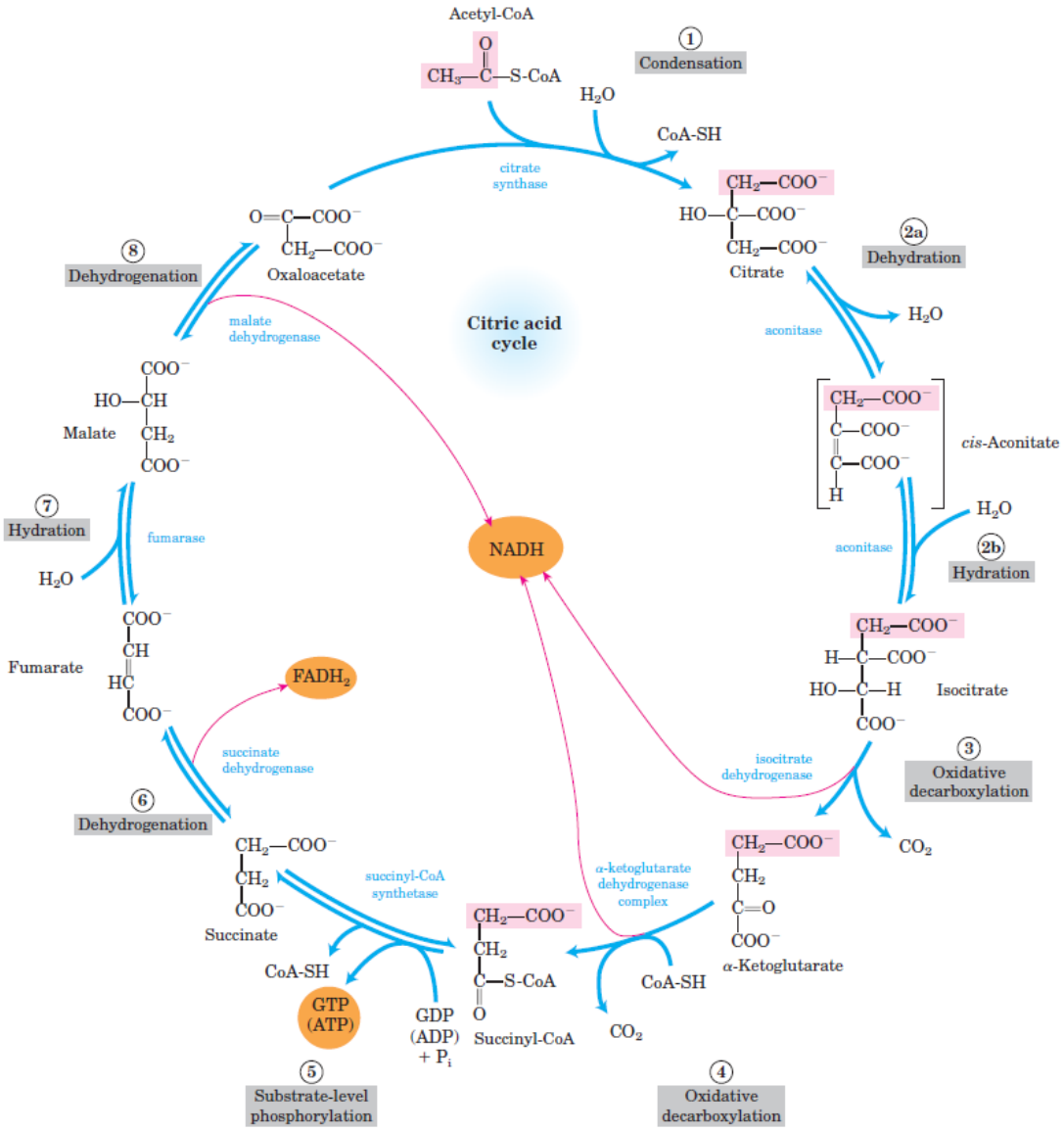


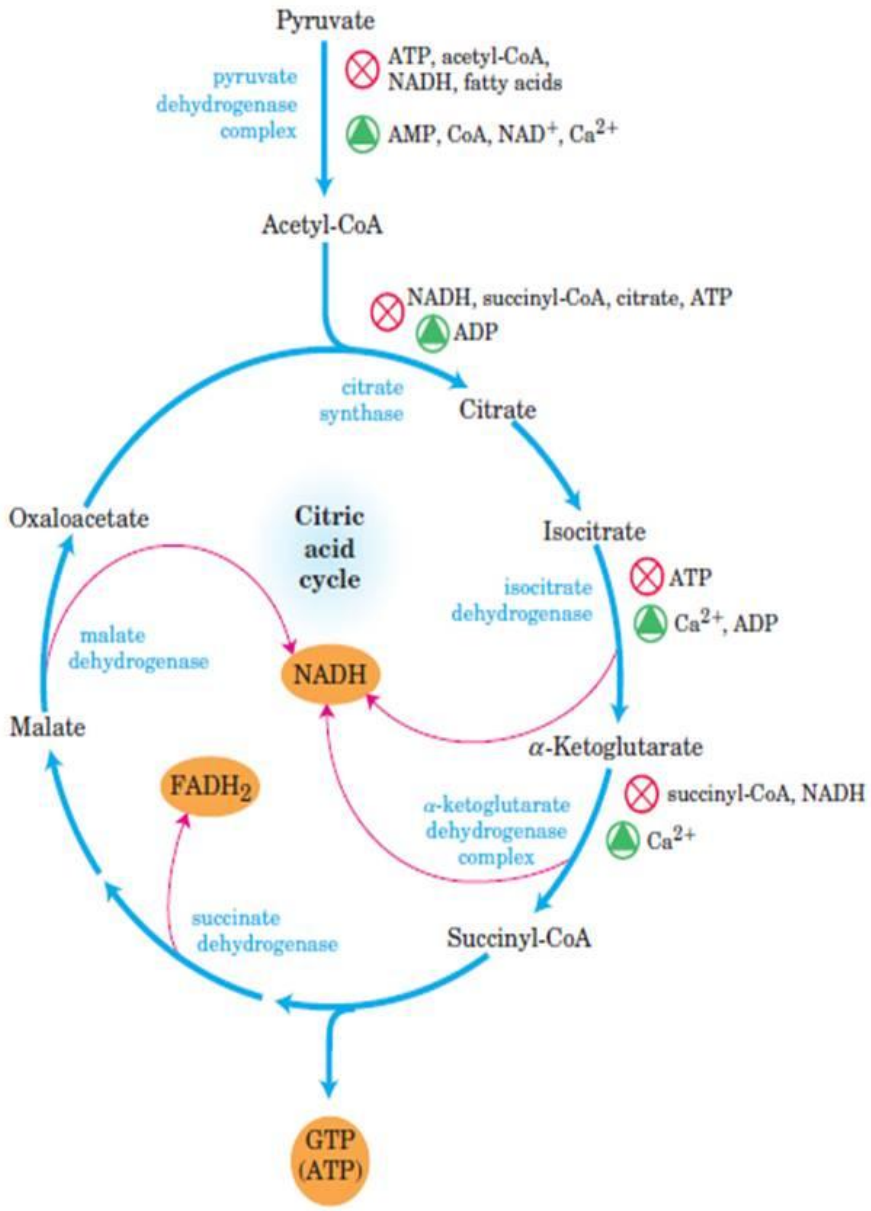
TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	*Number of ATP or reduced coenzyme directly formed*	*Number of ATP ultimately formed**
Glucose ⟶ glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate ⟶ fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate ⟶ 2 1,3-bisphosphoglycerate	2 NADH	3 or 5†
2 1,3-Bisphosphoglycerate ⟶ 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate ⟶ 2 pyruvate	2 ATP	2
2 Pyruvate ⟶ 2 acetyl-CoA	2 NADH	5
2 Isocitrate ⟶ 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate ⟶ 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA ⟶ 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate ⟶ 2 fumarate	2 $FADH_2$	3
2 Malate ⟶ 2 oxaloacetate	2 NADH	5
Total		30–32

* This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19-27 and 19-28.

REGULATION OF CITRIC ACID CYCLE:



1. ***The Production of Acetyl-CoA by the Pyruvate Dehydrogenase Complex Is Regulated***
   - The complex is strongly inhibited by ATP, as well as by acetyl-CoA and NADH, the products of the reaction.
   - The allosteric inhibition of pyruvate oxidation is greatly enhanced when long-chain fatty acids are available.
   - AMP, CoA, and NAD^+, all of which accumulate when too little acetate flows into the citric acid cycle, allosterically activate the pyruvate dehydrogenase complex.
   - Thus this enzyme activity is turned off when ample fuel is available in the form of fatty acids and acetyl-CoA and when the cell's ATP concentration and [NADH]/[NAD^+] ratio are high, and turned on when energy demands are high and greater flux of acetyl-CoA into the citric acid cycle is required.

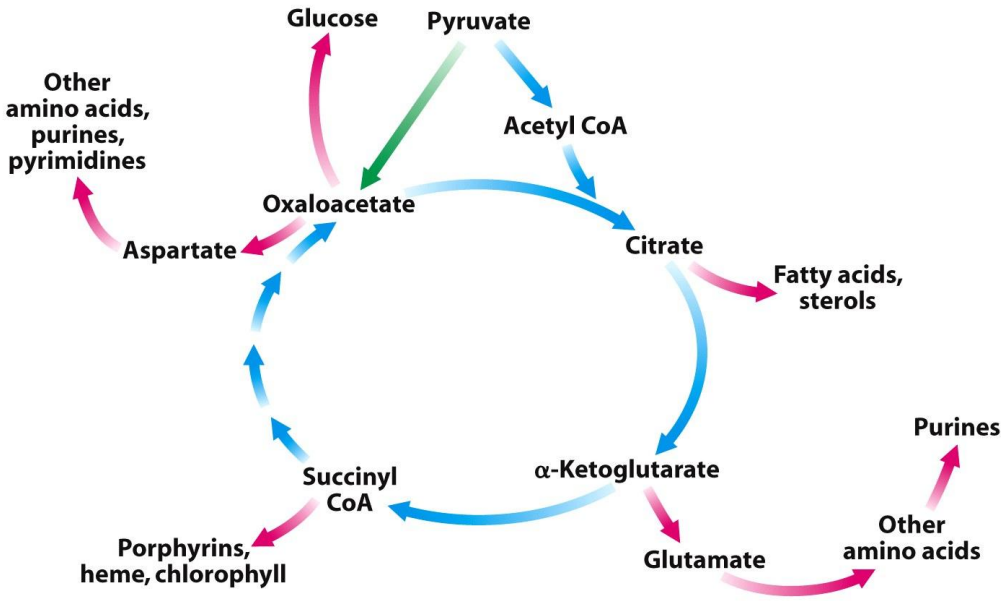
2. ***Three Enzymes of the Citric Acid Cycle Are Regulated***

- There are three strongly exergonic steps in the cycle, those catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.
- Three factors govern the rate of flux through the cycle:
  a) substrate availability
  b) inhibition by accumulating products
  c) allosteric feedback inhibition of early enzymes by later intermediates in the cycle
- Each can become the rate-limiting step under some circumstances.
- The availability of the substrates for **citrate synthase** (acetyl-CoA and oxaloacetate) varies with the metabolic circumstances and sometimes limits the rate of citrate formation.
- NADH, a product of the oxidation of isocitrate and α-ketoglutarate, accumulates under some conditions, and when the [NADH]/[NAD^+] ratio becomes large, both dehydrogenase reactions are severely inhibited by mass action.
- **Product accumulation inhibits** all three of the limiting steps of the cycle: succinyl-CoA inhibits α-ketoglutarate dehydrogenase (and also citrate synthase); citrate blocks citrate synthase; and the end product, ATP, inhibits both citrate synthase and isocitrate dehydrogenase. The inhibition of citrate synthase by ATP is relieved by ADP, an allosteric activator of this enzyme. Calcium ions, which in vertebrate muscle are the signal for contraction and the concomitant increased demand for ATP, activate both isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, as well as the pyruvate dehydrogenase complex.
- In short, the concentrations of substrates and intermediates of the citric acid cycle set the flux through this pathway at a rate that provides optimal concentrations of ATP and NADH.

AMPHIBOLIC NATURE OF CITRIC ACID CYCLE

Citric Acid Cycle Components Are Important Biosynthetic Intermediates

- In aerobic organisms, the citric acid cycle is an amphibolic pathway (i.e., it serves in both catabolic and anabolic processes). It not only functions in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, but also provides precursors for many biosynthetic pathways.
- By the action of several important auxiliary enzymes, certain intermediates of the citric acid cycle, particularly α-ketoglutarate and oxaloacetate, can be removed from the cycle to serve as precursors of amino acids. Aspartate and glutamate have the same carbon skeletons as oxaloacetate and α-ketoglutarate, respectively, and are synthesized from them by simple transamination. Through aspartate and glutamate the carbons of oxaloacetate and α-ketoglutarate are used to build other amino acids as well as purine and pyrimidine nucleotides.
- Oxaloacetate is converted into glucose in the process of gluconeogenesis.
- Succinyl-CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin) and electron carriers (in cytochromes).
- Given the number of biosynthetic products derived from citric acid cycle intermediates, this cycle clearly serves a critical role apart from its function in energy-yielding metabolism.

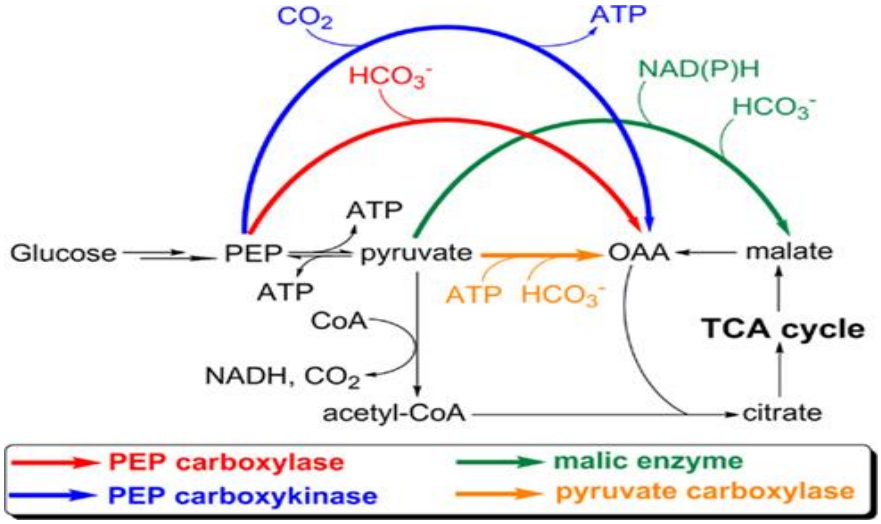


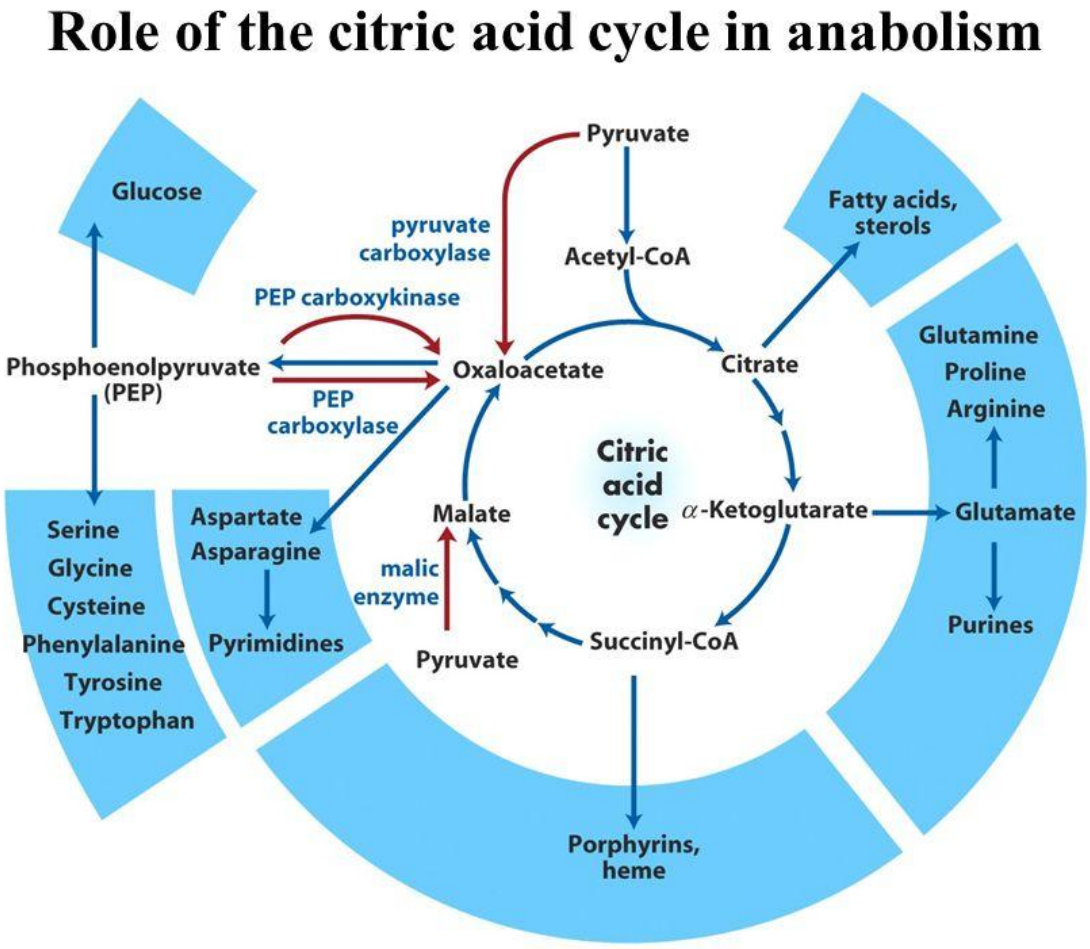
ANAPLEROTIC REACTIONS REPLENISH CITRIC ACID CYCLE INTERMEDIATES

When intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, the resulting decrease in the concentration of these intermediates would be expected to slow the flux through the citric acid cycle. However, the intermediates can be replenished by **anaplerotic reactions**. Under normal circumstances the reactions by which the cycle intermediates are drained away and those by which they are replenished are in dynamic balance, so that the concentrations of the citric acid cycle intermediates remain almost constant.

TABLE 16–2 Anaplerotic Reactions

Reaction	*Tissue(s)/organism(s)*
Pyruvate + HCO_3^- + ATP $\xrightleftharpoons{\text{pyruvate carboxylase}}$ oxaloacetate + ADP + P_i	Liver, kidney
Phosphoenolpyruvate + CO_2 + GDP $\xrightleftharpoons{\text{PEP carboxykinase}}$ oxaloacetate + GTP	Heart, skeletal muscle
Phosphoenolpyruvate + HCO_3^- $\xrightleftharpoons{\text{PEP carboxylase}}$ oxaloacetate + P_i	Higher plants, yeast, bacteria
Pyruvate + HCO_3^- + NAD(P)H $\xrightleftharpoons{\text{malic enzyme}}$ malate + $NAD(P)^+$	Widely distributed in eukaryotes and prokaryotes





The diagram also shows the anplerotic reactions.

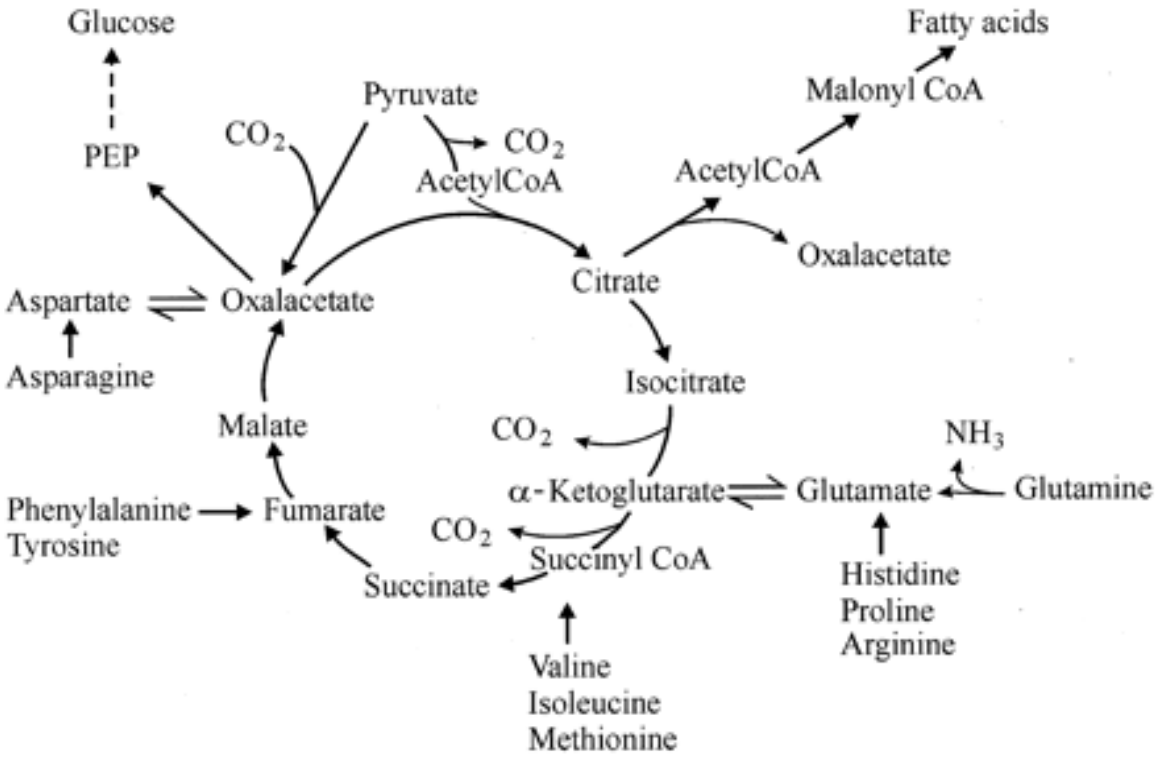


TABLE 16–4 ANAPLEROTIC REACTIONS OF THE TCA CYCLE

TCA Cycle Intermediate Produced	Non-TCA Cycle Reaction Generating the TCA Cycle Intermediate
α-Ketoglutarate	Glutamate + pyruvate → α-ketoglutarate + alanine (from protein breakdown)
Succinate	From glyoxylate cycle
Malate	Malic enzyme: pyruvate + CO_2 + NADPH + H^+ → malate + $NADP^+$
Oxaloacetate	Pyruvate carboxylase: pyruvate + CO_2 + ATP + H_2O → oxaloacetate + ADP + P_i
Oxaloacetate	Aspartate + pyruvate → oxaloacetate + alanine (from protein breakdown)