Chapter 16 The Citric Acid Cycle

- The pyruvate produced by glycolysis is oxidized to H_2O and CO_2.
- This aerobic phase of catabolism is called **respiration**.
- Catabolism of proteins, fats and carbohydrates occurs in the three stages of cellular respiration (**Fig. 16-1)**.

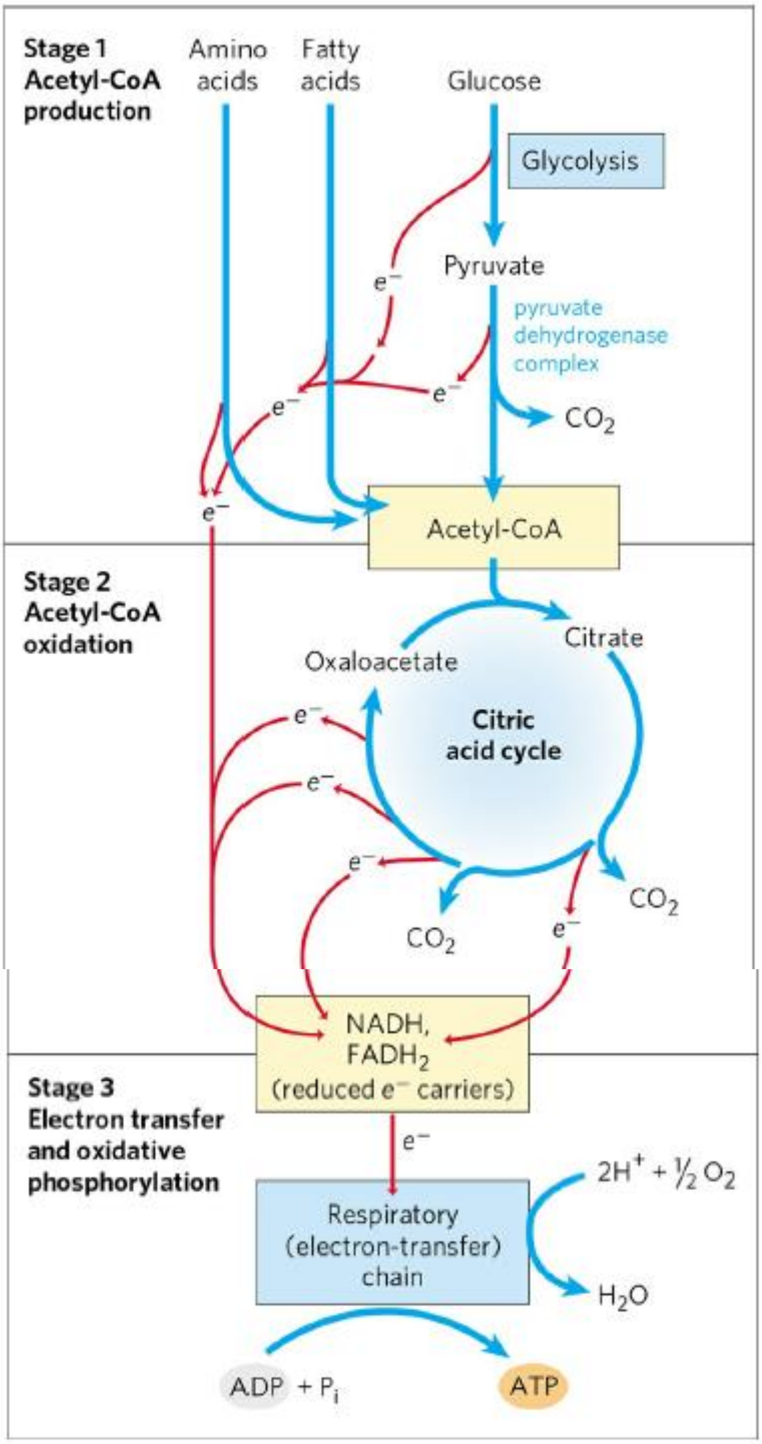


FIGURE 16-1 Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration. Stage 1: oxidation of fatty acids, glucose, and some amino acids yields acetyl-CoA. Stage 2: oxidation of acetyl groups in the citric acid cycle includes four steps in which electrons are abstracted. Stage 3: electrons carried by NADH and $FADH_2$ are funneled into a chain of mitochondrial (or, in bacteria, plasma membrane-bound) electron carriers—the respiratory chain—ultimately reducing O_2 to H_2O. This electron flow drives the production of ATP.

- Stage 1 : Oxidation of fatty acids, glucose and some amino acids yields acetly-CoA.
- Stage 2 : Oxidation of acetyl groups in the citric acid cycle yields CO_2 and energy.
  - The energy released is conserved in the reduced electron carriers NADH and $FADH_2$.
- Stage 3 : The electrons are transferred to O_2 (the final electron acceptor) via a chain of electron-carrying molecules known as the respiratory chain.
  - In the course of electron transfer, the energy is conserved in the form of ATP by a process called oxidative phosphorylation.
- Citric acid cycle is also called the **tricarboxylic acid (TCA) cycle** or the **Krebs cycle**.

16.1 Production of Acetyl-CoA (Activated Acetate)

- Pyruvate is oxidized to acetly-CoA and CO_2 by the **pyruvate dehydrogenase (PDH) complex** in the mitochondria of eukaryotic cells.
- The overall reaction is an oxidative decarboxylation (**Fig. 16-2**).

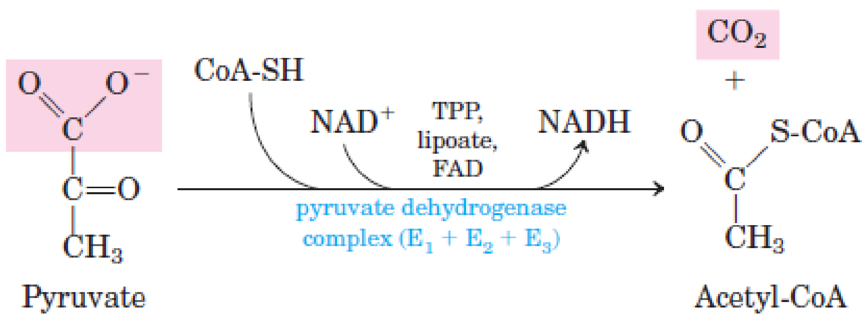


FIGURE 16-2 Overall reaction catalyzed by the pyruvate dehydrogenase complex. The five coenzymes participating in this reaction, and the three enzymes that make up the enzyme complex, are discussed in the text.

- Pyruvate dehydrogenase complex requires five coenzymes.
  - FAD
  - NAD^+
  - CoA : Coenzyme A (an acyl carrier) (**Fig. 16-3**).
  - TPP : Thiamine pyrophosphate
  - Lipoate

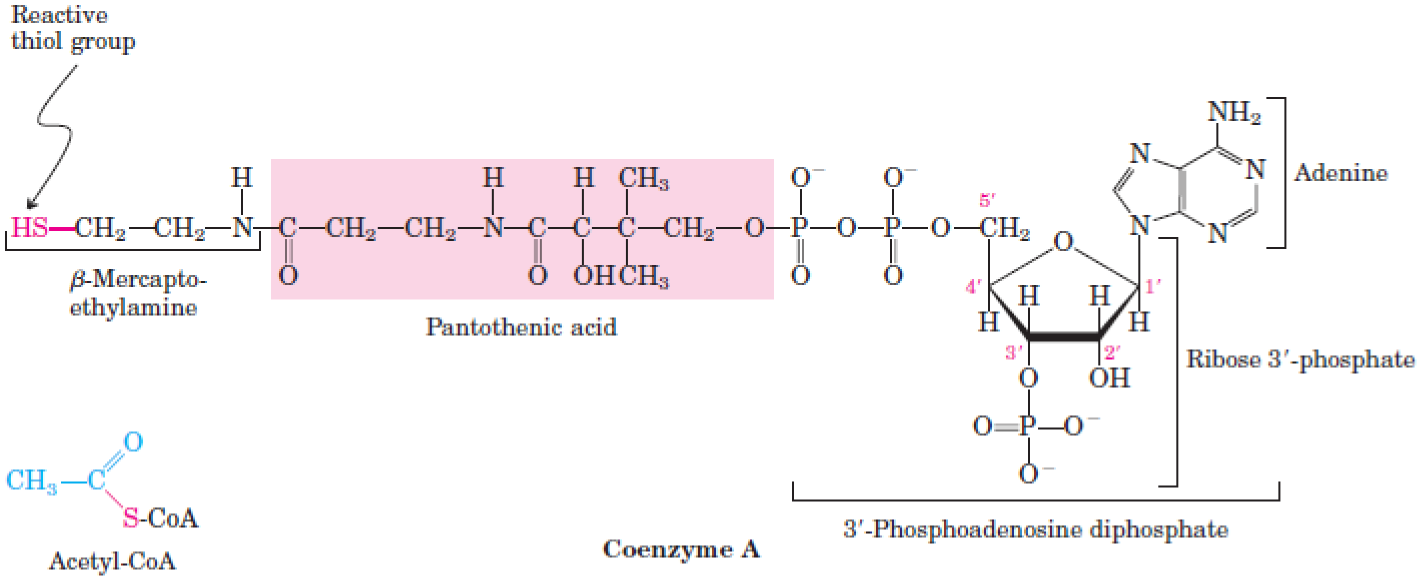


FIGURE 16-3 Coenzyme A (CoA).

- Pyruvate dehydrogenase complex consists of three distinct enzymes.
  - E_1 : Pyruvate dehydrogenase
  - E_2 : Dihydrolipoyl transacetylase
  - E_3 : Dihydrolipoyl dehydrogenase
- PDH complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate (**Fig. 16-6**).
- E_1 catalyzes first the decarboxylation of pyruvate, producing hydroxyethyl-TPP, and then the oxidation of the hydroxyethyl group to an acetyl group.

The electrons from this oxidation reduce the disulfide of lipoate bound to E_2, and the acetyl group is transferred into thioester linkage with one —SH group of reduced lipoate.

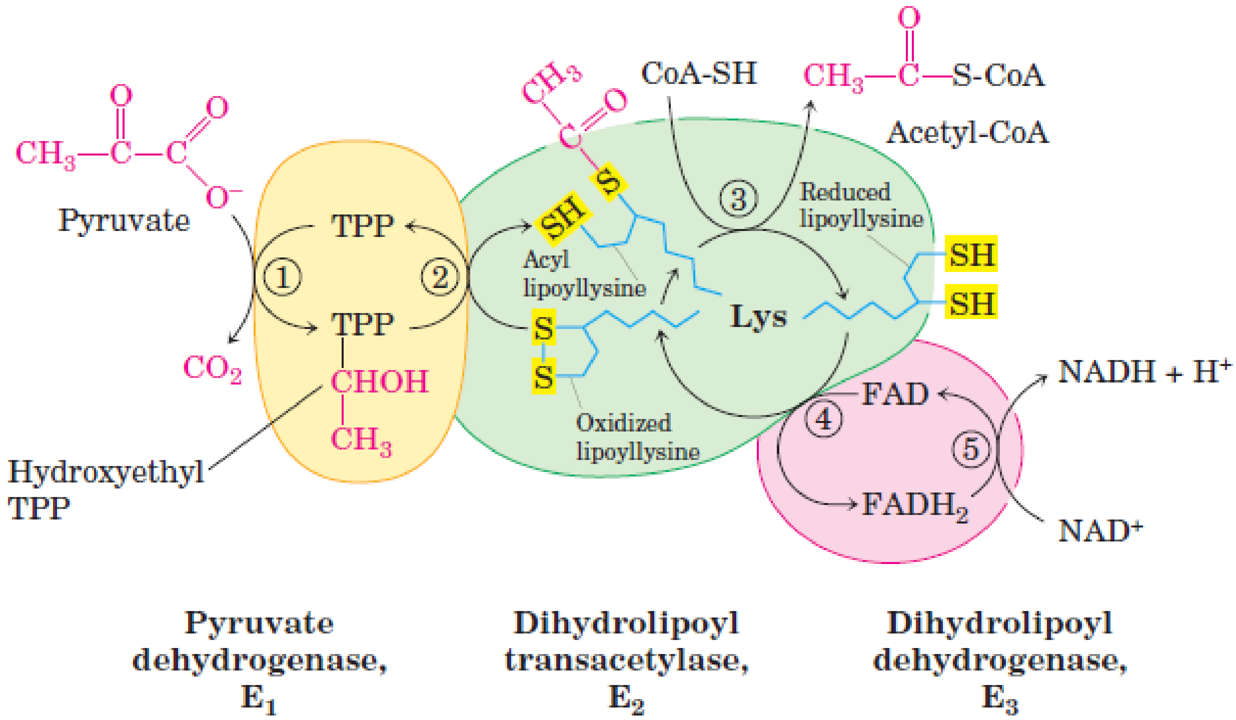


FIGURE 16-6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step 1 pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1) and is decarboxylated to the hydroxyethyl derivative (see Fig. 14-15). Pyruvate dehydrogenase also carries out step 2, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group. Step 3 is a transesterification in which the —SH group of CoA replaces the —SH group of E_2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step 4 dihydrolipoyl dehydrogenase (E_3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E_2 to the FAD prosthetic group of E_3, restoring the oxidized form of the lipoyllysyl group of E_2. In step 5 the reduced $FADH_2$ of E_3 transfers a hydride ion to NAD^+, forming NADH. The enzyme complex is now ready for another catalytic cycle. (Subunit colors correspond to those in Fig. 16-5b.)

- E_2 catalyzes the transfer of the acetyl group to coenzyme A, forming acetyl-CoA.
- E_3 catalyzes the regeneration of the disulfide (oxidized) form of lipoate; electrons pass first to FAD, then to NAD^+.

16.2 Reactions of the Citric Acid Cycle

- Oxidation of acetyl- CoA is carried out by the citric acid cycle (eight steps) (**Fig. 16-7)**.
- In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave; one molecule of oxaloacetate is used to form citrate and one molecule of oxaloacetate is regenerated.
- The energy of this oxidation is conserved in the reduced coenzymes NADH and $FADH_2$.
- Reactions occur in the mitochondria of eukaryotic cells.

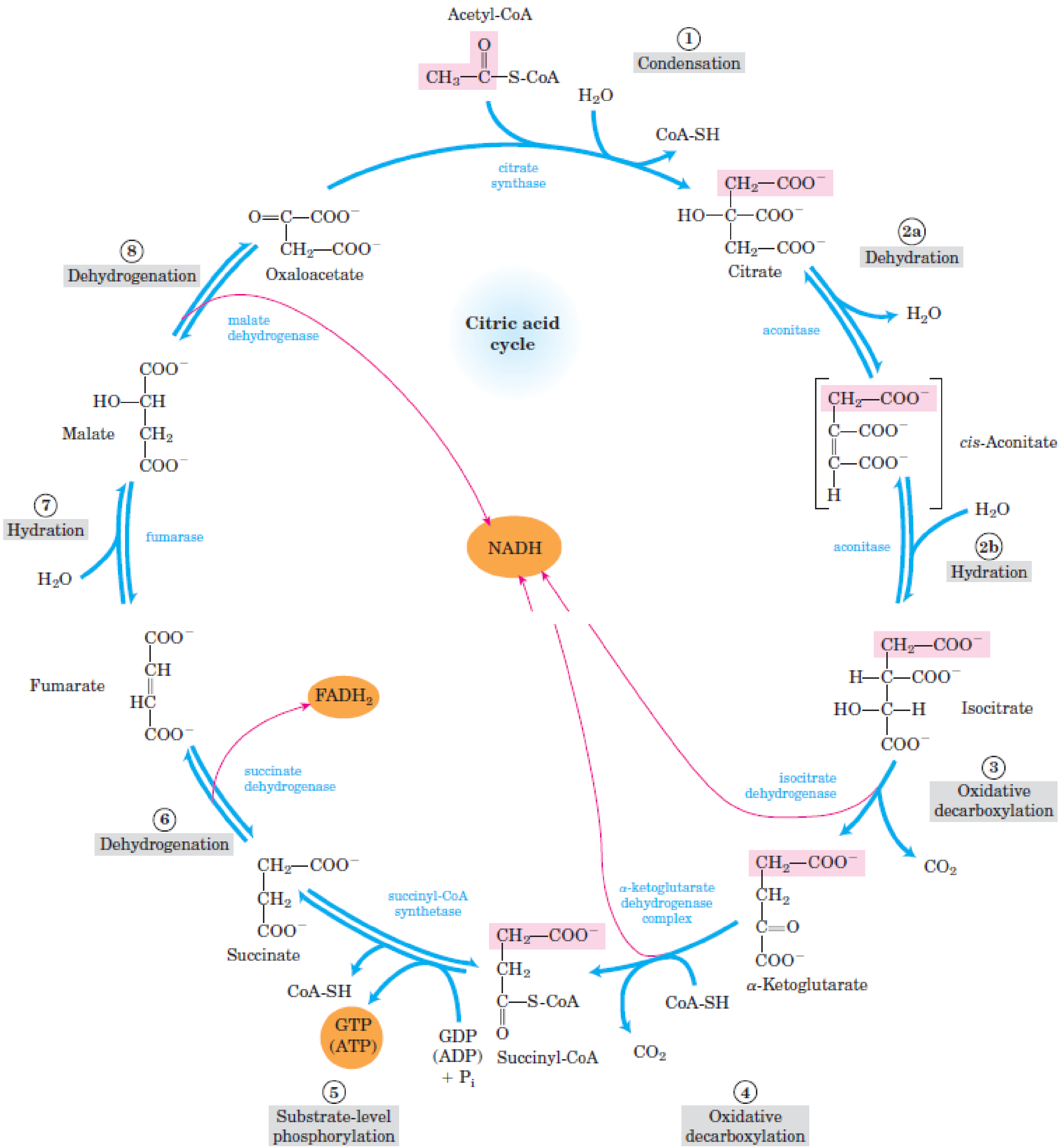
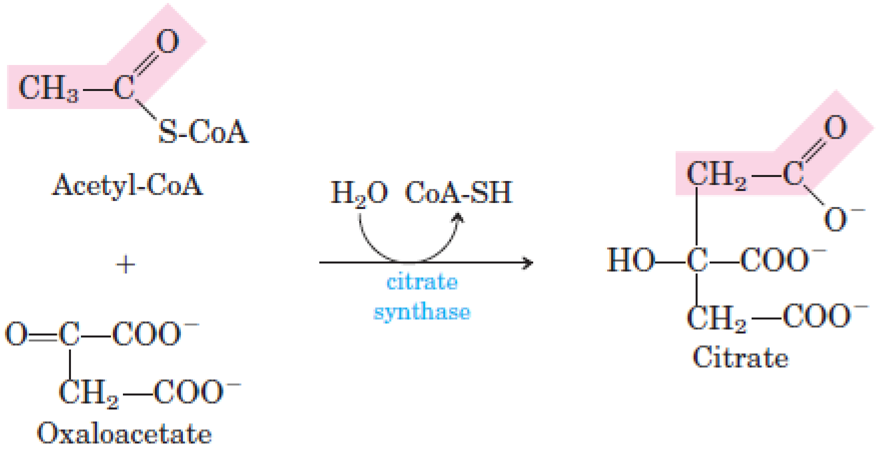
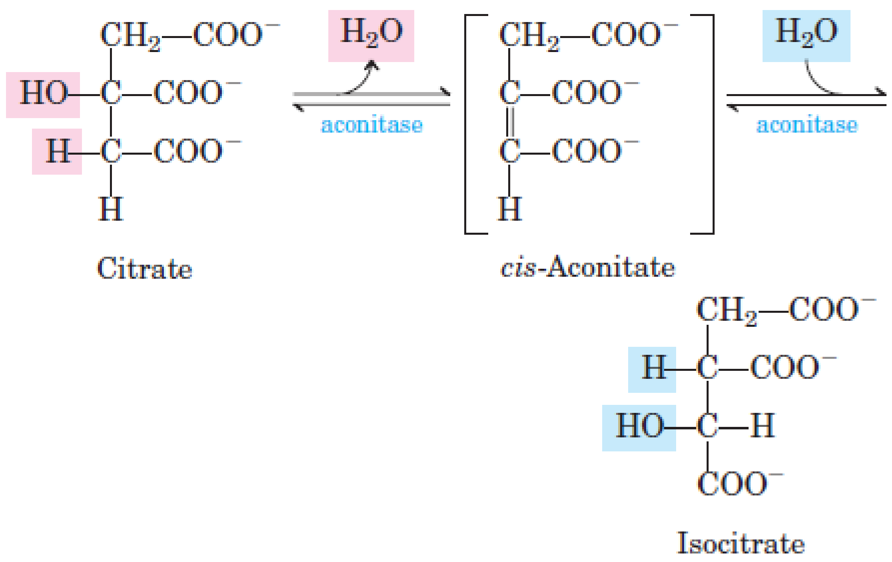


FIGURE 16-7 Reactions of the citric acid cycle.

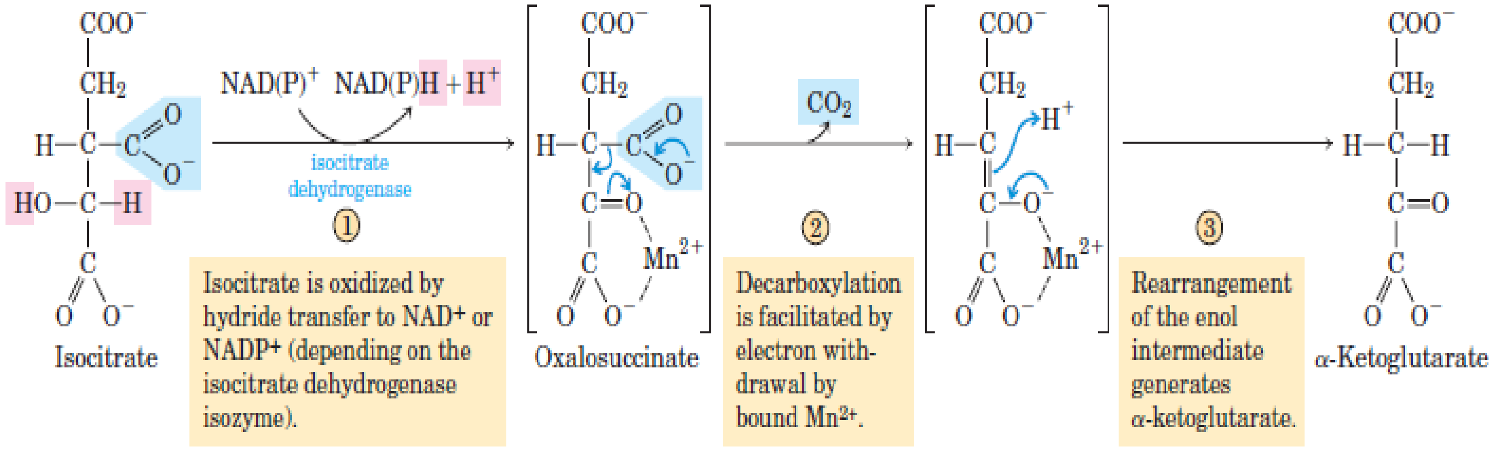
1 **Formation of Citrate** The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**, catalyzed by **citrate synthase**:



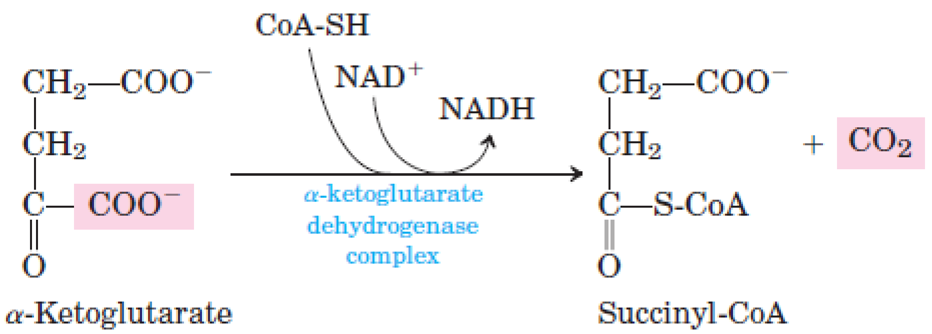
2 **Formation of Isocitrate via *cis*-Aconitate** The enzyme **aconitase** (more formally, **aconitate hydratase**) catalyzes the reversible transformation of citrate to **isocitrate**, through the intermediary formation of the tricarboxylic acid ***cis*-aconitate**, which normally does not dissociate from the active site.



3 Oxidation of Isocitrate to α-Ketoglutarate and CO_2 In the next step, **isocitrate dehydrogenase** catalyzes oxidative decarboxylation of isocitrate to form **α-ketoglutarate**

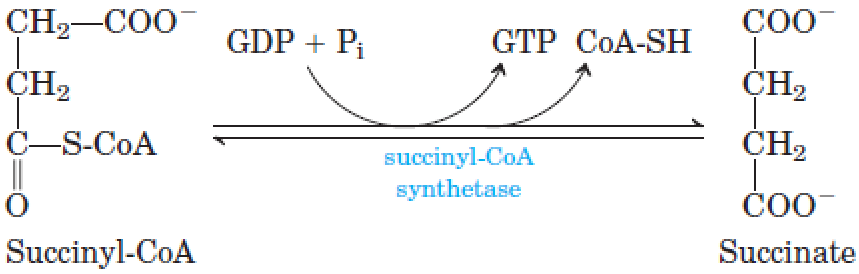


4 Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2 The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to **succinyl-CoA** and CO_2 by the action of the **α-ketoglutarate dehydrogenase complex**; NAD^+ serves as electron acceptor and CoA as the carrier of the succinyl group.



- α-ketoglutarate dehydrogenase complex (E_1+E_2+E_3) is similar to pyruvate dehydrogenase complex in both structure and function.

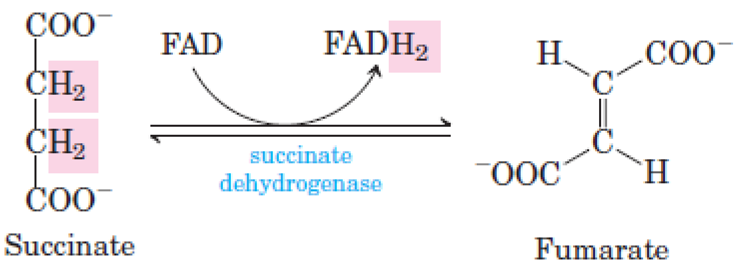
5 Conversion of Succinyl-CoA to Succinate Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis



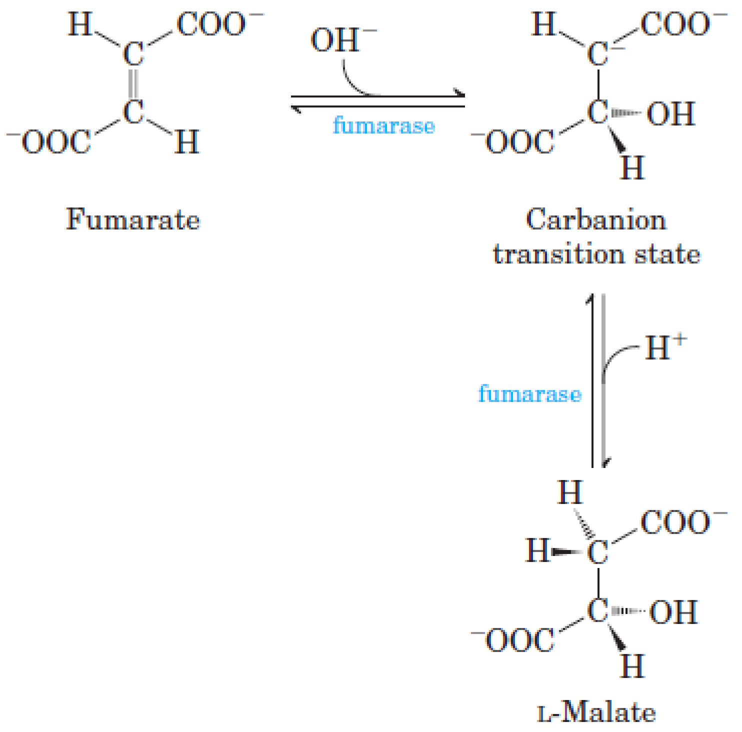
- A substrate-level phosphorylation occurs.
- The GTP formed by succinyl- CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by **nucleoside diphosphate kinase.**

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP}$$

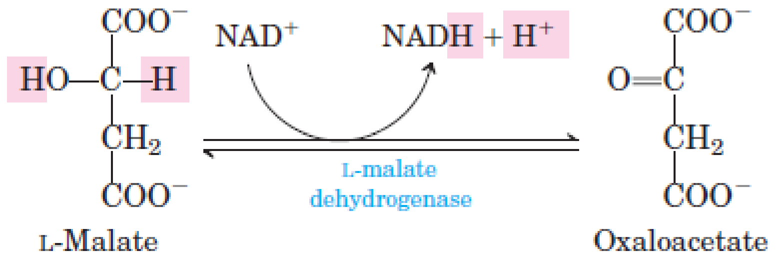
6 Oxidation of Succinate to Fumarate The succinate formed from succinyl-CoA is oxidized to **fumarate** by the flavoprotein **succinate dehydrogenase**:



7 Hydration of Fumarate to Malate The reversible hydration of fumarate to **L-malate** is catalyzed by **fumarase**



8 Oxidation of Malate to Oxaloacetate In the last reaction of the citric acid cycle, **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate, coupled to the reduction of NAD^+ to NADH:



The Energy of Oxidations in the Cycle Is Efficiently Conserved

- The energy released by these oxidations was conserved in the reduction of 3 NAD^+ and 1 FAD and the production of 1 ATP or GTP.
- At the end of the cycle a molecule of oxaloacetate was regenerated.

- Products of one turn of the citric acid cycle (**Fig. 16-14**).

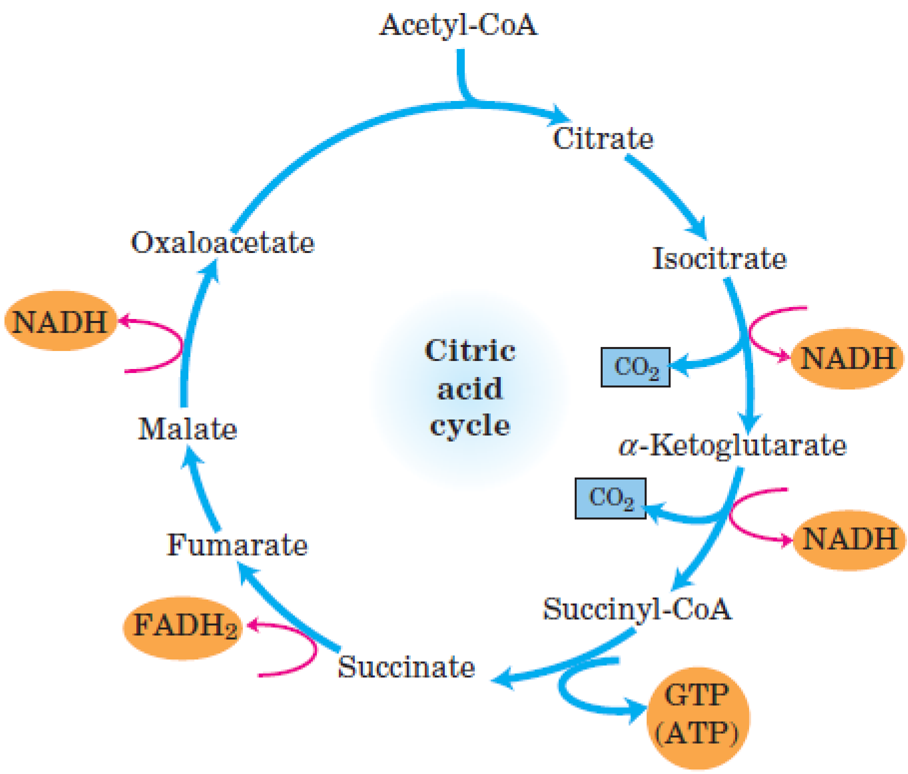


FIGURE 16-14 Products of one turn of the citric acid cycle. At each turn of the cycle, three NADH, one $FADH_2$, one GTP (or ATP), and two CO_2 are released in oxidative decarboxylation reactions. Here and in several of the following figures, all cycle reactions are shown as proceeding in one direction only, but keep in mind that most of the reactions are reversible

- 3 NADH, 1 $FADH_2$, 1 GTP (or ATP) and 2 CO_2 are released in the oxidative decarboxylation reactions of 1 acetyl-CoA.
- Stoichiometry of NADH, $FADH_2$ and ATP formation in the aerobic oxidation of 1 glucose

	NADH	$FADH_2$	ATP (or GTP)	CO_2
Glycolysis	2		2	
PDH Reaction	2			2
Citric Acid Cycle	6	2	2	4

- In oxidative phosphorylation,
  - passage of two electrons from NADH to O_2 yields about 2.5 ATP
  - passage of two electrons from $FADH_2$ to O_2 yields about 1.5 ATP
- After oxidative phosphorylation, 32 ATP are obtained per glucose.

Citric Acid Cycle Components Are Important Biosynthetic Intermediates

- Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways in anabolism (**Fig. 16-16).**

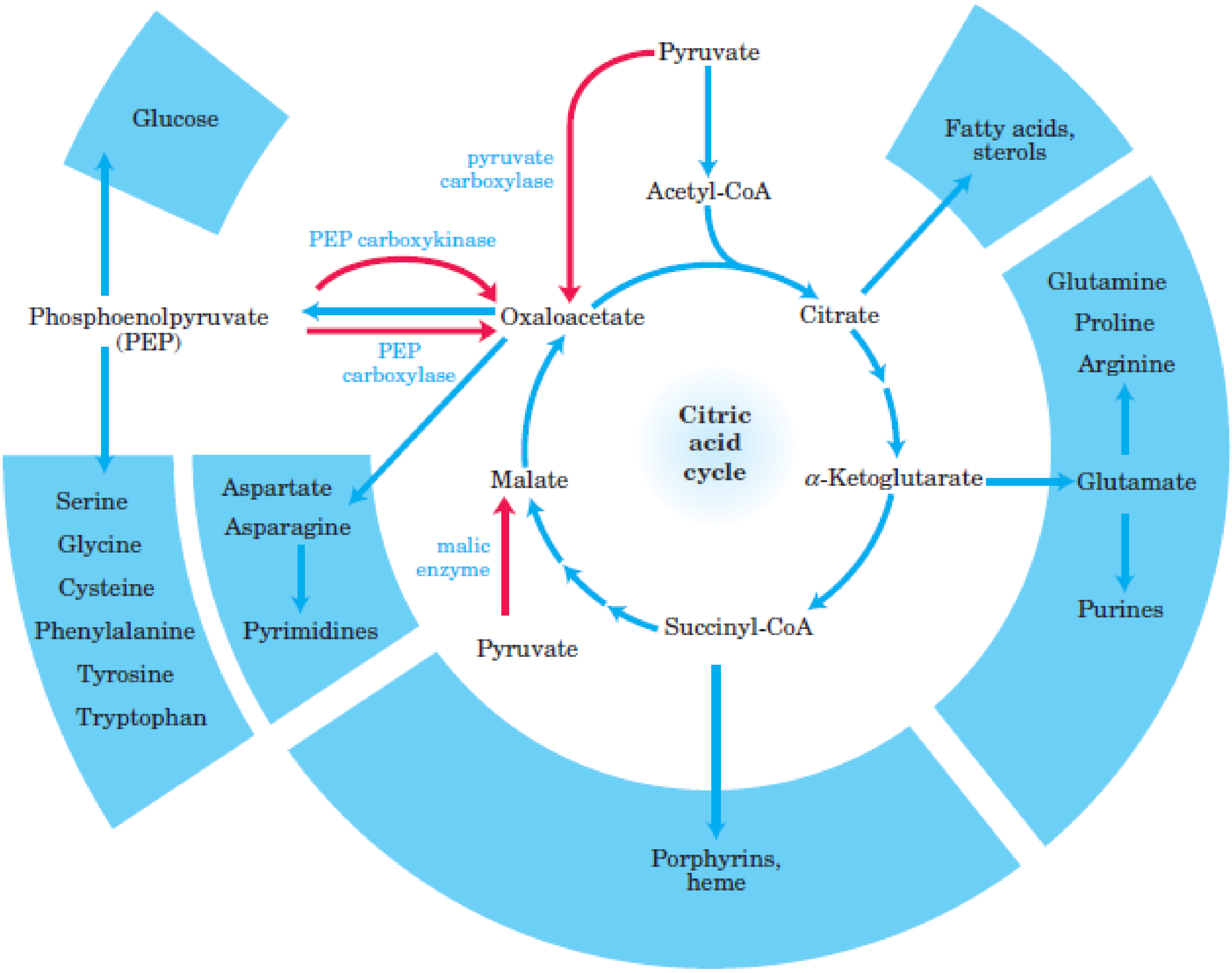


FIGURE 16-16 Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways.

16.3 Regulation of the Citric Acid Cycle

- Pyruvate dehydrogenase complex of mammals
  - is inhibited by ATP, acetyl-CoA, NADH and fatty acids
  - is activated by AMP, CoA, NAD^+ and Ca^{2+} **(Fig. 16-19)**.
- The citric acid cycle is regulated at its three exergonic steps catalyzed by citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex.
- Citrate synthase
  - is inhibited by NADH, succinyl-CoA, citrate and ATP
  - is activated by ADP **(Fig. 16-19)**.
- Isocitrate dehydrogenase
  - is inhibited by ATP
  - is activated by Ca^{2+} and ADP **(Fig. 16-19)**.
- α-ketoglutarate dehydrogenase complex
  - is inhibited by succinyl-CoA and NADH
  - is activated by Ca^{2+} **(Fig. 16-19)**.

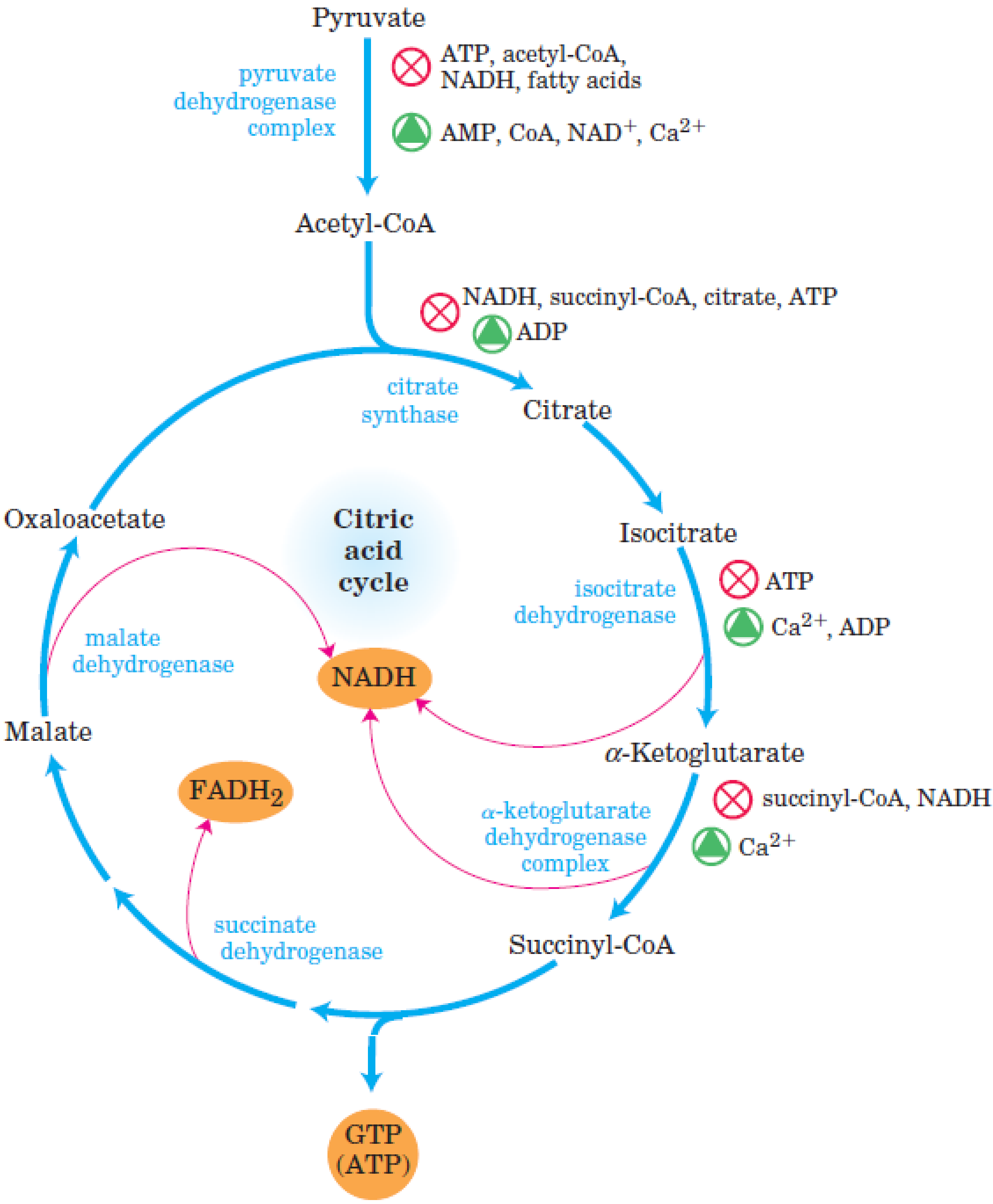


FIGURE 16-19 Regulation of metabolite flow from the PDH complex through the citric acid cycle in mammals.

16.4 The Glyoxylate Cycle

- Conversion of phosphoenolpyruvate to pyruvate and of pyruvate to acetyl-CoA are irreversible.

- Phosphoenolpyruvate can be synthesized from oxaloacetate in gluconeogenesis.
- A cell or organism is unable to convert fuels or metabolites that are degraded to acetate (fatty acids and certain amino acids) into carbohydrates.
- In many organisms other than vertebrates, the glyoxylate cycle serves as a mechanism for converting acetate to carbohydrate.
- In plants, the pathway takes place in glyoxysomes (specialized peroxisomes).
- The glyoxylate cycle produces four-carbon compounds from acetate (**Fig. 16-20)**.

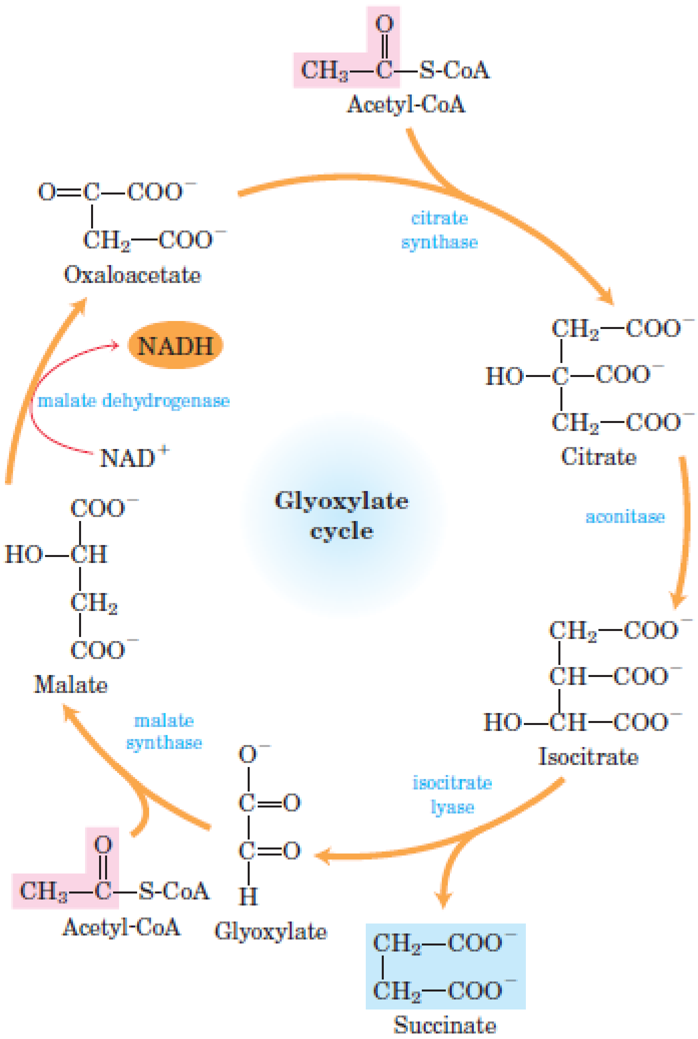


FIGURE 16–20 Glyoxylate cycle. The citrate synthase, aconitase, and malate dehydrogenase of the glyoxylate cycle are isozymes of the citric acid cycle enzymes; isocitrate lyase and malate synthase are unique to the glyoxylate cycle. Notice that two acetyl groups (pink) enter the cycle and four carbons leave as succinate (blue).

- Enzymes of the glyoxylate cycle catalyze the net conversion of acetate to succinate or other four-carbon intermediates of the citric acid cycle.
- Acetyl-CoA condenses with oxaloacetate to form citrate, and citrate is converted to isocitrate, exactly as in the citric acid cycle.
- The next step is not the breakdown of isocitrate by isocitrate dehydrogenase but the cleavage of isocitrate by **isocitrate lyase**, forming succinate and **glyoxylate**.
- The glyoxylate condenses with a second molecule of acetyl-CoA to yield malate by **malate synthase**.
- The malate is oxidized to oxaloacetate.
- Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule succinate, available for biosynthetic purposes.

$$2\ \text{Acetyl-CoA} + NAD^+ \longrightarrow \text{succinate} + 2\ \text{CoA} + NADH + H^+$$

- There is a relationship between the glyoxylate and citric acid cycle (**Fig. 16-24)**.
  - Succinate may be converted through fumarate and malate into oxaloacetate.
  - Oxaloacetate is converted to phosphoenolpyruvate and thus to glucose by gluconeogenesis.
- Vertebrates do not have the enzymes specific to the glyoxylate cycle.
  - They cannot bring about the net synthesis of glucose from (acetyl-CoA) lipids.
- Germinating seeds can convert the carbon of stored lipids into glucose.

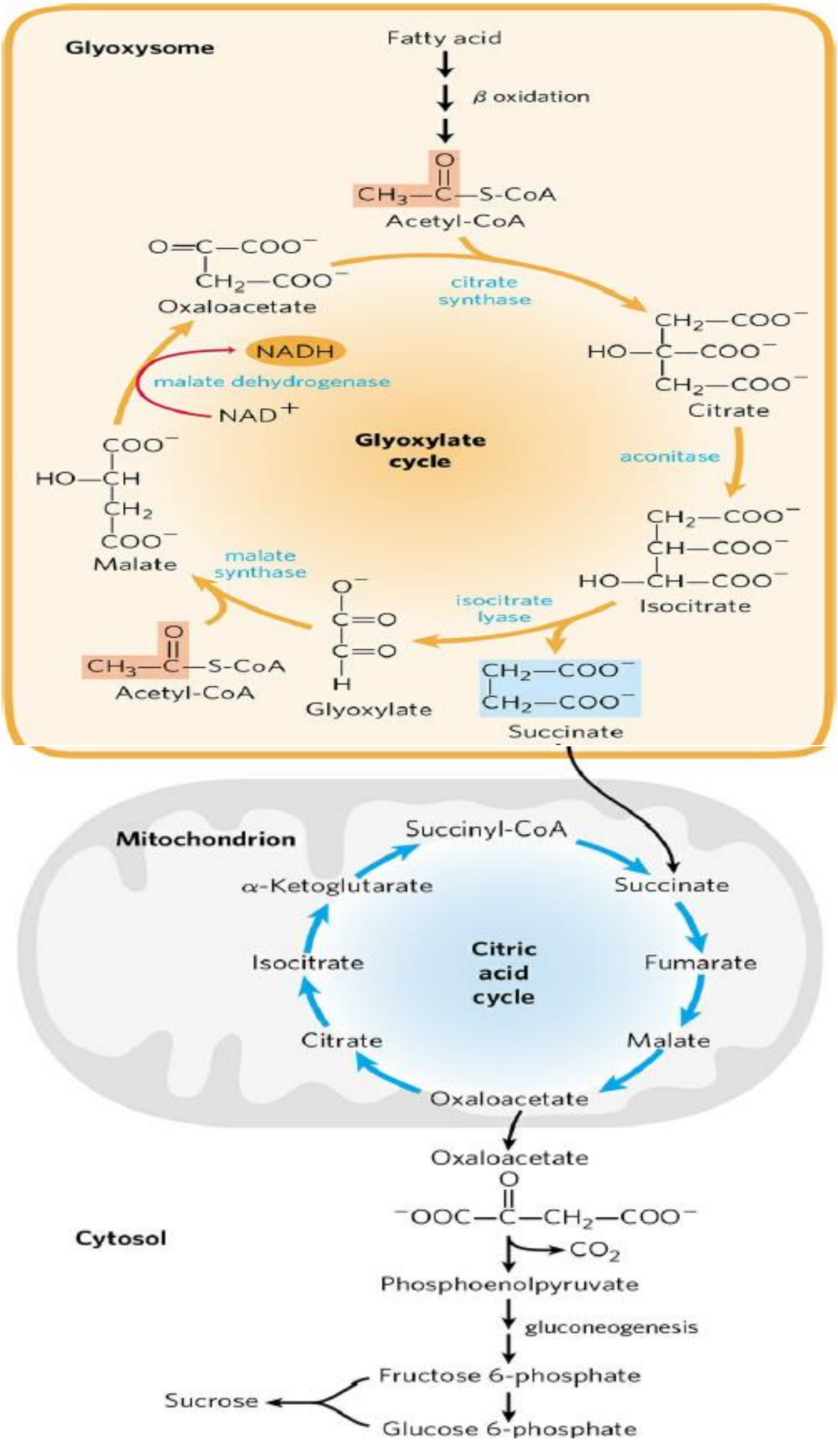


FIGURE 16-24 Relationship between the glyoxylate and citric acid cycles.