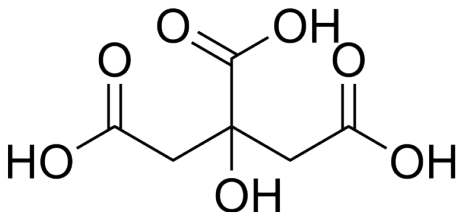
Main source used for taking this note: Essential Biochemistry - Charlotte Pratt & Kathleen Cornely

***Mitochondrial ETC Complexes:**

- **Complex I**: NADH dehydrogenase or NADH:ubiquinone oxidoreductase - initiating ETC by oxidizing NADH, transferring electrons to ubiquinone.
- **Complex II:** succinate dehydrogenase.
- **Complex III:** ubiquinol:cytochrome c oxidoreductase – transferring electrons from ubiquinol to cytochrome c.
- **Complex IV:** cytochrome c oxidase - final electron transfer to oxygen, forming water.
- **Complex V**: ATP synthase/F1F0-ATPase.



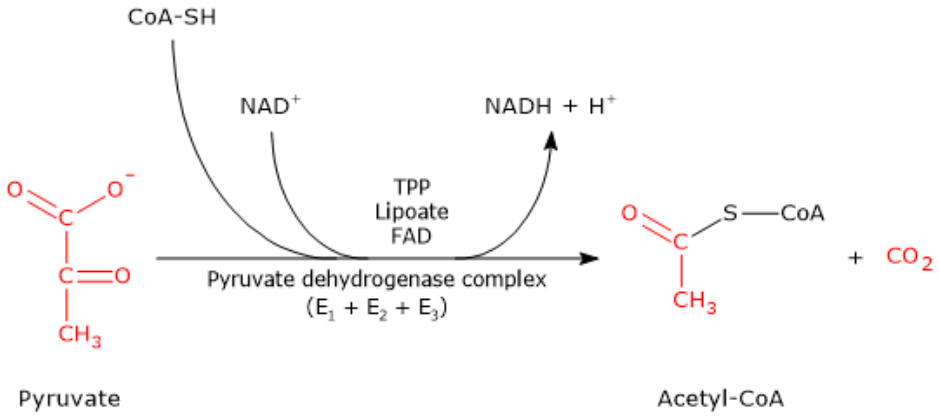
***Citric acid** is an organic compound with the chemical formula HO-$C(CH_2CO_2H)_2$. It occurs naturally in citrus fruits. In biochemistry, it is an intermediate in the citric acid cycle, which occurs in the metabolism of all aerobic organisms.

Input to TCA cycle: carbon atoms derived from amino acids, fatty acids, or carbohydrates. 8 reactions are involved.

PYRUVATE DEHYDROGENASE REACTION (RIGHT BEFORE TCA CYCLE)

The pyruvate dehydrogenase complex (~4600kDa) includes three types of enzymes (E1, E2, E3, containing 60 protein subunits) that collectively remove a carboxylate group from pyruvate (3-carbon) and produce acetyl-CoA and NADH. (Transferring acetyl unit to coenzyme A)

$$\text{pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{CO}_2 + \text{NADH}$$



Location: Mitochondrial matrix
Substrate: Pyruvate (3-carbon compound)
Steps:

- Decarboxylation: Removal of a carboxyl group from pyruvate with the help of thiamine pyrophosphate (TPP), a vitamin B1 derivative.
- Oxidation: The remaining two-carbon fragment is oxidized, transferring the energy to a swinging arm called lipoamide.
- Trans-acetylation: The acetyl group is transferred from lipoamide to CoA, forming acetyl-CoA.
- Reduction: Electrons from the oxidation are used to reduce NAD+ to NADH, which fuels the electron transport chain and ATP production.

Product: Acetyl-CoA
Significance: Connects glycolysis and citric acid cycle in cellular respiration.
Result: Acetyl-CoA enters the citric acid cycle for further energy extraction.

THE EIGHT REACTIONS OF TCA CYCLE

With the entry of **1 acetyl group** into the citric acid cycle,

- **2 fully oxidized CO2 molecules** are generated, indicating the loss of **4 pairs of electrons**.
- These electrons are then conveyed to **3 NAD+ molecules** and **1 ubiquinone (Q),**
- Resulting in the production of **3 NADH and 1 QH2**.

Consequently, the summarized equation for the citric acid cycle is as follows:

$$\text{acetyl-CoA} + \text{GDP} + P_i + 3\,\text{NAD}^+ + \text{Q} \rightarrow 2\,CO_2 + \text{CoA} + \text{GTP} + 3\,\text{NADH} + QH_2$$

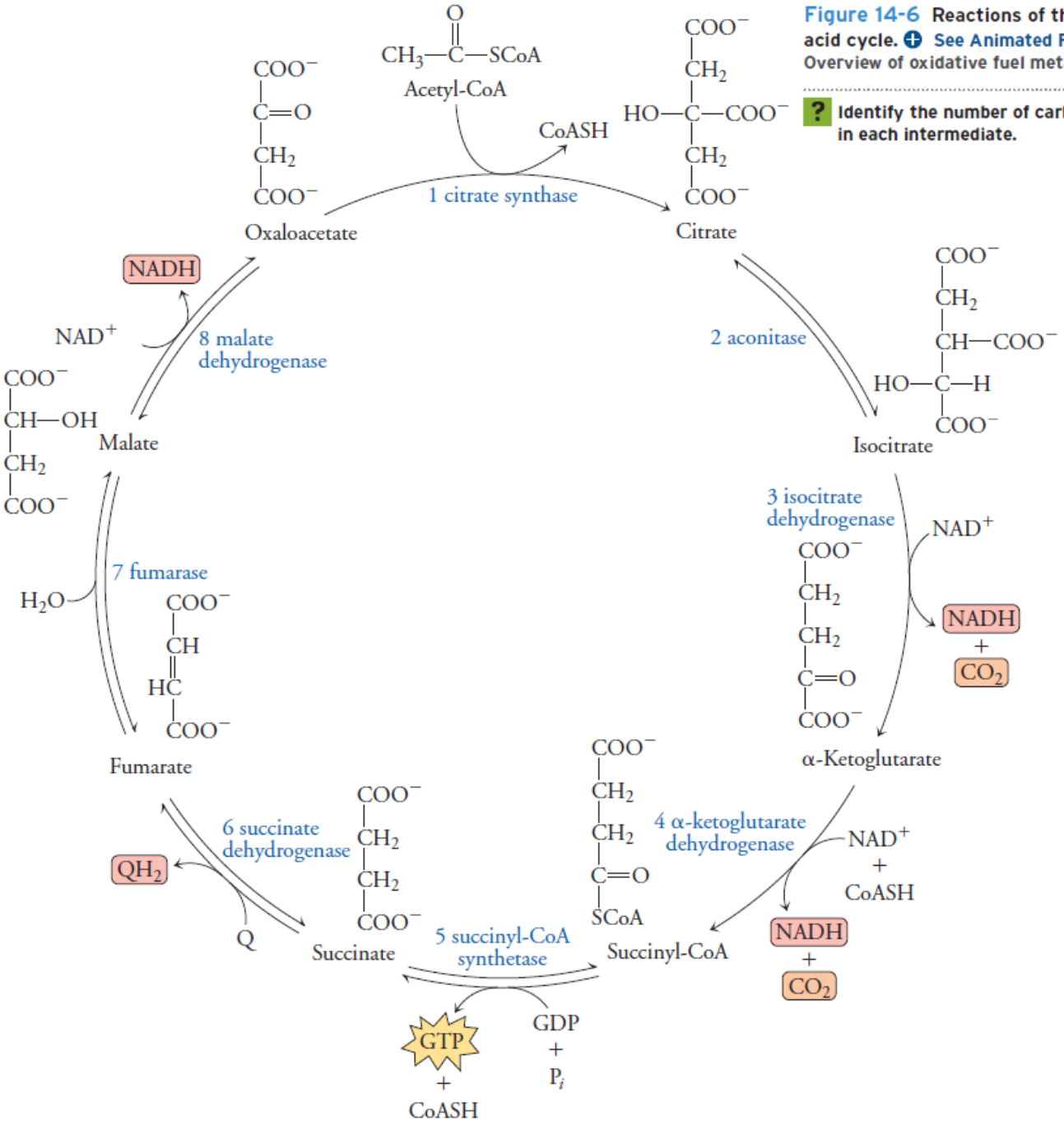
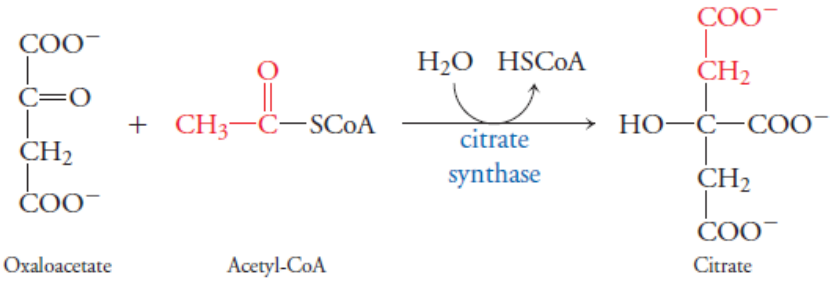


Figure 14-6 Reactions of the citric acid cycle. See Animated Figure. Overview of oxidative fuel metabolism.

? Identify the number of carbon atoms in each intermediate.

1. Citrate Synthase (Derived from its catalytic activity in forming citrate):

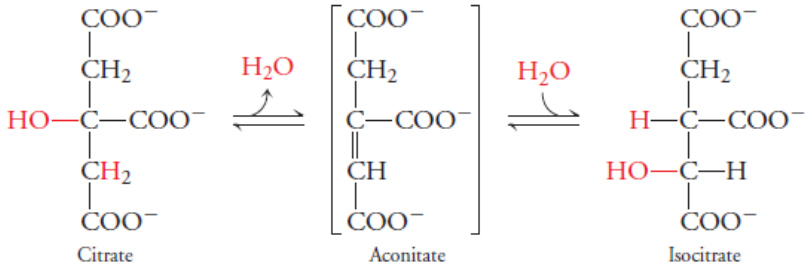
- Location: Mitochondrial matrix
- Reaction: **Acetyl-CoA** ($CH_3COSCoA$) + **Oxaloacetate** ($HO_2CCCH_2CO_2H$) → **Citrate** ($HO_2CCH_2CH(OH)CO_2H$) + CoA (HSCoA)



- Is the entry point for acetyl-CoA. Condenses a two-carbon acetyl group with a four-carbon oxaloacetate to form a six-carbon citrate.

2. Aconitase (Named after aconitic acid, an intermediate product):

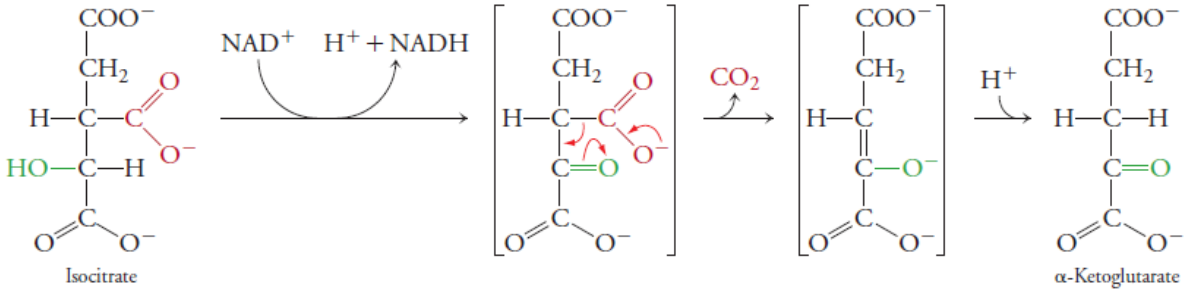
- Location: Mitochondrial matrix
- Reaction: Citrate ($HO_2CCH_2CH(OH)CO_2H$) → cis-Aconitate ($HO_2CCH=CHCO_2H$) → iso-Citrate ($HO_2CCH(OH)CH_2CO_2H$)



- Isomerizes citrate into two stereoisomers (cis-aconitate and iso-citrate) using iron-sulfur clusters. Regulates the cycle's rate by controlling citrate availability.

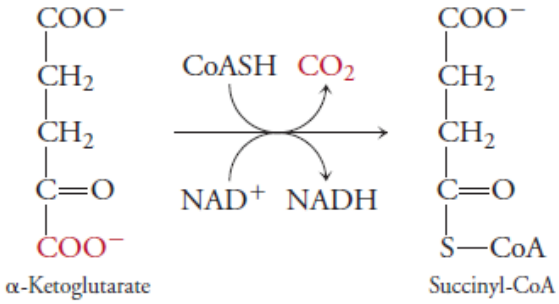
3. Isocitrate Dehydrogenase (removing hydrogen from iso-citrate):

- Location: Mitochondrial matrix
- Reaction: **iso-Citrate** ($HO_2CCH(OH)CH_2CO_2H$) + **NAD+** → **α-Ketoglutarate** ($HO_2CCH_2COCO_2H$) + CO_2 + **NADH**
- First decarboxylation step, generating a five-carbon α-ketoglutarate, releasing CO_2, and producing NADH, a high-energy electron carrier.



4. α-Ketoglutarate Dehydrogenase:

- Location: Mitochondrial matrix, **bound to inner membrane (part of ETC complex I)**
- Reaction: **α-Ketoglutarate** ($HO_2CCH_2COCO_2H$) + CoA (HSCoA) + **NAD+** + **FAD+** → **Succinyl-CoA** (CH_2CO-SCoA) + CO_2 + **NADH** + **$FADH_2$**



- Second decarboxylation and major ATP production step. Occurs within the ETC complex I, transferring electrons to NADH and $FADH_2$ for ATP generation via oxidative phosphorylation.
- The free energy of oxidizing α-ketoglutarate is conserved in the formation of the **thioester succinyl-CoA**.

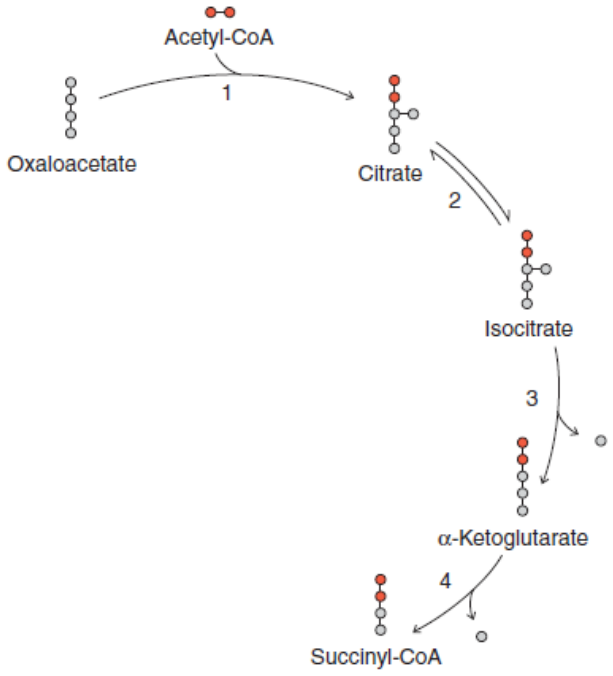


Figure 14-10 Fates of carbon atoms in the citric acid cycle. The two carbon atoms that are lost as CO_2 in the reactions catalyzed by isocitrate dehydrogenase (step 3) and α-ketoglutarate dehydrogenase (step 4) are not the same carbons that entered the cycle as acetyl-CoA (red). The acetyl carbons become part of oxaloacetate and are lost in subsequent rounds of the cycle.

5. Succinyl-CoA Synthetase (Combines substrate-level phosphorylation with CoA transfer):

- Reaction is reversible. The enzyme is named for the **reverse** reaction.
- Location: Mitochondrial matrix
- Reaction: **Succinyl-CoA** (CH_2CO-SCoA) + GDP/ADP + Pi → **Succinate** ($HOOCCH_2CH_2CO_2H$) + GTP/ATP + CoA (HSCoA)
- **Substrate-level phosphorylation, directly** generating one ATP through the **transfer of a phosphate group** to GDP/ADP (to distinguish it from oxidative phosphorylation and photophosphorylation).

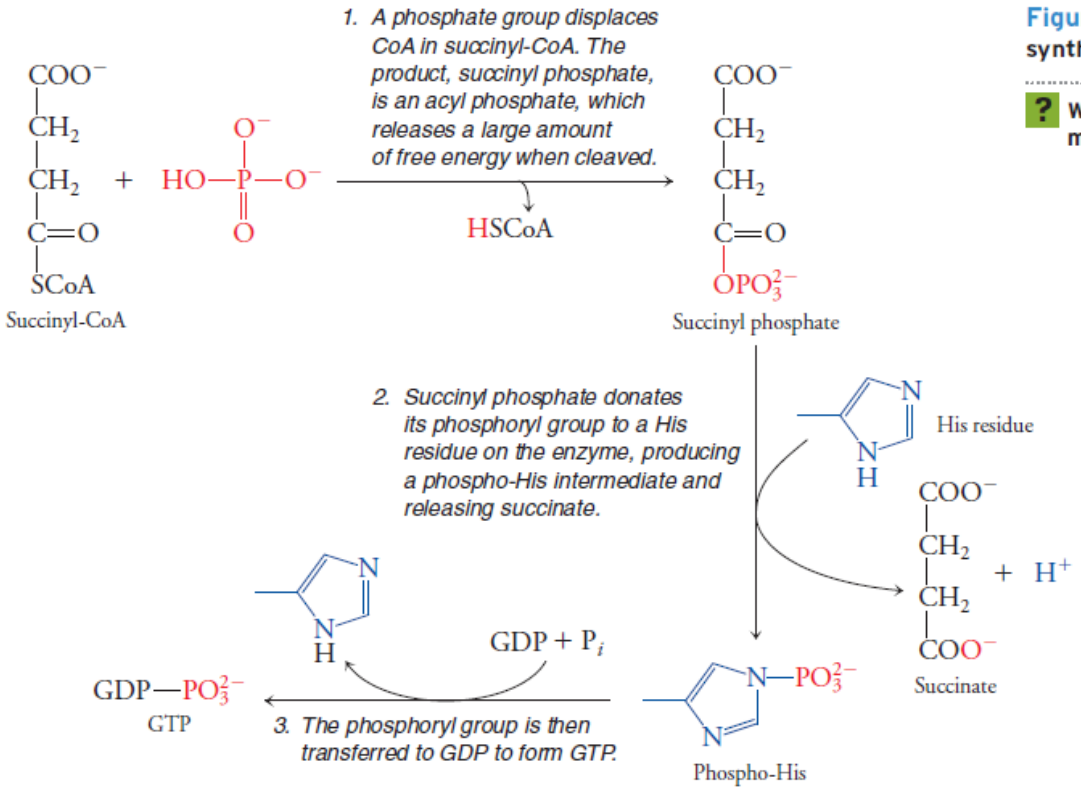
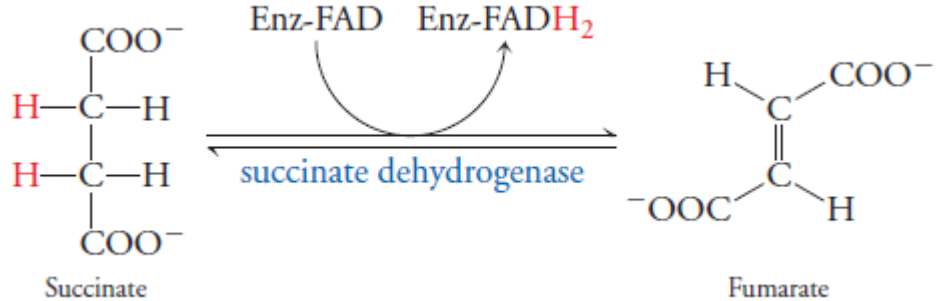


Figure 14-11 The succinyl-CoA synthetase reaction.

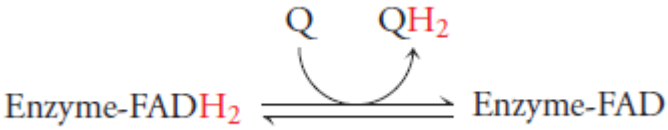
? What is the fate of the free CoA molecule?

6. Succinate Dehydrogenase (SDH – complex II, **other 7/8** TCA enzymes are soluble in matrix):

- Location: Mitochondrial inner membrane **(part of ETC complex II)**
- Reaction: Succinate ($HOOCCH_2CH_2CO_2H$) + **FAD+** → **Fumarate** ($HOOCCH{=}CHCO_2H$) + **$FADH_2$**
- Reversible between succinate and fumarate; require an FAD prosthetic group, which is reduced to FADH2.

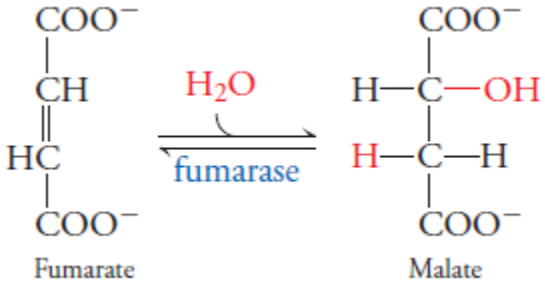


(To regenerate the enzyme,) the FADH2 group is reoxidized by the lipid-soluble electron carrier ubiquinone (Q). (Uniquinol: QH2)



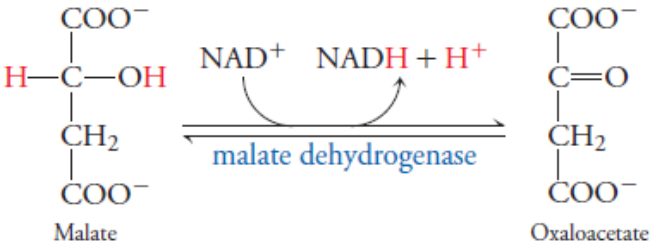
7. Fumarase (also fumarate hydratase) catalyzes a hydration reaction

- Location: Mitochondrial matrix
- Reaction: **Fumarate** ($HOOCCH=CHCO_2H$) → **L-Malate** ($HOOCCH_2CH(OH)CO_2H$)
- Hydrates fumarate (hydration of a double bond) to form malate, an isomerization step without energy input or output. Connects the TCA cycle to gluconeogenesis and other metabolic pathways.



8. Malate Dehydrogenase (Removes hydrogen from malate and regenerates oxaloacetate):

- Location: Mitochondrial matrix
- Reaction: L-Malate ($HOOCCH_2CH(OH)CO_2H$) + NAD+ → Oxaloacetate ($HO_2CCCH_2CO_2H$) + CO_2 + NADH
- NAD+ dependent oxidation reaction



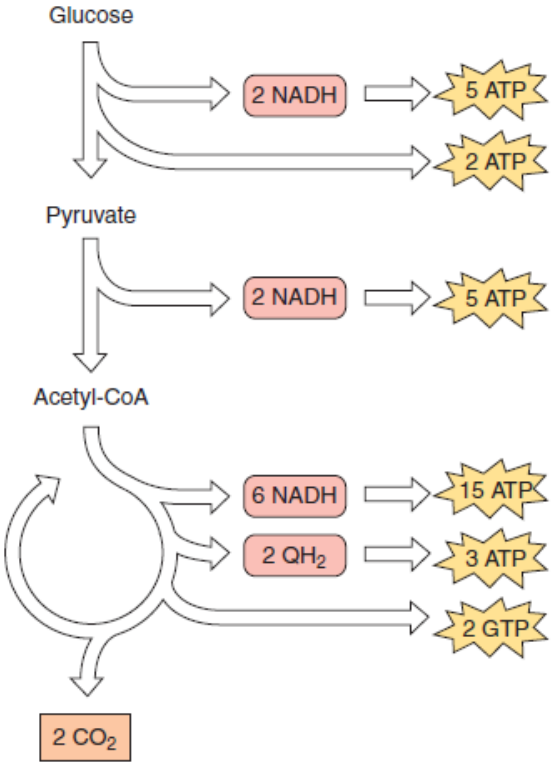
Summary/Other points:

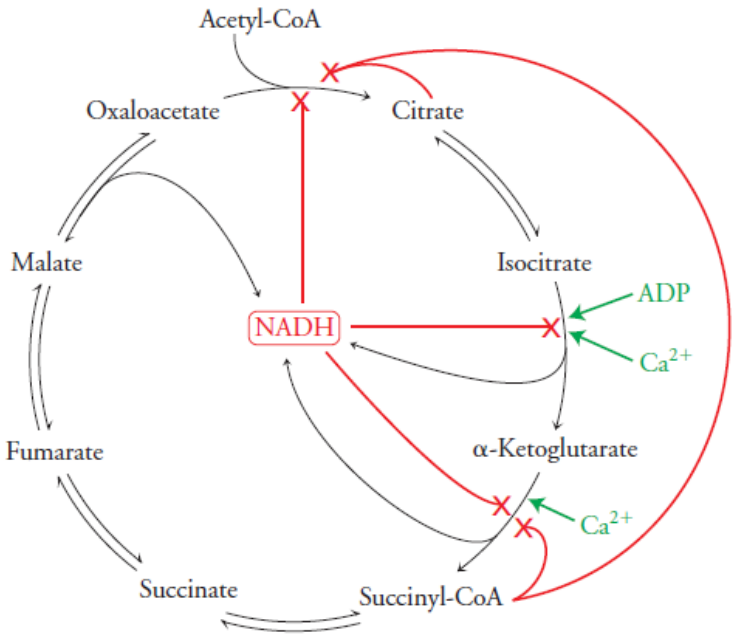
- The entire TCA pathway acts in a catalytic fashion to dispose of carbon atoms derived from amino acids, carbohydrates, and fatty acids.
- Muscle Energy Production:
    - Anaerobic operation: Produces only **2 ATP per glucose.**

- Aerobic conditions: Fully active citric acid cycle leads to **approximately 32 ATP equivalents** from a single glucose molecule.

Regulation of Citric Acid Cycle: Regulated at three metabolically irreversible steps:

- Citrate synthase (Reaction 1).
- Isocitrate dehydrogenase (Reaction 3).
- Alpha-ketoglutarate dehydrogenase (Reaction 4).





Regulation of TCA cycle.
Red: inhibition, Green: Activation

Pyruvate Entry:

- Pyruvate, the **end product of glycolysis**, undergoes an oxidative decarboxylation by the **pyruvate dehydrogenase complex.**
- This reaction yields **acetyl-CoA,** a two-carbon fuel for the citric acid cycle, along with **carbon dioxide** and **NADH**, an electron carrier molecule.

Citric Acid Cycle:

- The citric acid cycle, a **multistep enzymatic pathway**, functions as a central metabolic hub.
- It **condenses acetyl-CoA with oxaloacetate** to form citrate, initiating a series of oxidative transformations.
- These transformations extract the acetyl-CoA's two carbons, releasing them as two molecules of carbon dioxide.
- Simultaneously, electrons and protons are captured by **NAD+ and FAD**+, generating **NADH and FADH2**, high-energy electron carriers.

Electron Transfer and ATP Production:

- Electrons and protons from NADH and FADH2 enter the **electron transport chain**, fueling oxidative phosphorylation.
- This process couples electron transfer with ATP synthesis, generating the majority of cellular ATP.

Beyond Energy Generation:

- The citric acid cycle is not solely an energy-generating pathway.
- Intermediates serve as vital precursors for a diverse array of biomolecules, including amino acids, nucleotides, and heme groups.

Catalytic Regulation:

- The citric acid cycle operates as a **catalytic system**, with its rate dependent on the concentration of its components.
- Increased levels of intermediates and cofactors enhance the cycle's flux, amplifying its metabolic output.

Exercise and Cycle Activity:

- During exercise, skeletal muscle exhibits a **dramatic upregulation of the citric acid cycle**.
- Intermediates can increase 3-4 fold, while cycle flux can surge up to 100-fold due to elevated activities of key enzymes.
- This enhanced activity likely accommodates the increased pyruvate production associated with elevated glycolytic rates during exercise.

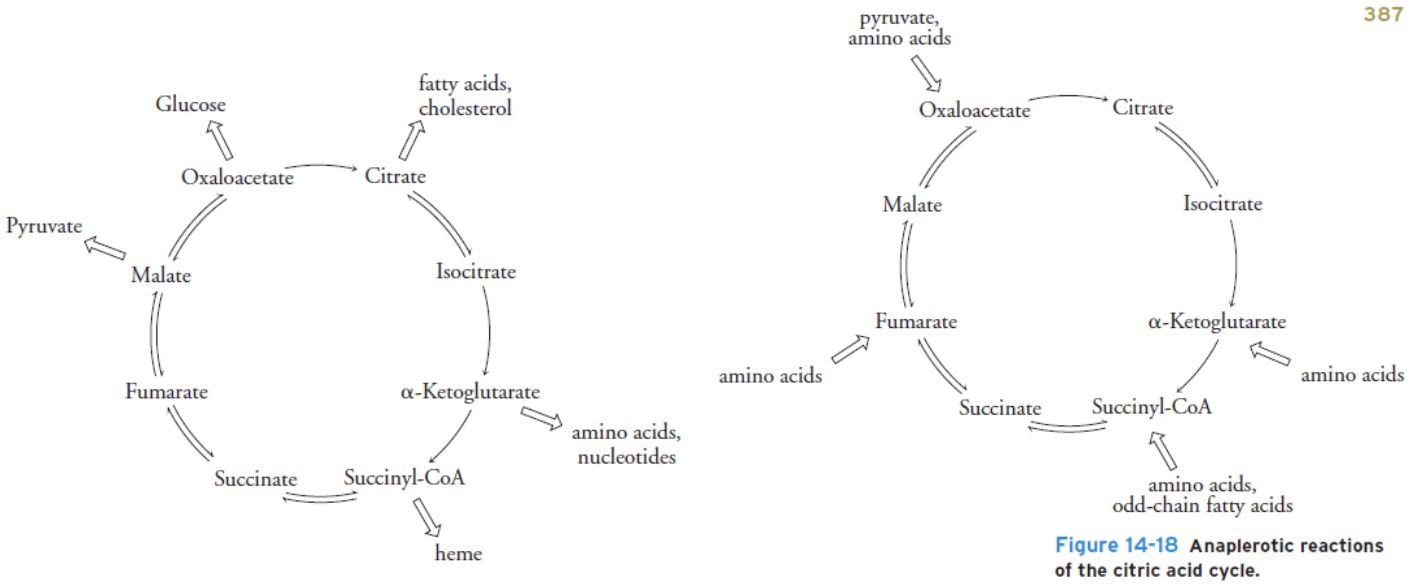


Figure 14-18 Anaplerotic reactions of the citric acid cycle.