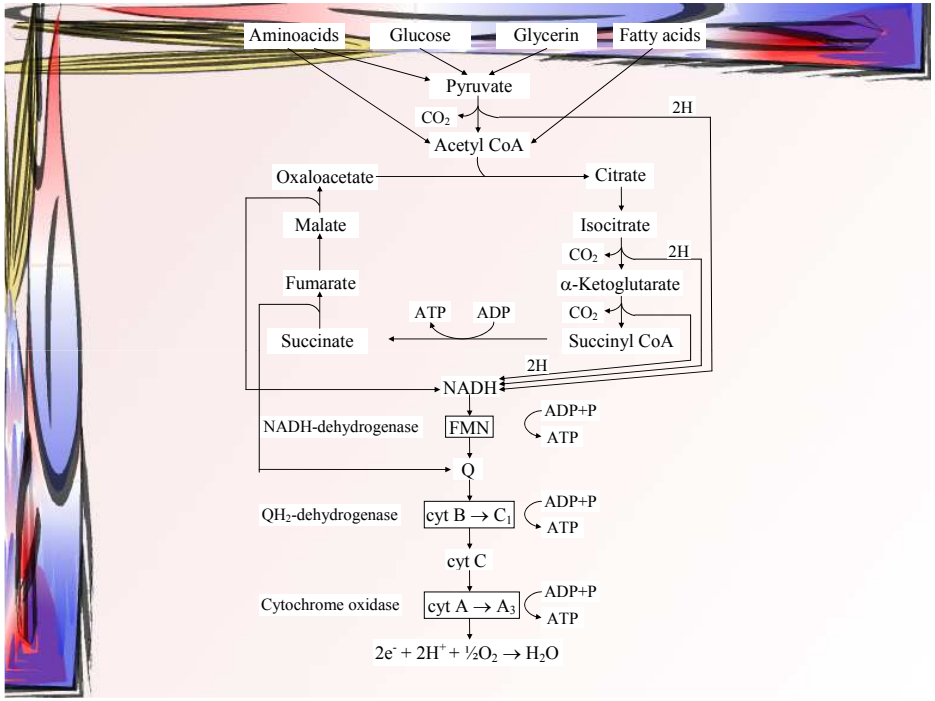
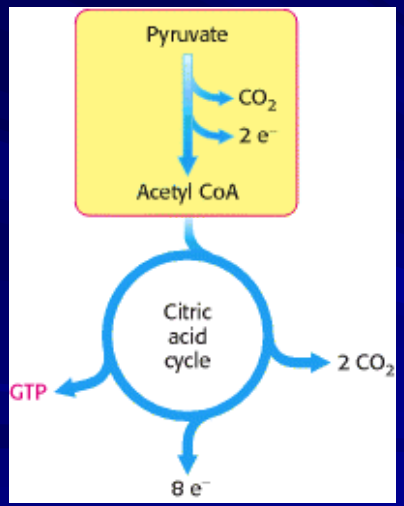
THE PYRUVATE DEHYDROGENASE (PDH) ENZYME COMPLEX

- **Function. PDH links glycolysis and the citric acid cycle.** PDH oxidizes pyruvate, the product of glycolysis, to CO_2 and acetyl coenzyme A (acetyl CoA), which is the substrate for the citric acid cycle.
- **Location.** PDH is located within the **mitochondrial matrix**.



THE PYRUVATE DEHYDROGENASE



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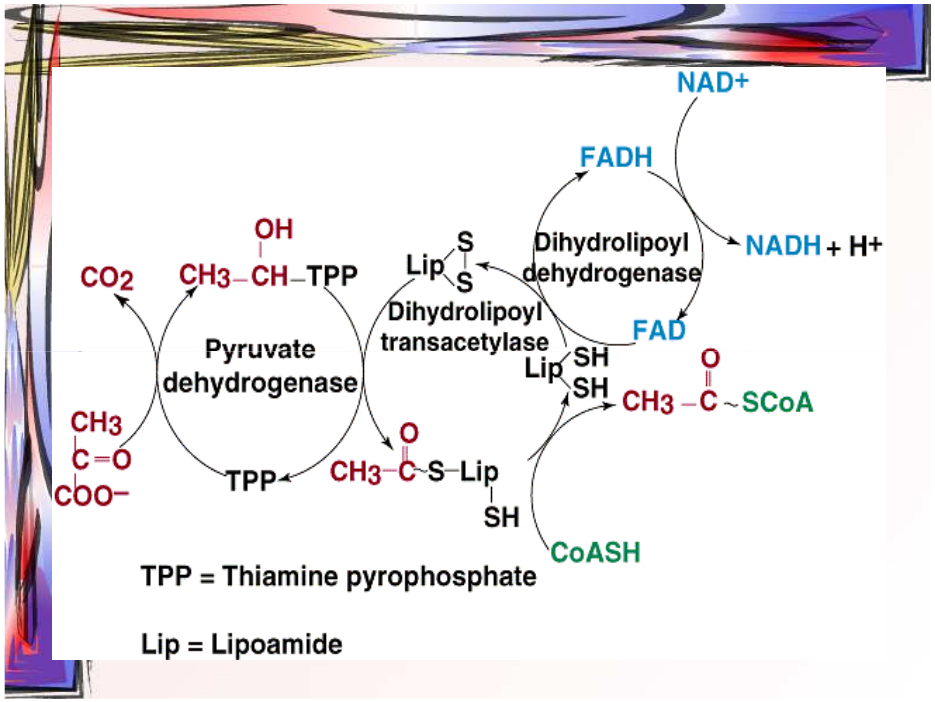
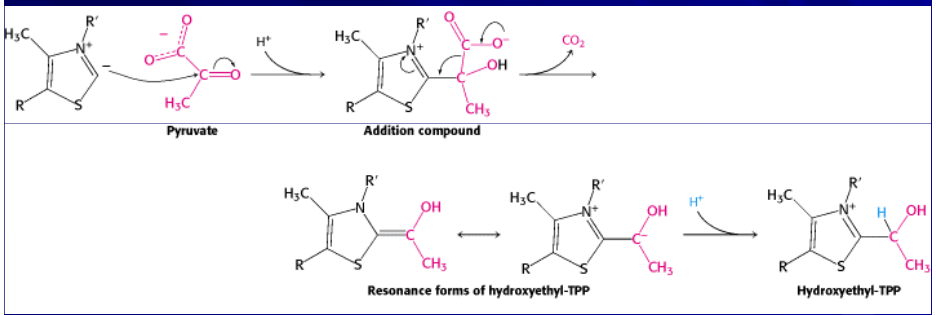
Four distinct enzymatic activities of PDH enzyme complex



Step 1: Pyruvate decarboxylase activity

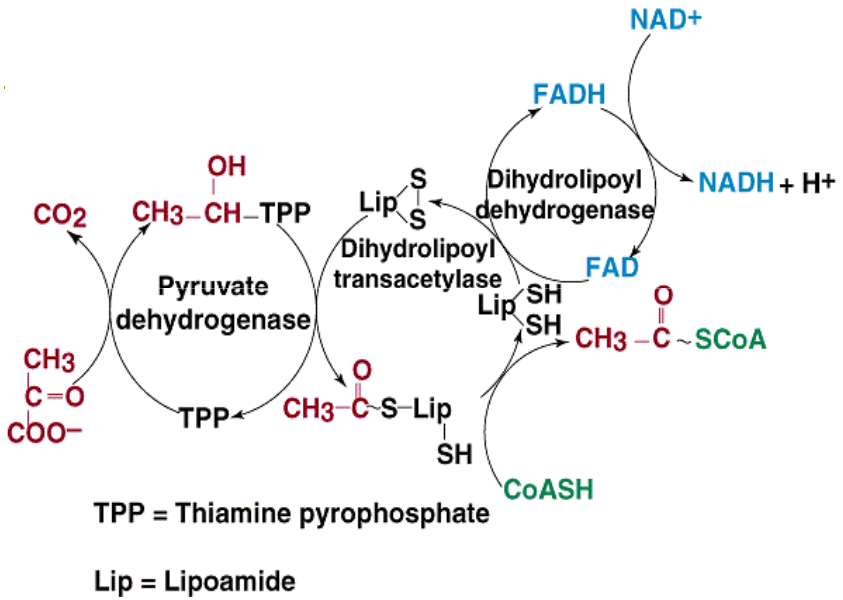
- This reaction is catalyzed by the E_1 subunit of PDH.
- The cofactor thiamine pyrophosphate (TPP) is required. Carbon dioxide is formed, and the substrate becomes covalently bound to TPP.

Mechanism of the Decarboxylation Reaction of E_1 , THe Pyruvate Dehydrogenase Component of the Pyruvate Dehydrogenese Complex



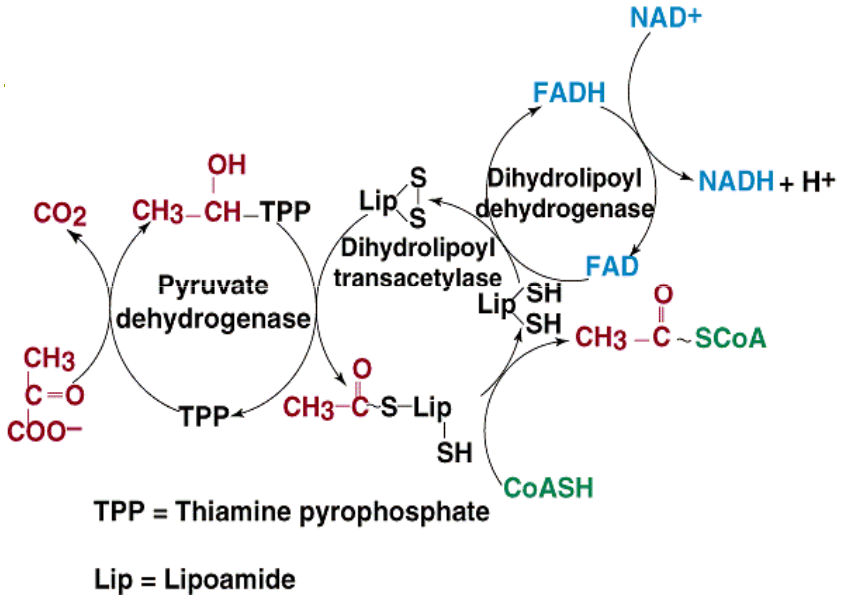
Step 2: Transfer of the two-carbon unit from E_1 to E_2

- **E_2 requires the coenzyme lipoic acid,** which is covalently attached to a lysine residue of the protein. This covalently bound form of lipoic acid is called **lipoamide.**
- In this step, the substrate is simultaneously oxidized to an acetyl group and transferred to the oxidized form of lipoamide. TPP is regenerated in this step.

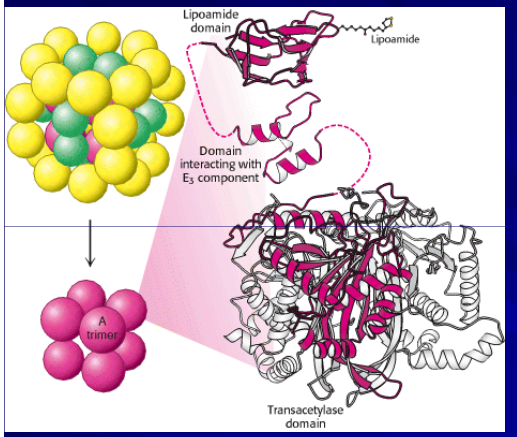


Step 3: Dihydrolipoyl transacetylase activity

- This reaction is catalyzed by the **E_2 subunit** of PDH.
- The reaction involves transfer of the acetyl group from lipoamide to coenzyme A to form acetyl CoA. Lipoamide is in the reduced state after this transfer.

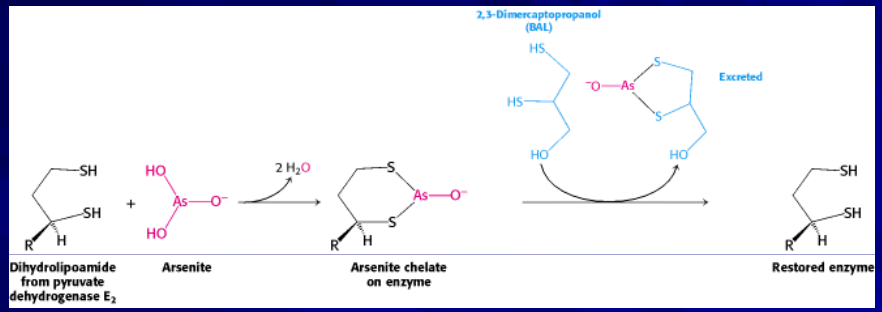


Structure of the Transacetylase (E_2) core



- Each red ball represents a trimer of three E_2 subunits.
- Each subunit consists of three domains:
  - a lipoamide-binding domain,
  - a small domain for interaction with E_3,
  - a large transacetylase catalytic domain.
- All three subunits of the transacetylase domain are shown in the ribbon representation, with one depicted in red

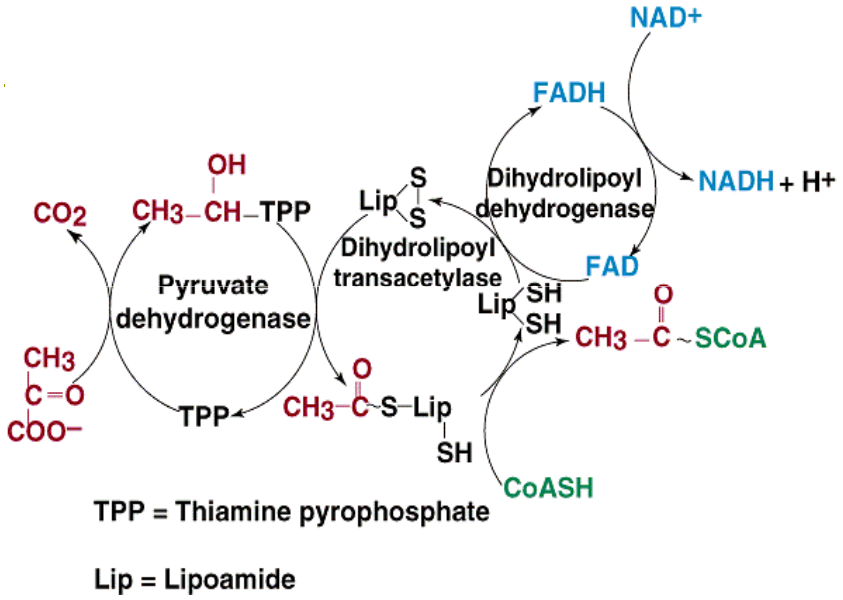
Arsenite Poisoning



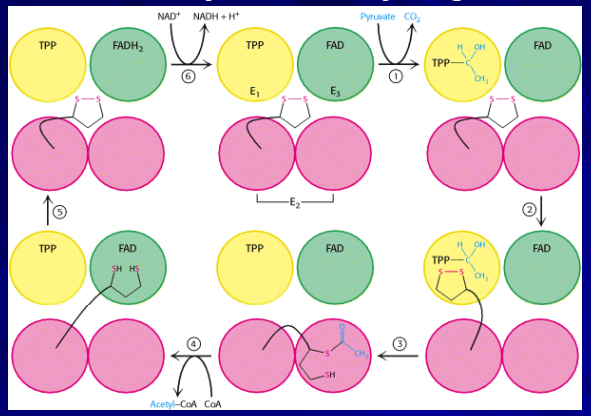
- Arsenite inhibits the pyruvate dehydrogenase complex by inactivating the dihydrolipoamide component of the transacetylase
- Some sulfhydryl reagents, such as 2,3-dimercaptoethanol, relieve the inhibition by forming a complex with the arsenite that can be excreted

Step 4: Dihydrolipoyl dehydrogenase activity

- This reaction is catalyzed by the E_3 subunit of PDH.
- Tightly bound flavin adenine dinucleotide (FAD) is a cofactor for E_3 in this reaction. FAD reoxidizes lipoamide and is reduced to $FADH_2$.

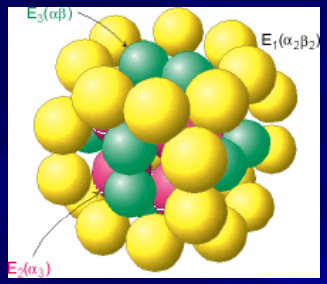


Reactions of the Pyruvate Dehydrogenase Complex

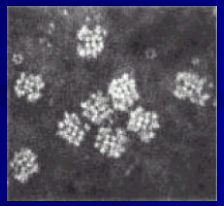


- At the top (center), the enzyme (represented by a yellow, a blue, and two red spheres) is unmodified and ready for a catalytic cycle.
- (1) Pyruvate is decarboxylated to form the hydroxyethyl TPP.
- (2) The dihydrolipoyl arm of E_2 moves into the active site of E_1.
- (3) E_1 catalyzes the transfer of the two-carbon group to the dihydrolipoyl group to form the acetyl-lipoyl complex.
- (4) E_2 catalyzes the transfer of the acetyl moiety to CoA to form the product acetyl CoA. The disulfhydryl lipoyl arm then swings to the active site of E_3. E_3 catalyzes
- (5) the reduction of the lipoic acid and
- (6) the transfer of the protons and electrons to NAD+ to complete the reaction cycle.

Schematic Representation of the Pyruvate Dehydrogenase Complex



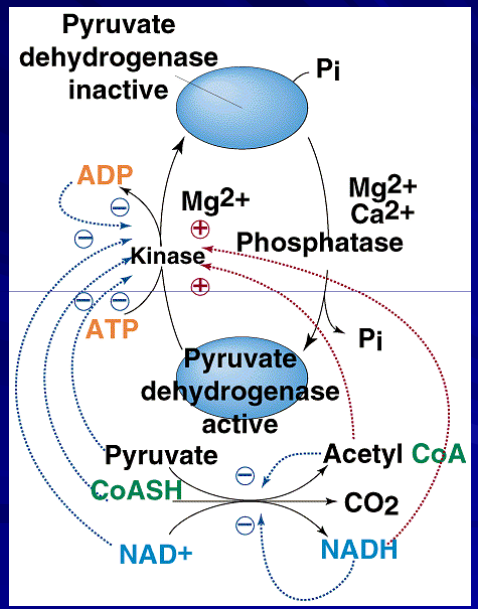
- The transacetylase core (E_2) is shown in red,
- The pyruvate dehydrogenase component (E_1) in yellow,
- The dihydrolipoyl dehydrogenase (E_3) in green.



- ***Electron Micrograph of the Pyruvate Dehydrogenase Complex From E. coli***

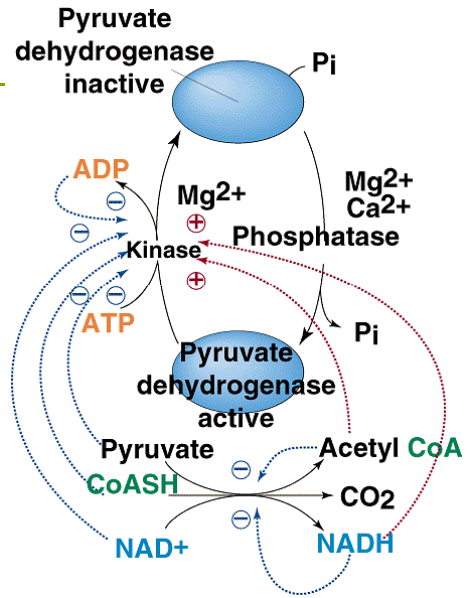
Mechanisms of PDH regulation

- **Product inhibition.** Both acetyl CoA and NADH inhibit PDH.
- **Availability of substrates.** Adequate concentrations of CoA and NAD^+ must be present.
- **Covalent modification**



Covalent modification in PDH regulation

- **PDH exists in two forms:**
  - Inactive, phosphorylated
  - Active, dephosphorylated
- **A protein kinase** that is tightly bound to the PDH complex yields the inactive form.
  - The kinase, which depends on magnesium ion (Mg^{2+}) and ATP, phosphorylates a serine residue PDH.
  - The protein kinase reaction is **stimulated by acetyl CoA and NADH.**
  - The protein kinase reaction is **inhibited by free CoA (CoASH), NAD+, and pyruvate.**
- **The complex is reactivated** by dephosphorylation by a **phosphoprotein phosphatase.** It is activated by increasing calcium concentrations in the mitochondria, which occur when ATP levels are low.



Hormonal regulation

- **Insulin** can activate PDH in adipose tissue.
- **Catecholamines** can activate PDH in cardiac muscle.

The PDH reaction is biologically irreversible. Thus, **it is not possible to make pyruvate from acetyl CoA.**

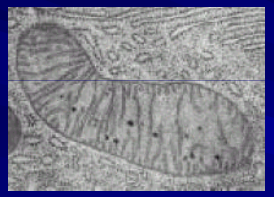
Genetic defects in PDH

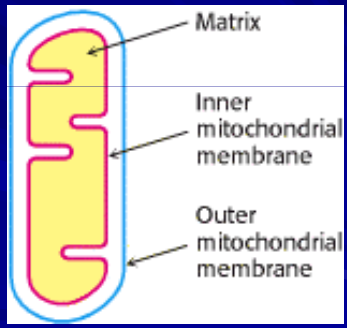
- **Results.** A defect in any of the protein subunits of PDH can result in a decrease or complete loss of activity. Severe cases are usually fatal.
- **Symptoms** include:
  - Lactic acidosis
  - Neurologic disorder
- **Treatments**
  - Administration of large doses of thiamine may be effective for certain defects in E_1 that reduce the affinity of the enzyme for TPP.
  - Administration of large doses of lipoic acid may be effective for defects in E_2 with reduced affinity for that compound.
  - A ketogenic diet high in fat and low in carbohydrate helps to lower the levels of pyruvate and lactate, which is formed from the excess pyruvate.

OVERVIEW OF THE CITRIC ACID CYCLE

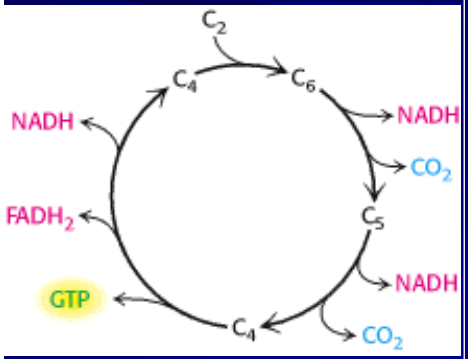
Description. The citric acid cycle (also known as the tricarboxylic acid cycle and the Krebs cycle) is a series of enzymatically catalyzed reactions that form a common pathway for the **final oxidation of all metabolic fuels** (i.e., carbohydrates, free fatty acids, ketone bodies, amino acids), which are catabolized to the substrate of the citric acid cycle (acetyl CoA)

Location. These reactions occur within the **mitochondrial matrix**



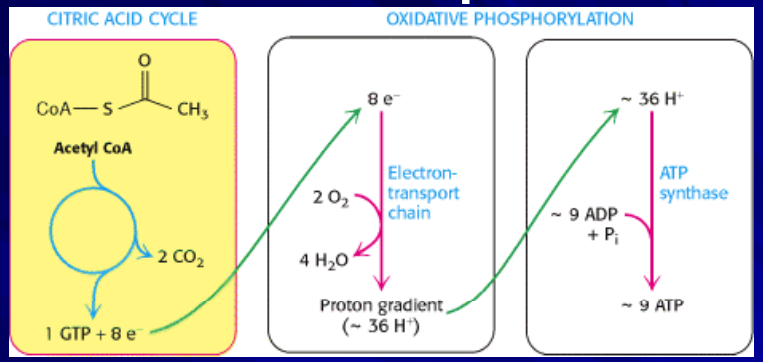


Functions



- The citric acid cycle is involved in both anabolic and catabolic processes.
  - **Anabolic reactions.** The intermediates of the citric acid cycle are used as precursors in the **biosynthesis** of many compounds.
  - **Catabolic reactions.** The cycle provides a means for the **degradation** of two-carbon acetyl residues, which are derived from carbohydrates, fatty acids, and amino acids.
- The citric acid cycle **provides much of the energy for respiration.** Electrons that are generated from the action of this cycle are transferred to the electron transport chain and used in the process of oxidative phosphorylation to generate ATP

Cellular Respiration



- **The citric acid cycle constitutes the first stage in cellular respiration, the removal of high-energy electrons from carbon fuels (left).**
- **These electrons reduce O_2 to generate a proton gradient (middle), which is used to synthesize ATP (right).**
- **The reduction of O_2 and the synthesis of ATP constitute oxidative phosphorylation**

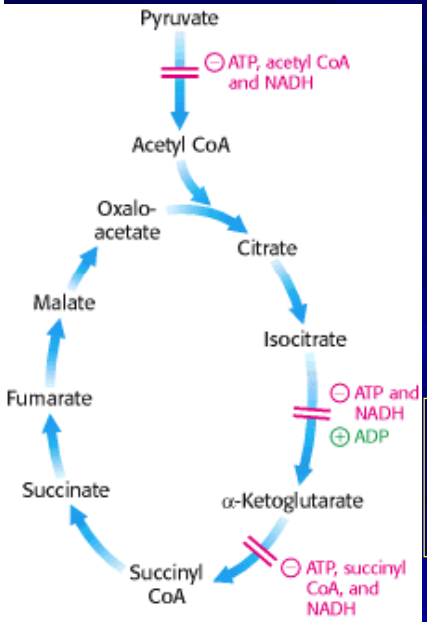
Stoichiometry

The net reaction of the citric acid cycle is:

Acetyl CoA + $3NAD^+$ + FAD + GDP + P_i + $2H_2O$ →
→ $2CO_2$ + 3NADH + $FADH_2$ + GTP + $2H^+$ + CoA

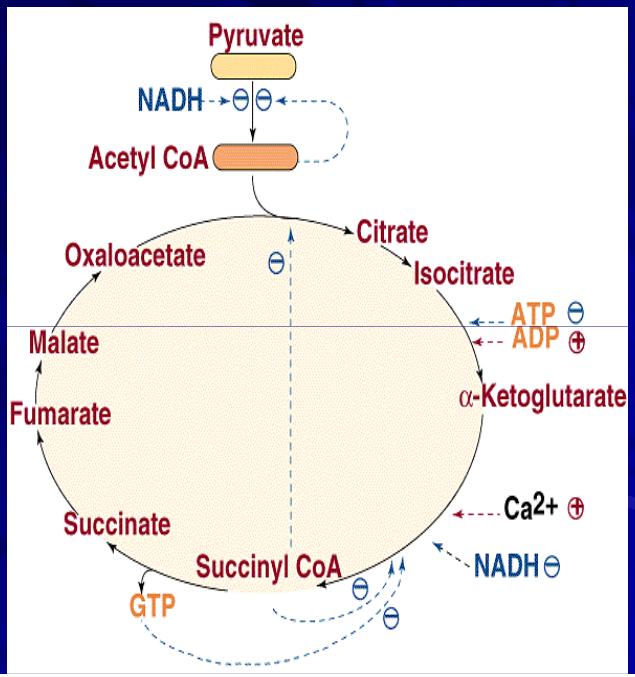
- Two carbon atoms enter the cycle as acetyl CoA and leave in the form of carbon dioxide.
- Four pairs of electrons are removed from the substrate; three pairs leave in the form of NADH, and one pair leaves as $FADH_2$.
- One high-energy phosphate bond is generated in the form of guanosine triphosphate (GTP).
- Although intermediates of the citric acid cycle may be interconverted, the cycle does not consume or produce solely from acetyl CoA any intermediate of the cycle.

Regulation

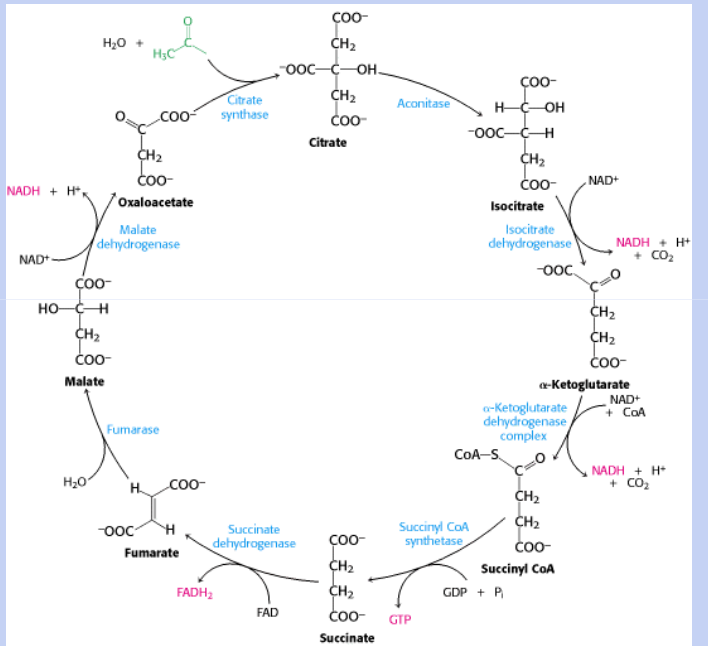


- There is no single enzyme of the citric acid cycle that serves as a point of regulation. Instead, multiple enzymes are allosterically regulated by the level of ATP and NADH, which reflects the energy state of the cell. This type of regulation by ATP levels is referred to as **respiratory control**

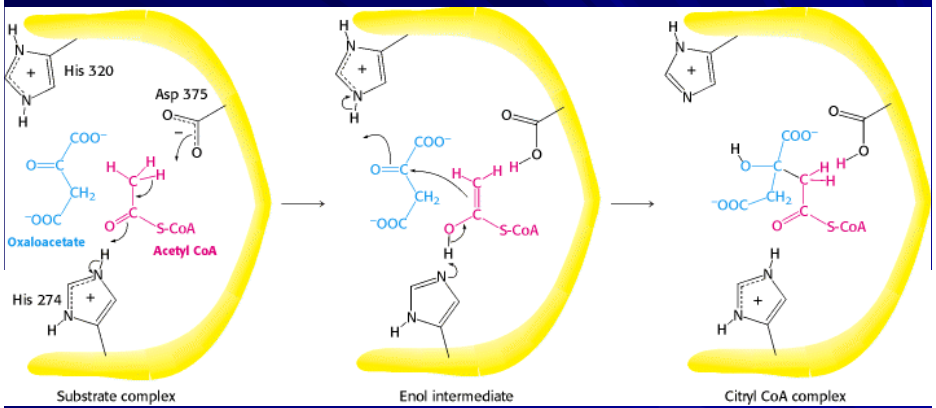
The key control points are the enzymes:
- **isocitrate dehydrogenase**
- **α-ketoglutarate dehydrogenase**



REACTIONS OF THE CITRIC ACID CYCLE



Acetyl CoA plus oxaloacetate to citrate and coenzyme A

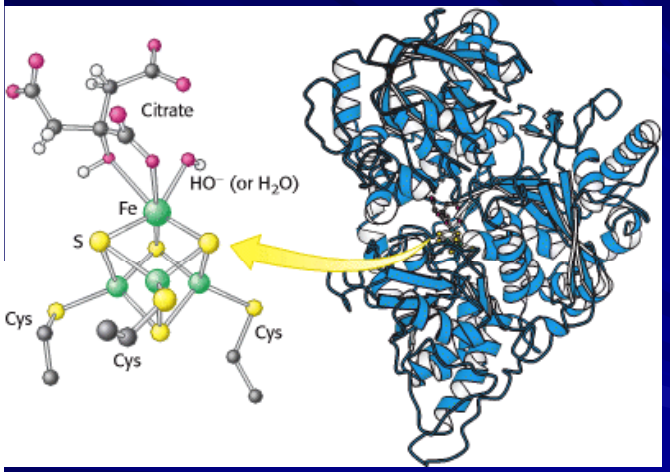


- This initial condensation reaction is catalyzed by **citrate synthase**
- The condensation of oxaloacetate and acetyl CoA proceeds through an enol intermediate.
- The subsequent hydrolysis of citryl CoA yields citrate and CoA

Regulation

- The reaction is inhibited by ATP, NADH, and succinyl CoA.
- The reaction is stimulated by adenosine monophosphate (AMP).

Citrate to isocitrate

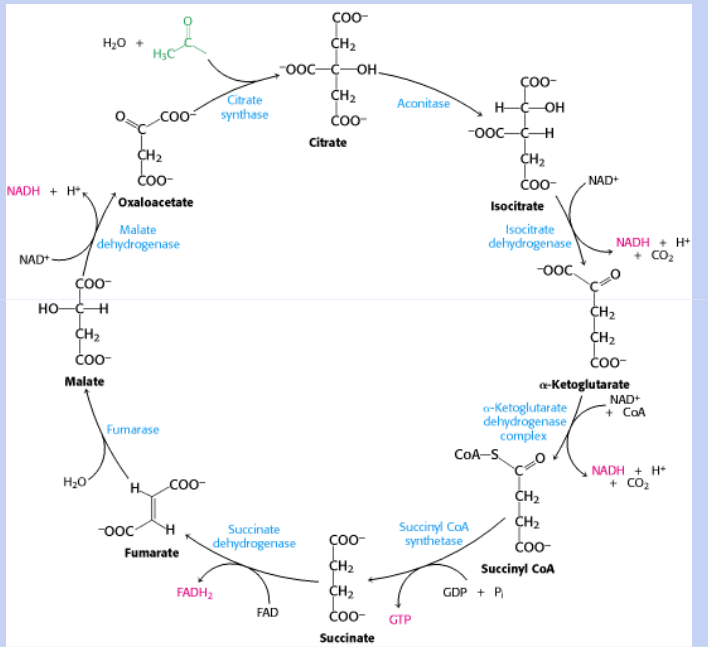


Binding of Citrate to the Iron-Sulfur Complex of Aconitase.

- **A 4Fe-4S iron-sulfur cluster is a component of the active site of aconitase.**
- **One of the iron atoms of the cluster binds to the carboxylate and hydroxyl groups of citrate**

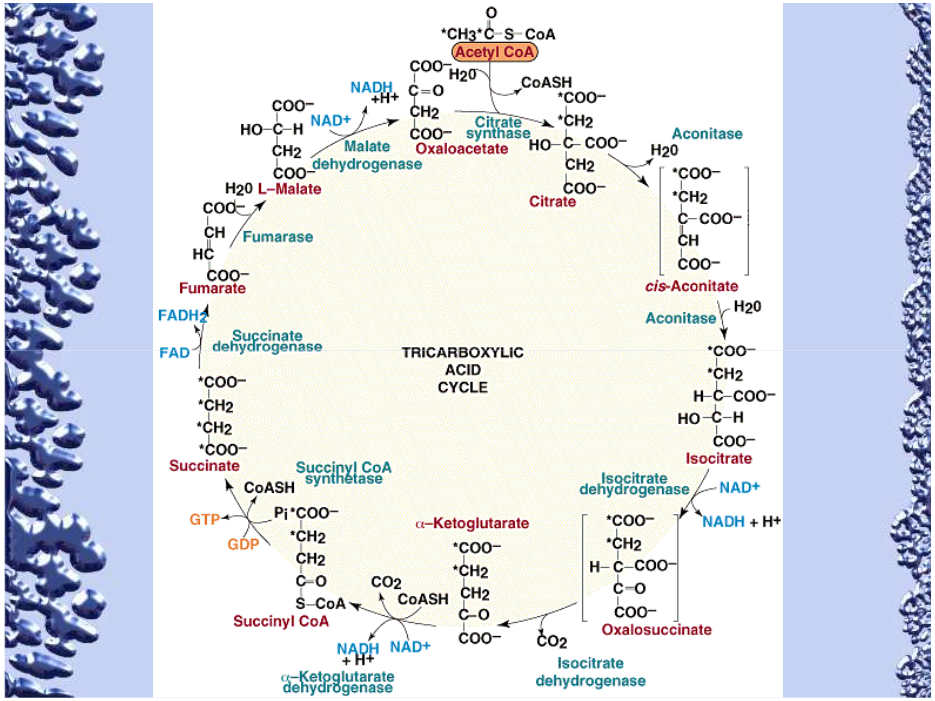
- This isomerization reaction is catalyzed by **aconitase**

REACTIONS OF THE CITRIC ACID CYCLE



Isocitrate to α-ketoglutarate and carbon dioxide

- This reaction is catalyzed by **isocitrate dehydrogenase.**
- In this oxidative decarboxylation reaction, **NAD^+ is reduced** to NADH.
- **Regulation**
  - This reaction is inhibited by ATP and NADH.
  - This reaction is stimulated by ADP.

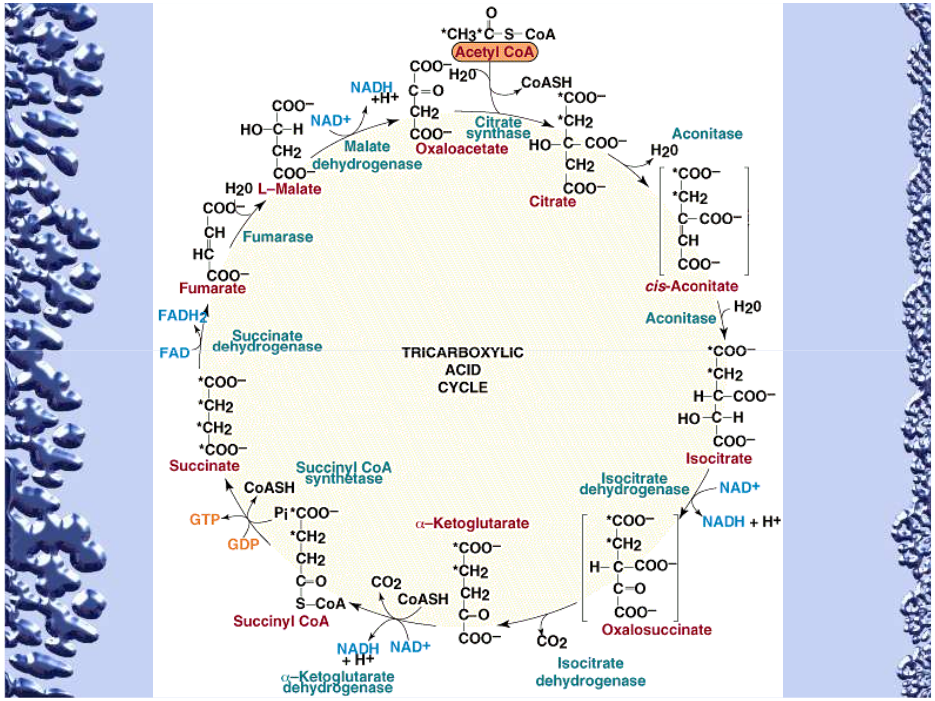


α-Ketoglutarate and CoA to succinyl CoA

- This reaction is catalyzed by **α-ketoglutarate dehydrogenase.**
  - **Prosthetic groups.** α-Ketoglutarate dehydrogenase is a multimeric protein that contains tightly bound TPP, lipoamide, and FAD.
  - **Structure.** α-Ketoglutarate dehydrogenase is very similar to PDH. In **fact, its E_3 subunit is identical to that of PDH.**
- In this oxidative decarboxylation reaction, NAD^+ is reduced to NADH and H^+, and CO_2 is released.
- The product, succinyl CoA, is an **energy-rich thioester** like acetyl CoA.
- The reaction is inhibited by ATP, NADH, and succinyl CoA.

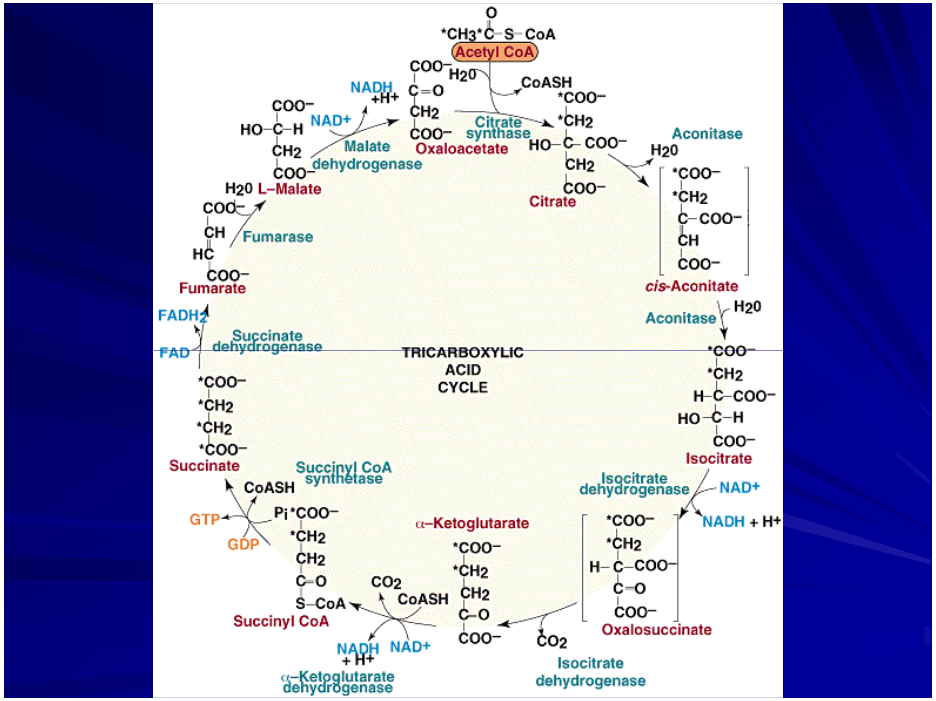
Succinyl CoA and GDP and inorganic phosphate (P_i) to succinate and GTP and CoA

- This reaction is catalyzed by **succinyl CoA synthetase.**
- This is a **substrate-level phosphorylation** with energy being conserved in the form of GTP.



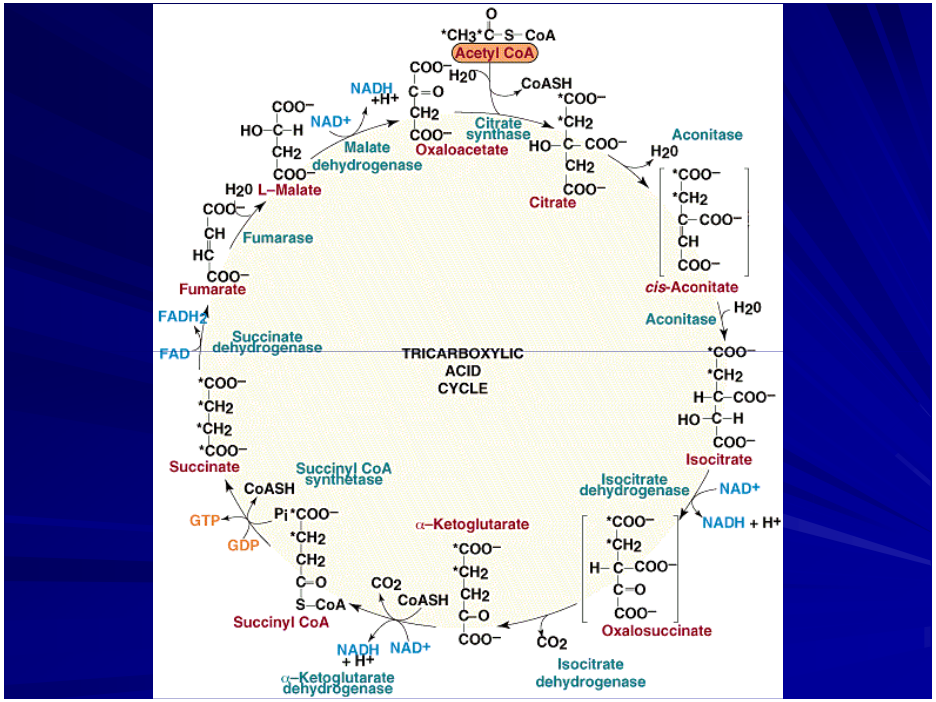
Succinate to fumarate

- This reaction is catalyzed by **succinate dehydrogenase (SDH)**
  - **Prosthetic groups.** SDH possesses three different types of **iron-sulfur centers and covalently bound FAD.**
  - SDH is tightly associated with the **inner mitochondrial membrane.**
- This is a dehydrogenation reaction during which **FAD is reduced to $FADH_2$.** $FADH_2$ is reoxidized by transferring electrons directly to the electron transport chain of the mitochondrial membrane



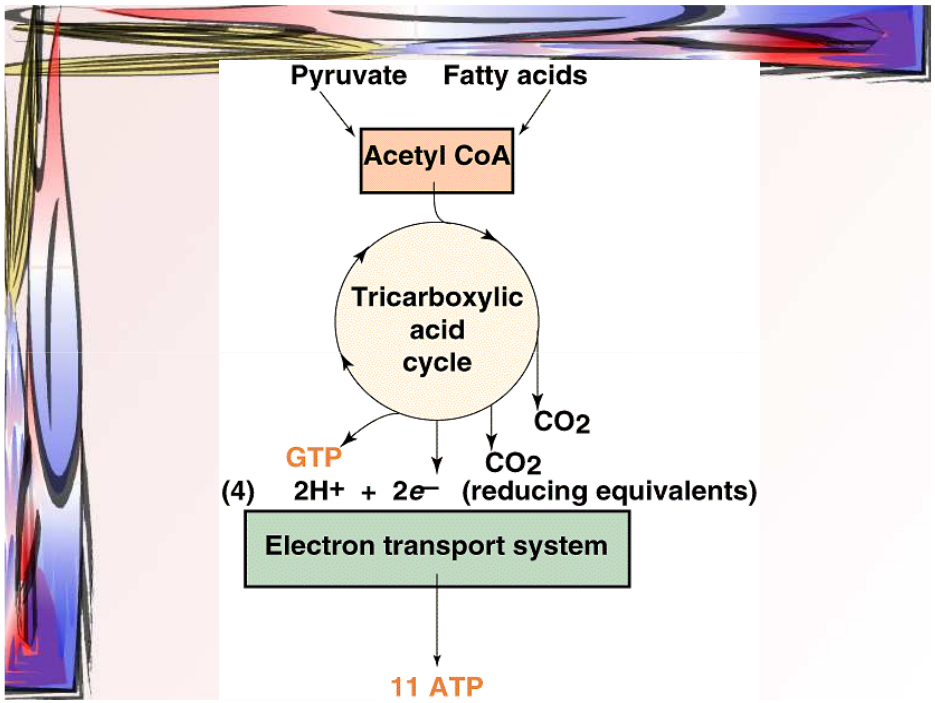
Fumarate to malate

- This hydration reaction is catalyzed by **fumarase**



Malate to oxaloacetate

- This reaction is catalyzed by **malate dehydrogenase.**
- This is a dehydrogenation reaction during which **NAD⁺ is reduced to NADH**



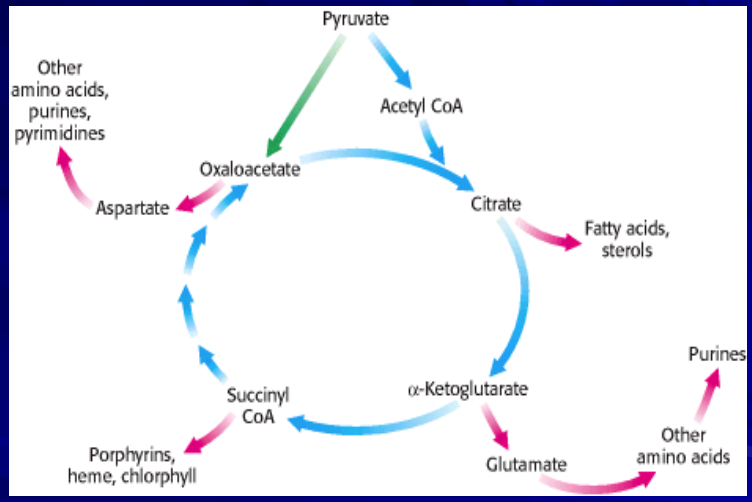
ANAPLEROTIC REACTIONS

- **Description.** Anaplerotic reactions can increase the concentration of citric acid cycle intermediates, allowing an increased rate of oxidation of two-carbon units As more intermediates are available, more moles of acetyl CoA can be processed The intermediates also may be used for other biosynthetic reactions and need to be replaced

Sources of carbon for anaplerotic reactions

- **Amino acid metabolism**
  - **Transaminases** form **α-ketoglutarate** and **oxaloacetate**, citric acid cycle mtermediates
  - **Glutamate dehydrogenase** also produces α-ketoglutarate
  - **Succinyl CoA** is formed from isoleucine, valine, methionine, and threonine
- **Pyruvate carboxylase** forms oxaloacetate from pyruvate It is a biotin dependent carboxylase

Biosynthetic Roles of the Citric Acid Cycle



- **Intermediates drawn off for biosyntheses (shown by red arrows) are replenished by the formation of oxaloacetate from pyruvate**