# METABOLISM II: Citric Acid Cycle

Date: October 10, 2007
Reading Assignment: **Textbook of Biochemistry, 6th ed.** , edited by Devlin, Chapt. 14, pp. 538-550. Some of the same material is also covered in **Molecular Biology of the Cell, 4th ed.**, by Alberts *et al.*, pp.99-102.

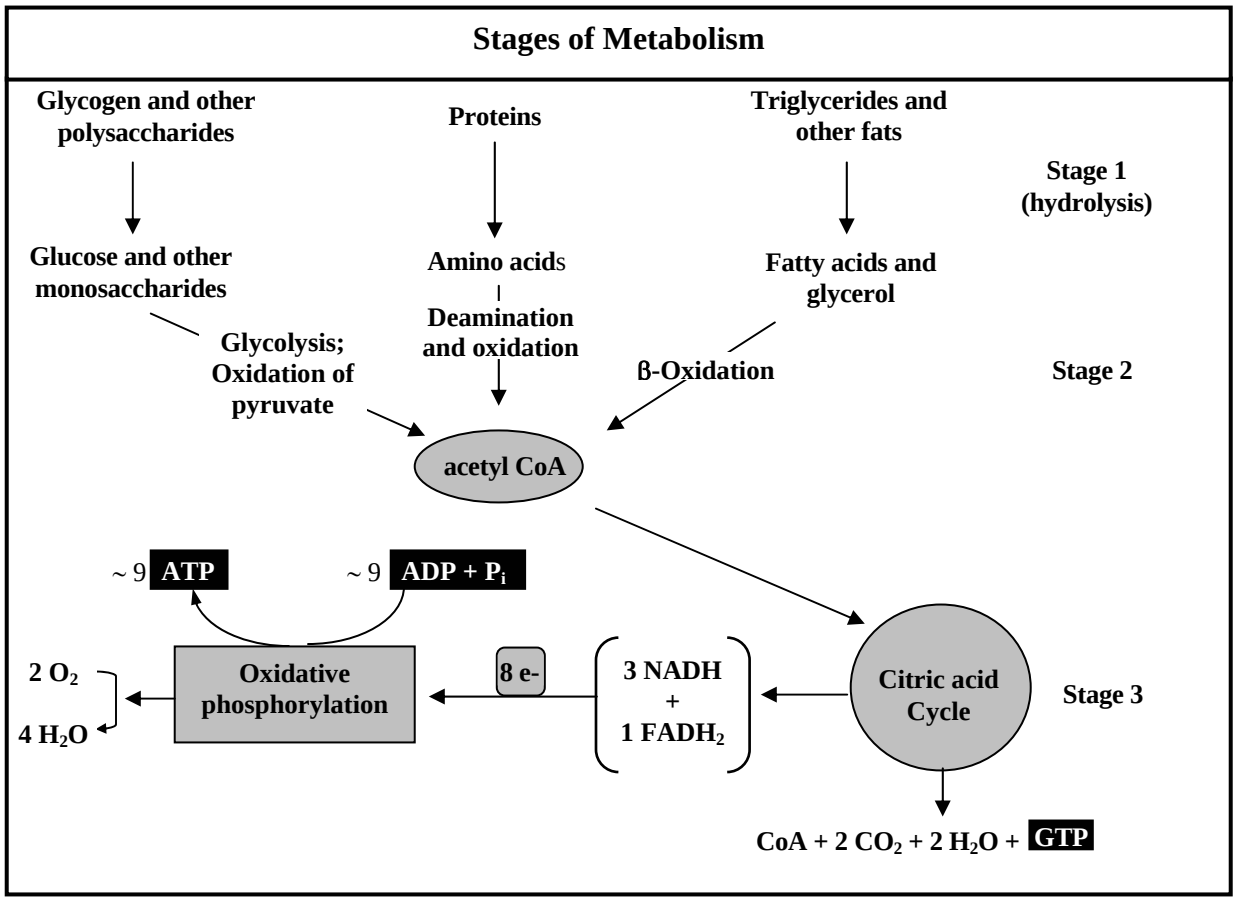
## KEY CONCEPTS AND *LEARNING OBJECTIVES*

1. The mitochondrial matrix contains a battery of enzymes that collectively catalyze the complete oxidation of a number of important metabolic fuels.
   - *a. Describe the three stages of catabolism of food.*
   - *b. Identify the major dietary constituents that give rise to acetyl-CoA*
   - *c. Describe the relationship between the oxidation of pyruvate and fatty acids, the formation of acetyl-CoA, and the generation of NADH and $FADH_2$.*
   - *d. Identify where in the cell pyruvate, fatty acids and acetyl-CoA are oxidized.*
   - *e. Draw the citric acid cycle showing the initial reaction in which citrate is formed, the steps that generate reduced cofactors (oxidation steps), and the step in which the high energy intermediate, GTP, is formed.*
   - *f. Predict the effect of increased levels of citric acid cycle intermediates on the rate of oxidation of aceetyl CoA and other substrates in the citric acid cycle.*

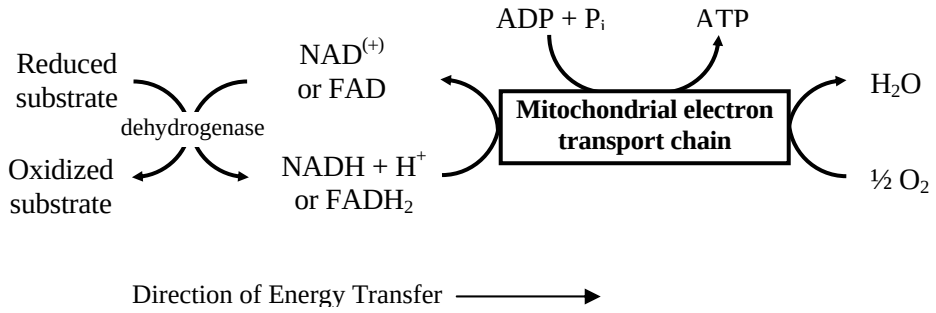
2. Pyruvate can be converted to glucose (gluconeogenesis) but in mammals it is not possible to make glucose from acetyl CoA. Consequently, pyruvate oxidation is inhibited when alternative fuels are available as an energy source.
   - *a. Describe the regulation of pyruvate dehydrogenase and identify key cellular constituents that influence its activity.*
   - *b. Explain how the regulatory properties of pyruvate dehydrogenase might limit pyruvate oxidation when other metabolic fuels are available.*

3. When an individual consumes more calories than are needed, excess food is converted to acetyl CoA and then to fat (fatty acid synthesis). Acetyl CoA is produced in the mitochondrial matrix, but fatty acid synthesis occurs in the cytosol. Acetyl CoA is transported out of mitochondria as citrate.
   - *a. Describe the regulation of the citric acid cycle, identifying key regulatory enzymes and the cellular constituents that influence their activities.*
   - *b. Explain how properties of the citric acid cycle and its regulation might inhibit the oxidation of citric acid so that it is available for export to the cytosol for fatty acid synthesis.*

1. The metabolic pathways by which foods are oxidized are collectively known as catabolism (Prepared by A. Frankfater).




2. NADH and $FADH_2$ produced during the oxidation of substrates in the mitochondrial matrix is reoxidized by molecular oxygen. The oxidations of NADH and $FADH_2$ are thermodynamically favorable, and the energy released is captured to make ATP (oxidative phosphorylation). (Prepared by A. Frankfater).



3. The formation and oxidation of acetyl CoA occur in the mitochondrial matrix produce NADH and $FADH_2$.

A. Pyruvate is formed from the oxidation of glucose in the cytosol and is oxidized to acetyl CoA in the mitochondrial matrix.

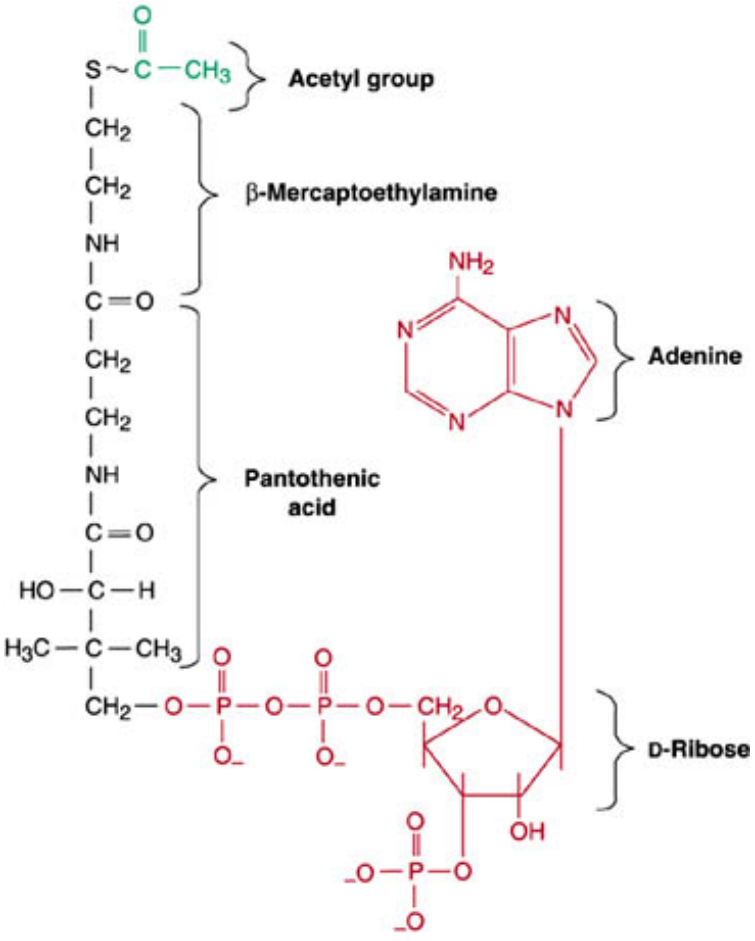
$$\text{Glucose} + 2\,NAD^{(+)} \xrightarrow{\text{glycolysis}} 2\,\text{pyruvate} + 2\,\text{NADH} + 2\,H^{(+)}\,\text{(cytosol)}$$

$$2\,\text{Pyruvate} + 2\,NAD^{(+)} + 2\,\text{CoA} \xrightarrow{\text{Pyruvate dehydrogenase}} 2\,\text{NADH} + 2\,H^{(+)} + 2\,\text{acetyl CoA (mitochondrial matrix)}$$

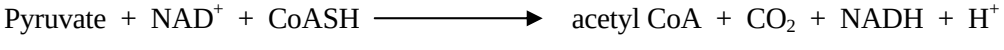
B. Fatty acids from dietary fats are joined to coenzyme A in the cytosol and are oxidized to acetyl CoA in the mitochondrial matrix. For example

$$\text{C-16 fatty acyl-CoA} + 7\,NAD^{(+)} + 7\,\text{FAD} + 7\,H_2O + \text{CoA} \xrightarrow{\text{mitochondrial fatty acid } \beta\text{-oxidation pathway}} 7\,\text{NADH} + 7\,H^{(+)} + 7\,FADH_2 + 8\,\text{acetyl CoA (mitochondrial matrix)}$$

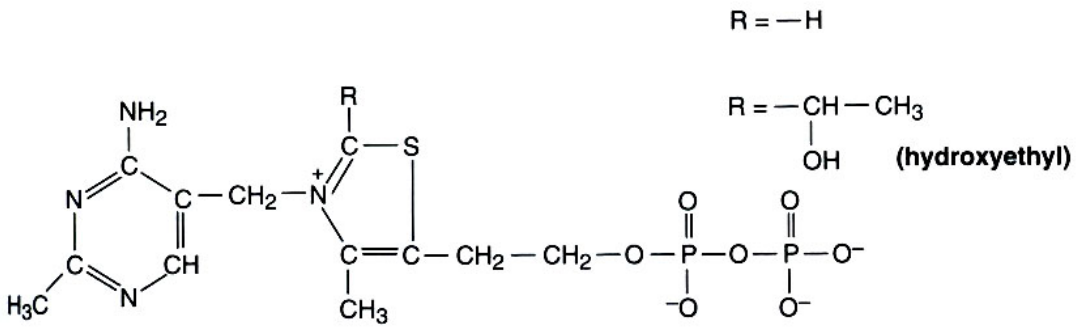
4. Coenzyme A is a universal carrier of acyl groups. Structure of Acetyl CoA (**Figs. 13-13** from **Textbook of Biochemistry, 5th ed.** , Devlin, editor, Wiley-Liss, publisher)



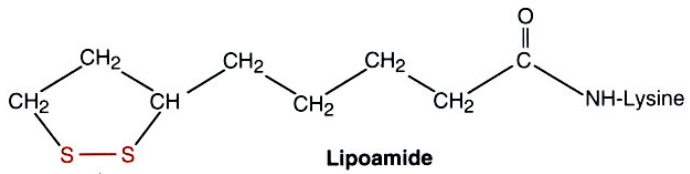
5. The conversion of pyruvate to acetyl CoA is catalyzed by a multienzyme complex called pyruvate dehydrogenase.



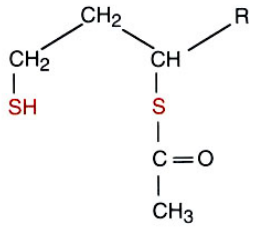

A. Five cofactors participate in the conversion of pyruvate to acetyl CoA, thiamine pyrophosphate, lipoamide, coenzyme A, FAD, and $NAD^+$. These are derived from the vitamins thiamine, lipoic acid, pantothenic acid, riboflavin and niacin, respectively.




**Thiamin pyrophosphate**

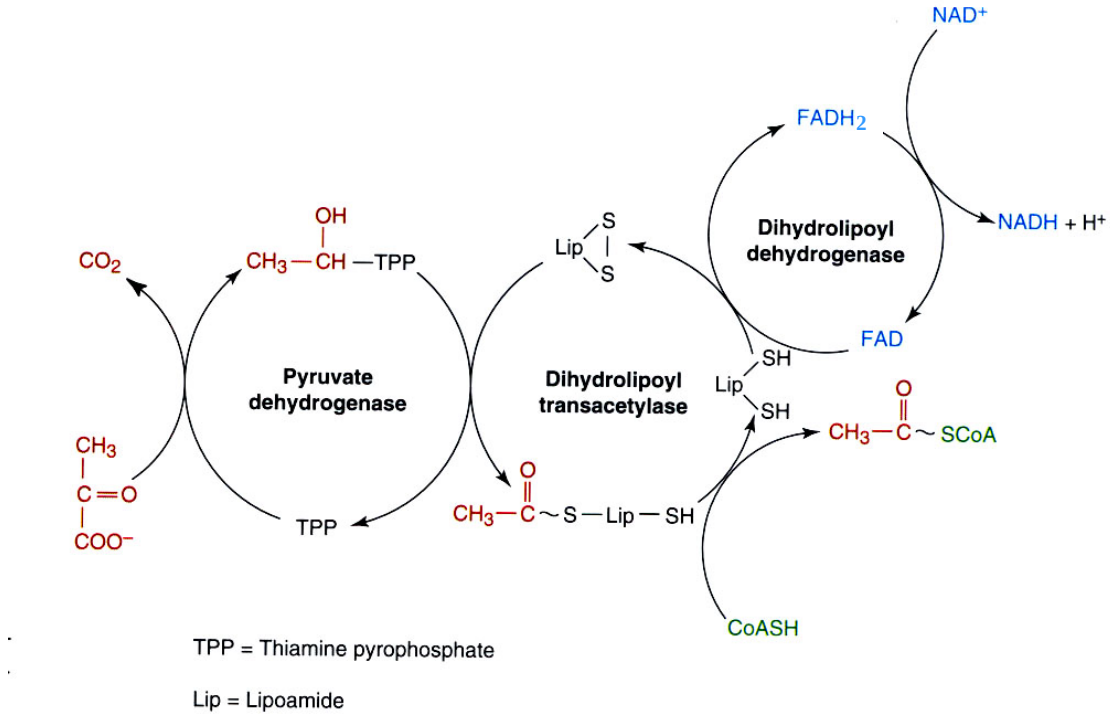



**Lipoamide (oxidized)**

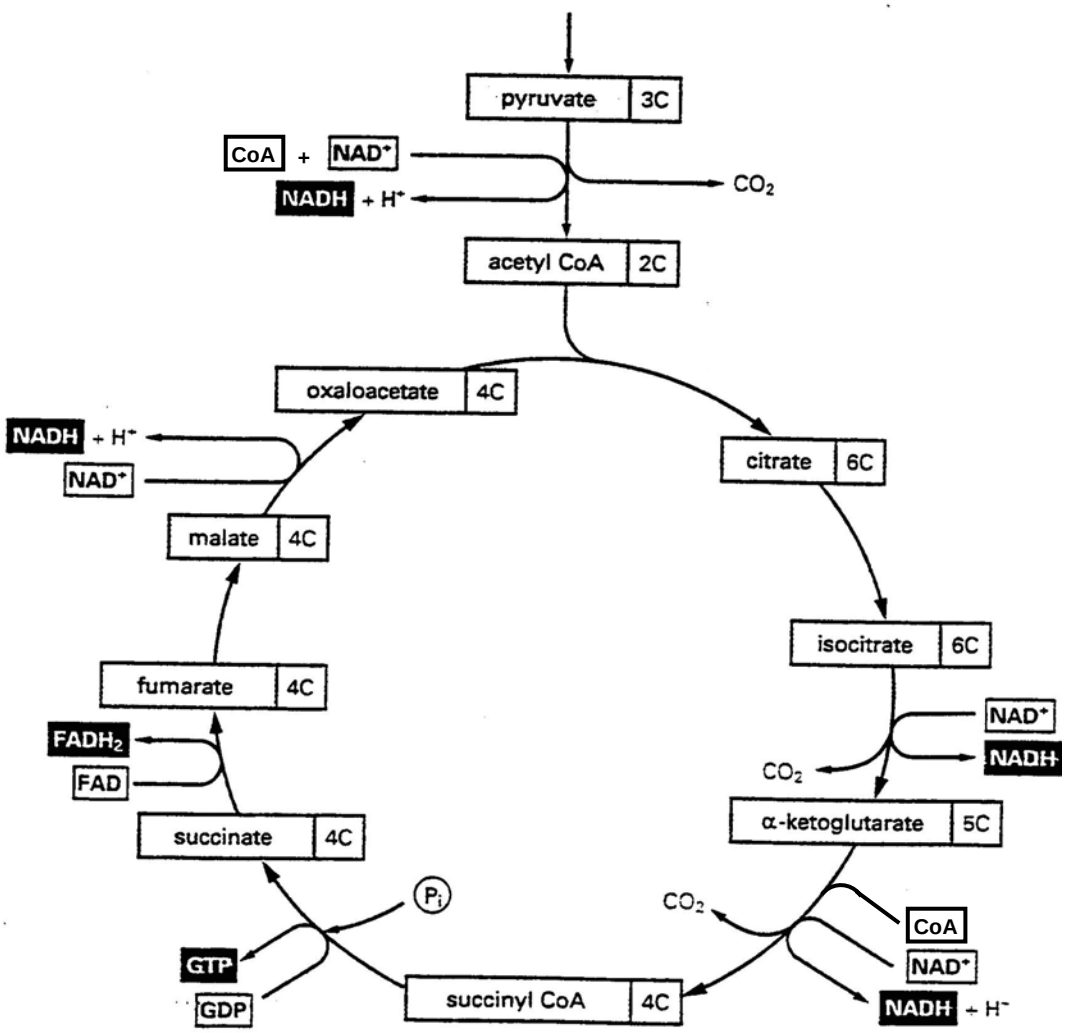


**(reduced/acetylated)**

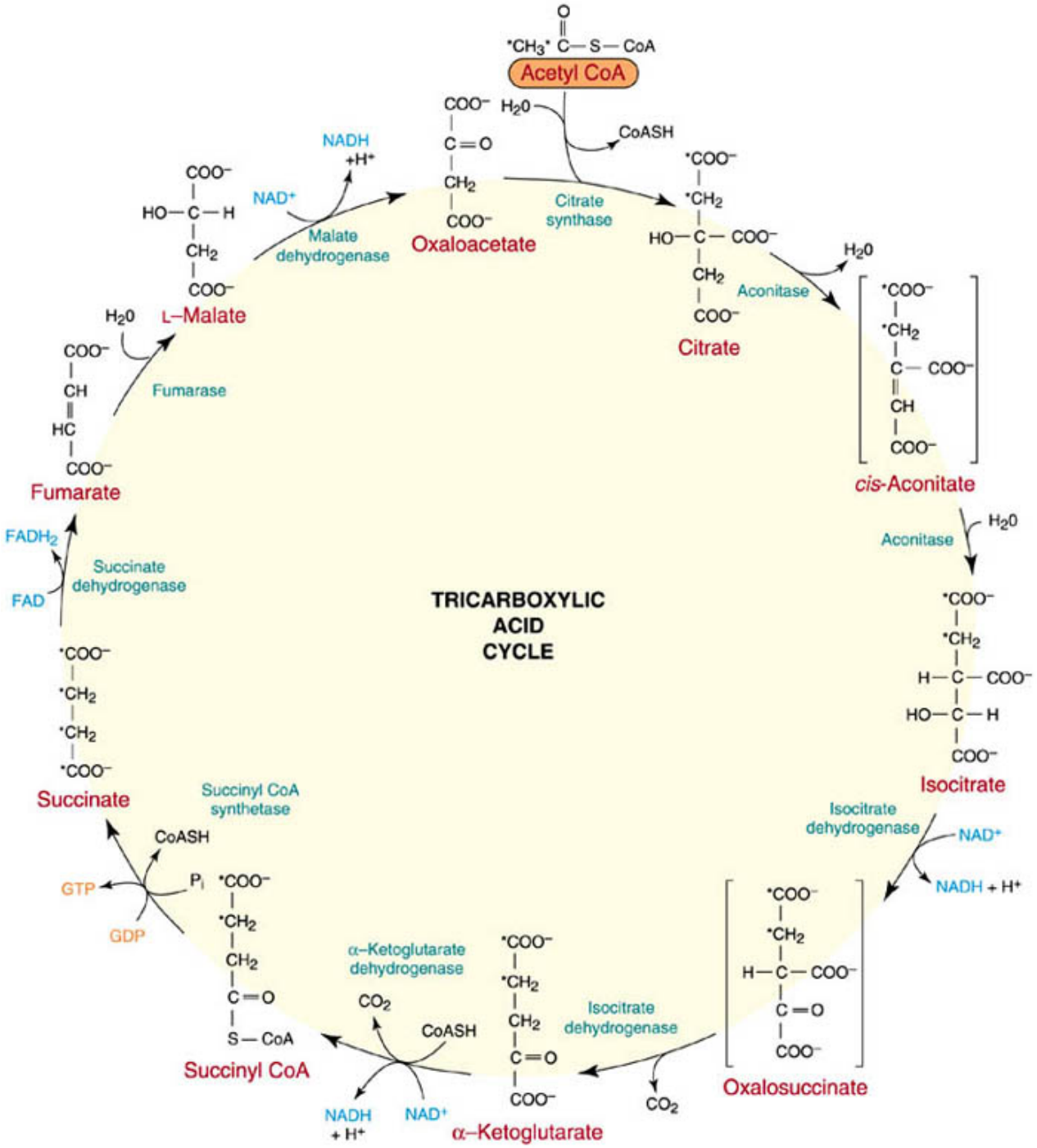
B. The reaction catalyzed by the multienzyme pyruvate dehydrogenase complex produces acetyl CoA. A similar reaction is catalyzed by the multienzyme α-ketoglutarate dehydrogenase of the citric acid cycle to yield succinyl CoA.



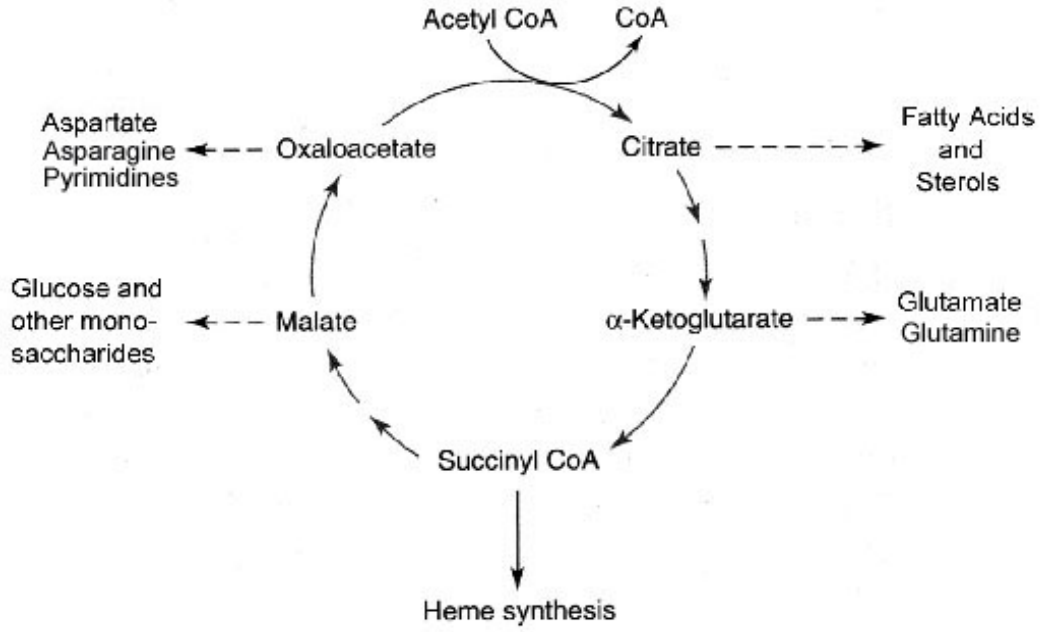
6. Overview of the Citric Acid Cycle (Modified by A. Frankfater)



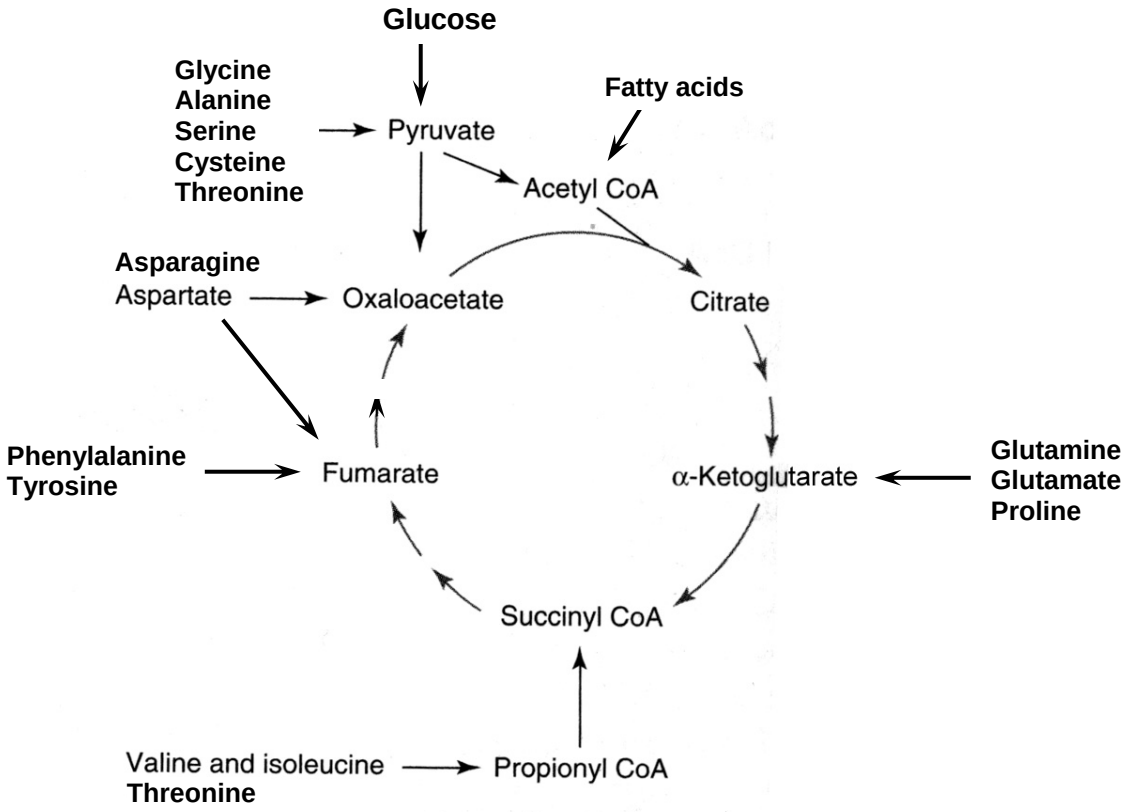
7. Reactions of the Citric Acid Cycle



8. Citric Acid Cycle Provides Intermediates for Biosynthetic Reactions

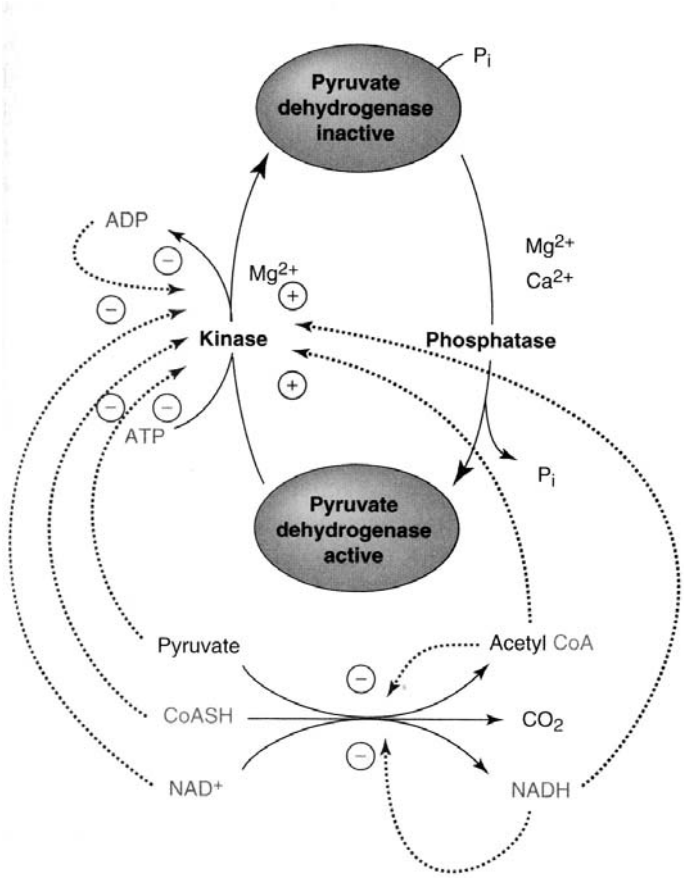



9. Citric Acid Cycle Intermediates can be Replenished by Anaplerotic (filling in) Reactions



10. Regulation of Pyruvate Oxidation and the Citric Acid Cycle

A. Pyruvate dehydrogenase is regulated by a combination of covalent modification (phosphorylation and dephosphorylation) and product inhibition.




Pyruvate dehydrogenase exists as a multiprotein complex containing a tightly-bound protein kinase and a tightly-bound phosphoprotein phosphatase. The kinase is stimulated to phosphorylate (inactivate) the dehydrogenase by the products of the reaction, acetyl CoA and NADH. The kinase is inhibited by the substrates in the reaction, pyruvate, Coenzyme A, and $NAD^+$, and by ADP (indicates the level of ATP is reduced). The phosphatase is stimulated by $Ca^{2+}$ to dephosphorylate (activate) the dehydrogenase.
In addition, but probably of less importance, the active (dephosphorylated) form of pyruvate dehydrogenase is reversibly inhibited by the products of the reaction, acetyl CoA and NADH.

B. Some enzymes of the Citric Acid Cycle are subject to allosteric regulation and/or product inhibition.