

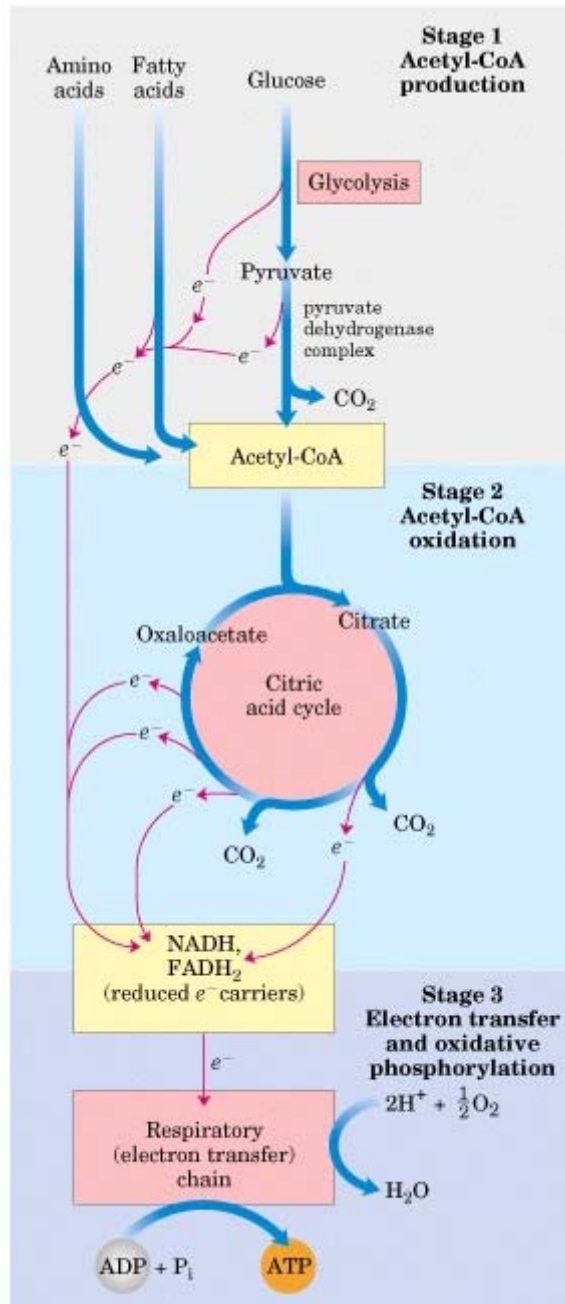
Citric Acid cycle or Tricarboxylic Acid cycle or Krebs Cycle

Overview and brief history

- Pyruvate Dehydrogenase Complex (PDC) and its control
- Reactions of TCA cycle or CAC
- Amphibolic nature of TCA cycle
- Regulation of TCA cycle
- Reactions of Glycolysis are localized in Cytosol, and do not require any oxygen.

whereas pyruvate dehydrogenase and TCA cycle reactions take place in mitochondria where oxygen is utilized to generate ATP by oxidative phosphorylation.

Consumption of oxygen (respiration) depends on the rate of PDC and TCA reactions.



In Cytosol

In Mitochondria

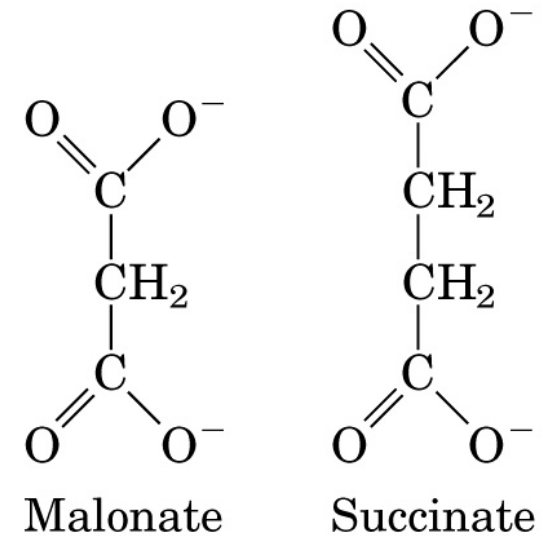
Historical perspective:

1930: Elucidation of Glycolysis

Study of oxidation of glucose in muscle, addition of Malonate inhibited the respiration (i.e. O₂ uptake).

Malonate is an inhibitor of Succinate oxidation to Fumerate

1935: Szent-Gyorgyi: demonstrated that little amounts (catalytic amounts) of succinate, fumerate, malate or oxaloacetate acelerated the rate of respiration.

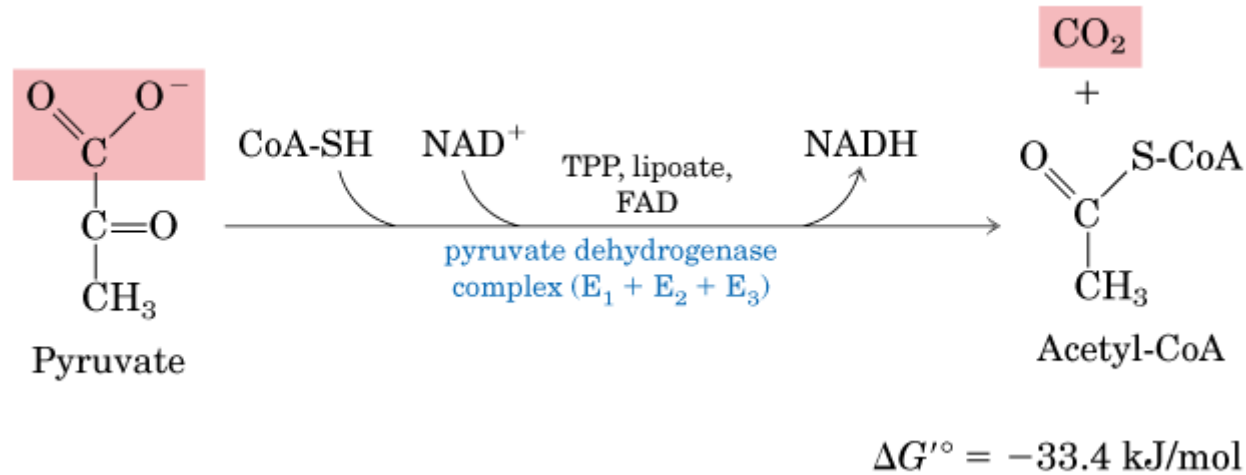


He also showed the sequence of inter-conversion:
Succinate --- Fumerate --- malate ---oxaloacetate.

1936: Martius & Knoop: Found the following sequence of reaction:
Citrate to cis-aconitase to Isocitrate to a Ketogluterate to succinate

1937: Krebs: Enzymatic conversion of Pyruvate + Oxaloacetate to citrate and CO₂
Discovered the cycle of these reactions and found it to be a major pathway for pyruvate oxidation in muscle.

Reaction of pyruvate dehydrogenase complex (PDC)



Reactions of TCA cycle: 8 reactions:

Citrate synthase

Aconitase

Iso-citrate dehydrogenase

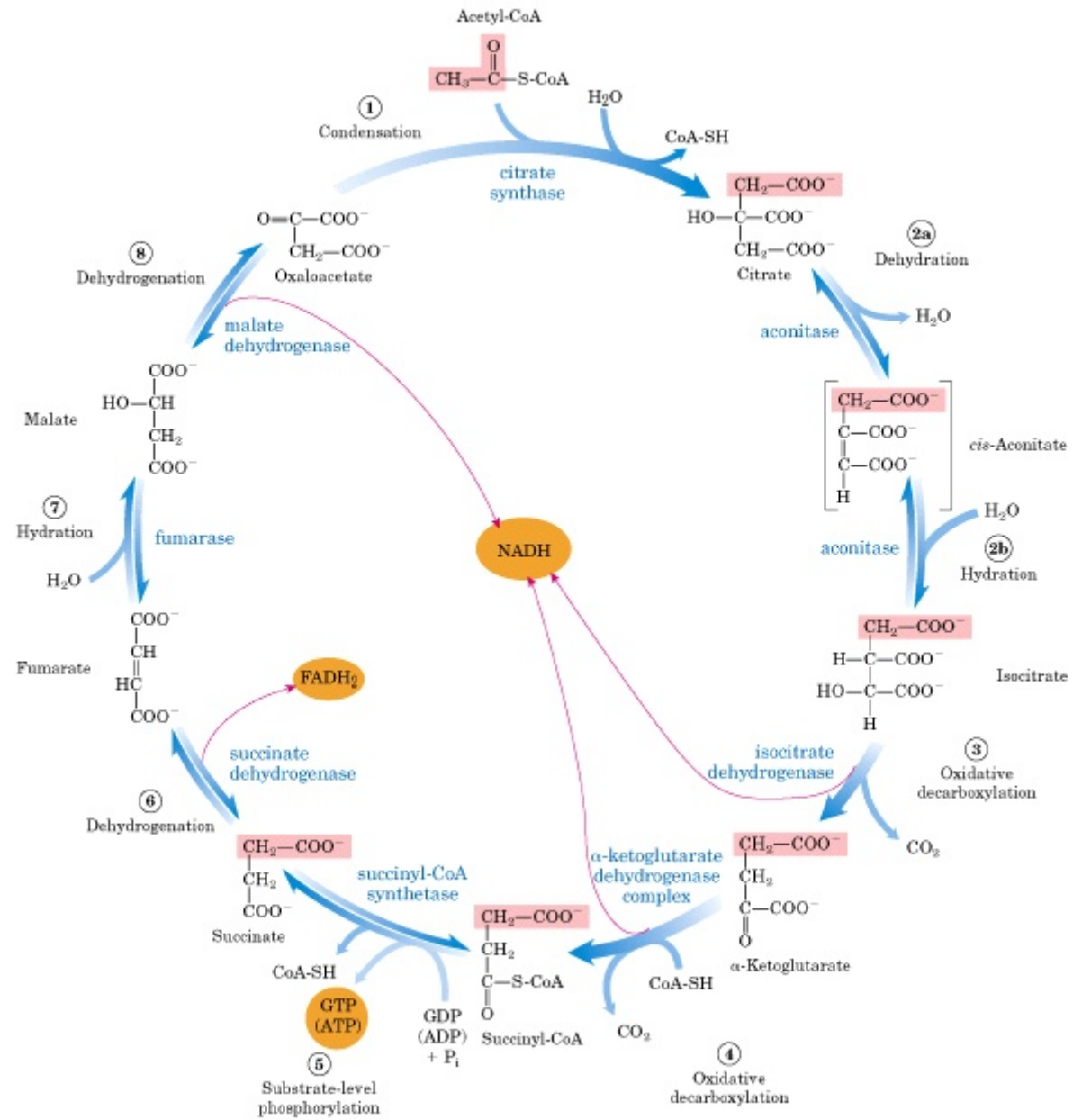
α ketoglutarate dehydrogenase

Succinyl-Coenzyme A synthetase

Succinate dehydrogenase

Fumerase

Malate dehydrogenase



Pyruvate dehydrogenase Complex (PDC)

It is a multi-enzyme complex containing three enzymes associated together non-covalently:

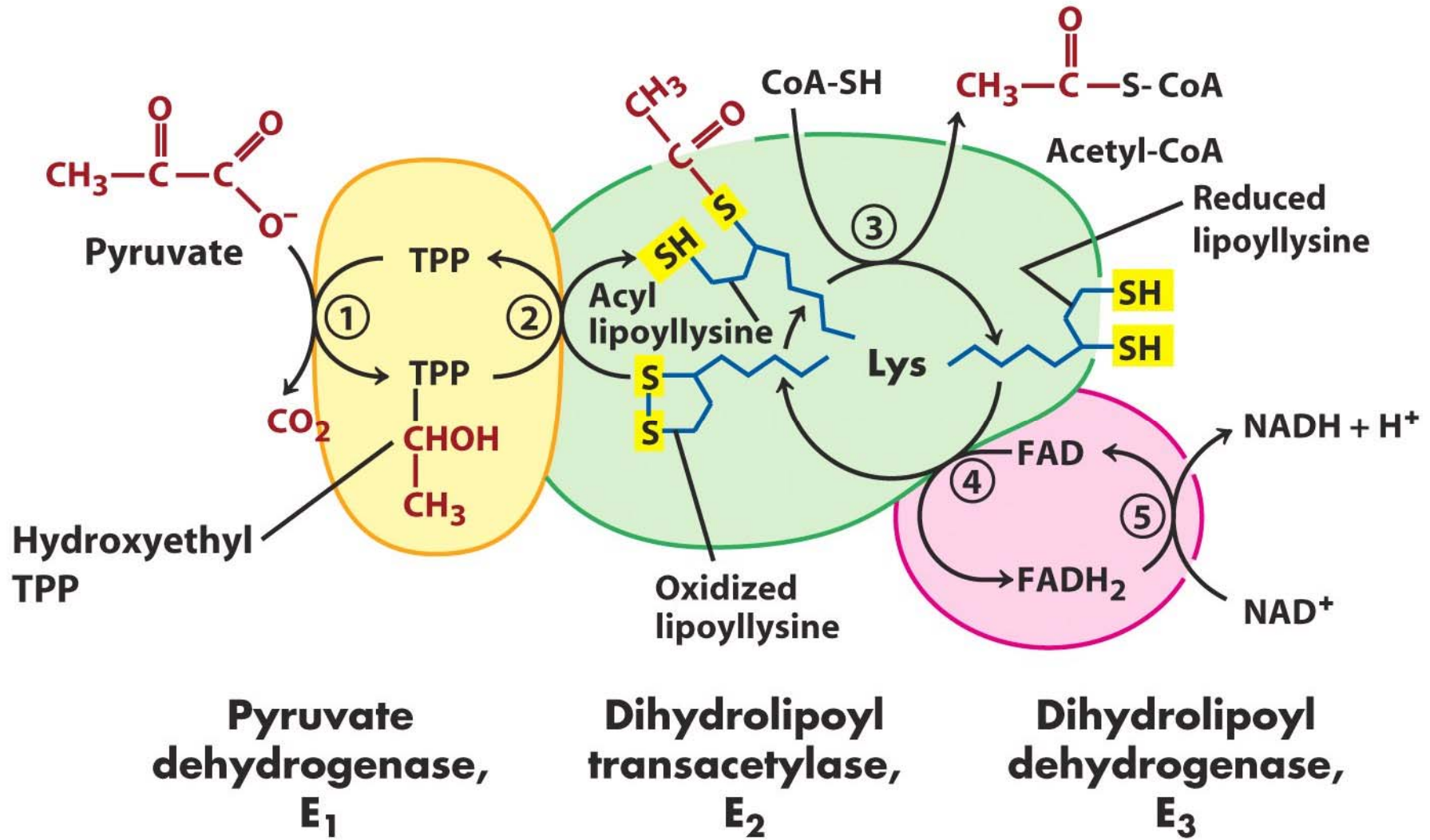
E-1 : Pyruvate dehydrogenase , uses Thiamine pyrophosphate as cofactor bound to E1

E-2 : Dihydrolipoyl transacetylase, Lipoic acid bound, CoA as substrate

E-3 : Dihydrolipoyl Dehydrogenase FAD bound, NAD⁺ as substrate

Advantages of multienzyme complex:

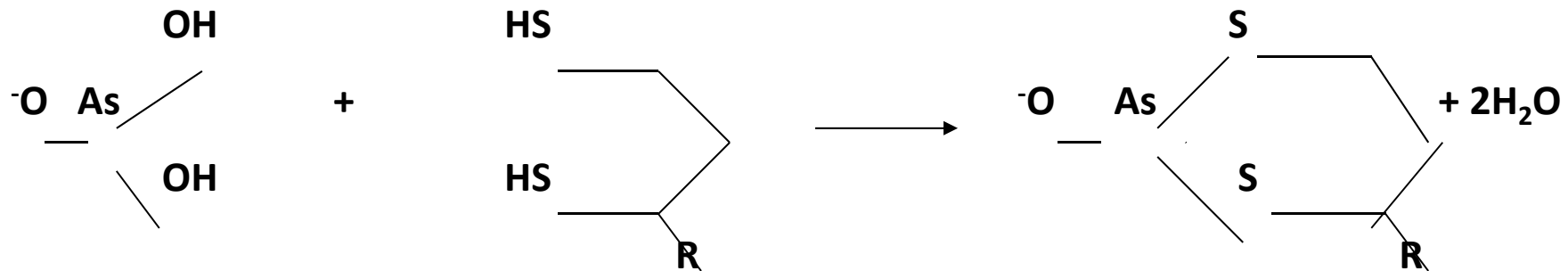
1. **Higher rate of reaction:** Because product of one enzyme acts as a substrate of other, and is available for the active site of next enzyme without much diffusion.
2. **Minimum side reaction.**
3. **Coordinated control.**



Thiamin (Vitamine B1) deficiency causes Beriberi:

Thiamine pyrophosphate (TPP) is an important cofactor of pyruvate dehydrogenase complex, or PDC a critical enzyme in glucose metabolism. Thiamine is neither synthesized nor stored in good amounts by most vertebrates. It is required in the diets of most vertebrates. Thiamine deficiency ultimately causes a fatal disease called Beriberi characterized by neurological disturbances, paralysis, atrophy of limbs and cardiac failure. Note that brain exclusively uses aerobic glucose catabolism for energy and PDC is very critical for aerobic catabolism. Therefore thiamine deficiency causes severe neurological symptoms.

Arsenic Poisoning: Arsenic compounds such as arsenite (AsO_3^{3-}) organic arsenicals are poisonous because they covalently bind to sulfhydryl compounds (SH- groups of proteins and cofactors). Dihydrolipoamide is a critical cofactor of PDC, and it has two-SH groups, which are important for the PDC reaction. These -SH groups are covalently inactivated by arsenic compounds as shown below;



Arsenic compounds in low doses are very toxic to microorganisms, therefore these compounds were used for the treatment of syphilis and other diseases in earlier days. Arsenicals were first antibiotics, but with a terrible side effects as they are eventually very toxic to humans.

Unfortunately and ignorantly, a common nineteenth century tonic, the Fowler's solution contained 10 mg/ml arsenite. This tonic must have been responsible for many deaths, including the death of the famous evolution scientist Charlse Darwin.

Arsenic Compound poisoning: Inactivation of E-2 of PDC, and other proteins.

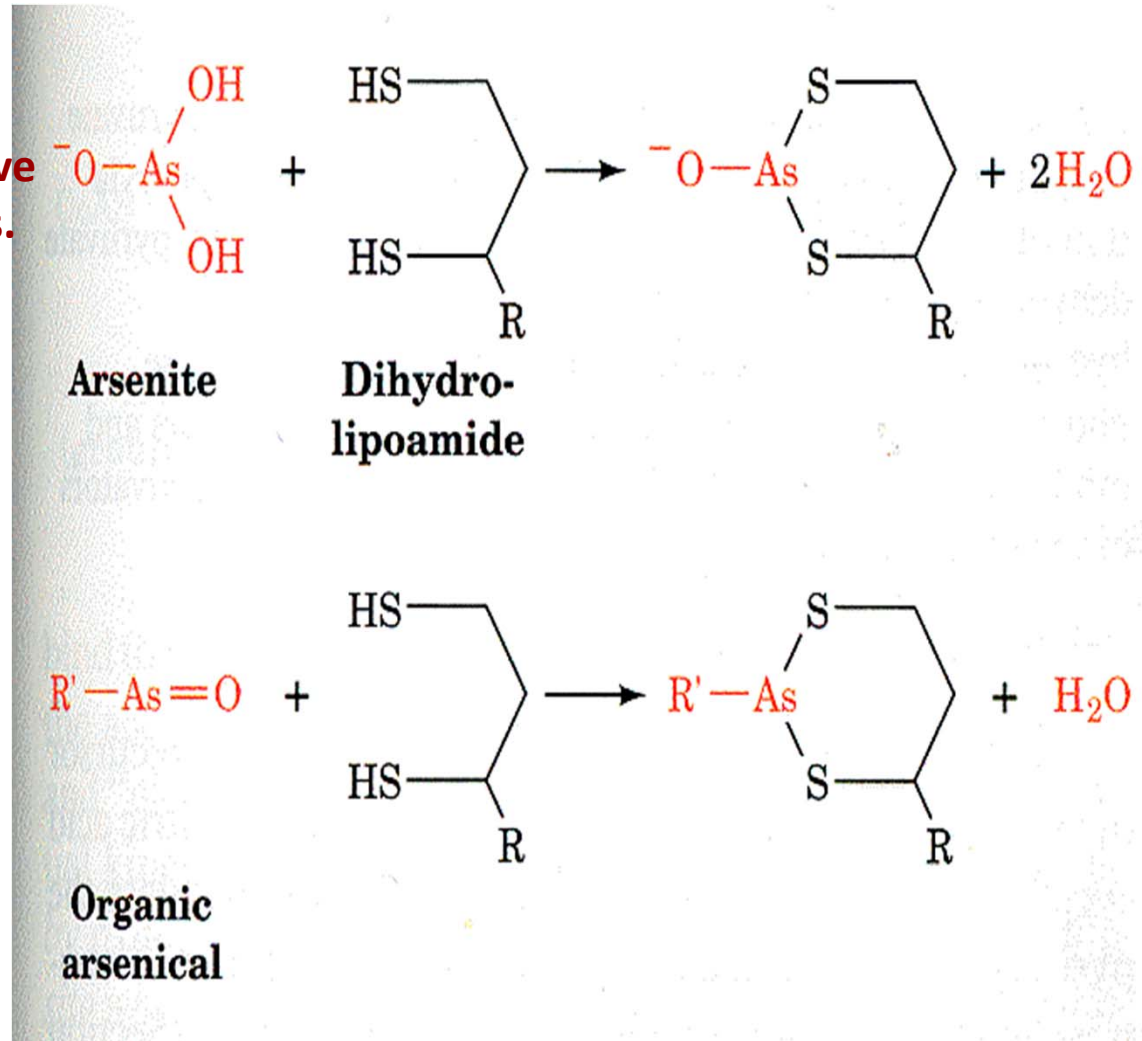
Organic Arsenicals were used as antibiotics for the treatment of syphilis and trypanosomiasis.

Micro-organisms are more sensitive to organic arsenicals than humans.

But these compounds had severe side effects and As-poisoning.

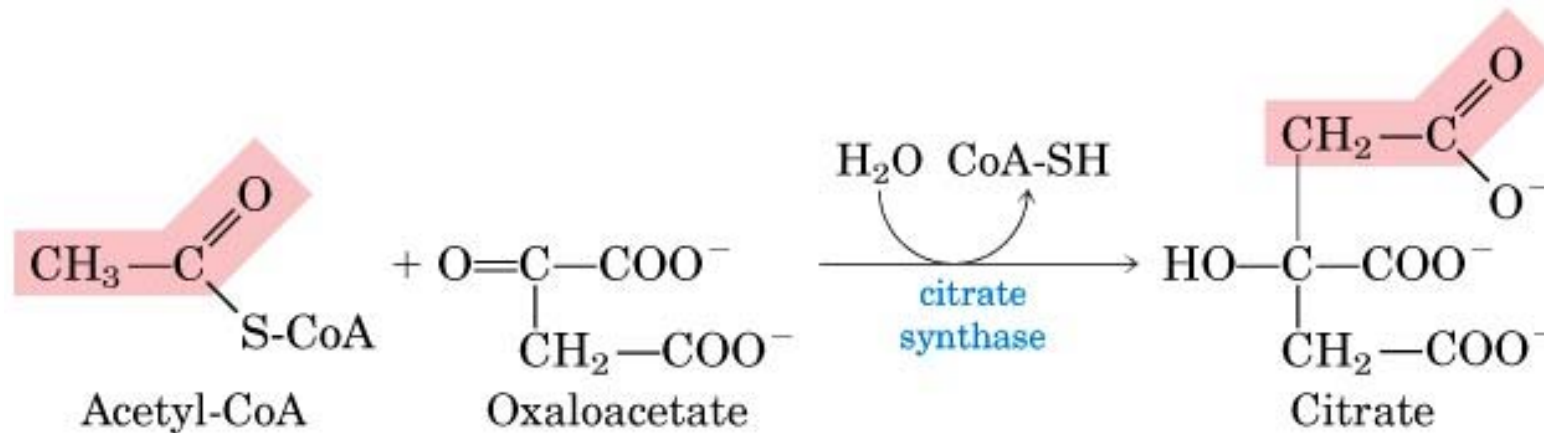
Fowler's solution, the famous 19th century tonic contained 10mg/ml As. Charles Darwin died of As poisoning by taking this tonic.

Napoleon Bonaparte's death was also suspected to be due to As poisoning.



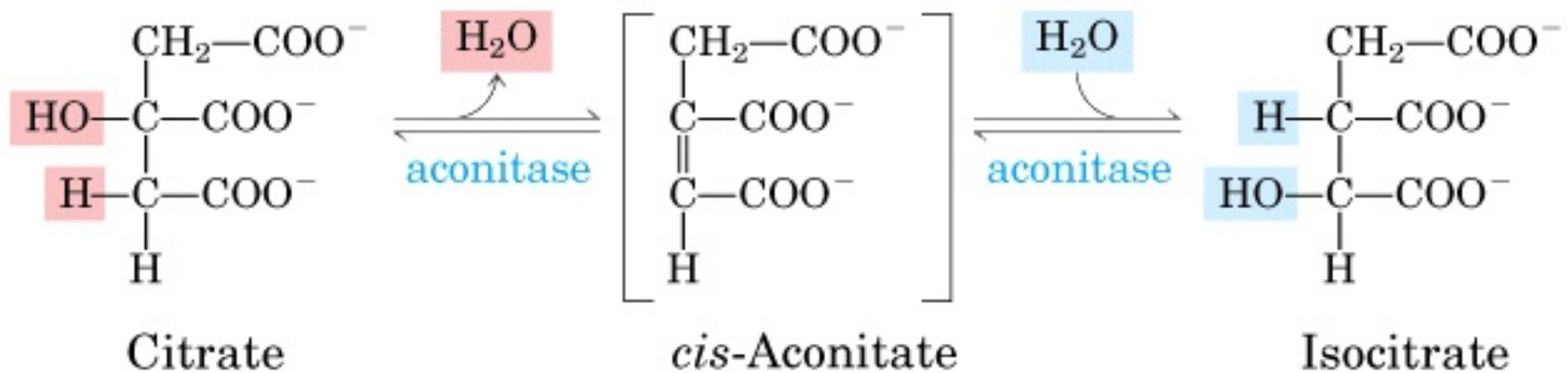
Reactions of Citric Acid Cycle

1. **Citrate synthase: Formation of Citroyl CoA intermediate.**
2. **Binding of Oxaloacetate to the enzyme results in conformational change which facilitates the binding of the next substrate, the acetyl Coenzyme A. There is a further conformational change which leads to formation of products. This mechanism of reaction is referred as induced fit model.**



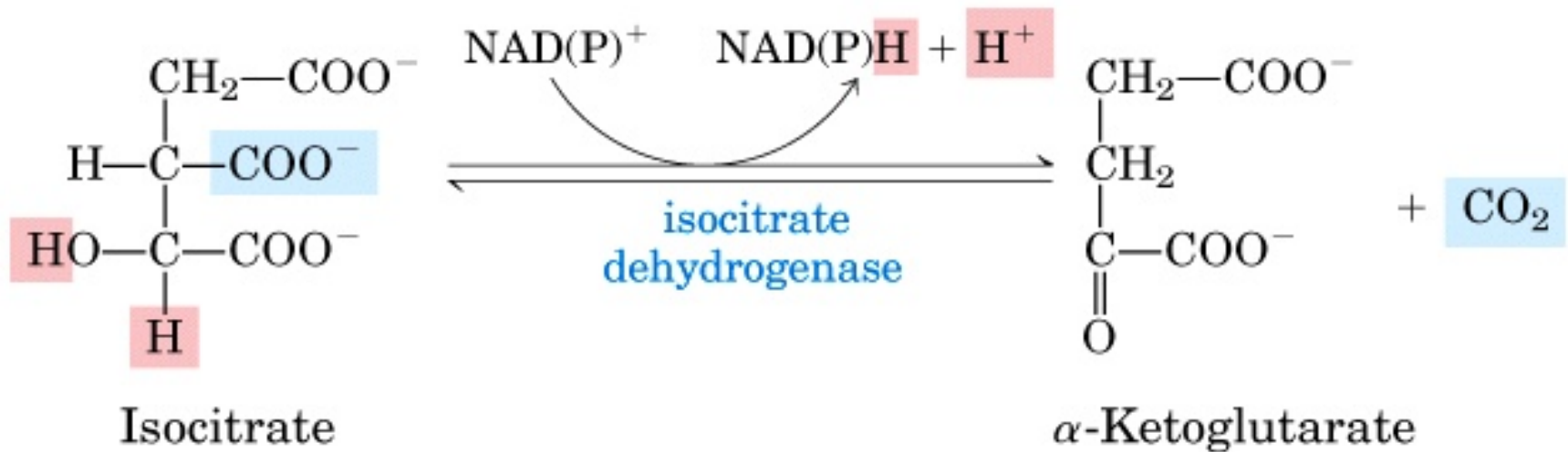
$$\Delta G'^{\circ} = -32.2 \text{ kJ/mol}$$

2. Aconitase: This enzyme catalyses the isomerization reaction by removing and then adding back the water (H and OH) to cis-aconitate in at different positions. Isocitrate is consumed rapidly by the next step thus deriving the reaction in forward direction.



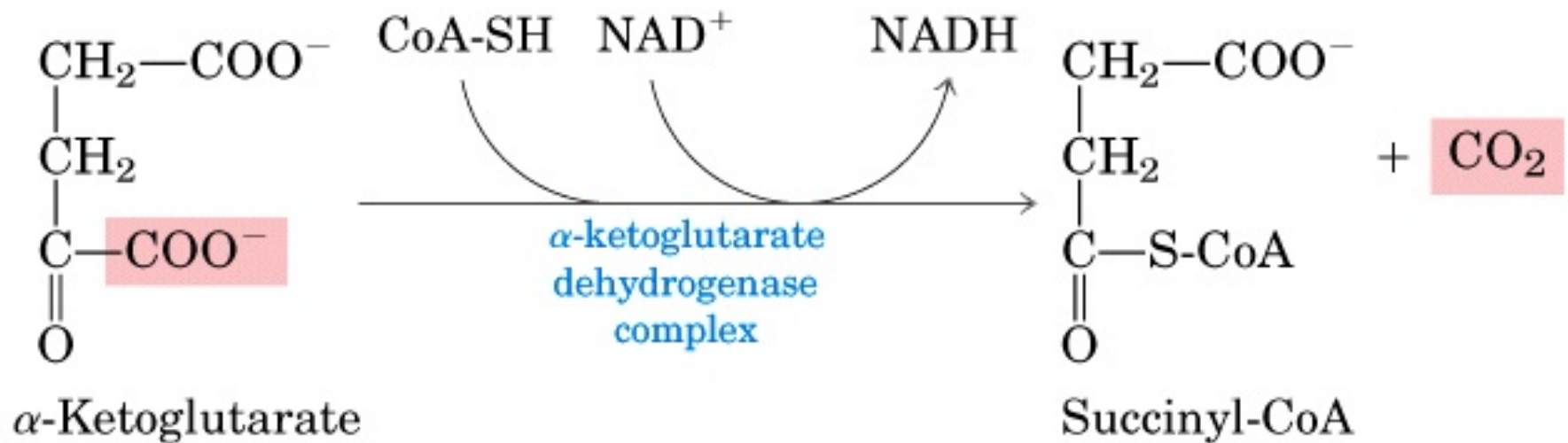
$$\Delta G'^{\circ} = 13.3 \text{ kJ/mol}$$

3. Isocitrate dehydrogenase: There are two isoforms of this enzyme, one uses NAD^+ and other uses NADP^+ as electron acceptor.



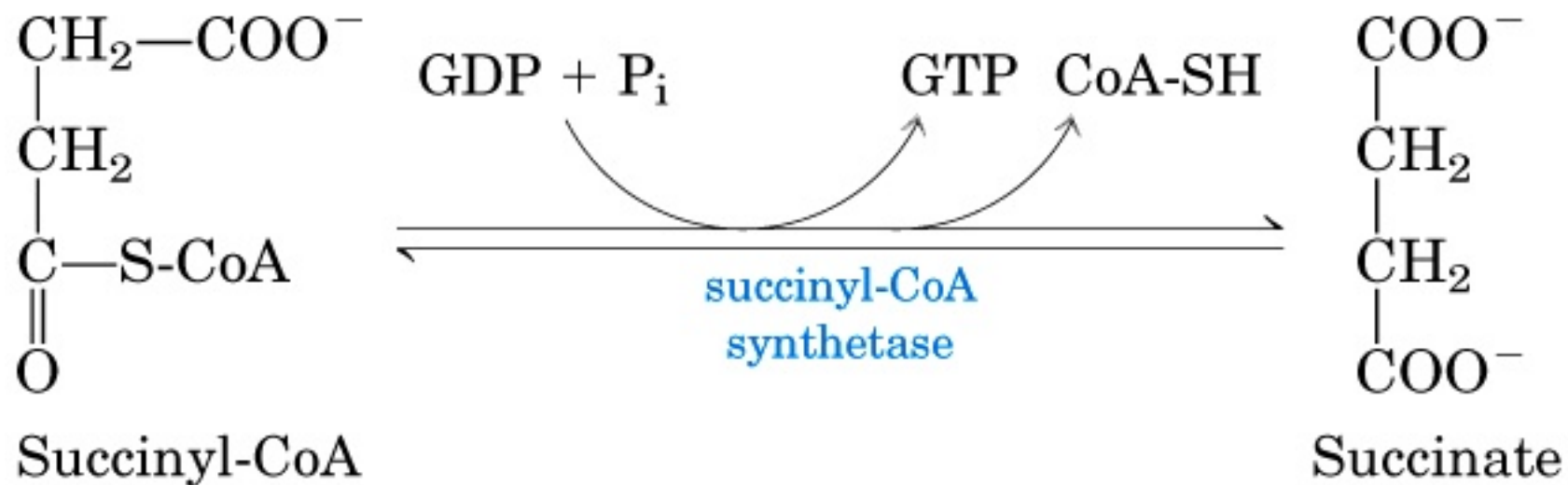
$$\Delta G'^{\circ} = -20.9 \text{ kJ/mol}$$

4. α -Ketoglutarate dehydrogenase: This is a complex of different enzymatic activities similar to the pyruvate dehydrogenase complex. It has the same mechanism of reaction with E1, E2 and E3 enzyme units. NAD^+ is an electron acceptor.



$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

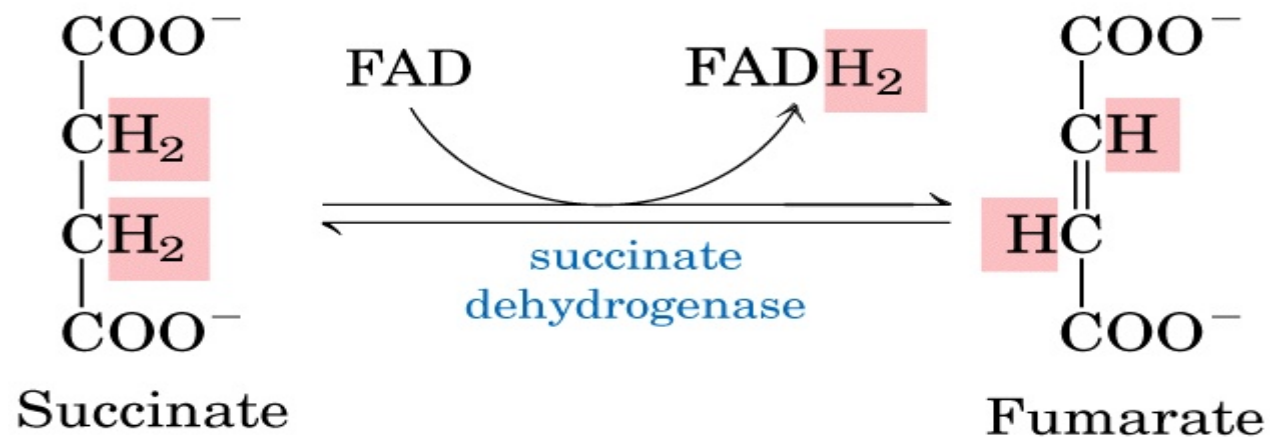
5. Succinyl CoA synthetase: Succinyl CoA, like Acetyl CoA has a thioester bond with very negative free energy of hydrolysis. In this reaction, the hydrolysis of the thioester bond leads to the formation of phosphoester bond with inorganic phosphate. This phosphate is transferred to Histidine residue of the enzyme and this high energy, unstable phosphate is finally transferred to GDP resulting in the generation of GTP.



$$\Delta G'^{\circ} = -2.9 \text{ kJ/mol}$$

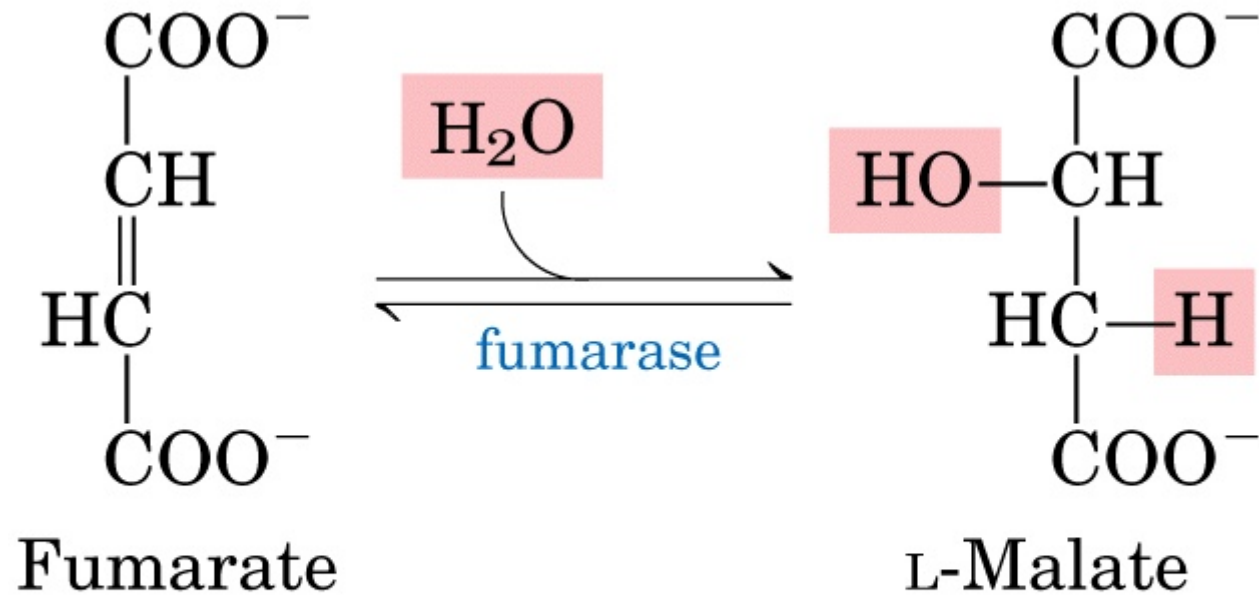
6. Succinate Dehydrogenase: Oxidation of succinate to fumarate. This is the only citric acid cycle enzyme that is tightly bound to the inner mitochondrial membrane. It is an FAD dependent enzyme.

Malonate has similar structure to Succinate, and it competitively inhibits SDH.



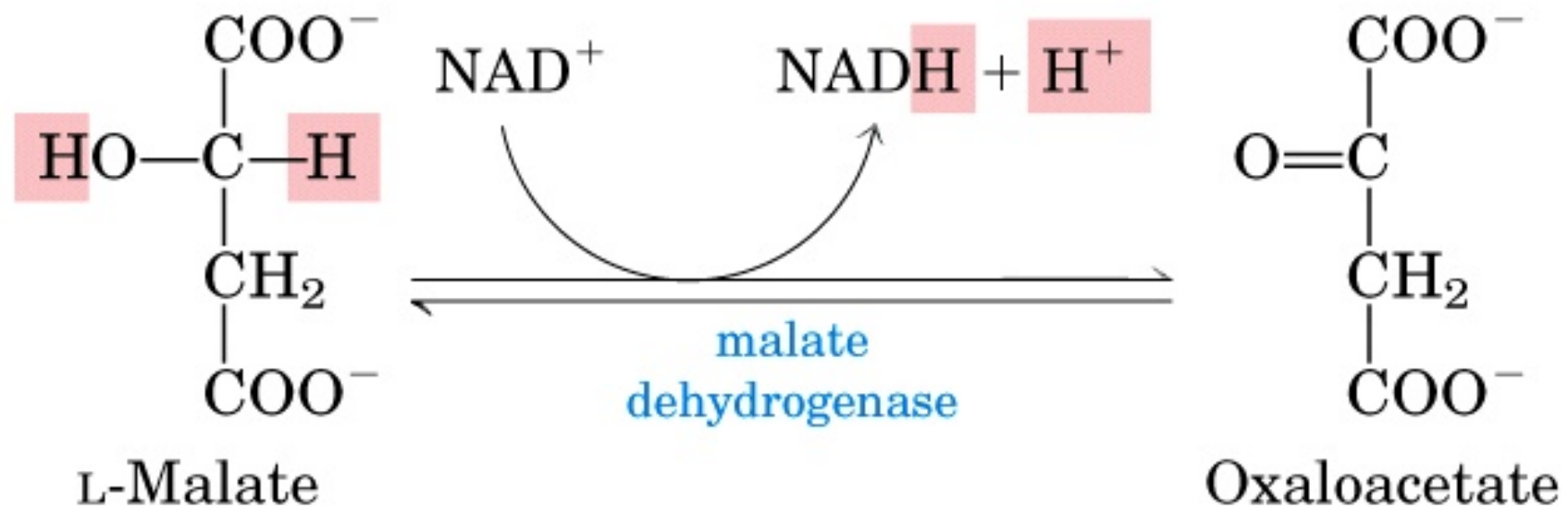
$$\Delta G'^{\circ} = 0 \text{ kJ/mol}$$

7. Fumarase: Hydration of Fumarate to malate: It is a highly stereospecific enzyme. Cis-Maleate (the cis form of fumarate is not recognized by this enzyme).



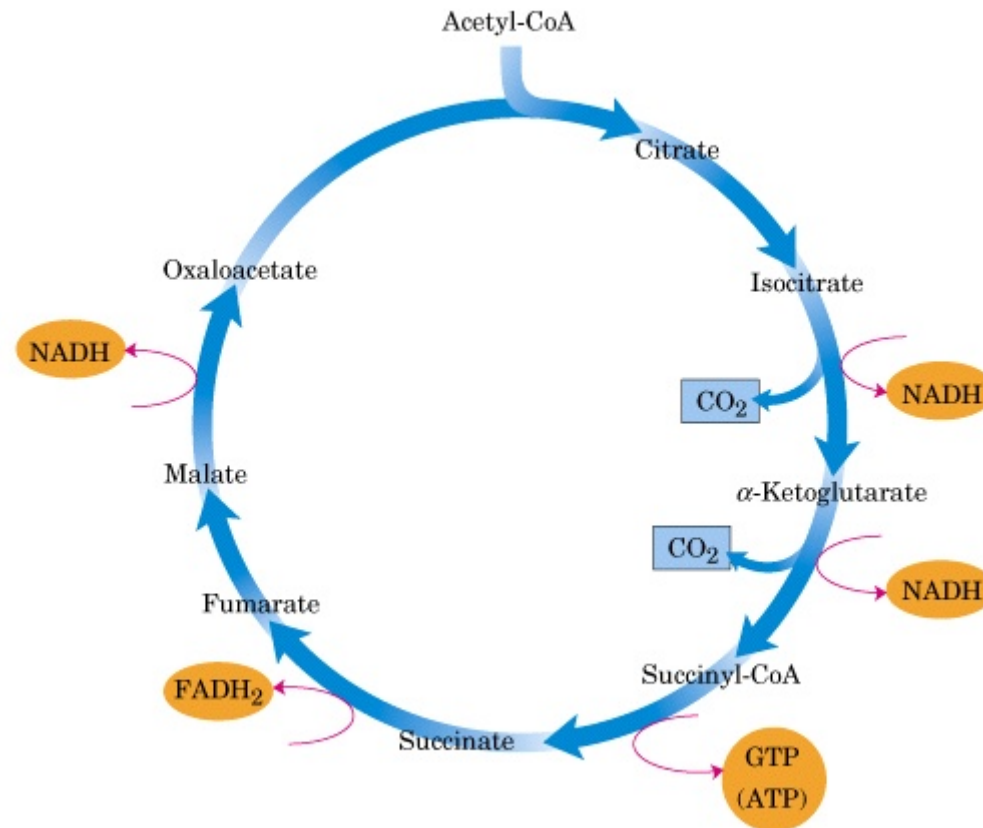
$$\Delta G'^{\circ} = -3.8 \text{ kJ/mol}$$

8. L-Malate dehydrogenase: Oxidation of malate to oxaloacetate: It is an NAD^+ dependent enzyme. Reaction is pulled in forward direction by the next reaction (citrate synthase reaction) as the oxaloacetate is depleted at a very fast rate.



$$\Delta G'^{\circ} = 29.7 \text{ kJ/mol}$$

Conservation of energy of oxidation in the CAC: The two carbon acetyl group generated in PDC reaction enter the CAC, and two molecules of CO₂ are released in one cycle. Thus there is complete oxidation of two carbons during one cycle. Although the two carbons which enter the cycle become the part of oxaloacetate, and are released as CO₂ only in the third round of the cycle. The energy released due to this oxidation is conserved in the reduction of **3 NAD⁺**, **1 FAD** molecule and synthesis of one **GTP** molecule which is converted to ATP.



Efficiency of Biochemical engine in Living Systems:

Oxidation of one glucose yields 2840 kJ/mole energy

Energy obtained by biological engine: $32\text{ATP} \times 30.5 \text{ kJ/Mol} = 976 \text{ kJ/mol}$

Thus 34% efficiency is obtained if calculations are done using standard conditions. But if concentrations in the cellular condition are taken in account, the efficiency is close to 65%.

table 16-1

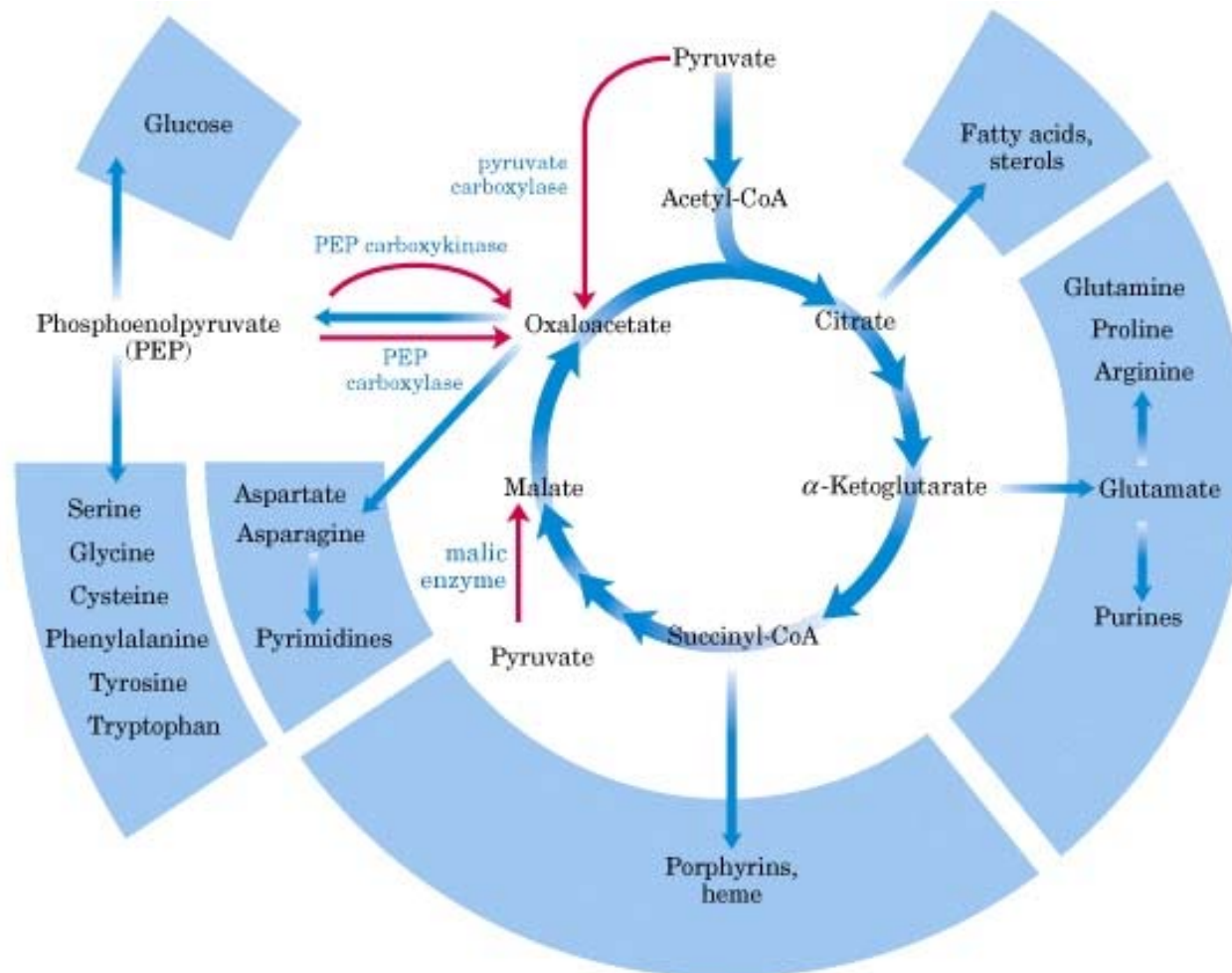
Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

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

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH₂. A negative value indicates consumption.

The amphibolic nature of Citric acid cycle: This pathway is utilized for the both catabolic reactions to generate energy as well as for anabolic reactions to generate metabolic intermediates for biosynthesis.

If the CAC intermediate are used for synthetic reactions, they are replenished by anaplerotic reactions in the cells (indicated by red colours).



Regulation of CAC:

Rate controlling enzymes:

Citrate synthetase

Isocitrate dehydrogenase

α -ketoglutarate dehydrogenase

Regulation of activity by:

Substrate availability

Product inhibition

Allosteric inhibition or activation by other intermediates

