

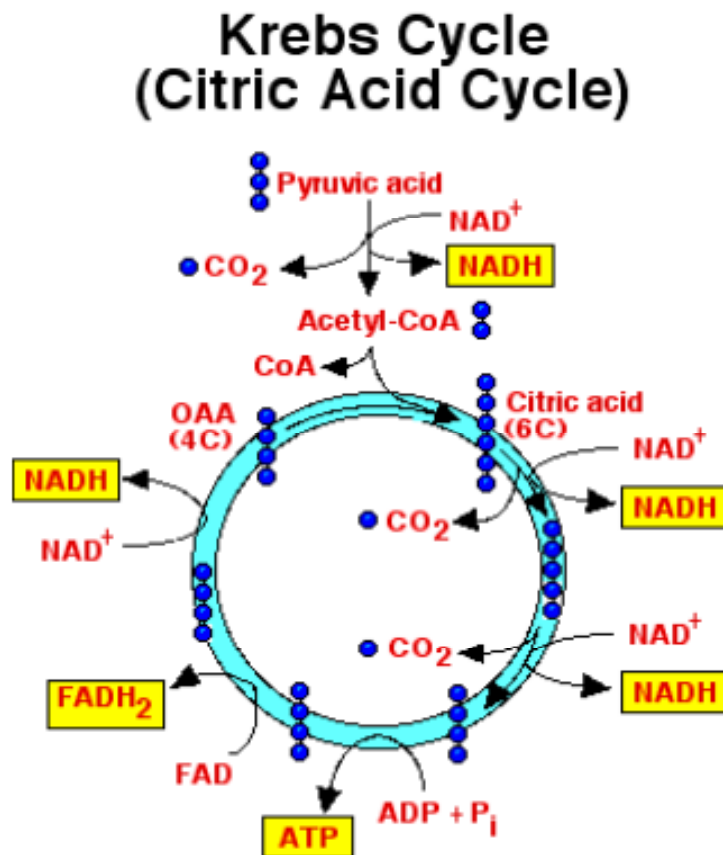
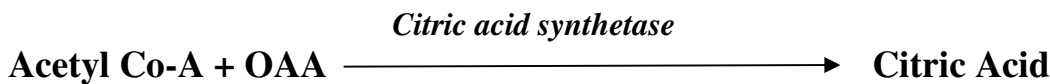
# Krebs' Cycle

The Krebs cycle is also known as the *citric acid cycle*. Citrate is a tricarboxylic acid, and the Krebs cycle is also known as the *tricarboxylic acid* (or *TCA*) cycle. It is 2<sup>nd</sup> part of aerobic respiration. It occurs in mitochondria.

Pyruvic acid (3-C compound) produced in glycolysis in cytosol is converted into Acetyl Co-A by release of one CO<sub>2</sub> molecule and formation of one molecule of NADH. The enzyme is Pyruvate dehydrogenase. Acetyl Co-A enters into mitochondria for the first reaction of Krebs' cycle.

## Step 1: Condensation

In step 1 of the Krebs cycle, the two-carbon compound, *acetyl-S-CoA*, participates in a condensation reaction with the four-carbon compound, *oxaloacetate*, to produce *citrate*.



## Step 2. Isomerization of Citrate

It involves moving the hydroxyl group in the citrate molecule by a sequential dehydration and hydration reaction, to form the *D-Isocitrate* isomer with *cis-Aconitase* as the intermediate. A single enzyme, *Aconitase*, performs this two-step process.



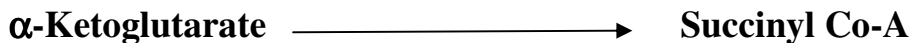
## Step 3: Generation of CO<sub>2</sub> by an NAD<sup>+</sup> linked enzyme

It is the first oxidative decarboxylation step of Krebs' Cycle. The reaction is catalyzed by the enzyme *Isocitrate dehydrogenase*. Isocitrate is converted to  $\alpha$ -Ketoglutaric acid. The reaction involves dehydrogenation to *Oxalosuccinate*, an unstable intermediate which spontaneously decarboxylates to give  *$\alpha$ -Ketoglutarate*. In addition to decarboxylation, this step produces a reduced nicotinamide adenine dinucleotide (NADH) cofactor.



## Step 4: A Second Oxidative Decarboxylation Step

This step is performed by a multi-enzyme complex, the  *$\alpha$ -Ketoglutarate Dehydrogenation Complex*.



## Summary of reactions till Step 4

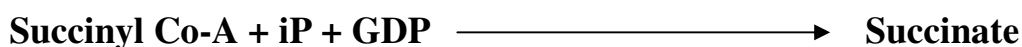
Two carbons have been added to *Oxaloacetate* by the action of *Citrate Synthase* (and *Acetyl-CoA*). Two carbons have been lost as CO<sub>2</sub> by oxidative decarboxylation steps. Two oxidized NAD<sup>+</sup> cofactors have been reduced to NADH.

In the remaining steps of the Krebs cycle, the Succinyl-CoA is converted back into the original substrate for the cycle: Oxaloacetate.

## Step 5: Substrate-Level Phosphorylation

Succinyl-CoA is a high potential energy molecule. The energy stored in this molecule is used to form a high energy phosphate bond in a Guanine nucleotide diphosphate (GDP) molecule.

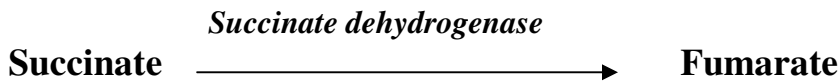
*Succinyl Co-A synthetase*



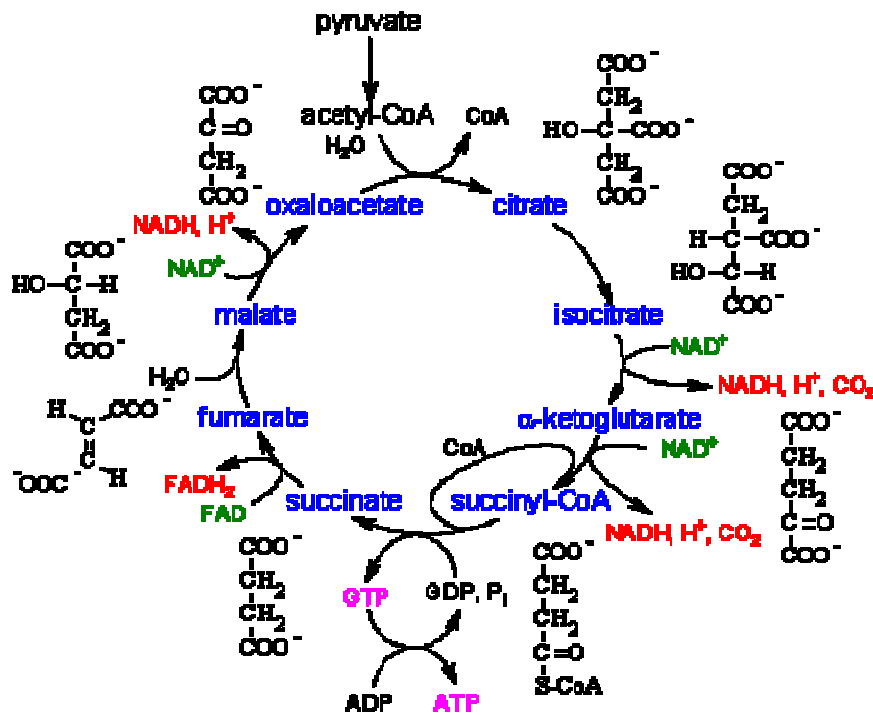
Most of the GTP formed is used in the formation of ATP, by the action of *Nucleoside Diphosphokinase*. In plants and bacteria ATP is formed in the *Succinyl-CoA Synthase* catalyzed reaction by phosphorylation of ADP directly. In animals, GDP is the substrate in the reaction with formation of GTP (which is then used to form ATP by *Nucleoside Diphosphokinase*).

### Step 6: Flavin-Dependent Dehydrogenation

The Succinate produced by *Succinyl CoA-Synthetase* in the prior reaction needs to be converted to Oxaloacetate to complete the Krebs cycle. Both Succinate and Oxaloacetate are 4-carbon compounds. The first step in the conversion is the dehydrogenation of Succinate to yield Fumarate.



In this reaction a C-C bond is being oxidized to produce a C=C bond. This oxidation is energetically more costly than oxidizing a C-O bond. The *redox coenzyme* for this reaction is FAD, and not  $\text{NAD}^+$  (FAD is a more powerful oxidizing agent compared to  $\text{NAD}^+$ ). FAD is covalently bound to the *Succinate Dehydrogenase* molecule.



Reactions at molecular level

### **Step 7: Hydration of a Carbon-Carbon Double Bond**

Fumarate undergoes a stereo-specific hydration of the C=C double bond, catalyzed by *Fumarate Hydratase* (also known as *Fumarase*), to produce L-Malate. *Fumarase* is the enzyme. Its hydrate Fumarate,

### **Step 8: A Dehydrogenation Reaction that Regenerates Oxaloacetate**

L-Malate (Malate) is dehydrogenated to produce Oxaloacetate by the enzyme *Malate Dehydrogenase*.

This is a highly endergonic reaction, and so, the equilibrium strongly favors the *reactants* over the products.

The step where acetyl Co-A condenses with OAA is a highly exergonic reaction catalyzed by *Citrate Synthase*. It keeps the level of Oxaloacetate low in the cell (mitochondrion) allowing the above reaction to proceed.

### **Summary of Krebs' Cycle in terms of ATP (energy gain)**

Total NADH in one Krebs' cycle

= 4 molecules

From one glucose molecule

= 8 NADH molecules.

*8 NADH molecules would yield  $8 \times 3 = 24$  ATP molecules in electron transport system.*

Total FADH<sub>2</sub> in one Krebs' cycle

= 1 molecule

From one glucose molecule

= 2 FADH<sub>2</sub> molecules.

*2 FADH<sub>2</sub> molecules would yield  $2 \times 2 = 4$  ATP in E.T.S.*

ATP production at substrate level is  $1 \times 2$

= 2 molecules from one glucose molecule.

*Total ATP generation from one glucose molecule in Krebs' cycle source*

*=  $24 + 4 + 2 = 30$  molecules.*