

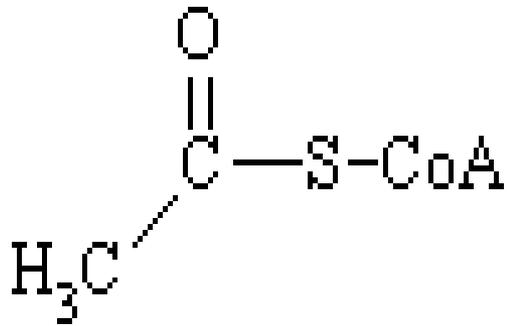
# **3. CITRIC ACID CYCLE**

- The citric acid cycle (Kreb's cycle, Tricarboxylic acid cycle) is a series of reactions in mitochondria that bring about the catabolism of acetyl residues, to CO<sub>2</sub> and water in aerobic condition.
- The hydrogen equivalents upon oxidation, lead to the release of most of the free energy which is captured as ATP of most of the available energy of tissue fuels.
- The acetyl residues are in the form of acetyl-CoA (CH<sub>3</sub>-CO~SCoA, active acetate), an ester of coenzyme A.
- Coenzyme A contains the vitamin pantothenic acid.

- The major function of the citric acid is to act as the final common pathway for the oxidation of carbohydrate, lipids, and protein.
- This is because glucose, fatty acids, and many amino acids are all metabolized to acetyl-CoA or intermediates of the cycle.

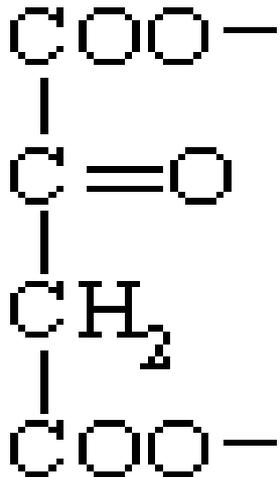
- It also plays a major role in gluconeogenesis, transamination, deamination, and lipogenesis.
- Several of these processes are carried out in many tissues but the liver is the only tissue in which all occur to a significant extent.
- Reactions of the citric acid cycle liberate reducing equivalents and CO<sub>2</sub>
- The reactions of citric acid cycle the following steps:

- **1. Condensation of acetyl-CoA with oxaloacetate to form citrate**
- The initial condensation of acetyl-CoA with oxaloacetate to form citrate is catalyzed by condensing enzyme, citrate synthase.

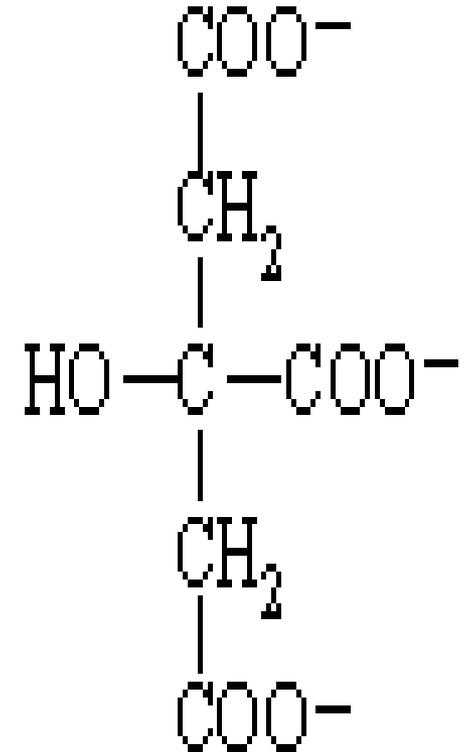
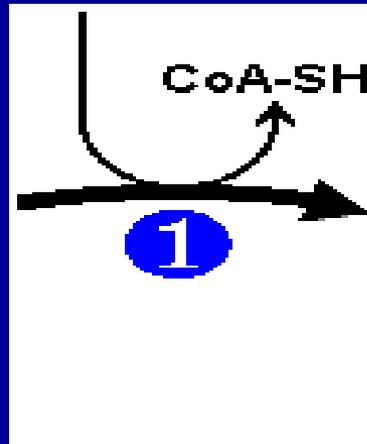


Acetyl CoA

Citrate synthase



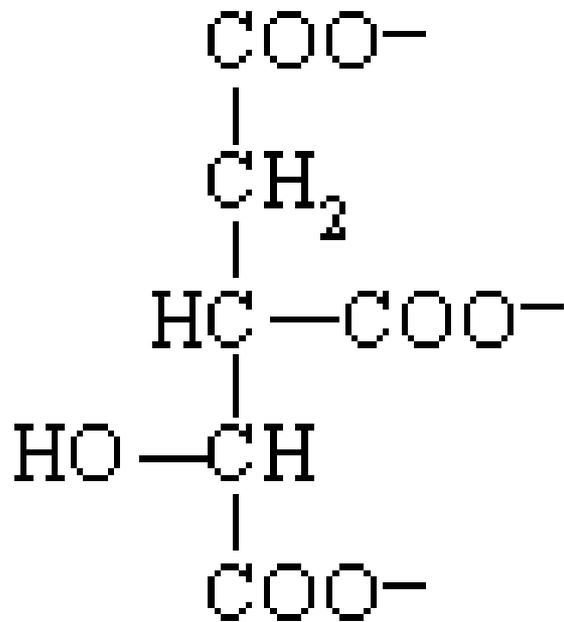
Oxaloacetate



Citrate

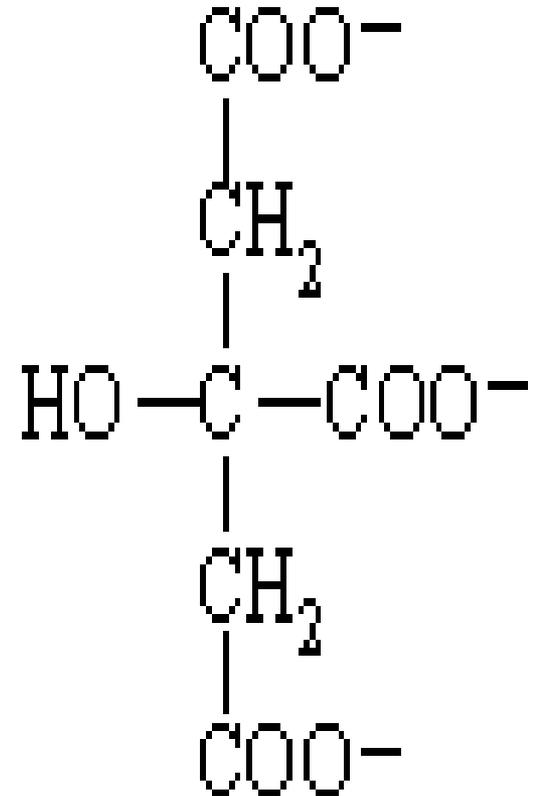
## 2. Conversion of citrate to isocitrate via *cis*-aconitate

- Citrate is converted to isocitrate by the enzyme aconitase (aconitate hydratase), which contains  $\text{Fe}^{2+}$ .
- This conversion takes place in two steps: dehydration to *cis*-aconitate and rehydration to isocitrate.



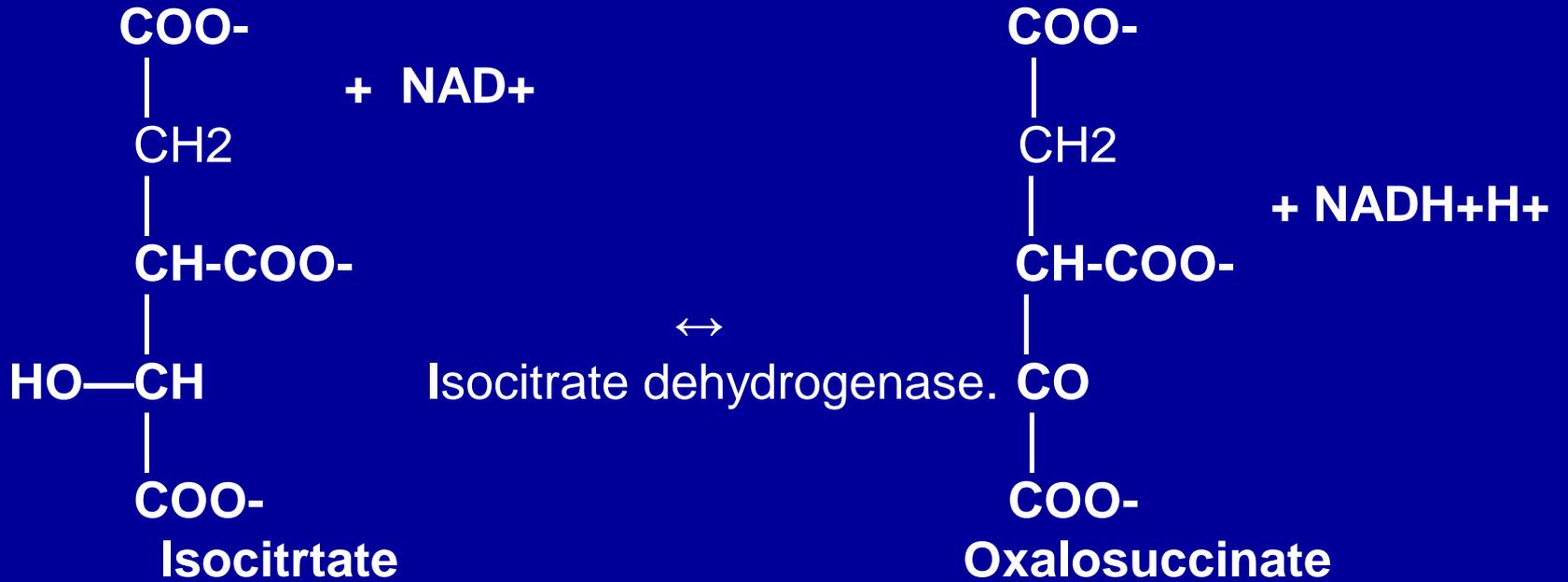
Citrate

Aconitase



Isocitrate

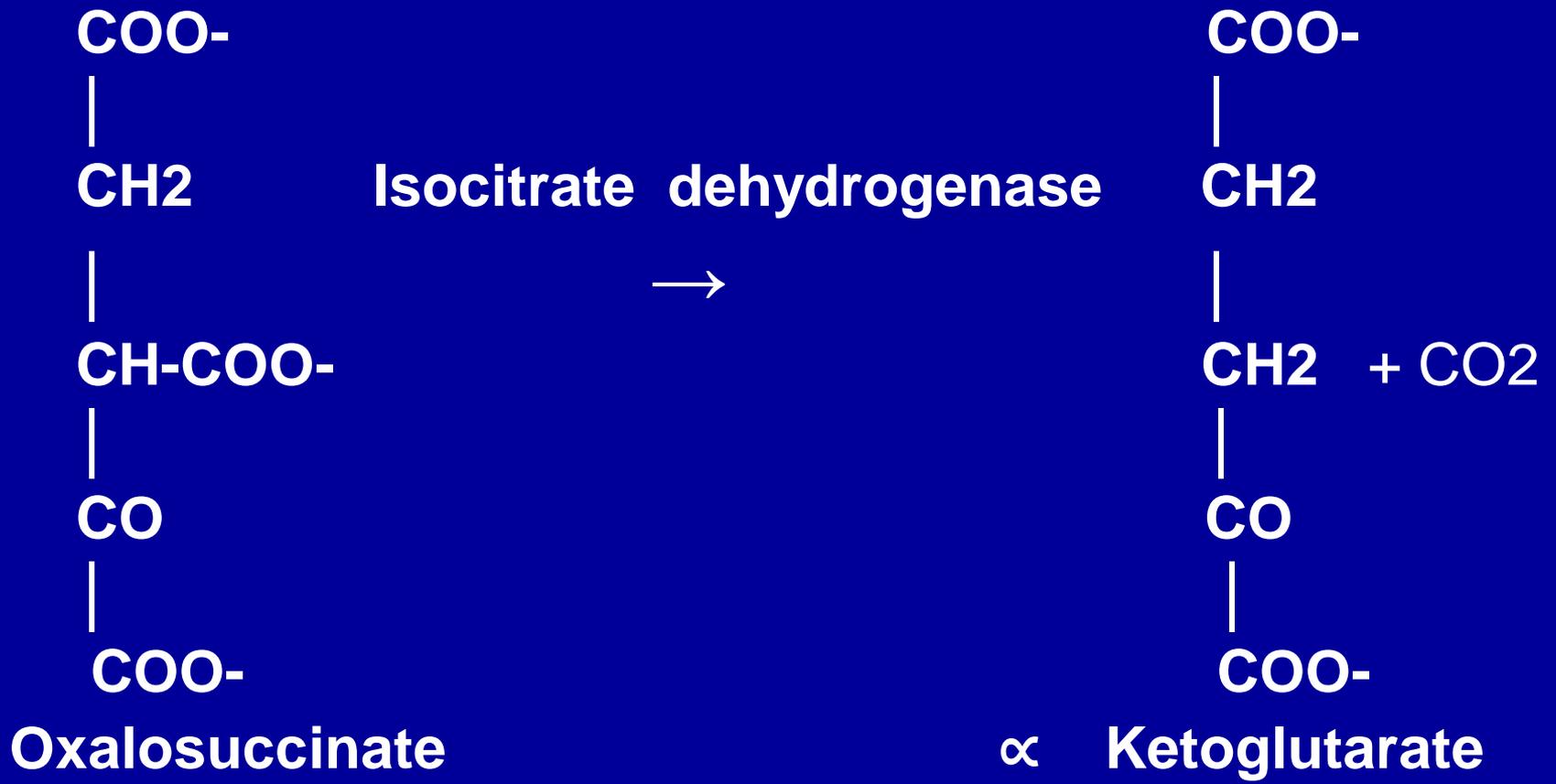
- **3. Dehydrogenation of isocitrate to**
- **oxalosuccinate**
  
- Isocitrate undergoes dehydrogenation in the presence of isocitrate dehydrogenase to form oxalosuccinate.
  
- The linked oxidation of isocitrate proceeds almost completely through the NAD<sup>+</sup> dependent enzyme isocitrate dehydrogenase.



## 4. Decarboxylation of oxalosuccinate to $\alpha$ -ketoglutarate

There follows decarboxylation of oxalosuccinate to  $\alpha$ -ketoglutarate, also catalyzed by isocitrate dehydrogenase.

A  $\text{CO}_2$  molecule is liberated.  $\text{Mn}^{2+}$  (or  $\text{Mg}^{2+}$ ) is an important component of the decarboxylation.



## 5. Decarboxylation of $\alpha$ -ketoglutarate to succinyl-CoA

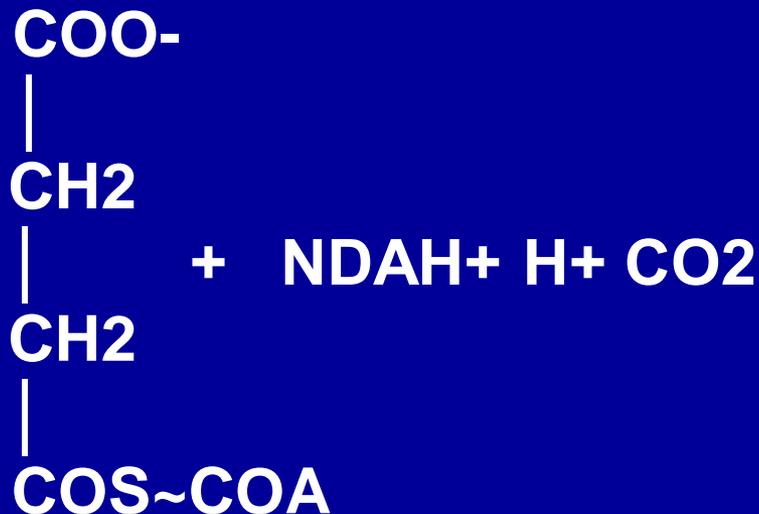
$\alpha$ -ketoglutarate undergoes oxidative decarboxylation.

The reaction is catalyzed by a  $\alpha$ -ketoglutarate dehydrogenase complex, which requires cofactors thiamin pyrophosphate, lipoate, NAD<sup>+</sup>, FAD and CoA results in the formation of succinyl-CoA, a high-energy thioester and NADH.

Arsenic inhibits the reaction, causing the substrate,  $\alpha$ -ketoglutarate to accumulate



$\alpha$  Ketoglutarate



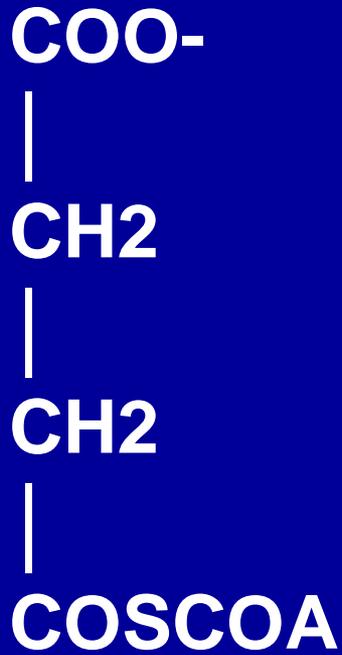
Succinyl CoA

- **6. Conversion of succinyl-CoA to succinate**

Succinyl-CoA is converted to succinate by the enzyme succinate thiokinase (succinyl-CoA synthetase).

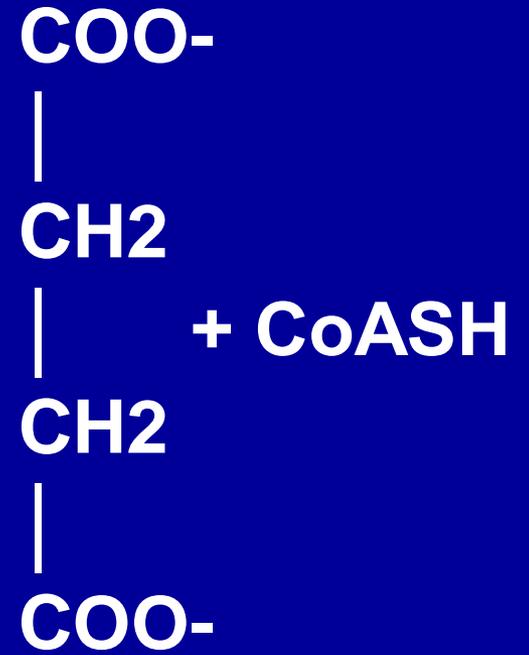
High-energy phosphate (ATP) is synthesized at the substrate level because the release of free energy from the oxidative decarboxylation of  $\alpha$ -ketoglutarate.

The reaction requires GDP or IDP which is converted to GTP or ITP in the presence of inorganic phosphate which then convert ADP to ATP.



**Succinyl CoA**

**Succinate thiokinase**



**Succinate**

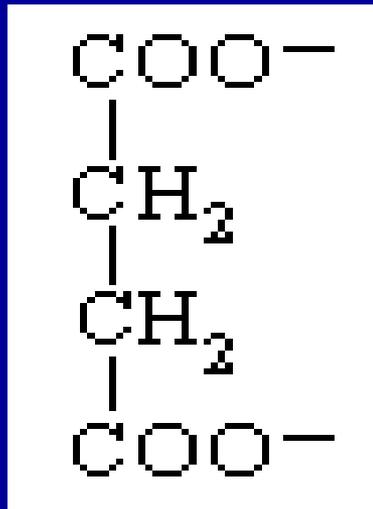
## 7. Dehydrogenation of succinate to fumarate

Succinate is metabolized further by undergoing a dehydrogenation catalyzed by succinate dehydrogenase.

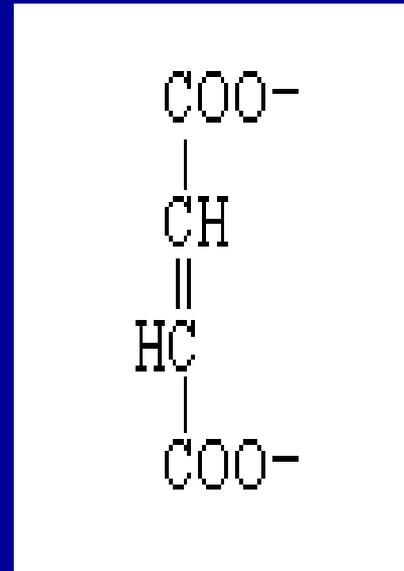
It is the only dehydrogenation in the citric acid cycle that involves the direct transfer of hydrogen from the substrate to a flavin protein without the participation of  $\text{NAD}^+$ .

The enzyme contains FAD and iron-sulfur (Fe:S) protein.

Fumarate is formed.



+ FAD<sup>+</sup>



+

FADH<sub>2</sub>

**Succinate**

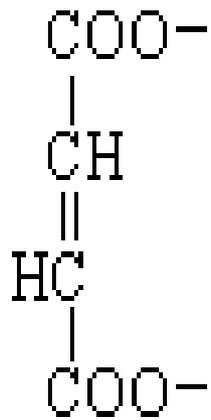
**Succinate dehydrogenase**

**Fumerate**

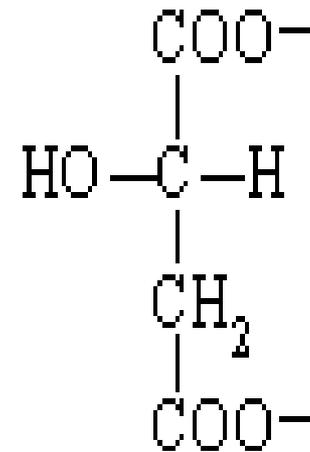
- **8. Addition of water to fumarate to give malate.**

Fumarase (fumarate hydratase) catalyzes the addition of water to fumarate to give malate.

In addition to being specific for the L-isomer of malate, fumarase catalyzes the addition of the elements of water to the double bond of fumarate in the *trans* configuration.



+ H<sub>2</sub>O



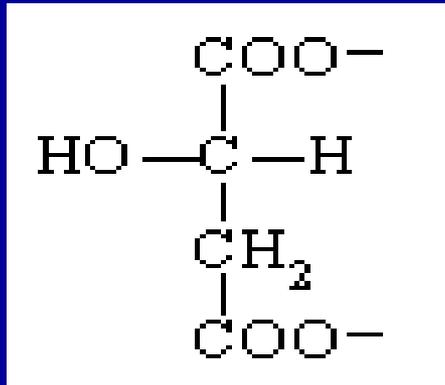
Fumarase

Fumerate

Malate

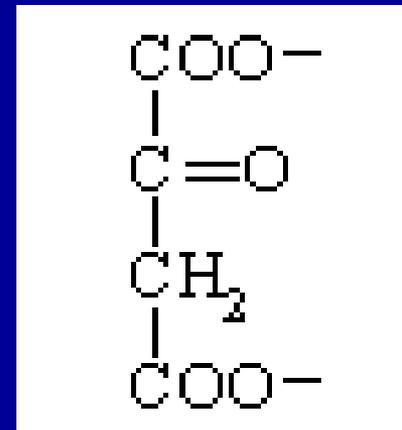
- **9. Dehydrogenation of malate to form oxaloacetate**
- Malate is converted to oxaloacetate by dehydrogenation catalysed by the enzyme malate dehydrogenase, a reaction requiring NAD<sup>+</sup>.

Malate  
dehydrogenase



Malate

+ NAD<sup>+</sup>



Oxaloacetate

+ NADH + H<sup>+</sup>

- One turn around the citric acid cycle is completed. An acetyl group, containing two carbon atoms, is fed into the cycle by combining it with oxaloacetate.
- At the end of the cycle a molecule of oxaloacetate was generated.
- The enzymes of the citric acid cycle, except for the  $\alpha$ -ketoglutarate and succinate dehydrogenase, are also found outside the mitochondria.

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- As a result of oxidations catalyzed by dehydrogenase enzymes of the citric acid cycle, three molecules of NADH and one molecule of FADH<sub>2</sub> are produced for each molecule of acetyl-CoA catabolized in one revolution of the cycle.
- These reducing equivalents are transferred to the respiratory chain in the inner mitochondrial membrane.

# Respiratory chain and ATP production

Pyruvate

Isocitrate

$ADP+P_i \rightarrow ATP$     $ADP+P_i \rightarrow ATP$

$\alpha$ ketoglutarate  $\rightarrow$  NAD  $\rightarrow$  FMN  $\rightarrow$  Co Q  $\rightarrow$  Cytb  $\rightarrow$  CytC1  $\rightarrow$

Malate

CytC  $\rightarrow$  Cyta  $\rightarrow$  Cyta3

$\downarrow$   
 $ADP+P_i \rightarrow ATP$

- During passage along the respiratory chain, reducing equivalents from each NADH generate three high-energy phosphate bonds by the esterification of ADP to ATP in the process of oxidative phosphorylation.
- However, FADH<sub>2</sub> produces only two high-energy phosphate bonds because it transfers its reducing power to Co Q, by passing the first site for oxidative phosphorylation in the respiratory chain.

- A further high-energy phosphate is generated at the level of the cycle itself (i.e., at substrate level) during the conversion of succinyl-CoA to succinate.
- Thus, 12 ATP molecules are generated for each turn of the cycle

# Energetics of Citric acid cycle

Name of enzyme	Reaction Catalyzed	No. of ATP formed
Isocitrate dehydrogenase	Respiratory chain oxidation of NADH +H <sup>+</sup>	3
$\alpha$ -Ketoglutarate dehydrogenase	Respiratory chain oxidation of NADH +H <sup>+</sup>	3
Succinate thiokinase	Phosphorylation at substrate level	1
Succinate dehydrogenase	Respiratory chain oxidation of FADH <sub>2</sub>	2
Malate dehydrogenase	Respiratory chain oxidation of NADH +H <sup>+</sup>	3
	Net	12

# Total number of ATP Produced during the complete oxidation of one molecules of glucose

- One molecule of glucose is converted to 2 molecules of pyruvate by glycolysis and the pyruvate is further converted to acetyl CoA by pyruvate dehydrogenase before entering into citric acid cycle.
- During this process two more molecules of NADH are available for oxidation by the electron transport cycle reoxidation route to yield 6 ATP molecules.
- $30+8=38$ ) ATP molecule per each glucose molecule.

- During the conversion of glucose to pyruvate two more molecules of ATP are available through substrate-linked phosphorylation.
- Thus the total number of ATP produced by the aerobic oxidation of glucose to carbon dioxide and water is 38
- $(12+3=15 \times 2)$

## **Significance of Citric acid cycle**

It acts as a common pathway for the oxidation of carbohydrate, lipid and protein because glucose, fatty acids and many amino acids are metabolised to acetyl-CoA Which is finally oxidized in the citric acid cycle.

The reducing equivalents in the form of hydrogen or electrons are formed by the action of specific dehydrogenase during the oxidation of acetyl CoA in the cycle.

- These reducing equivalents then enter the respiratory chain where large amounts of high energy phosphate are generated by the oxidative phosphorylation.
- The enzymes of citric acid cycle are located in the mitochondrial matrix.
- Either free or attached in the inner mitochondrial membrane which facilitates the transfer of reducing equivalents to the adjacent enzymes of electron transport chain which is situated in the inner mitochondrial membrane.

- Citric acid cycle is an amphibolic pathway as it functions not only in the oxidative degradation of carbohydrates, fatty acids and amino acids but also as a source of precursors for the anabolic process such as synthesis of fatty acid and amino acids.

