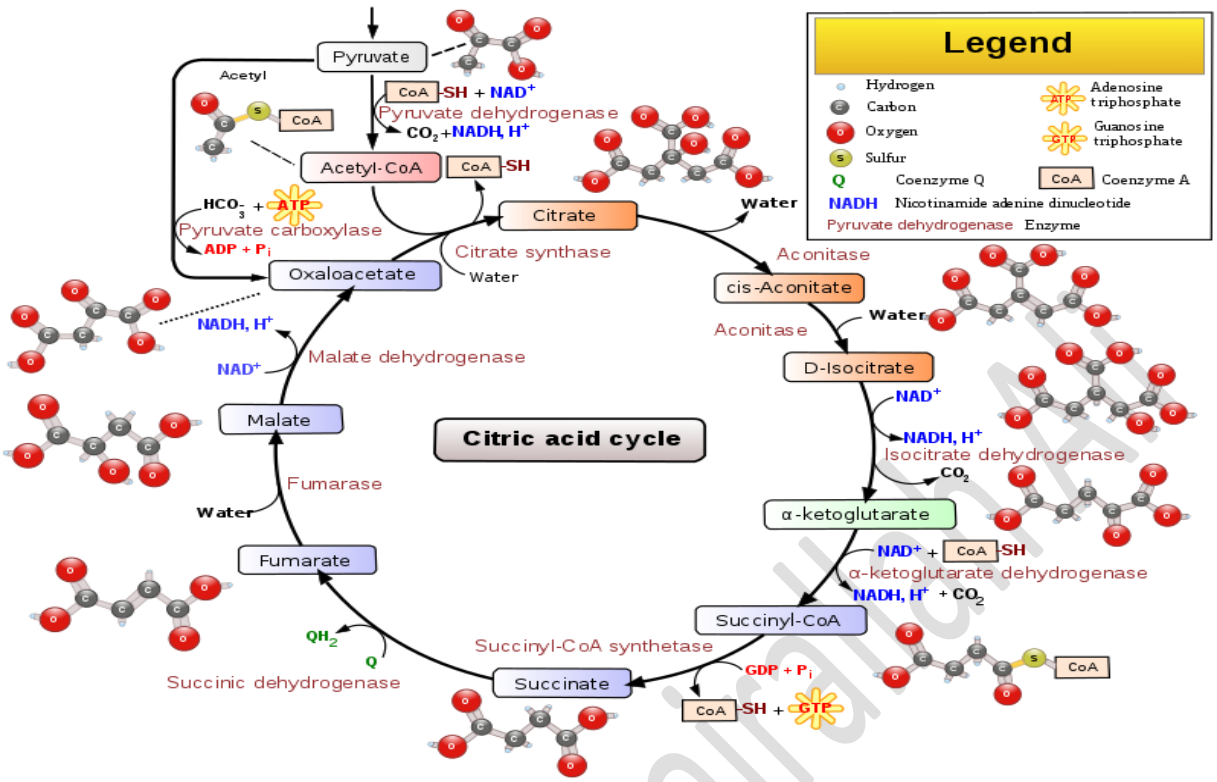
Citric acid cycle

The Krebs Cycle, also called the citric acid cycle, is the second major step in oxidative phosphorylation. After glycolysis breaks glucose into smaller 3-carbon molecules, the Krebs cycle transfers the energy from these molecules to electron carriers, which will be used in the electron transport chain to produce ATP.

The citric acid cycle (CAC)—also known as the Krebs cycle or the TCA cycle (tricarboxylic acid cycle) is a series of chemical reactions to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins. The Krebs cycle is used by organisms that respire (as opposed to organisms that ferment) to generate energy, either by anaerobic respiration or aerobic respiration. In addition, the cycle provides precursors of certain amino acids, as well as the reducing agent NADH, that are used in numerous other reactions. Its central importance to many biochemical pathways suggests that it was one of the earliest components of metabolism and may have originated abiogenically. Even though it is branded as a 'cycle', it is not necessary for metabolites to follow only one specific route.



In eukaryotic cells, the citric acid cycle occurs in the matrix of the mitochondrion. In prokaryotic cells, such as bacteria, which lack mitochondria, the citric acid cycle reaction sequence is performed in the cytosol with the proton gradient for ATP production being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion. The overall yield of energy-containing compounds from the citric acid cycle is three NADH, one FADH2, and one GTP.

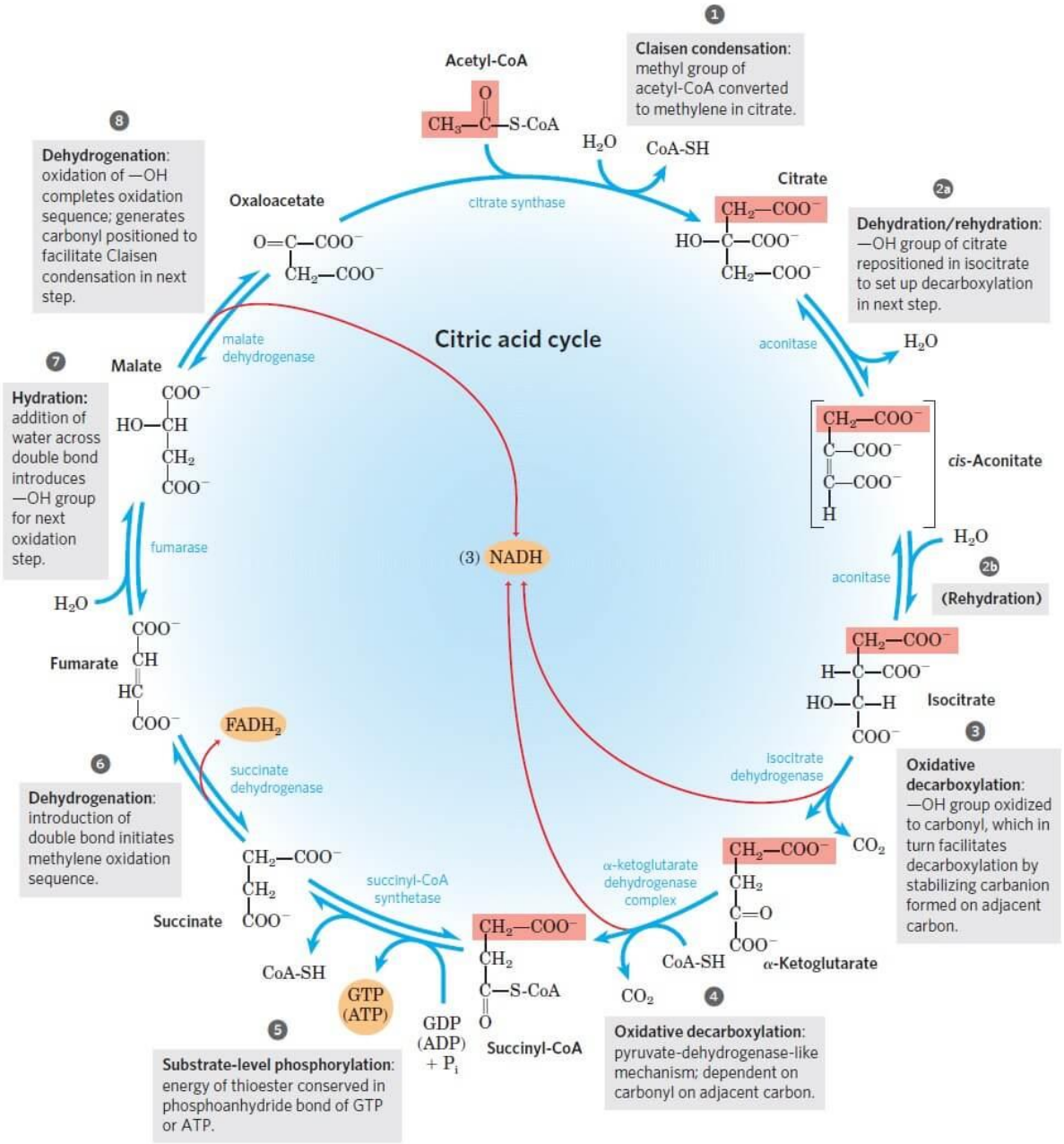
The citric acid cycle is a key metabolic pathway that connects carbohydrate, fat, and protein metabolism. The reactions of the cycle are carried out by eight enzymes that completely oxidize acetate (a two carbon molecule), in the form of acetyl-CoA, into two molecules each of carbon dioxide and water. Through catabolism of sugars, fats, and proteins, the two-carbon organic product acetyl-CoA is produced which enters the citric acid cycle. The reactions of the cycle also convert three equivalents of nicotinamide adenine dinucleotide (NAD+) into three equivalents of reduced NAD+ (NADH), one equivalent of flavin adenine dinucleotide (FAD) into one equivalent of FADH2, and one equivalent each of guanosine diphosphate (GDP) and inorganic phosphate (Pi) into

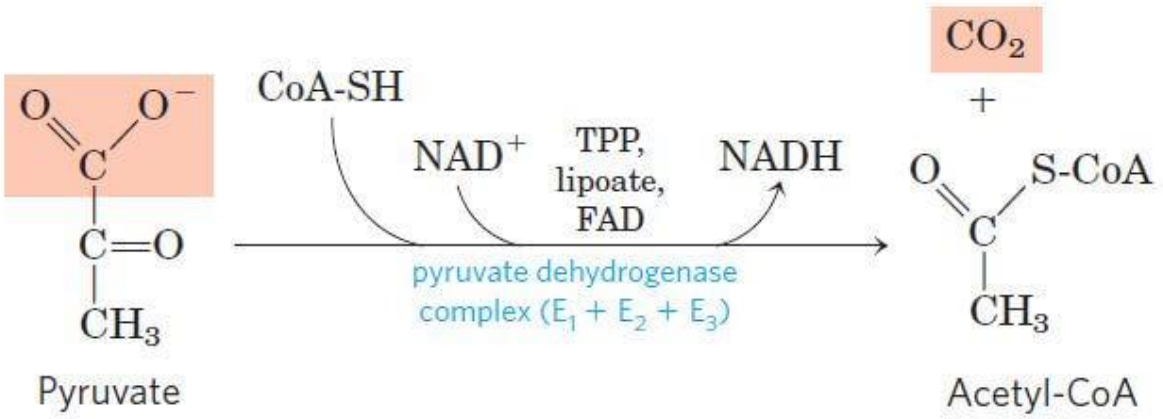
one equivalent of guanosine triphosphate (GTP). The NADH and FADH2 generated by the citric acid cycle are, in turn, used by the oxidative phosphorylation pathway to generate energy-rich ATP.

One of the primary sources of acetyl-CoA is from the breakdown of sugars by glycolysis which yield pyruvate that in turn is decarboxylated by the pyruvate dehydrogenase complex generating acetyl-CoA according to the following reaction scheme:

$$\text{pyruvate} + \text{HS-CoA} + \text{NAD}^+ \rightarrow \text{acetyl-CoA} + \text{NADH} + \text{CO}_2$$

The product of this reaction, acetyl-CoA, is the starting point for the citric acid cycle. Acetyl-CoA may also be obtained from the oxidation of fatty acids. Below is a schematic outline of the cycle:

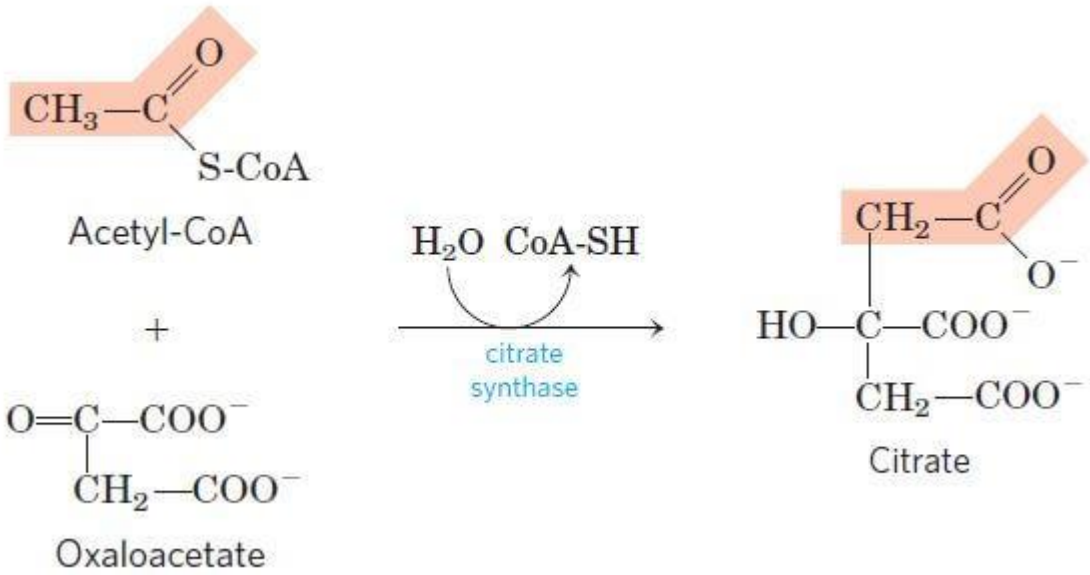

Citric acid cycle
1
Claisen condensation: methyl group of acetyl-CoA converted to methylene in citrate.
Acetyl-CoA
CH3—C—S-CoA
H2O
CoA-SH
Citrate
citrate synthase
Oxaloacetate
O=C—COO⁻
CH2—COO⁻
2a
Dehydration/rehydration: —OH group of citrate repositioned in isocitrate to set up decarboxylation in next step.
aconitase
H2O
cis-Aconitate
aconitase
H2O
2b
(Rehydration)
Isocitrate
3
Oxidative decarboxylation: —OH group oxidized to carbonyl, which in turn facilitates decarboxylation by stabilizing carbanion formed on adjacent carbon.
(3) NADH
CO2
Isocitrate dehydrogenase
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
CoA-SH
CO2
4
Oxidative decarboxylation: pyruvate-dehydrogenase-like mechanism; dependent on carbonyl on adjacent carbon.
Succinyl-CoA
GDP (ADP) + Pi
GTP (ATP)
CoA-SH
succinyl-CoA synthetase
5
Substrate-level phosphorylation: energy of thioester conserved in phosphoanhydride bond of GTP or ATP.
Succinate
FADH2
succinate dehydrogenase
6
Dehydrogenation: introduction of double bond initiates methylene oxidation sequence.
Fumarate
H2O
fumarase
7
Hydration: addition of water across double bond introduces —OH group for next oxidation step.
Malate
malate dehydrogenase
8
Dehydrogenation: oxidation of —OH completes oxidation sequence; generates carbonyl positioned to facilitate Claisen condensation in next step.



$\Delta G'^{\circ} = -33.4$ kJ/mol

Step 1: The first step is the condensation of acetyl CoA with 4-carbon compound oxaloacetate to form 6C citrate, coenzyme A is released. The reaction is catalysed **by *citrate synthase*.**

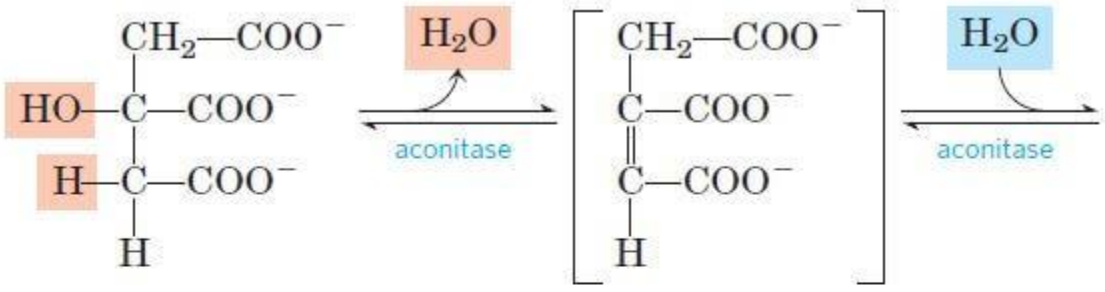
Formula: $acetyl - CoA + oxaloacetate + H_2O \xrightarrow{Citrate\ synthase} citrate + CoA - SH$



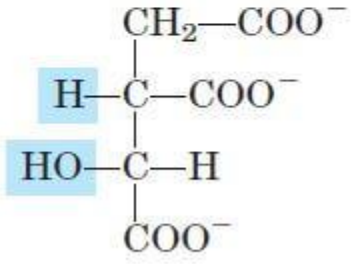
Step 2: Citrate is converted to its isomer, isocitrate. The enzyme ***aconitase*** catalyses this reaction.

Formula (a): $Citrate \xrightarrow{Aconitase} cis-Aconitate + H_2O$

(b): $cis-Aconitate + H_2O \xrightarrow{Aconitase} D-Isocitrate$



Citrate *cis*-Aconitate

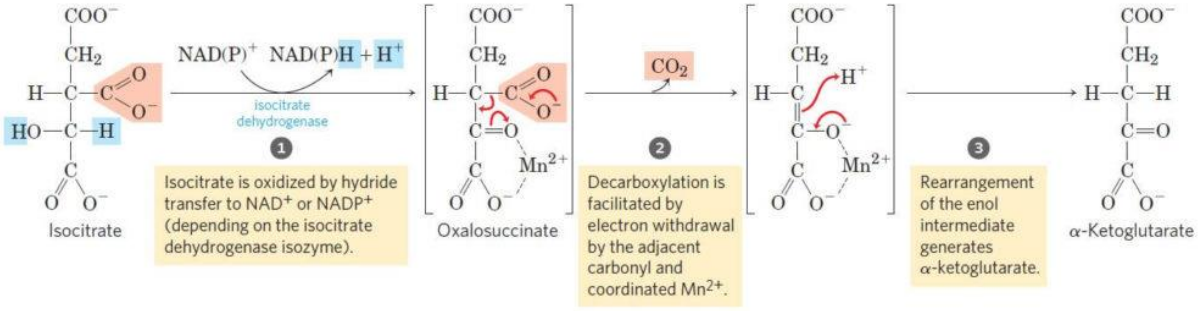


Isocitrate

Step 3: Isocitrate undergoes dehydrogenation and decarboxylation to form 5C **α**-ketoglutarate. A molecular form of CO_2 is released. ***Isocitrate dehydrogenase*** catalyses the reaction. It is an NAD^+ dependent enzyme. NAD^+ is converted to NADH.

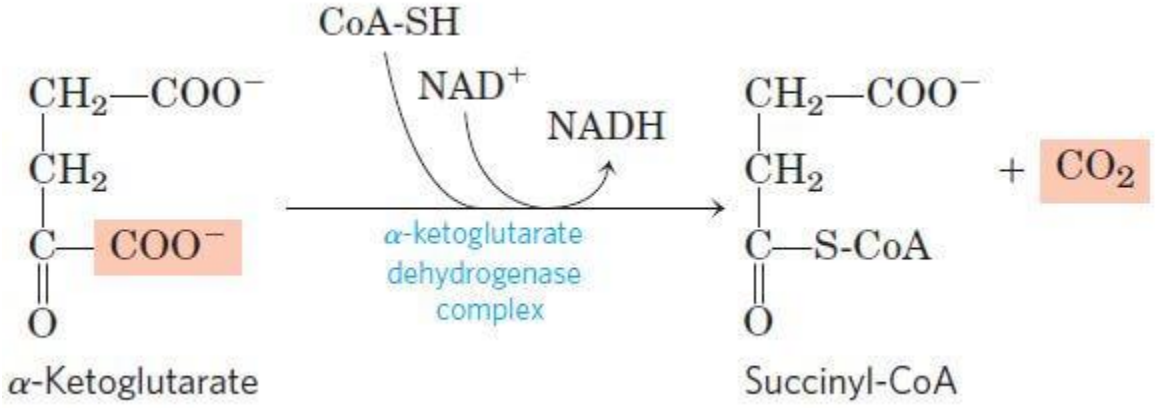
Formula: (a): $D-Isocitrate + NAD^+ \xrightarrow{Isocitrate\ dehydrogenase} Oxalosuccinate + NADH + H^+$

(b): $Oxalosuccinate \xrightarrow{Isocitrate\ dehydrogenase} \alpha-Ketoglutarate + CO_2$



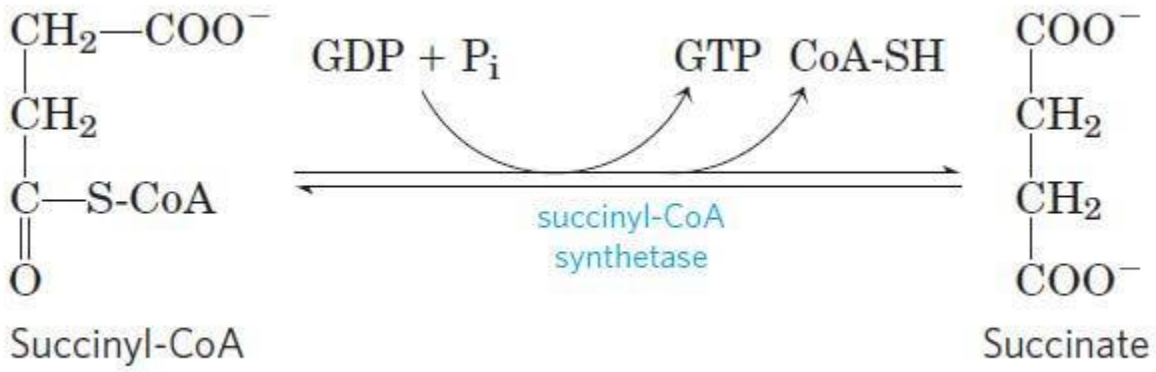
Step 4: α-ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by the ***α-ketoglutarate dehydrogenase*** **enzyme complex**. One molecule of CO_2 is released and NAD^+ is converted to NADH.

Formula: $$\alpha - Ketoglutarate + NAD^+ + CoA - SH \xrightarrow{\alpha-Ketoglutarate\ dehydrogenase} Succinyl - COA + CO_2 + NADH + H^+$$

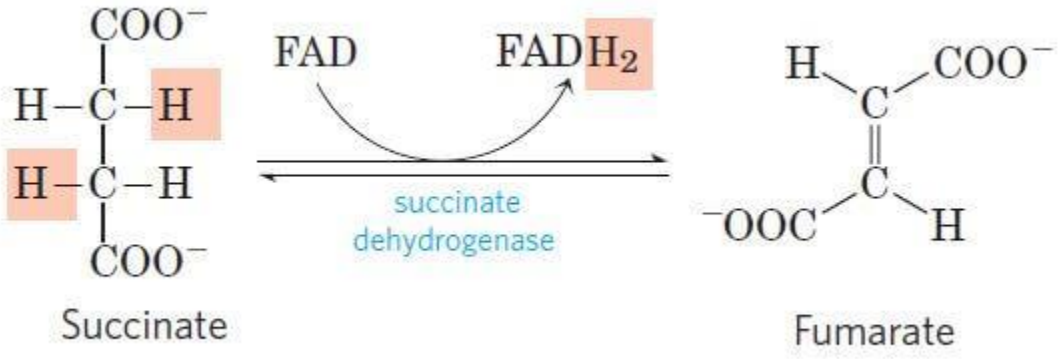


Step 5: Succinyl CoA forms succinate. The enzyme ***succinyl CoA synthetase*** catalyses the reaction. This is coupled with substrate-level phosphorylation of GDP to get GTP. GTP transfers its phosphate to ADP forming ATP.

Formula: $Succinyl - COA + GDP + P \xrightarrow{Succinyl-CoA\ synthetase} Succinate + CoA - SH + GTP$

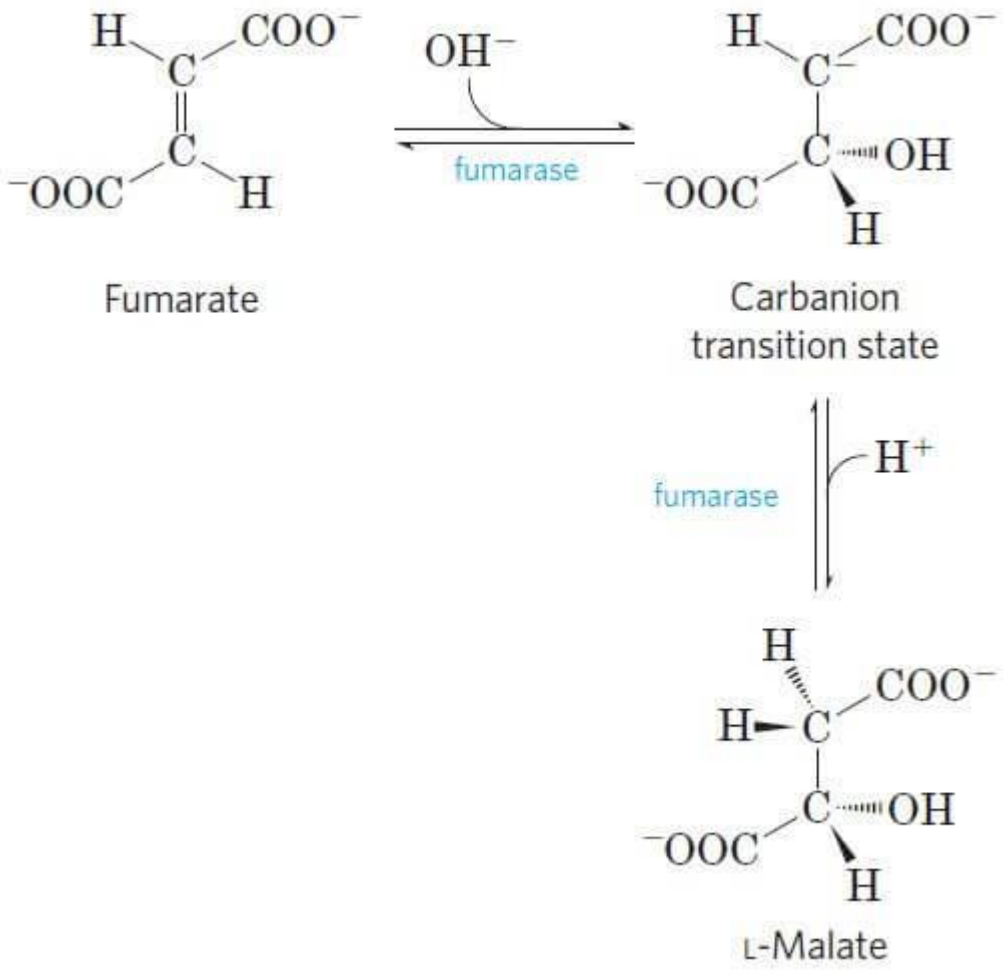


Step 6: Succinate is oxidised by the enzyme ***succinate dehydrogenase*** to fumarate. In the process, FAD is converted to $FADH_2$.



Formula: $Succinate + ubiquinone(Q) \xrightarrow{Succinate\ dehydrogenase} Fumarate + ubiquinol(QH_2)$

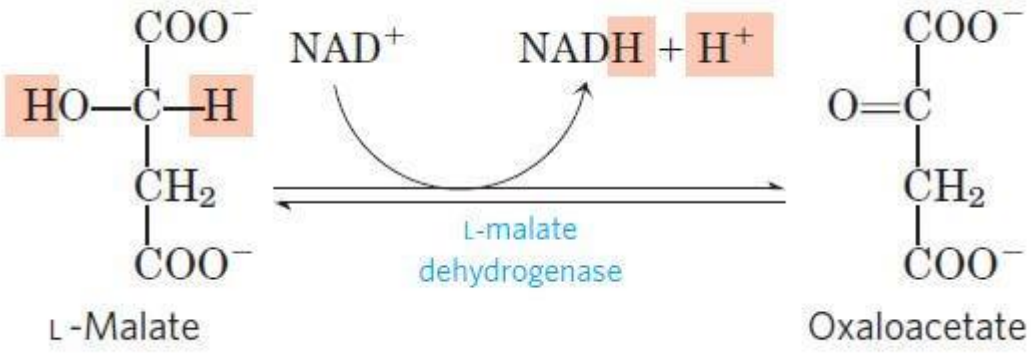
Step 7: Fumarate gets converted to malate by the addition of one H_2O. The enzyme catalysing this reaction is ***fumarase*.**

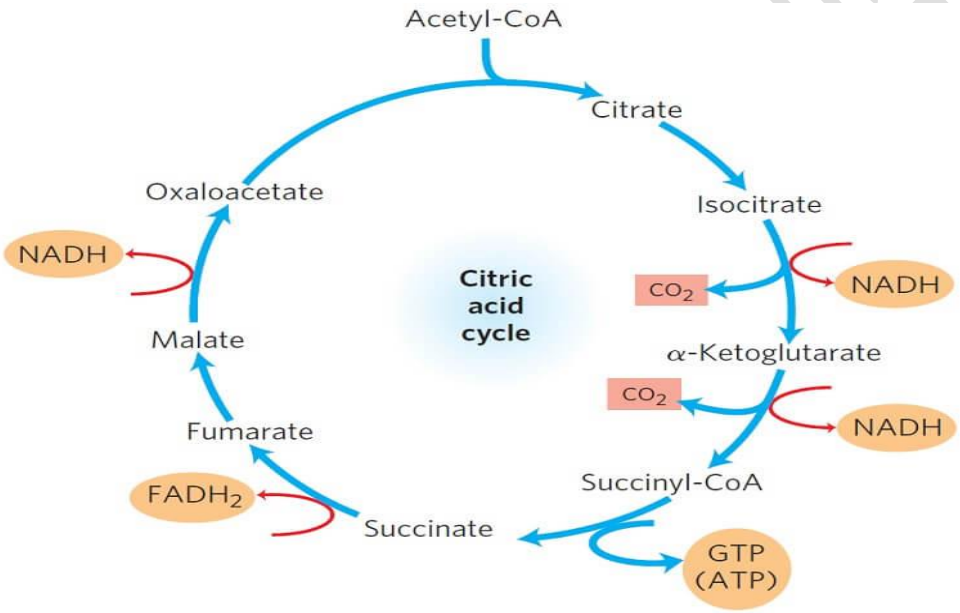


Formula: $Fumarate + H_2O \xrightarrow{Fumarase} Malate$

Step 8: Malate is dehydrogenated to form oxaloacetate, which combines with another molecule of acetyl CoA and starts the new cycle. Hydrogens removed, get transferred to NAD^+ forming NADH. ***Malate dehydrogenase*** catalyses the reaction.

Formula: $Malate + NAD^+ \xrightarrow[dehydrogenase]{Malate} Oxaloacetate + NADH + H^+$





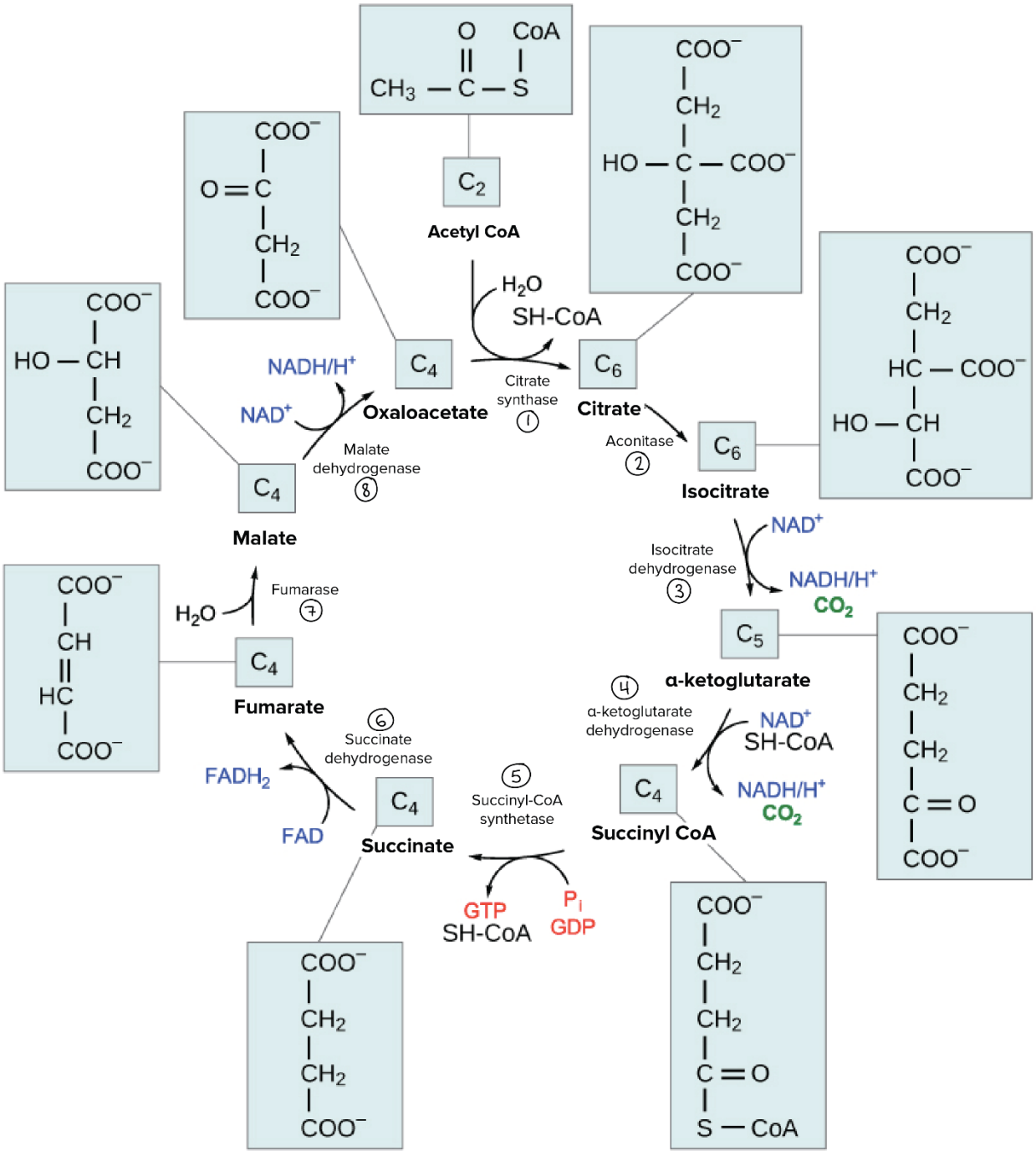
Process as a whole

Formula: $Acetyl - CoA + 3NAD^+ + Q + GDP + P + 2H_2O$

$$\xrightarrow[Substrates]{Enzymes +} CoA - SH + 3NADH + 3H^+ + QH_2 + GTP + 2CO_2$$

Reviewing the whole process, the Krebs cycle primarily transforms the acetyl group and water, into carbon dioxide and energized forms of the other reactants. Additionally, many of the enzymes and substrates are also intermediaries in other

biochemical reactions of both amino acids and fatty acids. the citric acid cycle is also part of the energy-releasing process of the metabolic oxidation of proteins and fatty acids as well. As a result the Krebs cycle is the primary method of cellular energy production.



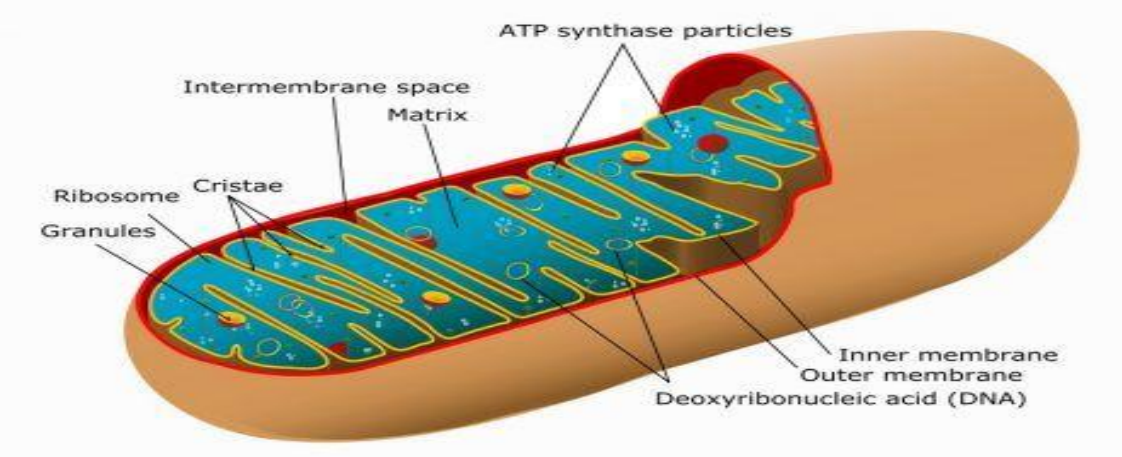
Conclusions

The Krebs cycle is both the central hub of cellular metabolism and one of Biology's prototypical biochemical processes. Since the Krebs cycle regulates and enables the cellular oxidation of glucose and plays a role in the metabolism of proteins and fats, it is the fuel source for cellular activity and therefore foundational for oxygen-based life. But the Krebs cycle also illustrates the role of biochemistry in biology as a field as well. It illustrates evolutionary biology, in that numerous organisms from single-cell to human beings share this same biochemistry. And it illustrates enzymatic reactions and biological conservation of resources and efficiency. As a result, the Krebs cycle is a critical part of science instruction to illustrate both how cellular biology works, and how biology as a field works to explore that biology.

Where does the Krebs cycle take place?

The TCA cycle was first observed in the muscle tissue of a pigeon. It takes place in all eukaryotic and prokaryotic cells. In eukaryotes, it occurs in the matrix of the mitochondrion. In prokaryotes, it takes place in the cytosol.

The Krebs cycle happens only within the mitochondrial matrix. Pyruvate is formed in the cytosol of the cell, then imported into the mitochondria. Here, it is converted to acetyl CoA and imported into the mitochondrial matrix. The mitochondrial matrix is the innermost part of the mitochondria. The graphic below shows the different parts of mitochondria.



The mitochondrial matrix has the required enzymes and environment for the complex reactions of the Krebs cycle to take place. Further, the products of the Krebs cycle drive the electron transport chain and oxidative phosphorylation, both of which occur in the inner mitochondrial membrane. The electron carriers will dump their electrons and protons into the chain, which ultimately drives the production of ATP. This molecule is then exported from the mitochondria as the main energy source for the cell.

Mitochondria are found in almost all organisms, especially multicellular organisms. Plants, animals, and fungi all use the Krebs cycle as an indispensable part of aerobic respiration.

Krebs cycle products

Before the Krebs cycle begins, a glucose molecule must be converted to acetyl-CoA. This process yields 2 acetyl-CoA molecules to be fed into the cycle. Thus, the cycle proceeds twice per original glucose, yielding twice the products shown below.

So, for every 1 pyruvate molecule added, the Krebs cycle will produce:

- 2 molecules of CO_2
- 3 molecules of NADH
- 1 molecule of $FADH_2$
- 1 molecule of GTP

double A molecule of glucose contains 2 pyruvate molecules, so 1 glucose molecule will produce the amount of products listed above as it moves through the Krebs cycle. These products will then be converted to ATP in later stages of aerobic respiration. **Carbon dioxide is the only "waste" product and must be removed from the cell.** Large organisms must remove carbon dioxide from all their cells. In these animals, carbon dioxide is typically exchanged in the gills or lungs for oxygen, which helps drive the final stages of aerobic respiration.

Products of the citric acid cycle

Each citric acid cycle forms the following products:

2 molecules of CO2 are released. Removal of CO2 or decarboxylation of citric acid takes place at two places:

In the conversion of isocitrate (6C) to **α**-ketoglutarate (5C)

In the conversion of **α**-ketoglutarate (5C) to succinyl CoA (4C)

1 ATP is produced in the conversion of succinyl CoA to succinate

3 NAD+ are reduced to NADH and 1 FAD+ is converted to FADH2 in the following reactions:

Isocitrate to **α**-ketoglutarate → NADH

α-ketoglutarate to succinyl CoA → NADH

Succinate to fumarate → FADH2

Malate to Oxaloacetate → NADH

Note that 2 molecules of Acetyl CoA are produced from oxidative decarboxylation of 2 pyruvates so two cycles are required per glucose molecule.

To summarize, for complete oxidation of a glucose molecule, Krebs cycle yields 4 CO2, 6NADH, 2 FADH2 and 2 ATPs.

Each molecule of NADH can form 2-3 ATPs and each FADH2 gives 2 ATPs on oxidation in the electron transport chain.

Key TCA cycle enzymes

- Malic dehydrogenase
- α-Ketoglutarate dehydrogenase
- Citrate synthase
- Fumarase
- Aconitase

TCA cycle applications

These TCA-related metabolic applications are commonly studied using stable isotope-labeled compounds and mass spectrometry:

- Lipid Metabolism
- Amino Acid Metabolism
- Protein Metabolism (Turnover)
- Glucose Metabolism
- Energy Expenditure

- Metabolomics

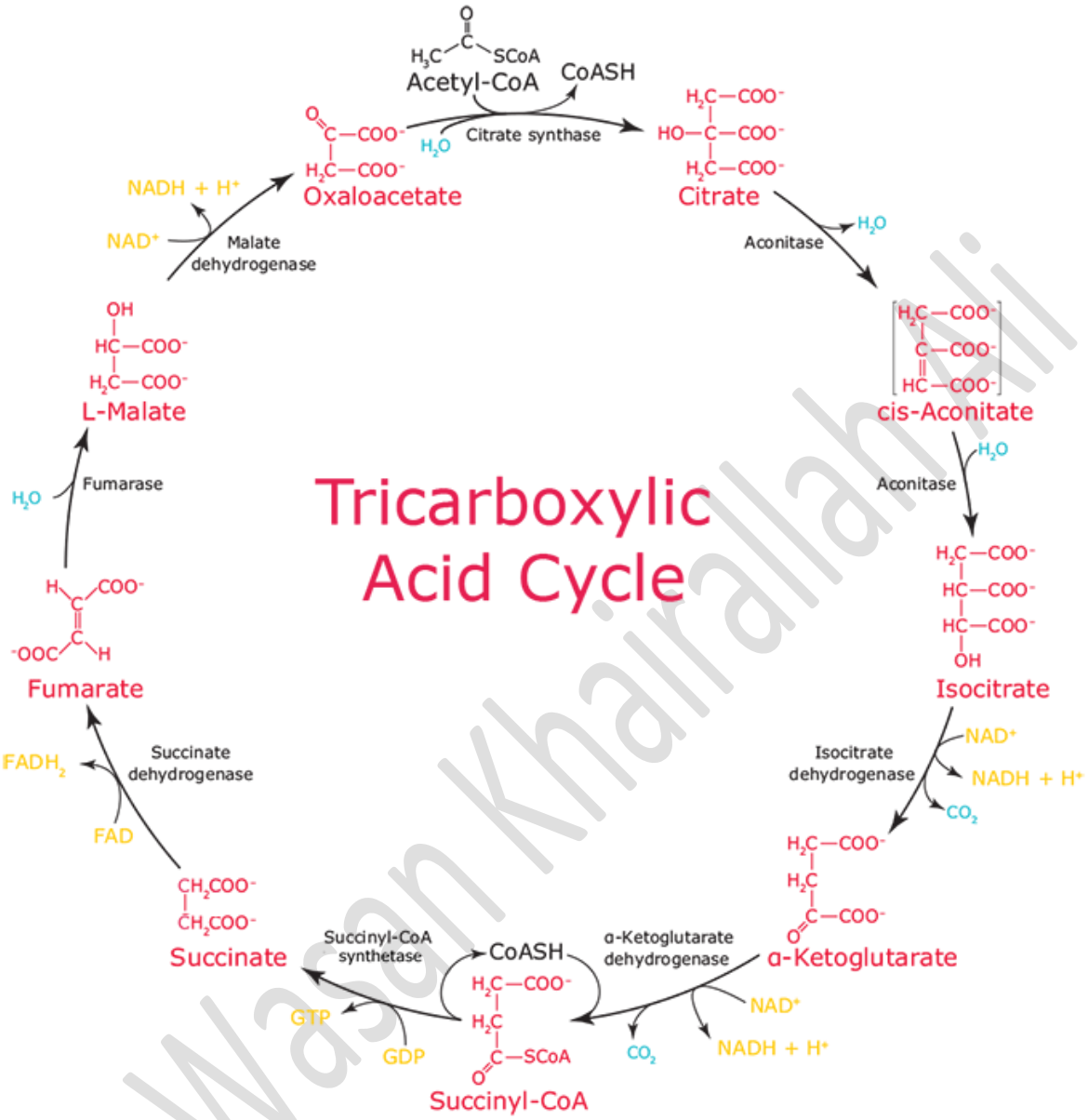
We sell TCA metabolites and enzymes for pinpointing metabolic processes as well as stable isotope-tagged compounds that can aid in measuring rates of whole body metabolism or glucose metabolism. Stable isotope-enriched compounds are metabolically similar to natural homologues, making them safe to use as tracers.

Energy Extraction via the Krebs Cycle

Theoretical Yield: 24 Molecules of ATP / Molecule of Glucose

Practical Yield: 20 Molecules of ATP / Molecule of Glucose

The Krebs cycle is the primary metabolic pathway through which aerobic energy is released from carbohydrates, proteins, and fats in a useable form. When measuring the energy production of the Krebs cycle, the output is measured in molecules of ATP (Adenosine triphosphate) per molecule of glucose. In total, the theoretical (and typical textbook) yield of cellular respiration (including the Krebs cycle) from one molecule of glucose is 38 molecules of ATP, but in practice the actual yield is closer to 30-32 ATP. Since one molecule of glucose produces two molecules of Acetyl–CoA, the Krebs cycle's energy output is usually expressed as the product of the two cycles necessary to breakdown both Acetyl–CoA's. Two Krebs cycles create two GTP, Guanosine triphosphate, which can be readily converted into 2 ATP. The other energy-producing products of the Krebs cycle (NADH, and QH2) theoretically generate an additional 22 ATP, but in practice produce closer to 18 ATP via the mitochondrial electron transport chain. This practical difference results from energy lost from the active transport of various reactants as well as the leakage of electrons within the electron transport chain.



Significance of Krebs Cycle

- Krebs cycle or Citric acid cycle is the final pathway of oxidation of glucose, fats and amino acids
- Many animals are dependent on nutrients other than glucose as an energy source
- Amino acids (metabolic product of proteins) are deaminated and get converted to pyruvate and other intermediates of the Krebs cycle. They enter the cycle and get metabolised e.g. alanine is converted to pyruvate, glutamate to **α**-ketoglutarate, aspartate to oxaloacetate on deamination
- Fatty acids undergo ***β***-oxidation to form acetyl CoA, which enters the Krebs cycle
- It is the major source of ATP production in the cells. A large amount of energy is produced after complete oxidation of nutrients
- It plays an important role in gluconeogenesis and lipogenesis and interconversion of amino acids
- Many intermediate compounds are used in the synthesis of amino acids, nucleotides, cytochromes and chlorophylls, etc.
- Vitamins play an important role in the citric acid cycle. Riboflavin, niacin, thiamin and pantothenic acid as a part of various enzymes cofactors (FAD, NAD) and coenzyme A
- Regulation of Krebs cycle depends on the supply of NAD^+ and utilization of ATP in physical and chemical work
- The genetic defects of the Krebs cycle enzymes are associated with neural damage
- As most of the biological processes occur in the liver to a significant extent, damage to liver cells has a lot of repercussions. Hyperammonemia occurs in liver diseases and leads to convulsions

and coma. This is due to reduced ATP generation as a result of the withdrawal of **α**-ketoglutarate and formation of glutamate, which forms glutamine.

Why Krebs Cycle Is Called the Citric Acid Cycle?

Krebs cycle is also referred to as the Citric Acid Cycle. Citric acid is the first product formed in the cycle.