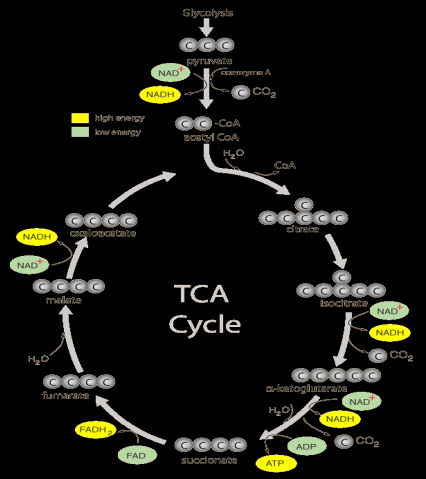
TCA CYCLE STEPS REGULATION AND SIGNIFICANCE



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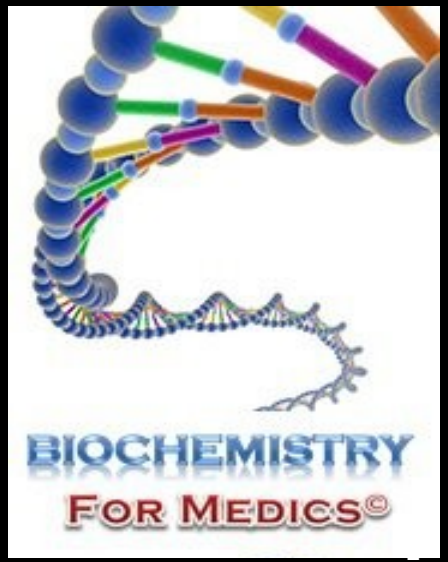


Introduction

- The citric acid cycle is the central metabolic hub of the cell.
- It is the *final common pathway for the oxidation of fuel molecule such as* amino acids, fatty acids, and carbohydrates.
- In eukaryotes, the reactions of the citric acid cycle take place inside mitochondria, in contrast with those of glycolysis, which take place in the cytosol.

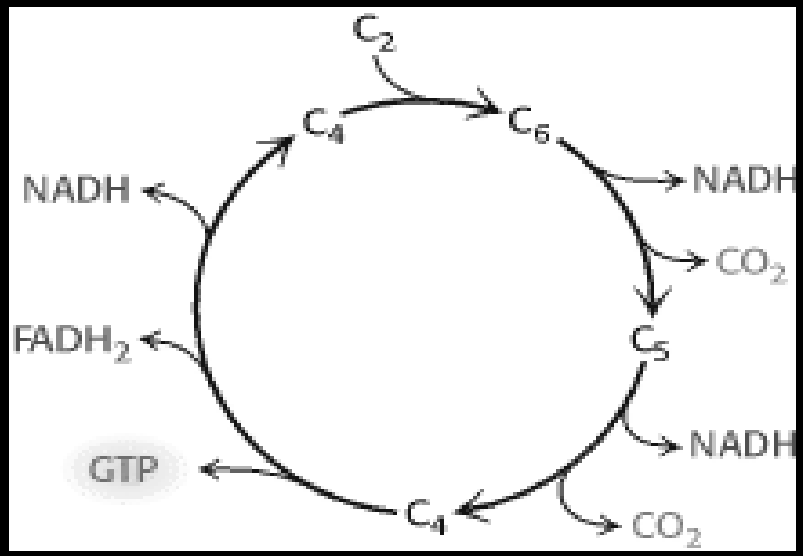
Overview of the Citric Acid Cycle

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) includes a series of oxidation-reduction reactions in mitochondria that result in the oxidation of an acetyl group to two molecules of carbon dioxide and reduce the coenzymes that are reoxidized through the electron transport chain, linked to the formation of ATP.

Overview of the Citric Acid Cycle

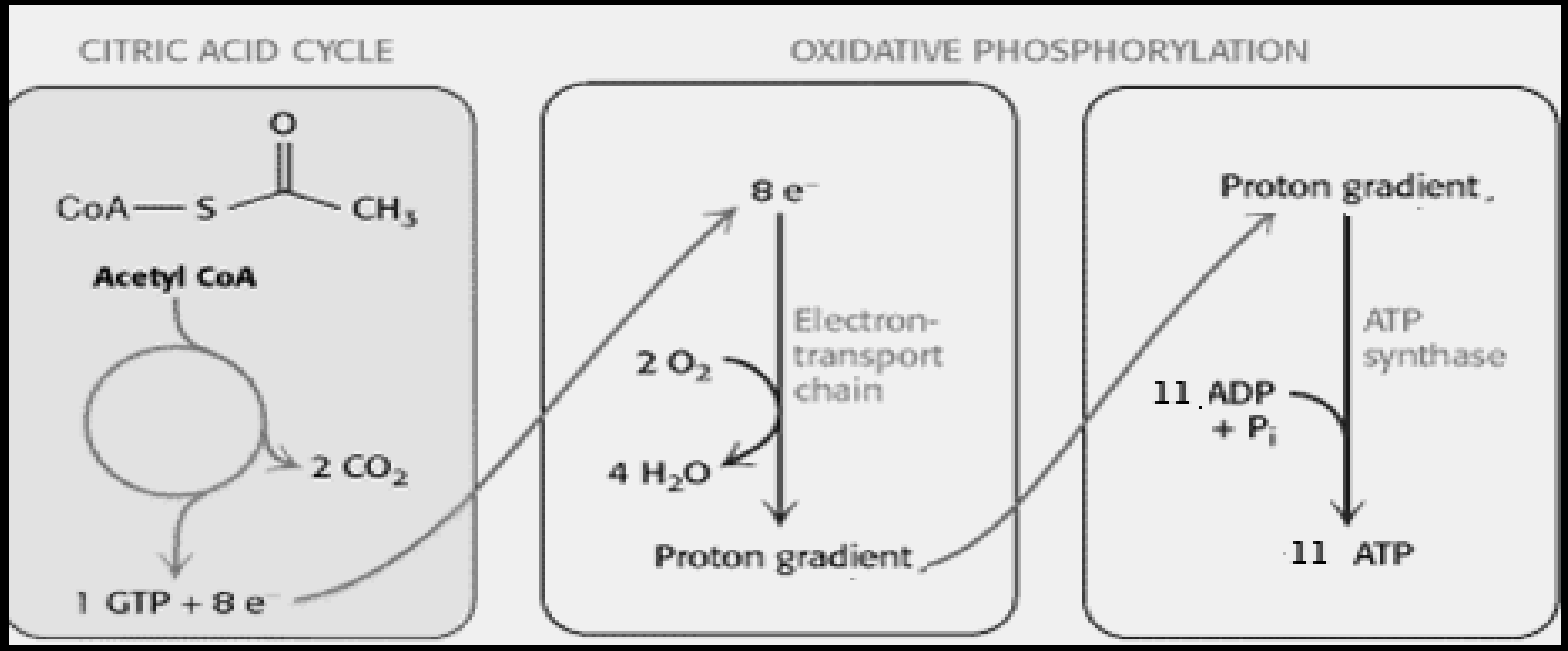
- A four- carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid (citrate).
- An isomer of citrate is then oxidatively decarboxylated.
- The resulting five-carbon compound (α-ketoglutarate) also is oxidatively decarboxylated to yield a four carbon compound (succinate).
- Oxaloacetate is then regenerated from succinate.
- Two carbon atoms enter the cycle as an acetyl unit and two carbon atoms leave the cycle in the form of two molecules of carbon dioxide.

Overview of the Citric Acid Cycle



- Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD+), whereas one pair of hydrogen atoms (hence, two electrons) are transferred to one molecule of flavin adenine dinucleotide (FAD) .
- The function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.

Citric acid cycle and requirement of oxygen



Oxygen is required for the citric acid cycle indirectly in as much as it is the electron acceptor at the end of the electron-transport chain, necessary to regenerate NAD+ and FAD.

Citric acid cycle and requirement of oxygen (contd.)

- The citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant.
- Instead, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to form NADH and FADH2 .
- In *oxidative phosphorylation*, electrons released in the reoxidation of NADH and FADH2 flow through a series of membrane proteins (referred to as the *electron-transport chain*) to generate a proton gradient across the membrane
- The citric acid cycle, in conjunction with oxidative phosphorylation, provides the vast majority of energy used by aerobic cells in human beings, greater than 95%.

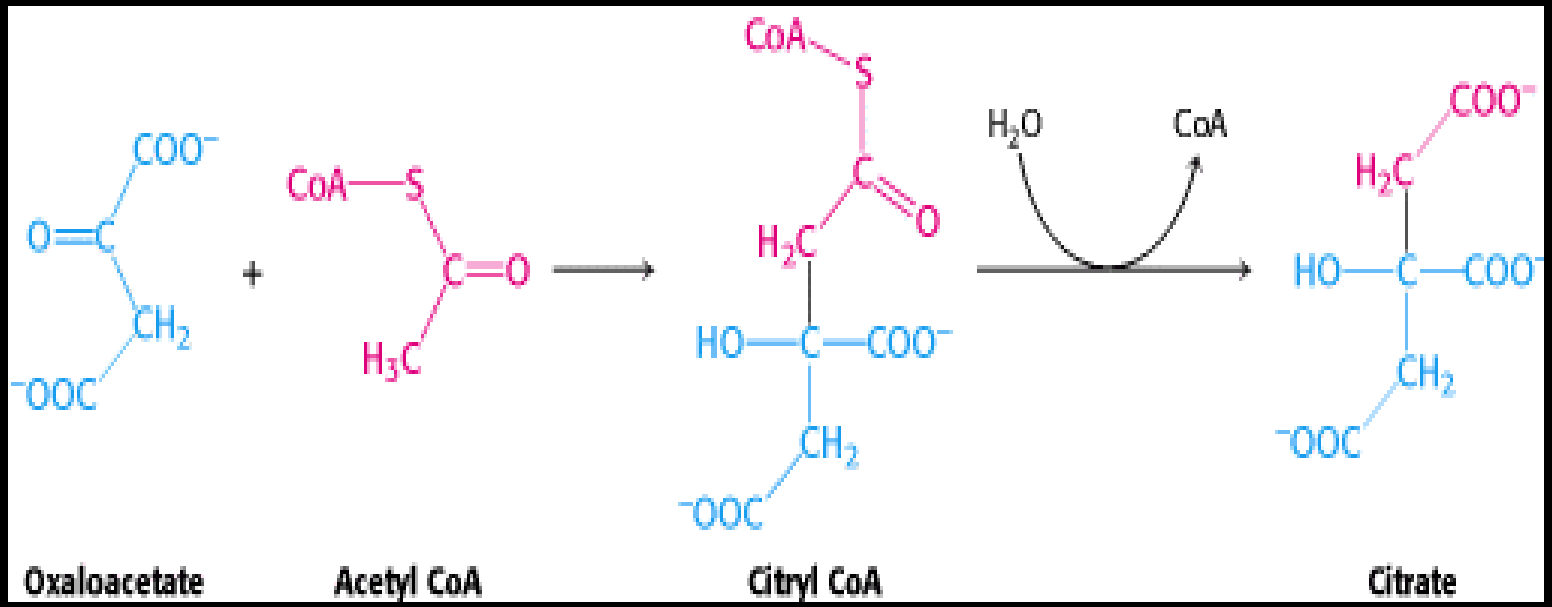
Role of oxaloacetate in citric acid cycle

- The four-carbon molecule, oxaloacetate that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle.
- The oxaloacetate acts catalytically: it participates in the oxidation of the acetyl group but is itself regenerated.
- Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl molecules.

Reactions of the Citric Acid Cycle

- **Step-1 Formation of Citrate-** The citric acid cycle begins with the condensation of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA. Oxaloacetate reacts with acetyl CoA and H2O to yield citrate and CoA.
- This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by *citrate synthase.*

Step-1-Formation of Citrate

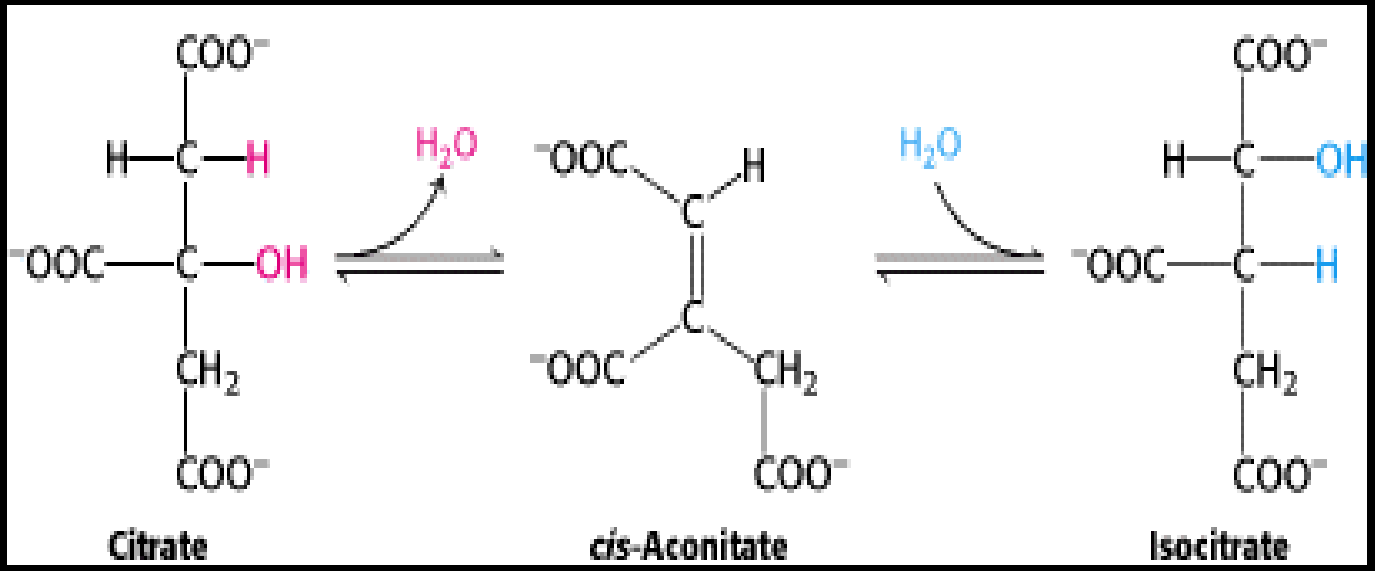


Oxaloacetate first condenses with acetyl CoA to form citryl CoA, which is then hydrolyzed to citrate and CoA.

Step-2-Formation of Isocitrate

- Citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation.
- The isomerization of citrate is accomplished by a dehydration step followed by a hydration step.
- The result is an interchange of a hydrogen atom and a hydroxyl group.
- The enzyme catalyzing both steps is called Aconitase because cis-aconitate is an intermediate.

Step-2-Formation of Isocitrate (contd.)



Aconitase is an *iron-sulfur protein*, or *nonheme iron protein.* It contains four iron atoms that are not incorporated as part of a heme group.

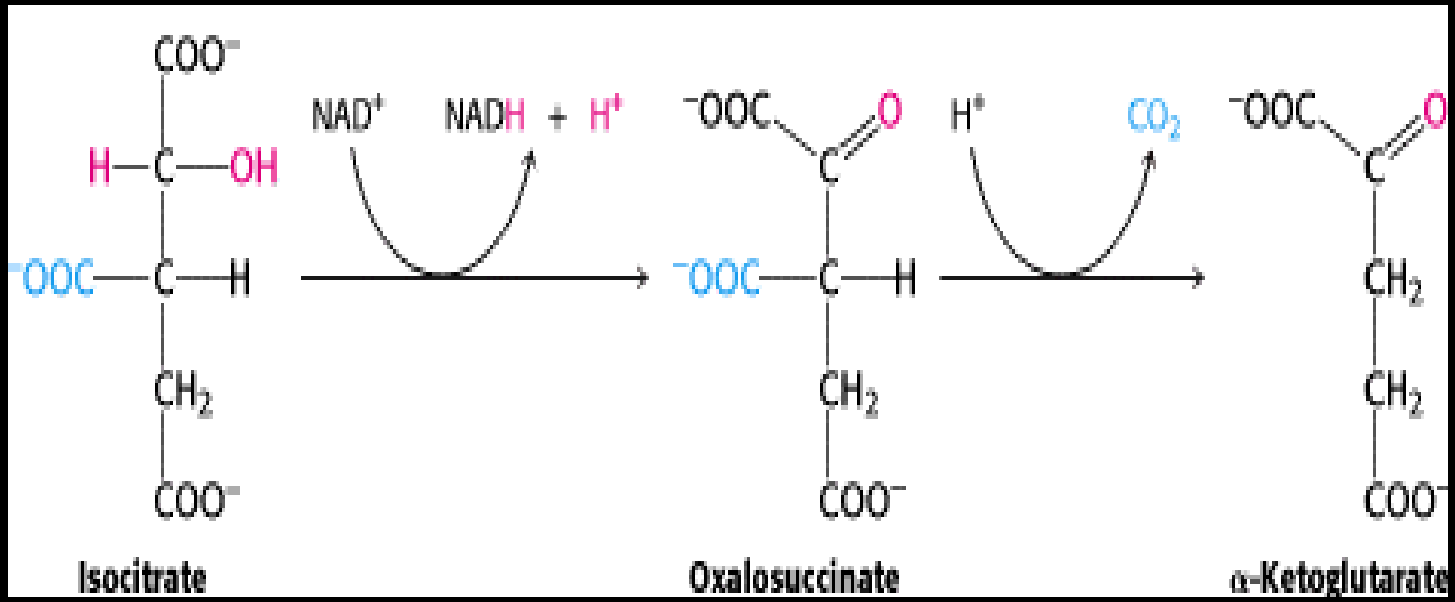
Step-2-Formation of Isocitrate (contd.)

- The poison Fluoroacetate is toxic, because fluoroacetyl-CoA condenses with oxaloacetate to form fluorocitrate, which inhibits Aconitase, causing citrate to accumulate.
- The mode of inhibition is suicidal inhibition

Step-3- Formation of α- Keto Glutarate

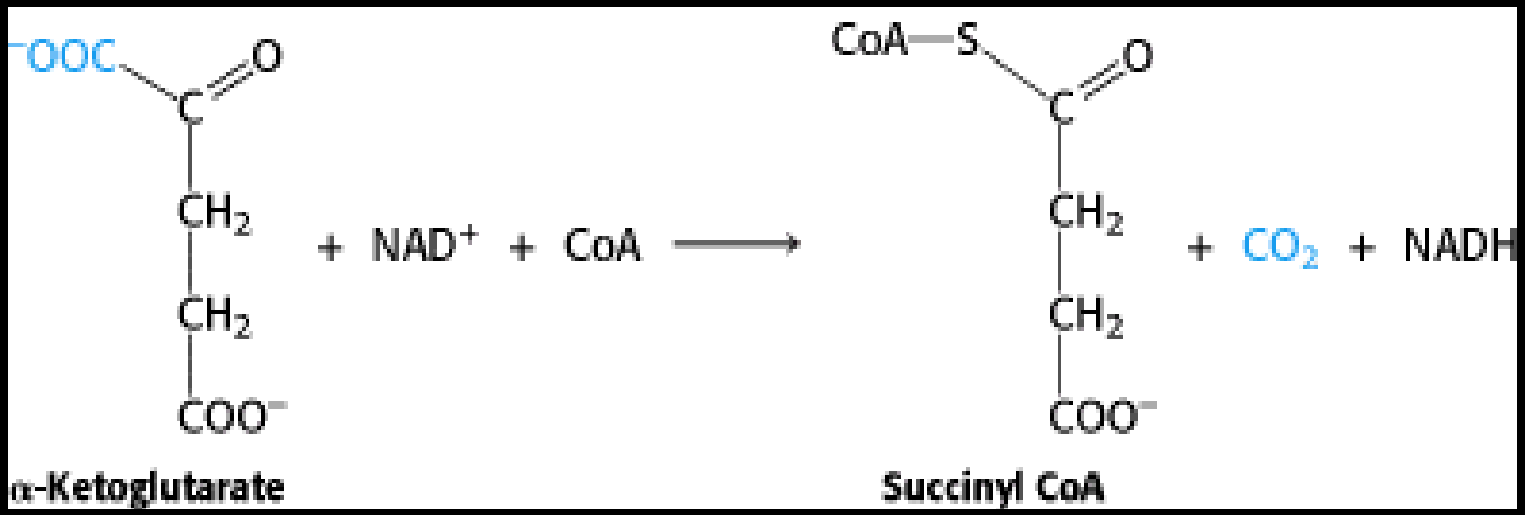
- Isocitrate undergoes dehydrogenation catalyzed by **isocitrate dehydrogenase** to form, initially, Oxalo succinate, which remains enzyme-bound and undergoes decarboxylation to **α** -ketoglutarate.
- The decarboxylation requires Mg^{++} or Mn^{++} ions.
- There are three isoenzymes of isocitrate dehydrogenase.
- One, which uses NAD^{+}, is found only in mitochondria.
- The other two use $NADP^{+}$ and are found in mitochondria and the cytosol.

Step-3- Formation of α- Keto Glutarate (contd.)



Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+-dependent enzyme.

Step-4-Formation of Succinyl Co A



The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of Succinyl CoA from α-ketoglutarate.

Step-4-Formation of Succinyl Co A(contd.)

- α-Ketoglutarate undergoes **oxidative decarboxylation** in a reaction catalyzed by a multi-enzyme complex similar to that involved in the oxidative decarboxylation of pyruvate.
- The α-**-ketoglutarate dehydrogenase complex** requires the same cofactors as the pyruvate dehydrogenase complex—thiamine diphosphate, lipoate, NAD^+, FAD, and CoA—and results in the formation of succinyl-CoA.

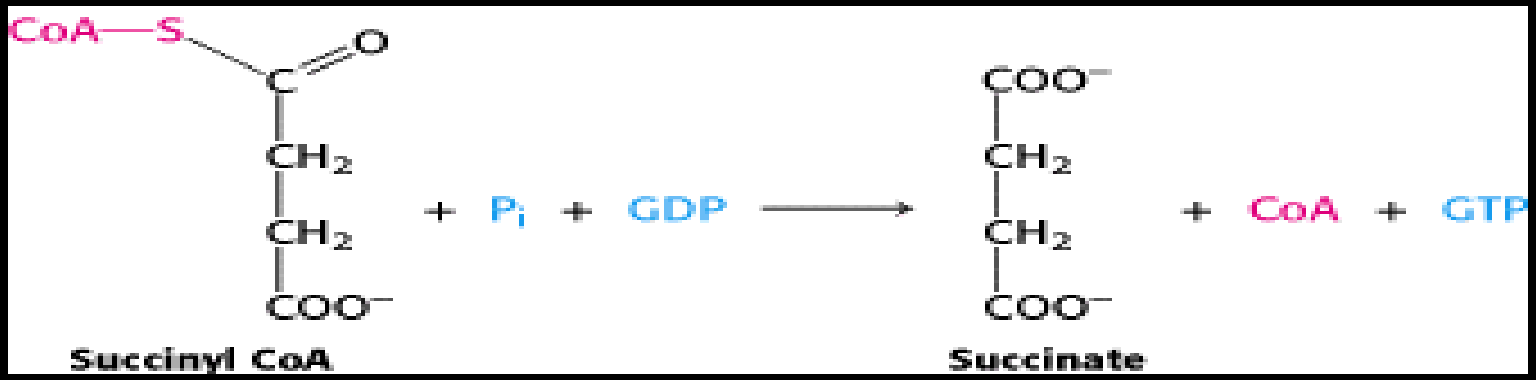
Step-4-Formation of Succinyl Co A

- The equilibrium of this reaction is so much in favor of succinyl-CoA formation that it must be considered to be physiologically unidirectional.
- As in the case of pyruvate oxidation, arsenite inhibits the reaction, causing the substrate, α -ketoglutarate, to accumulate.

Step-5- Formation of Succinate

- Succinyl CoA is an energy-rich thioester compound
- The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually GDP.
- This reaction is catalyzed by succinyl CoA synthetase (succinate thiokinase).

Step-5- Formation of Succinate



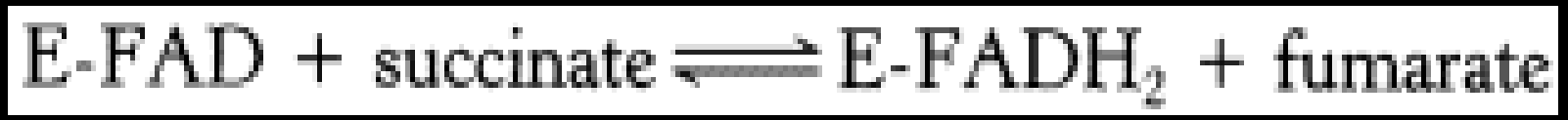
- This is the only example in the citric acid cycle of substrate level phosphorylation.
- Tissues in which gluconeogenesis occurs (the liver and kidney) contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for ADP

Step-5- Formation of Succinate

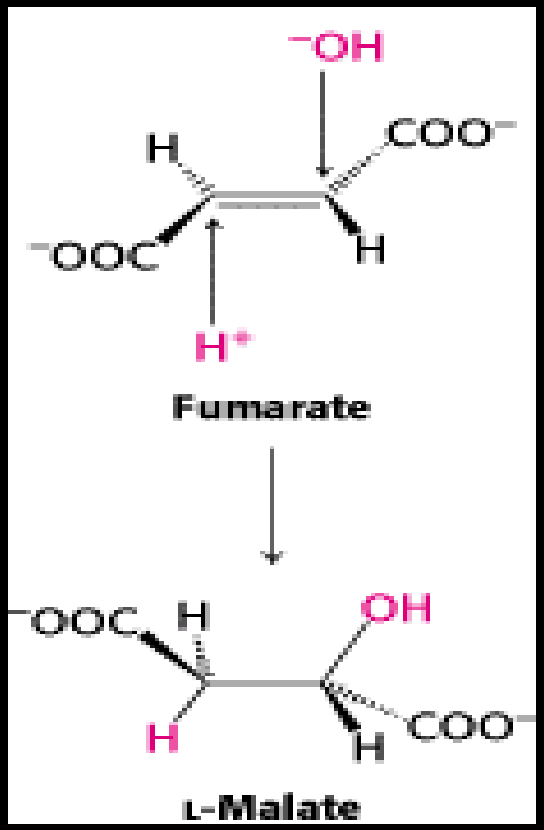
- The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis, and provides a regulatory link between citric acid cycle activity and the withdrawal of oxaloacetate for gluconeogenesis. Nongluconeogenic tissues have only the isoenzyme that uses ADP.

Step-6- Formation of Fumarate

- The first dehydrogenation reaction, forming fumarate, is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane.
- The enzyme contains FAD and iron-sulfur (Fe:S) protein, and directly reduces ubiquinone in the electron transport chain.



Step-7- Formation of Malate



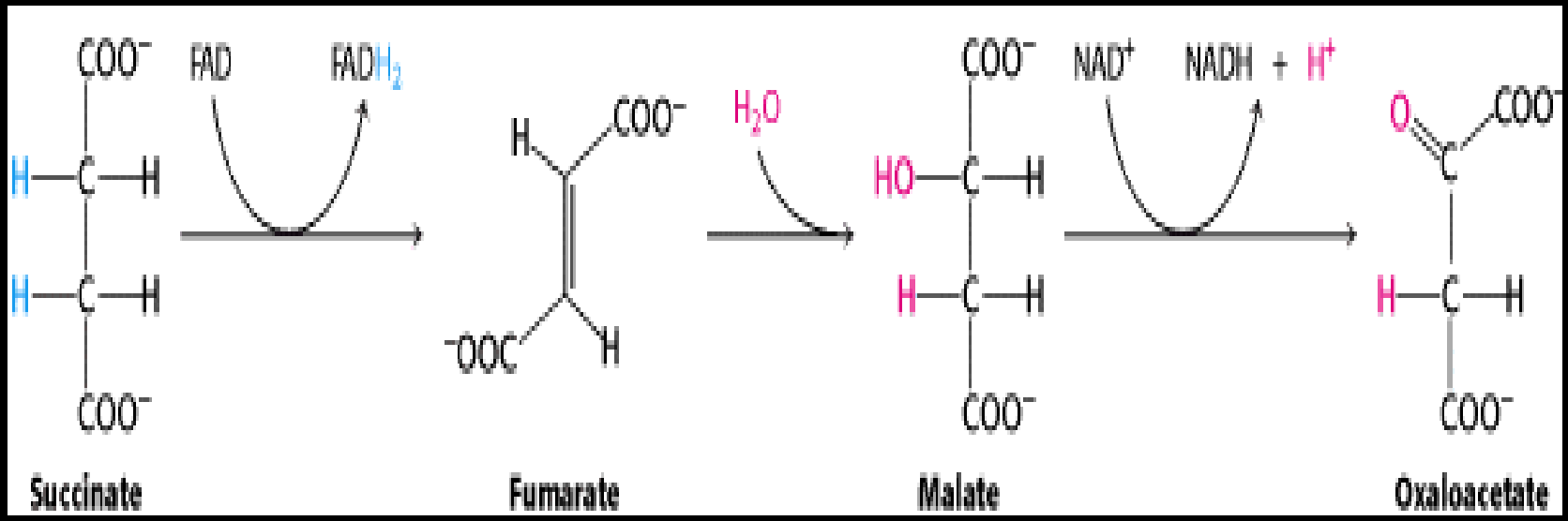
Fumarase (fumarate hydratase) catalyzes the addition of water across the double bond of fumarate, yielding malate.

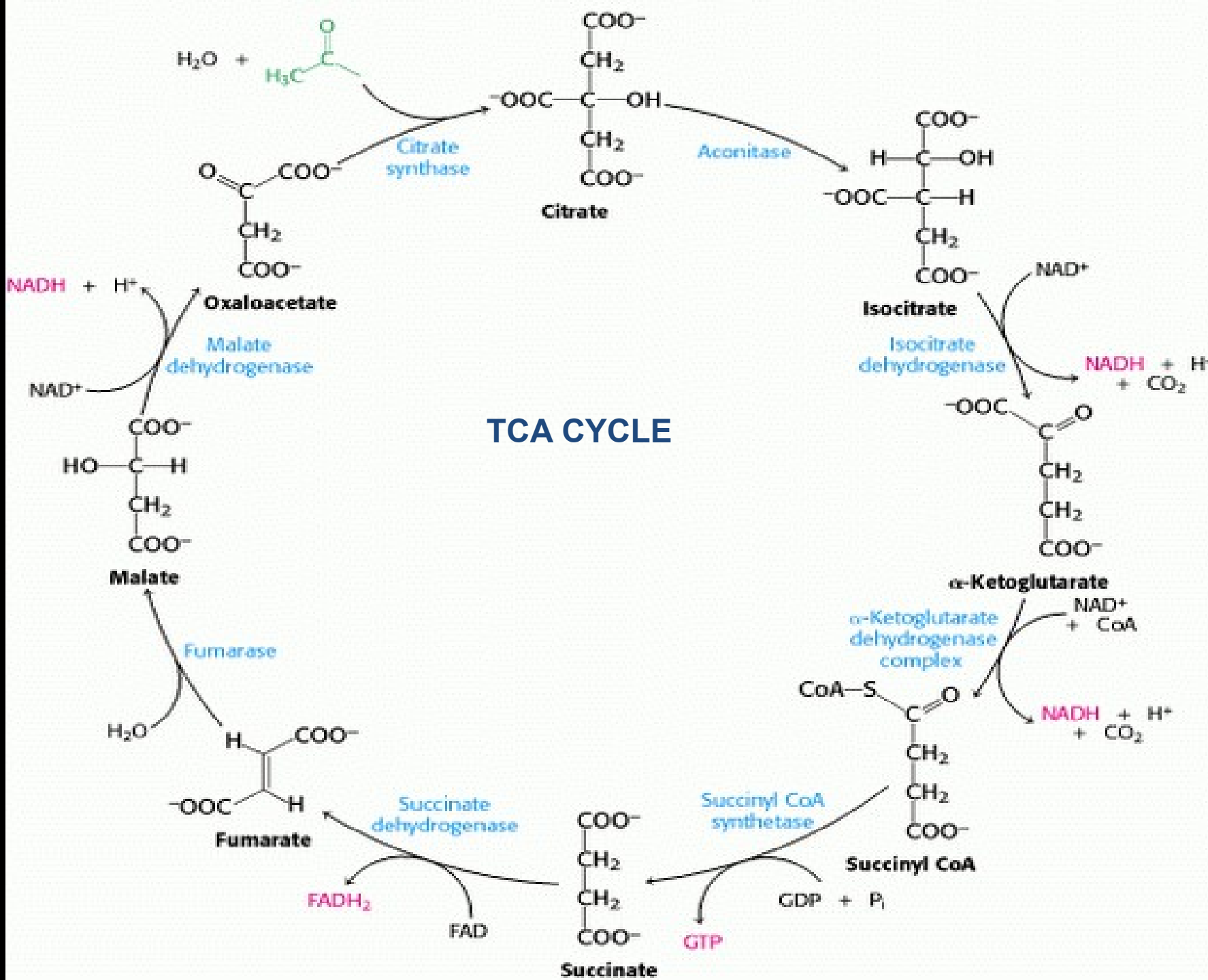
Step-8- Regeneration of oxaloacetate

- Malate is converted to oxaloacetate by **malate dehydrogenase,** a reaction requiring NAD^+.
- Although the equilibrium of this reaction strongly favors malate, the net flux is to oxaloacetate because of the continual removal of oxaloacetate (to form citrate, as a substrate for gluconeogenesis, or to undergo transamination to aspartate) and also the continual reoxidation of NADH.

Step-8- Regeneration of oxaloacetate

$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$




TCA CYCLE
Citrate
Aconitase
Isocitrate
Isocitrate dehydrogenase
NAD+
NADH + H+ + CO2
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
NAD+ + CoA
NADH + H+ + CO2
Succinyl CoA
Succinyl CoA synthetase
GDP + Pi
GTP
Succinate
Succinate dehydrogenase
FADH2
FAD
Fumarase
H2O
Fumarate
Malate
Malate dehydrogenase
NADH + H+
NAD+
Oxaloacetate
Citrate synthase
H2O +

Energy yield per Acetyl co A per turn of cycle

- As a result of oxidations catalyzed by the dehydrogenases of the citric acid cycle, three molecules of NADH and one of FADH2 are produced for each molecule of acetyl-CoA catabolized in one turn of the cycle.
- These reducing equivalents are transferred to the respiratory chain, where reoxidation of each NADH results in formation of 3, and 2 ATP of FADH2.
- Consequently, 11 high-transfer-potential phosphoryl groups are generated when the electron-transport chain oxidizes 3 molecules of NADH and 1 molecule of FADH2,
- In addition, 1 ATP (or GTP) is formed by substrate-level phosphorylation catalyzed by succinate thiokinase.

Energy yield per Acetyl co A per turn of cycle

- 1 acetate unit generates approximately 12 molecules of ATP.
- In dramatic contrast, only 2 molecules of ATP are generated per molecule of glucose (which generates 2 molecules of acetyl CoA) by anaerobic glycolysis.
- Molecular oxygen does not participate directly in the citric acid cycle.
- However, the cycle operates only under aerobic conditions because NAD+ and FAD can be regenerated in the mitochondrion only by the transfer of electrons to molecular oxygen.

Regulation of the TCA cycle

- Regulation of the TCA cycle like that of glycolysis occurs at both the level of entry of substrates into the cycle as well as at the key reactions of the cycle.
- Fuel enters the TCA cycle primarily as acetyl-CoA. The generation of acetyl-CoA from carbohydrates is, therefore, a major control point of the cycle.
- This is the reaction catalyzed by the PDH complex.

Regulation of the TCA cycle (contd.)

1) **Regulation of PDH Complex**

a) **Allosteric modification-**PDH complex is inhibited by acetyl-CoA and NADH and activated by non-acetylated CoA (CoASH) and NAD+.

b) **Covalent modification-**The pyruvate dehydrogenase activities of the PDH complex are regulated by their state of phosphorylation. This modification is carried out by a specific kinase (PDH kinase) and the phosphates are removed by a specific phosphatase (PDH phosphatase).

PDH kinase is activated by NADH and acetyl-CoA and inhibited by pyruvate, ADP, CoASH, Ca2+ and Mg2+.

The PDH phosphatase, in contrast, is activated by Mg2+ and Ca2+.

Regulation of the TCA cycle (contd.)

2) **Regulation of TCA cycle enzymes**

   The most likely sites for regulations are the nonequilibrium reactions catalyzed citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The dehydrogenases are activated by Ca2+, which increases in concentration during muscular contraction and secretion, when there is increased energy demand.

Regulation of TCA cycle enzymes

a) **Citrate synthase-** There is allosteric inhibition of citrate synthase by ATP and long-chain fatty acyl-CoA.

b) **Isocitrate dehydrogenase-** is allosterically stimulated by ADP, which enhances the enzyme's affinity for substrates. In contrast, NADH inhibits iso-citrate dehydrogenase by directly displacing NAD+. ATP, too, is inhibitory.

Regulation of TCA cycle enzymes

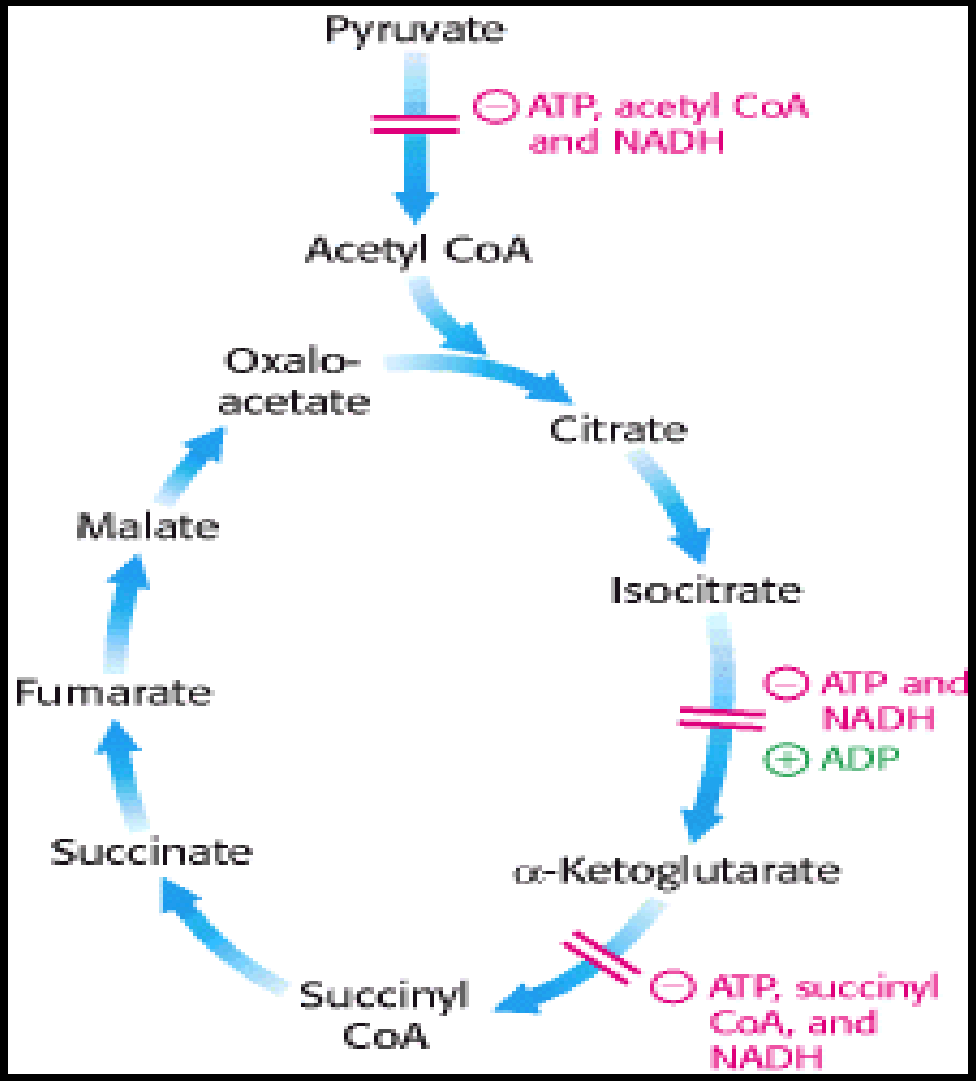
c) α-ketoglutarate dehydrogenase -α- Ketoglutarate dehydrogenase is inhibited by succinyl CoA and NADH. In addition, α-ketoglutarate dehydrogenase is inhibited by a high energy charge. Thus, the rate of the cycle is reduced when the cell has a high level of ATP.

d) Succinate dehydrogenase is inhibited by oxaloacetate, and the availability of oxaloacetate, as controlled by malate dehydrogenase, depends on the [NADH]/[NAD+] ratio.

TCA cycle and cellular ratio of NAD+/NADH

- Since three reactions of the TCA cycle as well as PDH utilize NAD^+ as co-factor, the cellular ratio of NAD^+/NADH has a major impact on the flux of carbon through the TCA cycle.
- The activity of TCA cycle is immediately dependent on the supply of NAD+, which in turn, because of the tight coupling between oxidation and phosphorylation, is dependent on the availability of ADP and hence, ultimately on the rate of utilization of ATP in chemical and physical work.
- Thus, **respiratory control** via the respiratory chain and oxidative phosphorylation primarily regulates citric acid cycle activity.

Regulation of TCA cycle- Summary



Excess of ATP depicts energy rich state of the cell, hence TCA cycle is inhibited while reverse occurs when the cell is in a low energy state with excess of ADP.

Significance of TCA Cycle

- The citric acid cycle is not only a pathway for oxidation of two-carbon units, but is also a major pathway for interconversion of metabolites arising from **transamination** and **deamination** of amino acids, and providing the substrates for amino acid synthesis by transamination, as well as for **gluconeogenesis and fatty acid synthesis.**
- Because it functions in both oxidative and synthetic processes, it is **amphibolic**.

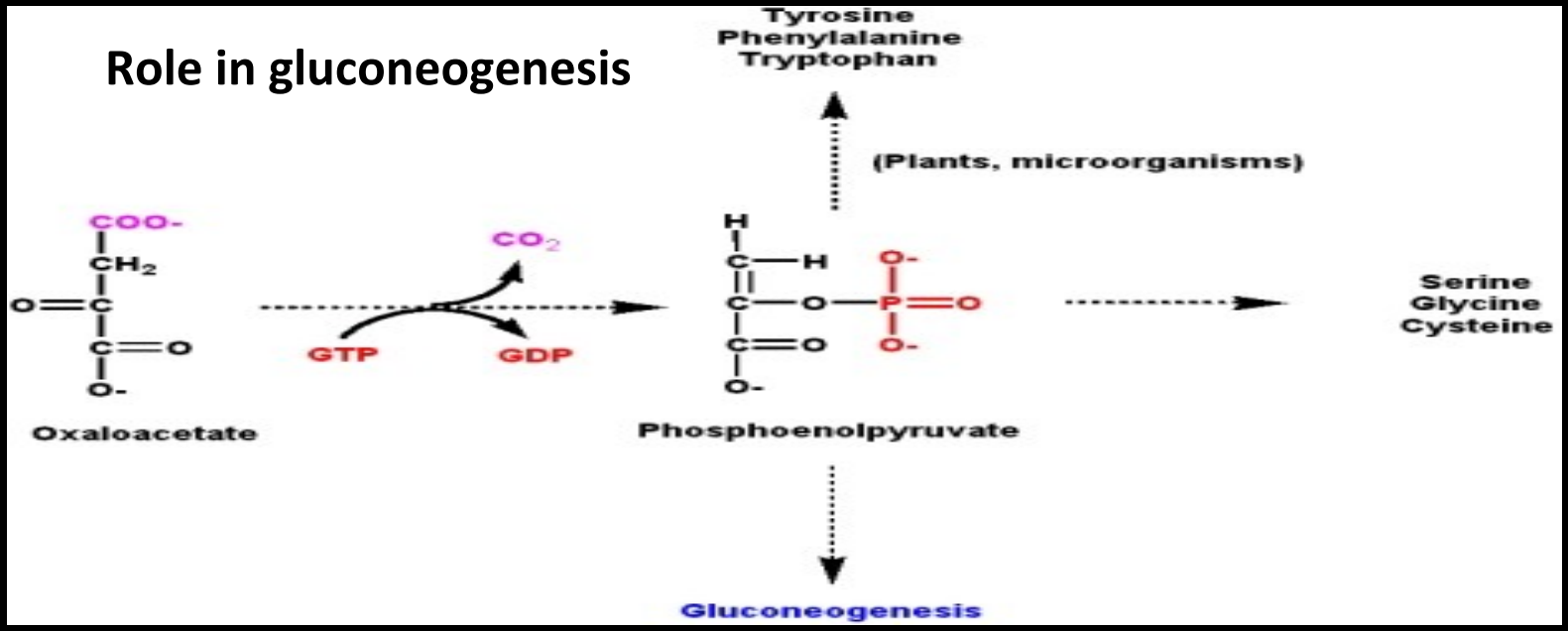
A) Catabolic role OF TCA Cycle

- The citric acid cycle is the final common pathway for the oxidation of carbohydrate, lipid, and protein because glucose, fatty acids, and most amino acids are metabolized to acetyl-CoA or intermediates of the cycle.
- The function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.
- 1 acetate unit generates approximately 12 molecules of ATP per turn of the cycle.

B) Anabolic role of TCA cycle

- As a major metabolic hub of the cell, the citric acid cycle also provides intermediates for biosynthesis of various compounds.
- ***i) Role in Gluconeogenesis***- All the intermediates of the cycle are potentially **glucogenic,** since they can give rise to oxaloacetate, and hence net production of glucose (in the liver and kidney, the organs that carry out gluconeogenesis.

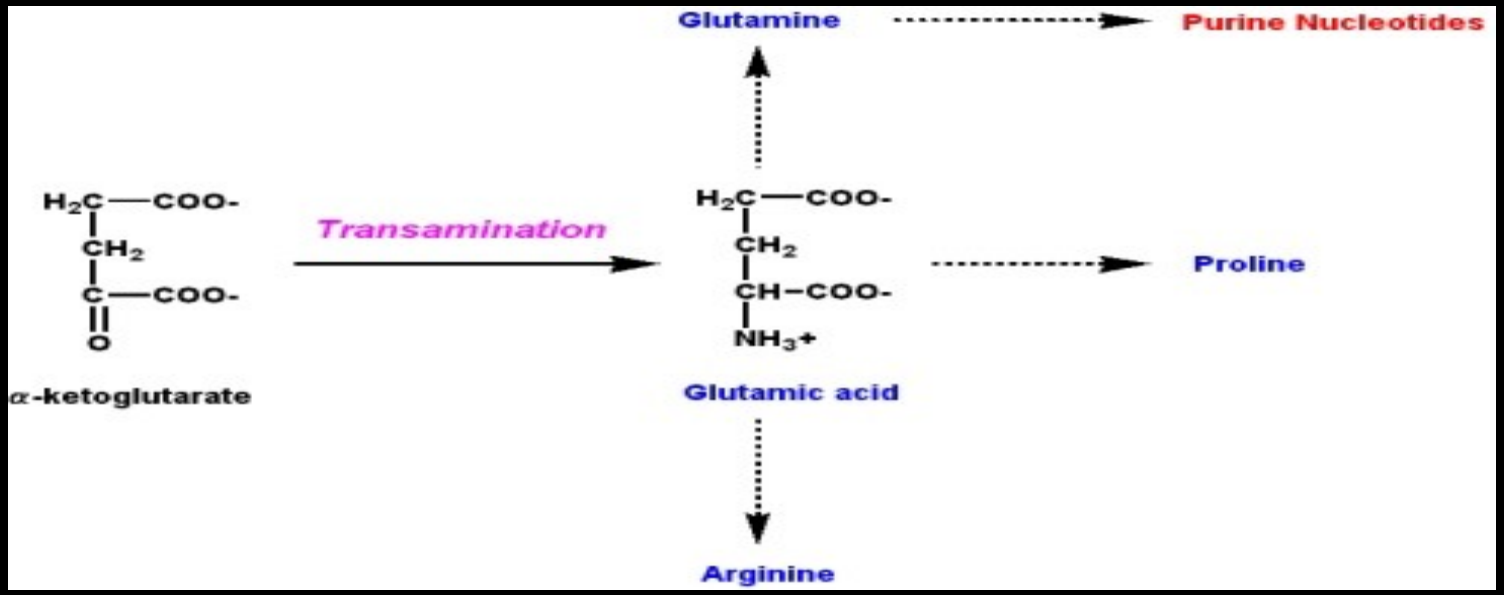
B) Anabolic role of TCA cycle



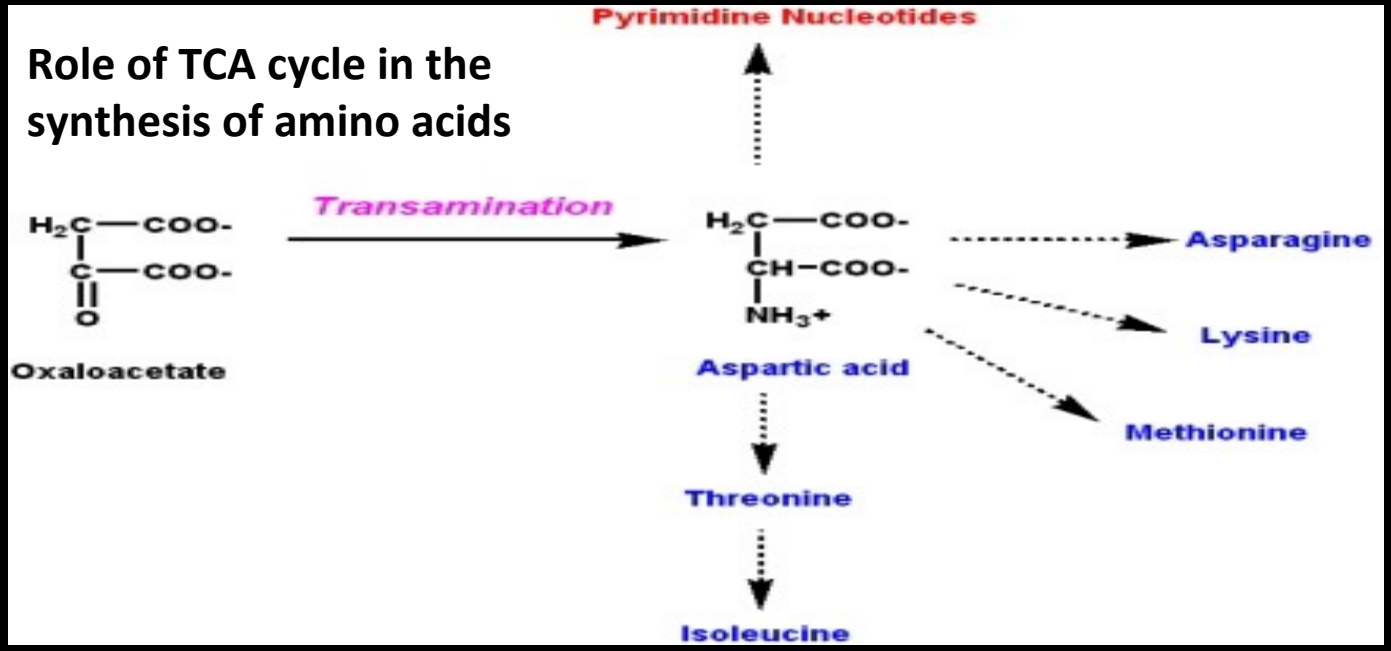
- The key enzyme that catalyzes net transfer out of the cycle into gluconeogenesis is **phospho-enol-pyruvate carboxy kinase,** which catalyzes the decarboxylation of oxaloacetate to phosphoenolpyruvate, with GTP acting as the phosphate donor.

ii) Role in synthesis of nonessential amino acids

Since the transamination reactions are reversible, the cycle also serves as a source of carbon skeletons for the synthesis of some amino acids like Alanine, aspartate, Asparagine Glutamate , glutamine etc.



Role of TCA cycle in the synthesis of amino acids



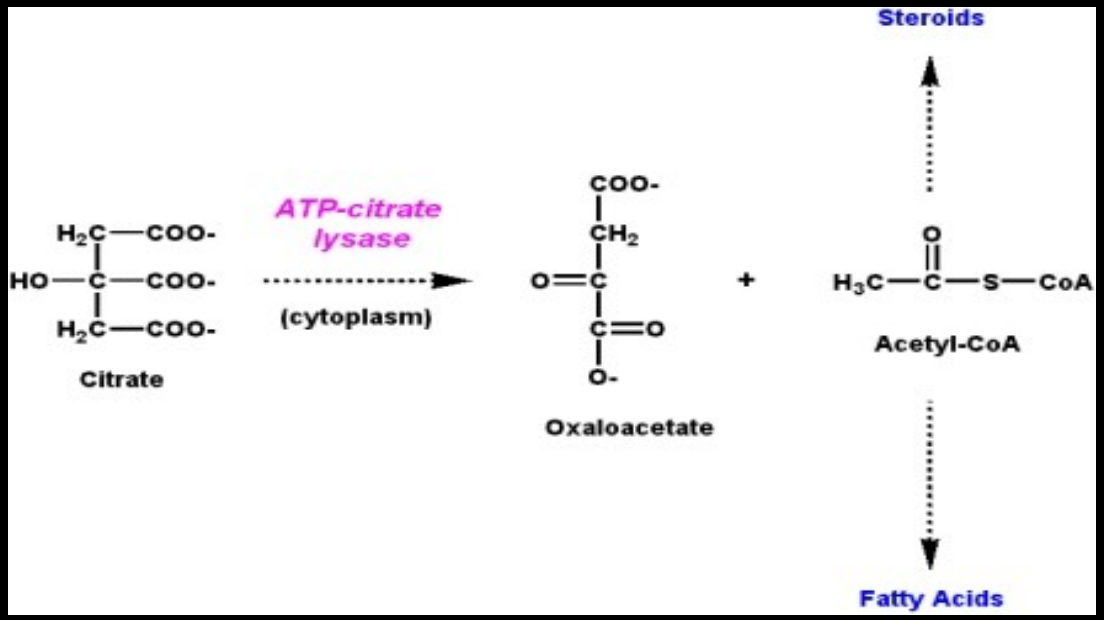
Aspartic acid is a precursor of Asparagine, Lysine, Methionine, Threonine and Isoleucine. These amino acid except Asparagine are essential amino acids, they are synthesized only in plants.

Role of TCA cycle in fatty acid synthesis

iii) Role in fatty acid synthesis- Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase, is the major substrate for long-chain fatty acid synthesis .

Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol, and cleaved in a reaction catalyzed by **ATP-citrate lyase.**

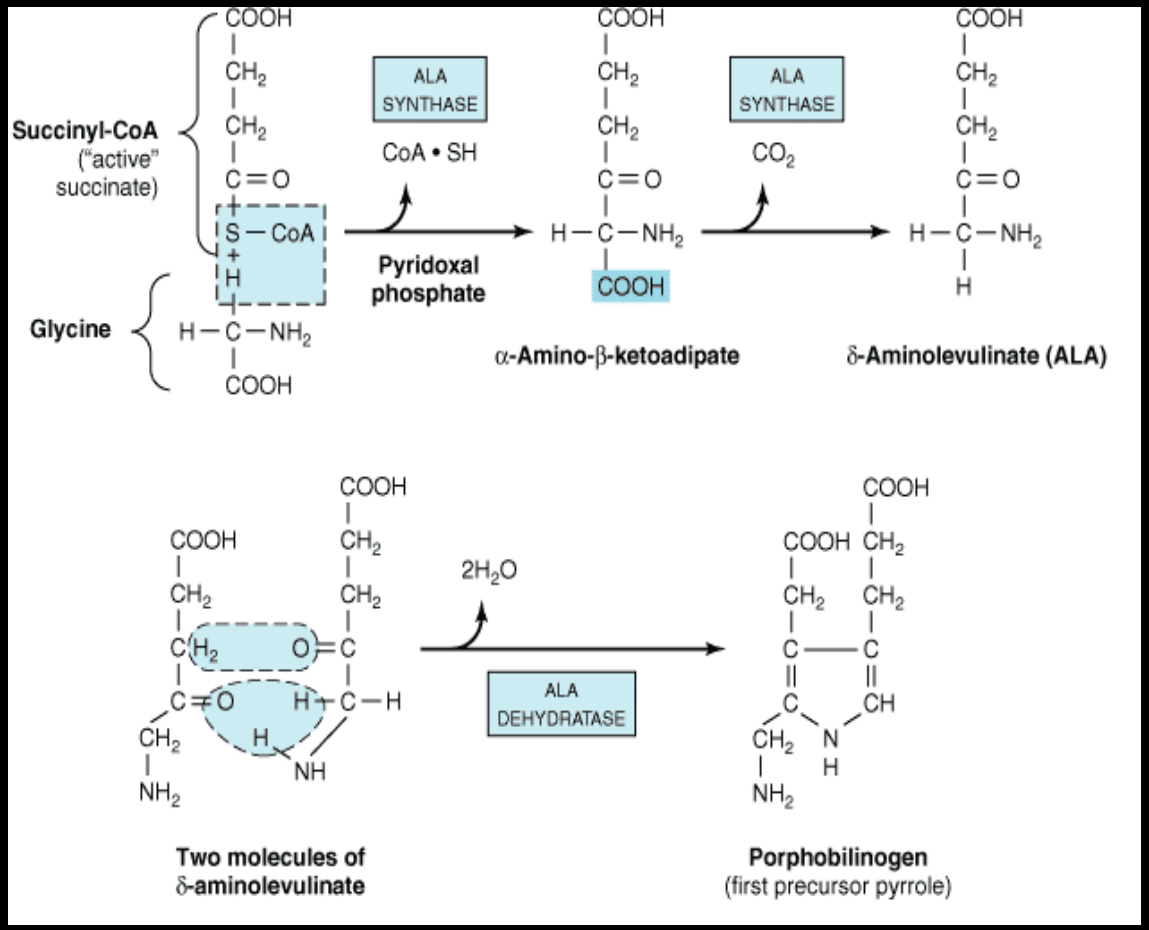
Role of TCA cycle in fatty acid synthesis



- Citrate is transported out of the mitochondrion when Aconitase is saturated with its substrate.
- This ensures that citrate is used for fatty acid synthesis only when there is an adequate amount to ensure continued activity of the cycle.

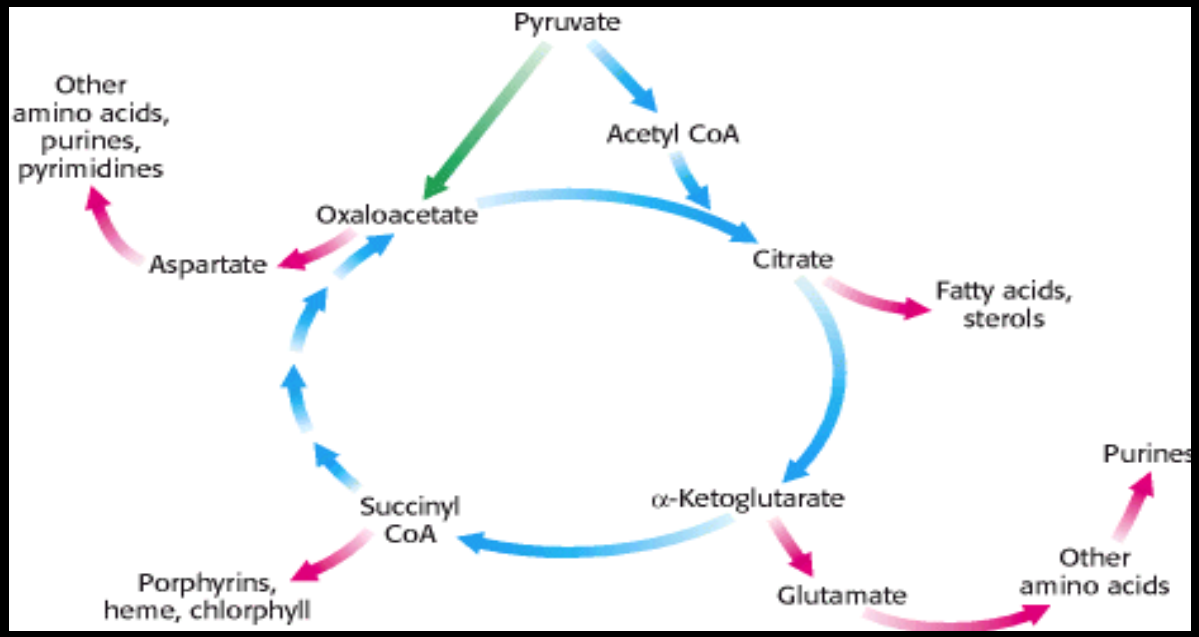
Acetyl co A can also be used for the synthesis of cholesterol, steroids etc.

iv) Role in Heme synthesis



- Succinyl co A condenses with amino acid Glycine to form Alpha amino beta keto Adipic acid, which is the first step of haem biosynthesis.

v) Role in purine and pyrimidine synthesis



Glutamate and Aspartate derived from TCA cycle are utilized for the synthesis of purines and pyrimidines.

Role of vitamins

- **Riboflavin,** in the form of flavin adenine dinucleotide (FAD), a cofactor for succinate dehydrogenase
- **Niacin,** in the form of nicotinamide adenine dinucleotide (NAD), the electron acceptor for isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase;
- **Thiamine(vitamin B1) ,** as thiamine pyro phosphate, the coenzyme for decarboxylation in the α -ketoglutarate dehydrogenase reaction;
- **Pantothenic acid,** as part of coenzyme A such as acetyl-CoA and succinyl-CoA and
- **Biotin-** in CO2 fixation reaction to compensate oxaloacetate concentration.

Anaplerotic reactions

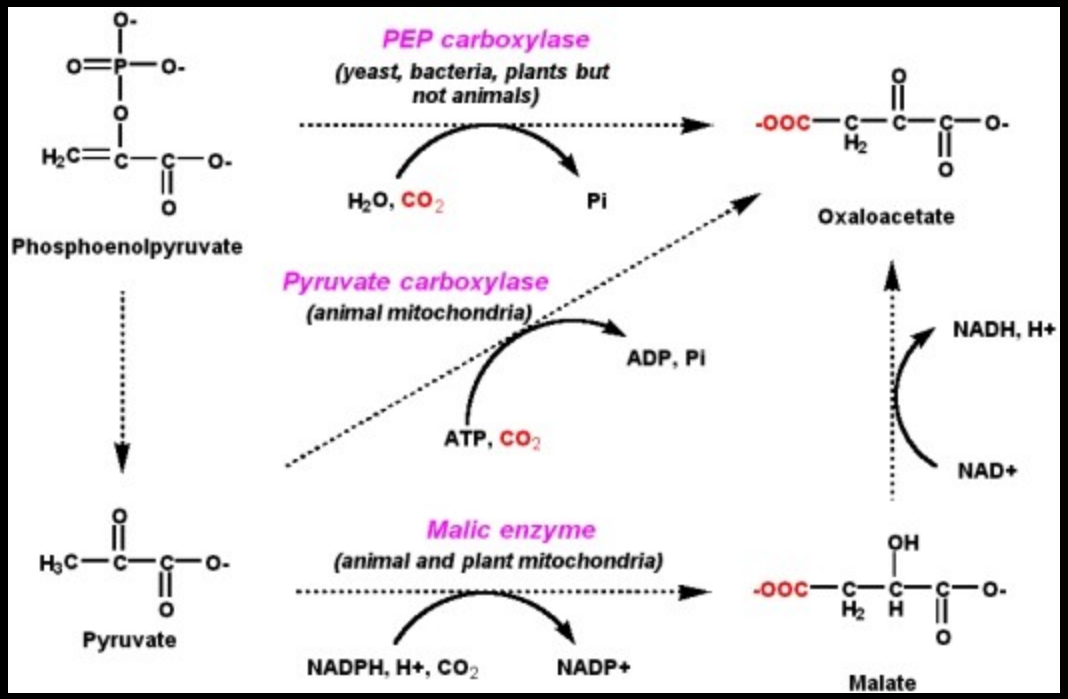
- Anaplerosis is the act of replenishing TCA cycle intermediates that have been extracted for biosynthesis (in what are called **cataplerotic reactions**).
- The TCA Cycle is a hub of metabolism, with central importance in both energy production and biosynthesis.
- Therefore, it is crucial for the cell to regulate concentrations of TCA Cycle metabolites in the mitochondria.
- Anaplerotic flux must balance cataplerotic flux in order to retain homeostasis of cellular metabolism

Anaplerotic reactions

1) Formation of oxaloacetate from pyruvate

- In case oxaloacetate is converted into amino acids for protein synthesis or used for gluconeogenesis and, subsequently, the energy needs of the cell rise.
- The citric acid cycle will operate to a reduced extent unless new oxaloacetate is formed, because acetyl CoA cannot enter the cycle unless it condenses with oxaloacetate.
- Even though oxaloacetate is recycled, a minimal level must be maintained to allow the cycle to function.

Anaplerotic reactions



Oxaloacetate is formed by –
a)Carboxylation of pyruvate, by pyruvate carboxylase
b)Through formation of malate from pyruvate by Malic enzyme
c)From malate to oxaloacetate by malate dehydrogenase

Mammals lack the enzymes for the net conversion of acetyl CoA into oxaloacetate or any other citric acid cycle intermediate.

Anaplerotic reactions

2) **Formation of oxaloacetate from Aspartate-** Oxaloacetate can also be formed from Aspartate by transamination reaction.
3) **Formation of Alpha keto glutarate-** Alpha ketoglutarate can be formed from Glutamate dehydrogenase or from transamination reactions.
4) **Formation of Succinyl co A** – Succinyl co A can be produced from the oxidation of odd chain fatty acid and from the metabolism of methionine and isoleucine (through carboxylation of Propionyl co A to Methyl malonyl co A and then Succinyl co A)

Fats burn in the flame of carbohydrates?

- fats burn in the flame of carbohydrates means fats can only be oxidized in the presence of carbohydrates.
- Acetyl co A represents fat component, since the major source is fatty acid oxidation.
- Acetyl co A is completely oxidized in the TCA cycle in the presence of oxaloacetate.

Fats burn in the flame of carbohydrates?

- Pyruvate is mainly used up for Anaplerotic reactions to compensate for oxaloacetate concentration.
- Thus without carbohydrates (Pyruvate), there would be no Anaplerotic reactions to replenish the TCA-cycle components.
- With a diet of fats only, the acetyl CoA from fatty acid degradation would not get oxidized and build up due to non functioning of TCA cycle.
- Thus fats can burn only in the flame of carbohydrates.

For further reading

Follow the links

http://www.namrata.co/tca-cycle-lecture-1/

http://www.namrata.co/significance-of-tca-cycle/