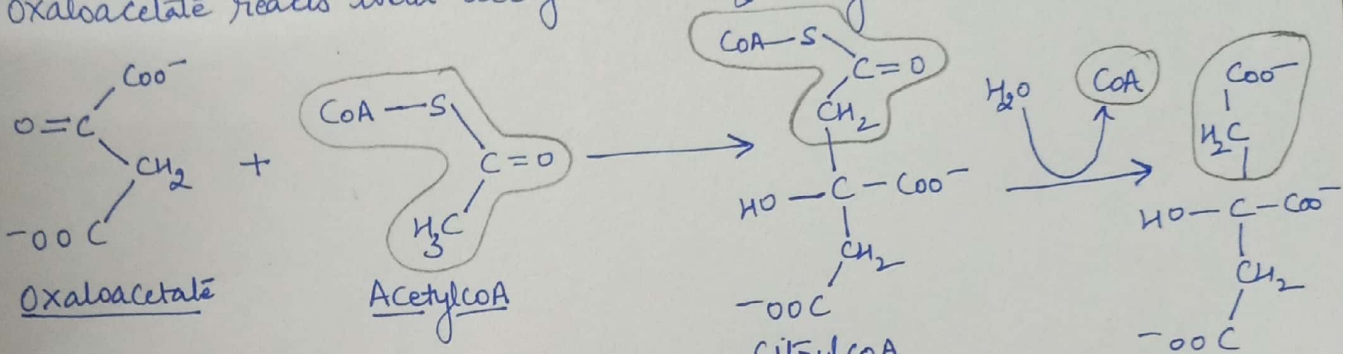
THE CITRIC ACID CYCLE oxidizes two-carbon units.

The conversion of Pyruvate into acetyl CoA by the Pyruvate dehydrogenase complex is the link between glycolysis and cellular respiration because acetyl CoA is the fuel for the citric acid cycle.

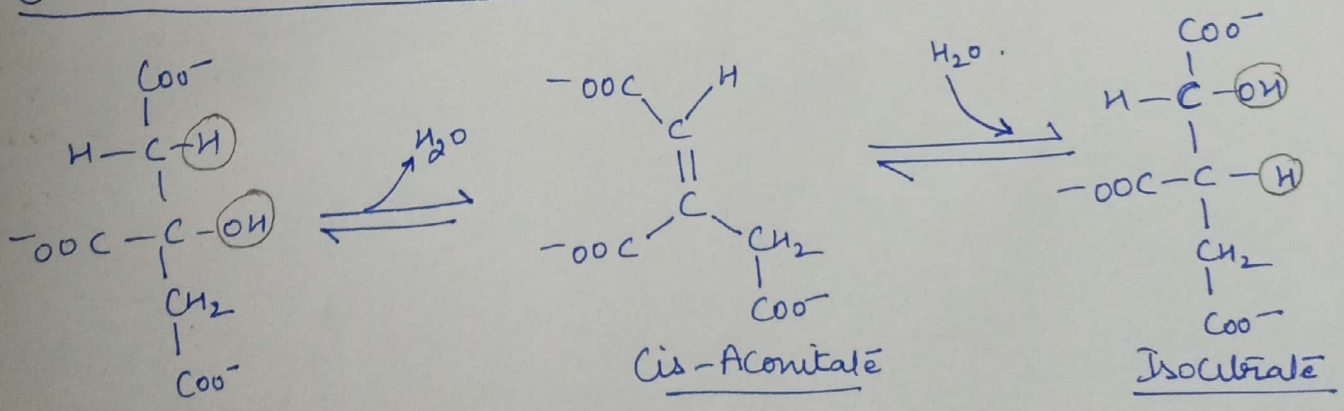
Citrate Synthase forms Citrate from Oxaloacetate and Acetyl CoA.

The Citrate 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 H_2O to yield citrate and CoA.



This reaction, which is an aldol condensation followed by a hydrolysis, is catalyzed by **Citrate synthase**. Oxaloacetate first condenses with acetyl CoA to form Citryl CoA, an energy-rich molecule because it contains the thioester bond that originated in acetyl CoA. The hydrolysis of Citryl CoA thioester to citrate and CoA drives the overall reaction far in the direction of the synthesis of citrate.

Citrate is Isomerized into Iso Citrate

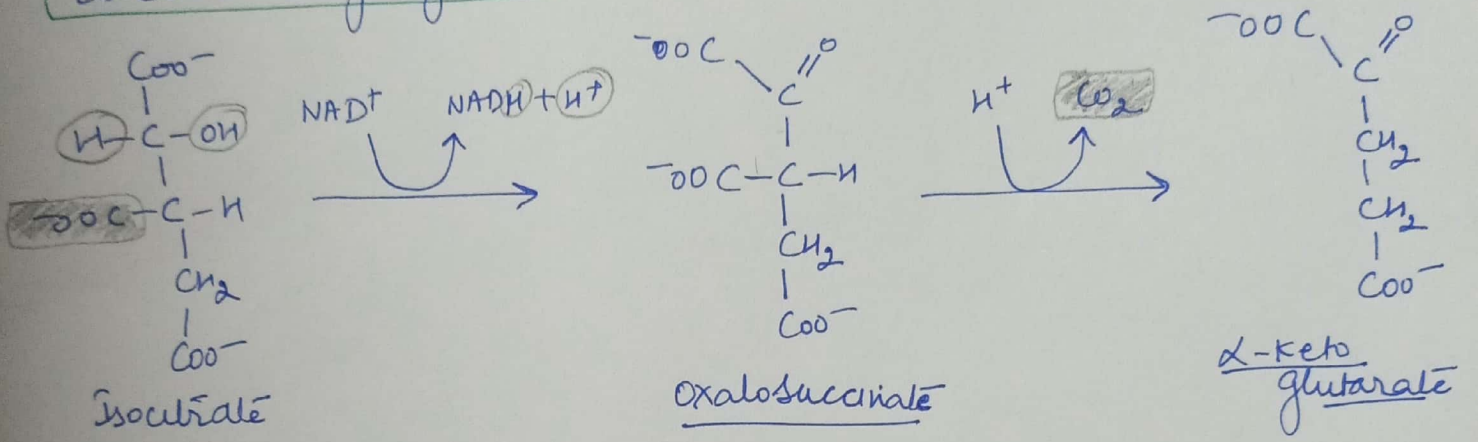


The hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylation that follows. Thus, citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation.

The isomerisation of citrate is accomplished by a dehydration step followed by a hydration step. The result is an interchange of an H and an OH. The enzyme catalyzing both steps is called **aconitase** because cis-aconitate is an intermediate.

Isocitrate is oxidised and decarboxylated to α-ketoglutarate.

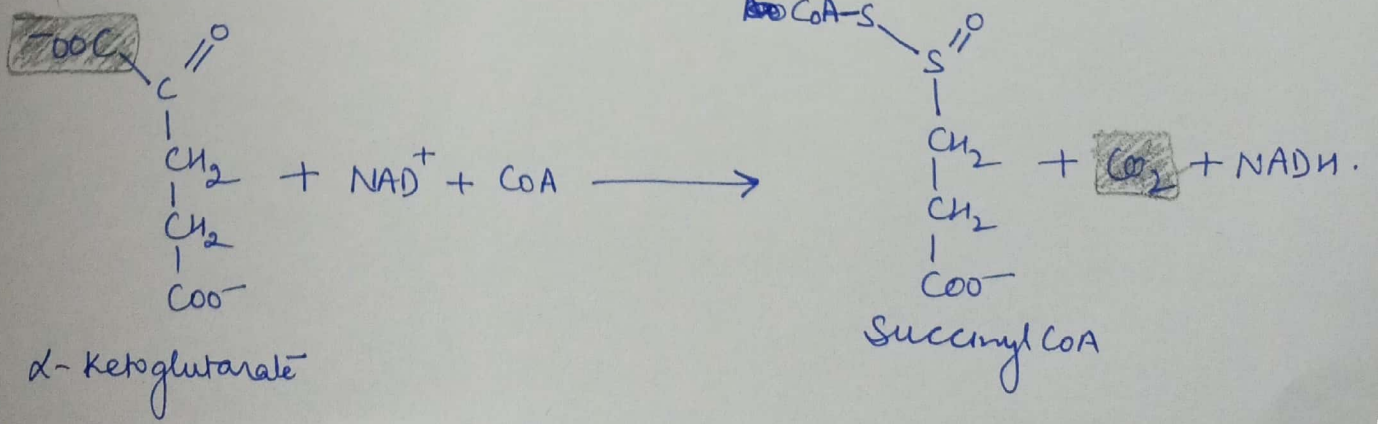
First of four oxidation-reduction reactions in the citric acid cycle. The oxidative decarboxylation of isocitrate is catalysed by enzyme **Isocitrate dehydrogenase**.



The intermediate in this reaction is Oxalosuccinate, an unstable β-keto acid. While bound to the enzyme, it loses CO_2 to form α-ketoglutarate.

Succinyl CoA is formed by the oxidative decarboxylation of α-ketoglutarate

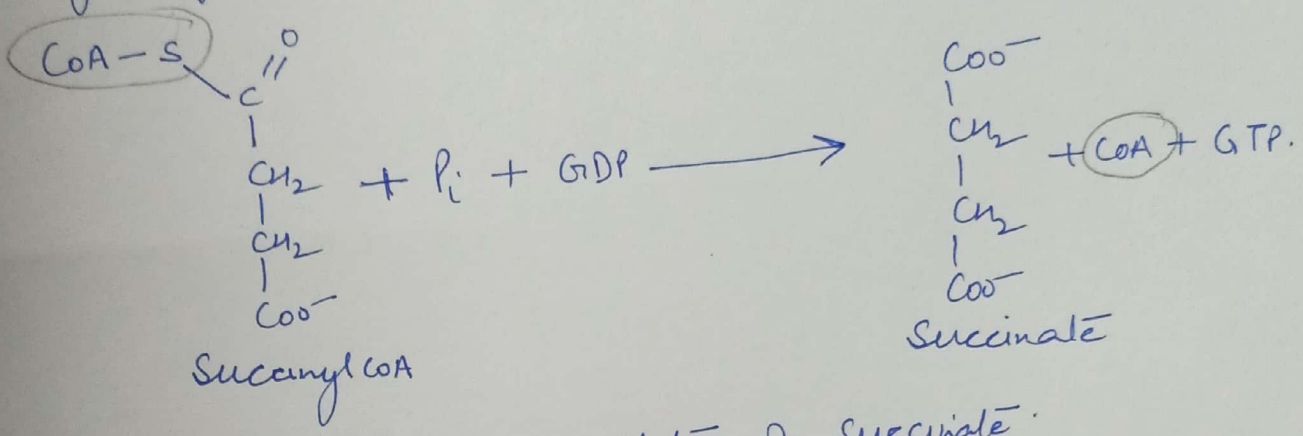
The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of succinyl CoA from α-ketoglutarate.



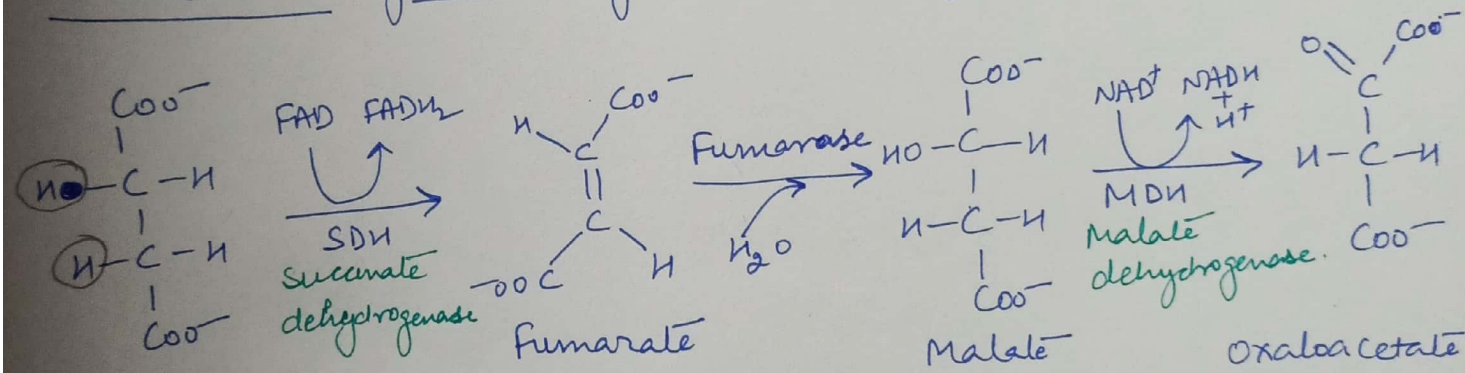
This reaction is catalyzed by the **α-ketoglutarate dehydrogenase** complex.

A Compound with high ~~energy~~ Phosphoryl-transfer potential is generated from Succinyl CoA.

Succinyl CoA is an energy-rich thio ester compound. The cleavage of the thioester bond of Succinyl CoA is coupled to the phosphorylation of a purine nucleotide diphosphate, usually GDP. This reaction is catalysed by enzyme **Succinyl CoA synthetase** (Succinate thiokinase).



Oxaloacetate is regenerated by the oxidation of Succinate.



A methylene group (CH_2) is converted into a Carbonyl group (C=O) in three step:- an oxidation, a hydration and a second oxidation reaction. Oxaloacetate is thereby regenerated for another round of the cycle, and more energy is extracted in the form of $FADH_2$ and NADH.

Succinate is oxidised to fumarate by Succinate dehydrogenase. The hydrogen acceptor is FAD rather than ~~the~~ NAD^+, which is used in the other three oxidation reactions in the cycle. FAD is the hydrogen acceptor in this reaction because the free-energy change is

insufficient to reduce NAD^+. FAD is nearly always the electron acceptor in oxidations that remove two hydrogen atoms from a substrate. Succinate dehydrogenase is an iron-sulfur protein and is embedded in the inner mitochondrial membrane. Succinate dehydrogenase is directly associated with the electron transport chain (ETC), the link between the Citric acid cycle and ATP formation.

$FADH_2$ produced by the oxidation of succinate does not dissociate from the enzyme, in contrast with NADH produced in other oxidation-reduction reactions. Rather, two electrons are transferred from $FADH_2$ directly to iron-sulfur clusters of the enzyme, which in turn passes the electron to coenzyme Q (CoQ). Coenzyme Q, an important membrane of the electron transport chain, passes electrons to the ultimate acceptor, molecular oxygen.

The next step is the hydration of fumarate to form L-malate. Fumarase catalyzes a stereospecific trans addition of H^+ and OH^-. The OH^- group adds to only one side of the double bond of fumarate, hence, only the L-isomer of malate is formed.

Finally, malate is oxidized to form oxaloacetate, This reaction is catalyzed by malate dehydrogenase, and NAD^+ is again the hydrogen acceptor.

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

The standard free energy for this reaction, unlike that for the other steps in the citric acid cycle, is significantly +ve. The oxidation of malate is driven by the use of the products - Oxaloacetate by Citrate synthase and NADH by the ETC.

The Citric acid cycle produces High-transfer-potential electrons, GTP and CO_2

$$\text{Acetyl CoA} + 3NAD^+ + FAD^+ + GDP + P_i + 2H_2O \longrightarrow 2CO_2 + 3NADH + FADH_2 + GTP + 2H^+ + CoA.$$

Entry to the CAC and metabolism through it is highly regulated! ⑤

① The enzyme Pyruvate dehydrogenase is regulated allosterically and by reversible phosphorylation.

The activity of Pyruvate dehydrogenase complex is regulated stringently.

* The high conc. of reaction products such as Acetyle CoA, NADH inhibit enzymes – Transacetylase and dihydrolipoyl dehydrogenase respectively.
* Phosphorylation of the Pyruvate dehydrogenase by a specific kinase enzyme switch off the activity of Pyruvate dehydrogenase. And in contrast phosphatase activity of Pyruvate dehydrogenase reactivates it again!

Under rest conditions of muscles the ratio of NADH/NAD, Acetyl CoA/CoA, and ATP/ADP will be high, thus this high ratio promotes phosphorylation thereby leading to switching off the activity of Pyruvate dehydrogenase. Where as during ~~excessive~~ exercise or high muscle activity both ↑ADP, ↑Pyruvate conc. activates Pyruvate dehydrogenase by inhibiting kinases activity.

↳ The phosphorylation is stimulated by Calcium ions (Ca^{++}), as the cytoplasmic Ca^{++} level rises, the mitochondrial Ca^{++} level also rises which in turn activates phosphatases thereby enhancing Pyruvate dehydrogenase activity.

HORMONES ROLE.
- In liver epinephrine → ↑(Inc.) conc. of Ca^{++}.
- Insulin on liver and adipose tissue → ↑(Inc.) Phosphatase activity

② Other Controls of Citric acid cycle

(i) Isocitrate dehydrogenase – Allosterically stimulated by ADP, which enhances enzyme's affinity for substrates. The binding of Isocitrate, NAD^+, ADP and Mg^{++} mutually and cooperatively increases this enzyme's activity.

(ii) α-Ketoglutarate dehydrogenase activity is regulated in simila

manner as enzyme Pyruvate dehydrogenase complex. The enzyme α-ketoglutarate dehydrogenase is inhibited by succinyl coA and high conc. of ATP.

Biosynthetic roles of Citric acid cycle