

KREBS CYCLE (TRICARBOXYLIC ACID CYCLE, TCA)

- ✓ Krebs cycle after Sir Hans Krebs.
- ✓ Called **citric acid cycle** because citrate was the product of the first reaction
- ✓ The most common name is **TCA cycle**, due to involvement of tricarboxylic acids (citrate and isocitrate).
- The TCA cycle accounts for over Two thirds of the ATP generated from fuel oxidation.

❖ **It is the common final catabolic pathway,**

oxidation of fatty acids

glucose
 amino acids
 acetate
 ketone bodies

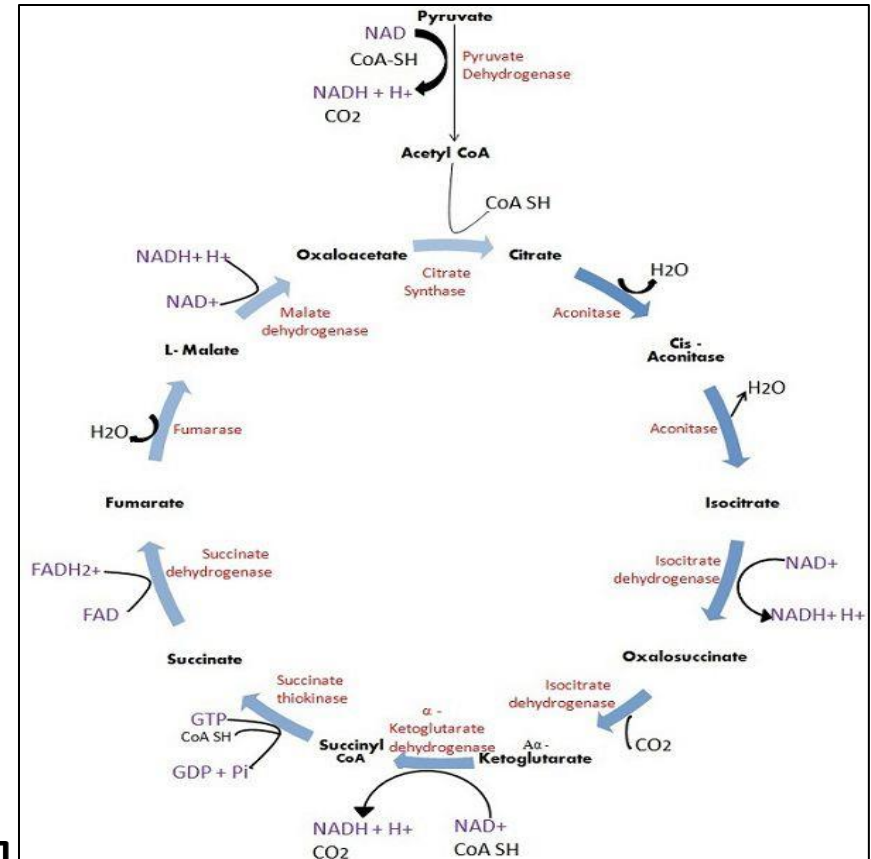
all generate
Acetyl CoA

substrate for the TCA

Thus, TCA cycle is central to energy generation from cellular respiration.

- ✓ Acetyl CoA is oxidized to **Two molecules of CO₂** and the energy is conserved as **NADH, FADH₂, and GTP**
- ✓ **NADH and FADH₂** subsequently donate electrons to O₂ via the **electron transport chain**, with the generation of ATP (**oxidative phosphorylation**).

- I. The first step, condensation of **acetyl CoA** and **oxaloacetate** to form **citrate** catalyzed by **Citrate Synthase**
- II. **Citrate** is modified to **isocitrate** by **Aconitase** enzyme.



III. Isocitrate undergoes oxidative decarboxylation by **Isocitrate Dehydrogenase** to form **α -ketoglutarate**. **Isocitrate dehydrogenase** is **NAD**-dependent enzyme. This reaction leads to release of the **first CO₂** and formation of **NADH**.

IV. α -ketoglutarate undergoes oxidative decarboxylation catalyzed by **α -Ketoglutarate Dehydrogenase Complex**. **Succinyl CoA** is formed.

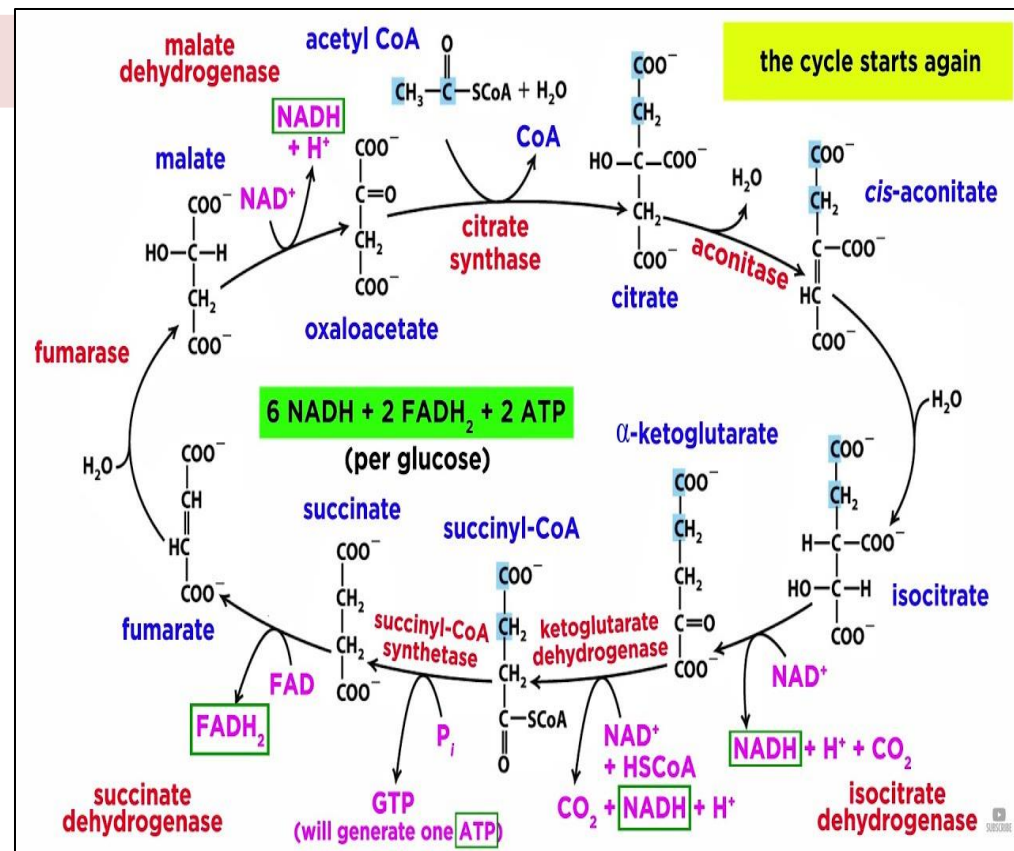
- ✓ This reaction is very similar to the reaction catalyzed by the **PDH (pre-Krebs)**.
- ✓ **α -ketoglutarate dehydrogenase complex** is a complex formed of **Three enzymes** bound together (**α -ketoglutarate dehydrogenase**, **dihyrolipoyl transacetylase** and **dihydrolipoyl dehydrogenase**) and **Five coenzymes** (**Thiamine pyrophosphate (TPP)**, **Lipoic acid**, **FAD** and **NAD**, **Coenzyme A**).
- ✓ **α -ketoglutarate dehydrogenase complex** leads to release of the **second CO₂**, and formation of **NADH**.
- ✓ However, α -ketoglutarate dehydrogenase does not subjected to covalent modification by hormones like PDH.
- ✓ This reaction is inhibited by Arsenite (an inhibitor of lipoic acid-requiring enzymes).

V. Succinyl CoA is converted to **succinate** by **Succinate Thiokinase** (or **Succinyl CoA Synthetase**).

- ✓ **مهم جداً** This reaction is an example of substrate level phosphorylation.

*substrate level phosphorylation is the formation of a high-energy phosphate bond (ATP or GTP) **without** the use of molecular **O₂** or the **ETC** (it is **NOT** an oxidative phosphorylation).*

- ✓ The high-energy phosphate bond of GTP is energetically **equivalent** to that of ATP.



VI. Succinate is oxidized by **Succinate Dehydrogenase** (a part of inner mitochondrial membrane) to form fumarate.

- ✓ **مهم جداً** This is the only step in TCA cycle where **FAD** is the cofactor for the oxidation reaction.
- ✓ Succinate dehydrogenase is competitively **Inhibited by Malonate**.

VII. Fumarate by the action **fumarase** is hydrated leading to the formation of malate.

VIII. Malate is converted to oxaloacetate, to close the cycle. The reaction is catalyzed by the **Malate Dehydrogenase** enzyme and **NADH** is produced.

To summarize: in the citric acid cycle, the two carbons of acetyl CoA are oxidized and released in the form of **2 CO₂ + 3 NADH + one FADH₂ + one GTP**.

ENERGY YIELD OF TCA

Each NADH will produce 3 ATP $\longrightarrow 3 \text{ NADH} \times 3 = 9 \text{ ATP}$ } By oxidative phosphorylation In ETC
Each FADH₂ will produce 2 ATP $\longrightarrow 1 \text{ FADH}_2 \times 2 = 2 \text{ ATP}$
An extra **ATP (GTP)** is produced in the cycle by the substrate level phosphorylation .

سؤال مهم the final yield of energy per molecule of **Acetyl CoA** oxidized in the TCA cycle equals **12 ATP**.

سؤال مهم In which reactions

- The **complete oxidation** of one molecule of **Glucose** under **Aerobic conditions** will yield **36-38 ATP** as follows:
Aerobic glycolysis (**6-8 ATP**) according to shuttle
Oxidative decarboxylation of 2 pyruvates (**6 ATP**) by PDH
Krebs cycle for 2 molecules of acetyl CoA (**12 X 2 = 24 ATP**)

- Oxidation of Glucose under anaerobic conditions will only yield **2 ATP**.

N.B Four members of the vitamin B-complex (in the form of their coenzymes) play an important role in the TCA cycle

1- Niacin (vit B3) in the form of **NAD⁺**

2-Riboflavin (vit B2) in the form of **FAD**

3-Thiamin (vitamin B1) in the form of **TPP**

4-pantothenic acid as a part of **coenzyme A (CoASH)**

Note: All the coenzymes of the TCA cycle, except for lipoic acid, are derived from vitamins

THE IMPORTANCE AND THE AMPHIBOLIC NATURE OF THE TCA

Although Krebs cycle is mainly Catabolic, it has some Anabolic features (It is an Amphibolic cycle).

I- The Catabolic nature

TCA cycle is **the final common Catabolic pathway** for oxidation of metabolic intermediates derived from carbohydrates, lipids and proteins.

II- The Anabolic nature

-Some intermediates of TCA cycle participate in synthesis of other important biomolecules in different pathways in different cell types.

❖ Anabolic intermediates:

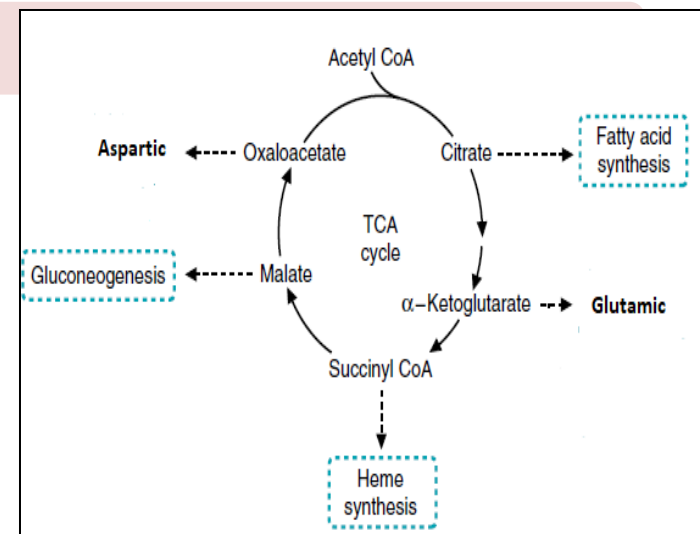
(1) After a high carbohydrate meal, Citrate efflux from mitochondria and cleavage to Acetyl CoA for cytosolic Fatty acid synthesis (lipogenesis).

(2) During fasting, gluconeogenic precursors are converted to Malate, which leaves the mitochondria for cytosolic part of gluconeogenesis.

(3) The liver also uses TCA cycle intermediates to synthesize Non-essential Amino Acids. For example:

- α -ketoglutarate is converted to glutamate
 - oxaloacetate to aspartate
 - Pyruvate to alanine
- } **Transamination reactions.**

(1) Succinyl CoA can be utilized to form Heme in cells of the liver and bone marrow.



Important notes

- All the enzymes are in the **matrix** of the mitochondria except **succinate dehydrogenase**, which is in the inner mitochondrial membrane.
- **Succinate thiokinase (Succinyl CoA synthetase)** catalyzes a **substrate-level phosphorylation** of GDP to GTP.
- Deficiency of any of the enzymes of the TCA has not been reported, since it is incompatible with life.