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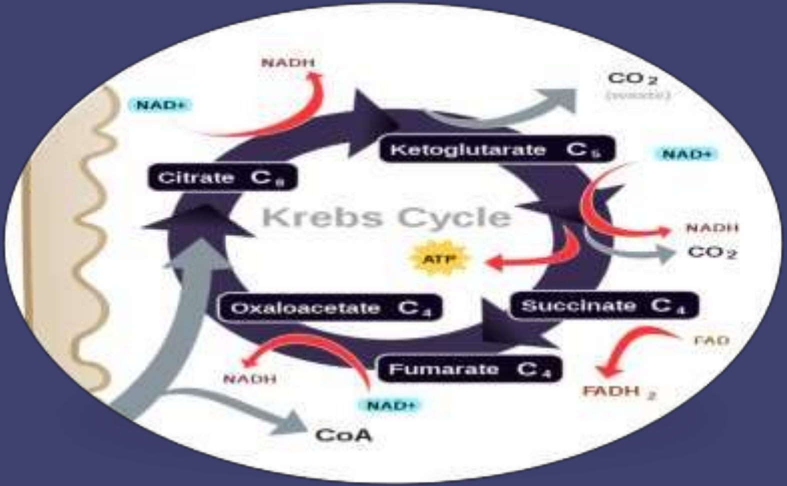
College Of Medicine

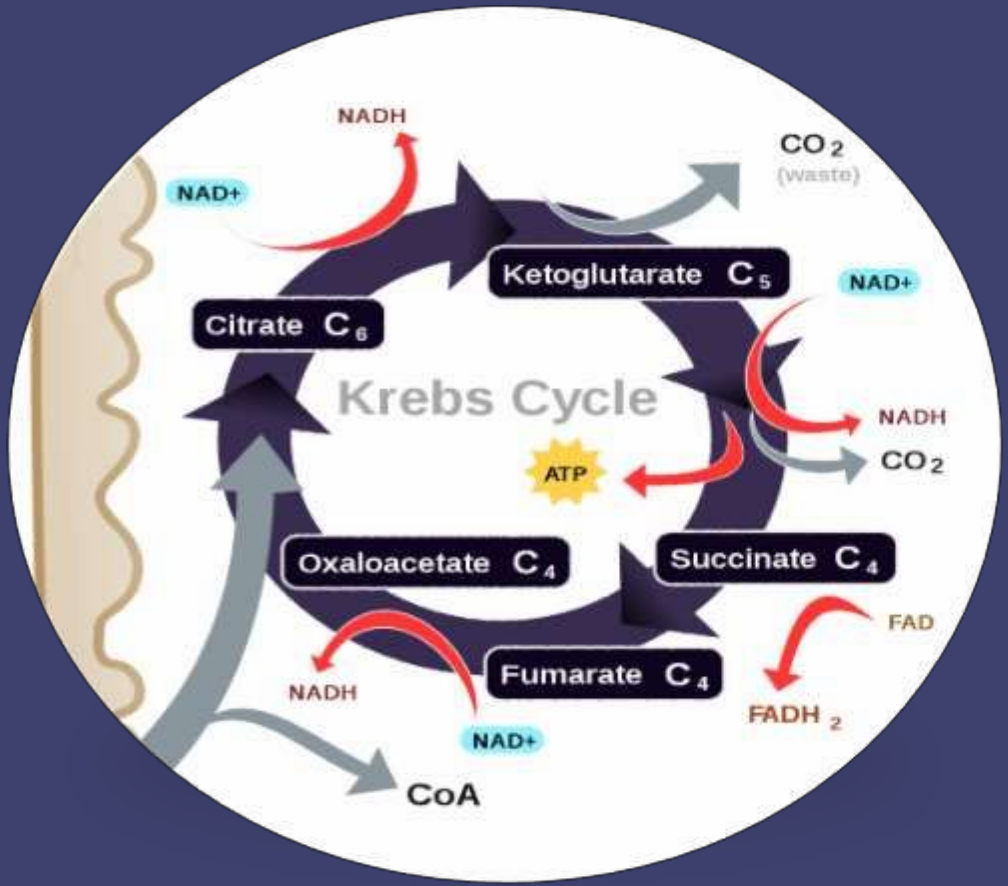
Citric acid cycle (TCA)



By Assistance Professor Dr. Ajile A. Alzamily

CITRIC ACID CYCLE

- Introduction
- Site
- Pathway
- Energetics
- Regulation
- Clinical importance



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.

Definition :

- The citric acid cycle is a series of reactions that brings about catabolism of acetyl-coA liberating **reducing equivalents** which upon oxidation through respiratory chain of mitochondria, **generate ATP**.
- It plays a central role in the breakdown or **catabolism** of organic fuel molecules—i.e **glucose** and some other sugars, fatty acids, and some amino acids. Before these rather large molecules can enter the TCA cycle they must be degraded into a two-carbon compound called acetyl coenzyme A (acetyl CoA). Once fed into the TCA cycle, acetyl CoA is converted into **carbon dioxide** and energy.

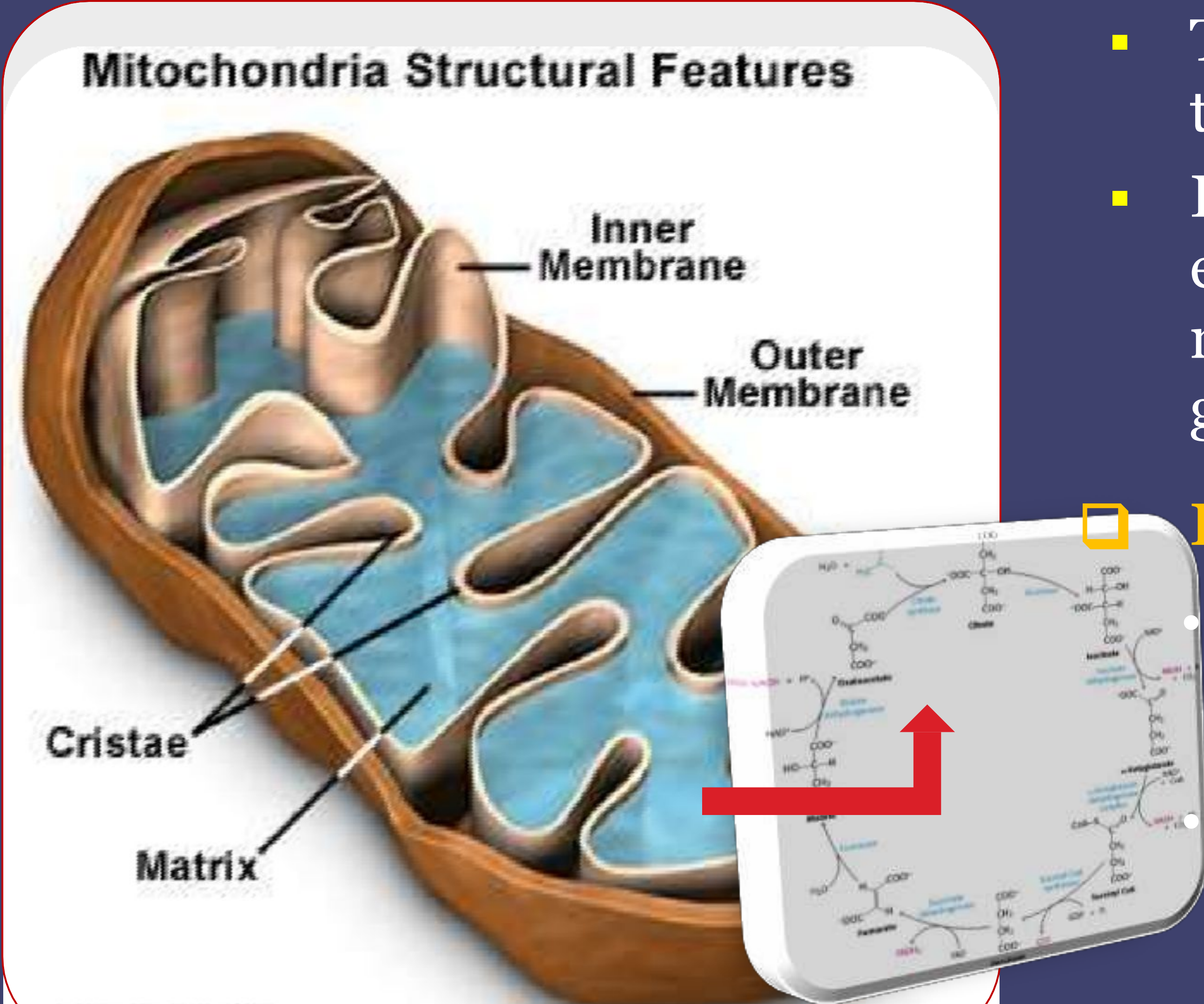


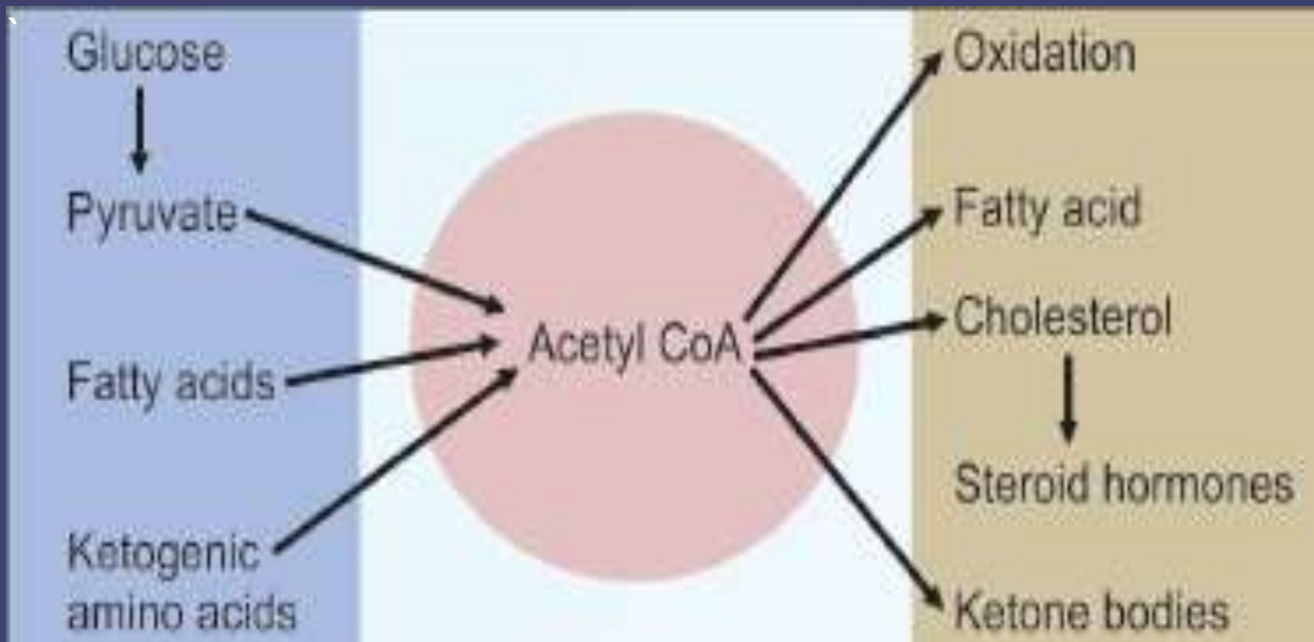
Figure 1

- Takes place in the **matrix** of the mitochondria. (Figure 1)
- It happens once for every pyruvate molecule in glycolysis….

Purpose

- Conversion of Acetyl-CoA to CO_2
- Generates reducing equivalents (NADH + H^+, FADH2) & GTP to be oxidized in the respiratory chain to generate ATP

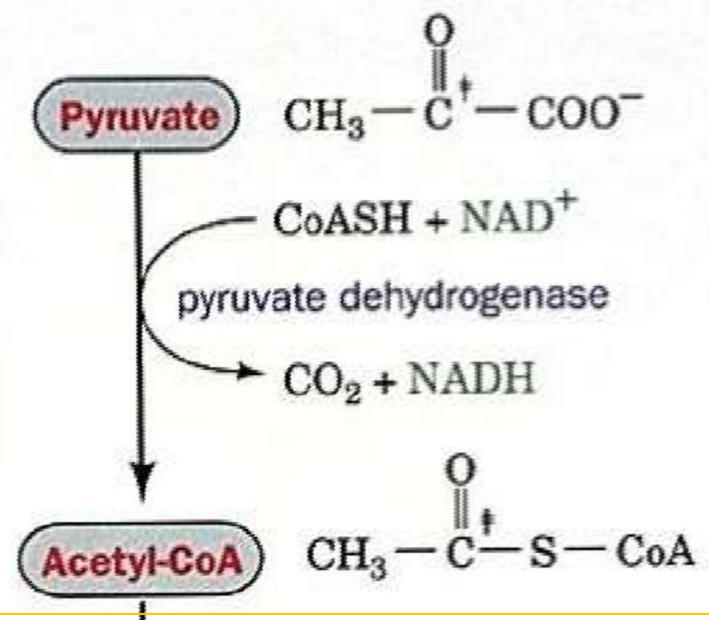
OVERVIEW

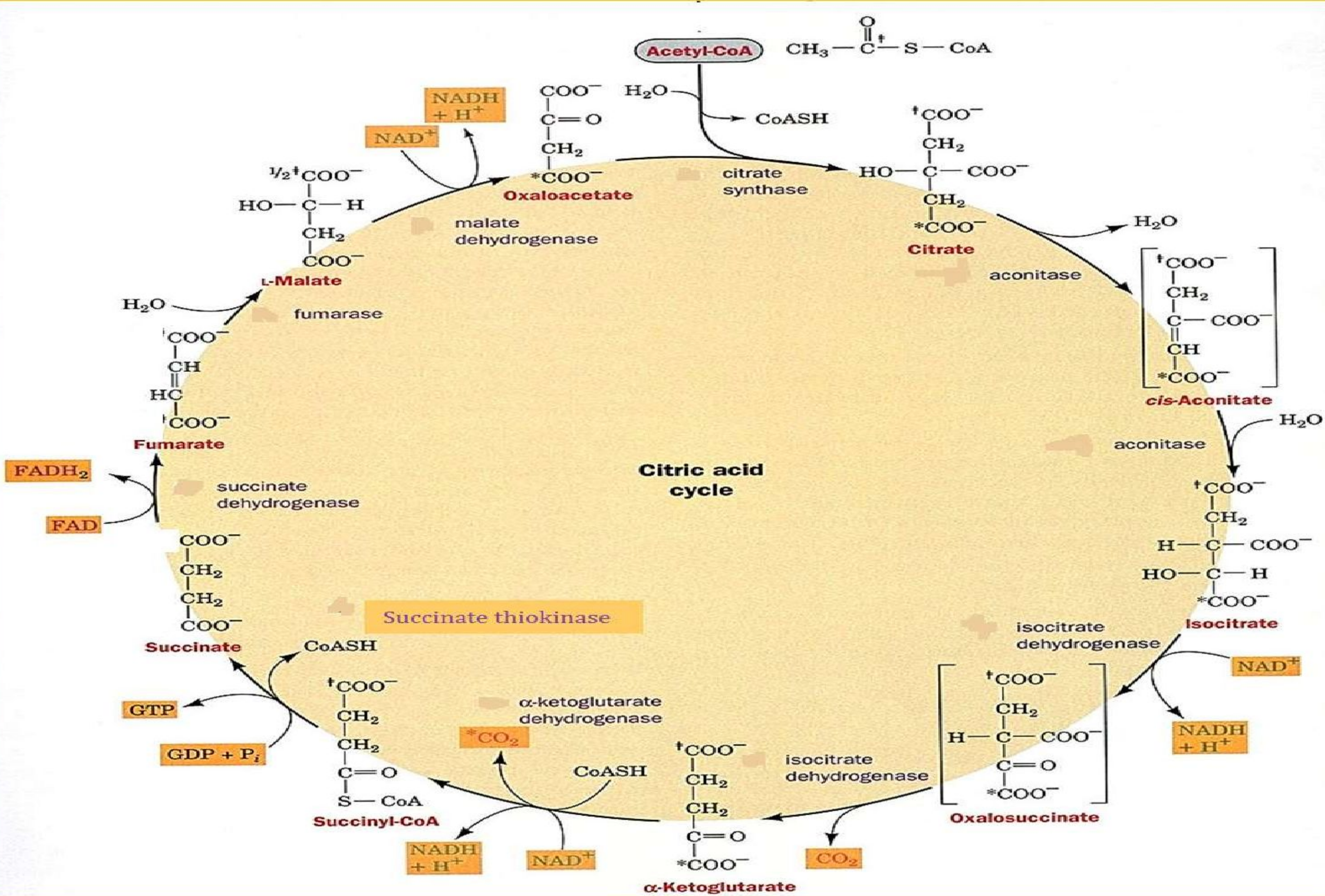


- Acetyl coA, the precursor for fatty acid synthesis is produced from pyruvate, ketogeni amino acids, fatty acid oxidation and by alcohol metabolism.

- It is a substrate for TCA cycle and a precursor fo fatty acids, ketone bodies and sterols.

STEPS INVOLVED IN TCA CYCLE



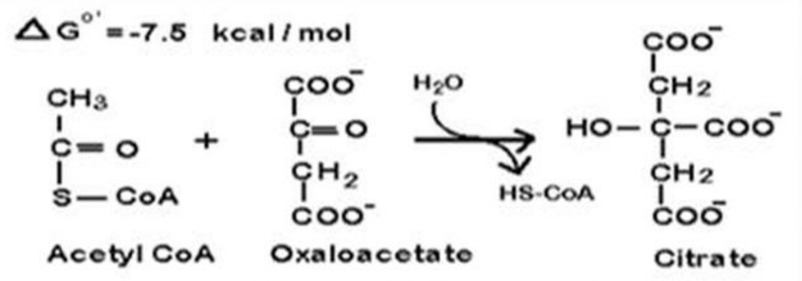

Acetyl-CoA
CH3—C—S—CoA
H2O
CoASH
citrate synthase
Citrate
H2O
aconitase
cis-Aconitate
H2O
aconitase
Isocitrate
isocitrate dehydrogenase
NAD+
NADH + H+
Oxalosuccinate
CO2
isocitrate dehydrogenase
α-Ketoglutarate
α-ketoglutarate dehydrogenase
CoASH
NAD+
NADH + H+
*CO2
Succinyl-CoA
CoASH
GTP
GDP + Pi
Succinate thiokinase
Succinate
succinate dehydrogenase
FAD
FADH2
Fumarate
fumarase
H2O
L-Malate
malate dehydrogenase
NAD+
NADH + H+
Oxaloacetate
Citric acid cycle

Reactions of TCA Cycle

➢ **Step:1 Formation of citrate** (Condensation alalddual)

- Oxaloacetate condenses with acetyl CoA to form Citrate, catalysed by the enzyme citrate synthase
- Inhibited by:
- ATP, NADH, Citrate - competitive inhibitor of oxaloacetate.

- Only step in TCA cycle that involves the formation of a C−C bond



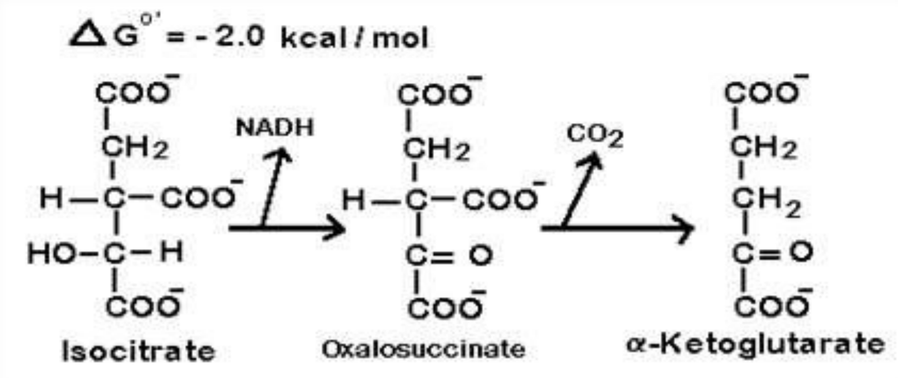
The first step is the step the organization to speed tri-carboxylic acid cycle. Alaoxalit interaction is used, a compound produced from the TCA cycle to produce Citrate , so the entry to the acetyl COA cycle does not lead to the formation of the compound or intermediate cycle consumption.

Steps 2 & 3 Citrate is isomerized to isocitrate

- Citrate is isomerized to isocitrate by the enzyme aconitase
- This is achieved in a two stage reaction of dehydration followed by hydration through the formation of an intermediate -cis-aconiase

Isocitrate Dehydrogenase

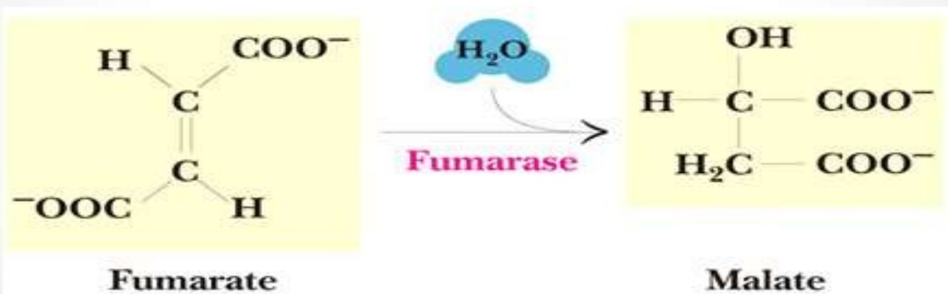
Oxidation and the removal the carboxyl of Citrate.



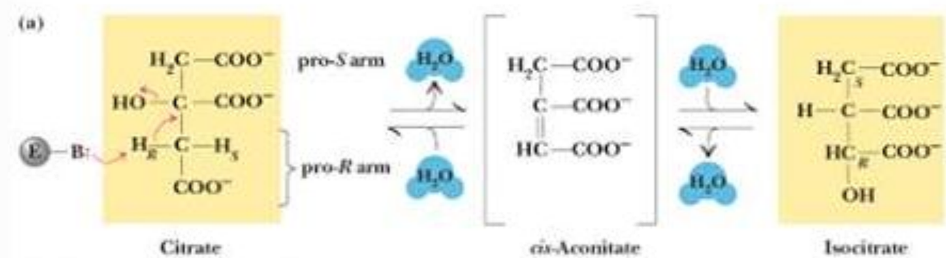
Steps 4 & 5 Formation of α-ketoglutarate

- Isocitrate dehydrogenase (ICDH) catalyses the conversion of (oxidative decarboxylation) of isocitrate to oxalosuccinate & then to α-ketoglutarate.
- The formation of NADH & the liberation of CO_2 occure at this stage.
- Stimulated (cooperative) by isocitrate, NAD^+, Mg^{2+}, ADP, Ca^{2+} (links with contraction).
- Inhibited by NADH & ATP

Add water to Fumarase



Aconitase(Symmetry citrate)



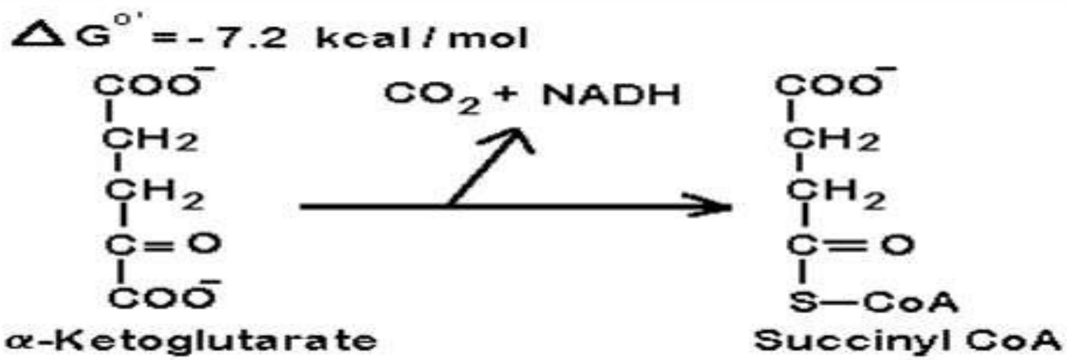
Aconitase removes the pro-R H of the pro-R arm of citrate

Step: 6 Conversion of α-ketoglutarate to succinyl CoA

- Occurs through oxidative decarboxylation, catalysed by α-ketoglutarate dehydrogenase complex.
- α-ketoglutarate dehydrogenase is an multienzyme complex.
- At this stage of TCA cycle, second NADH is produced & the second CO_2 is liberated.

α -Ketoglutarate Dehydrogenase

Oxidative stress Deletion carboxyl groups of α -ketoglutarate.

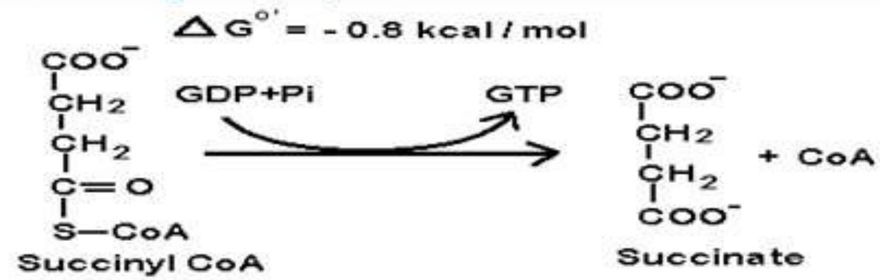


Step: 7 Formation of succinate

- Succinyl CoA is converted to succinate by succinate thiokinase.
- This reaction is coupled with the phosphorylation of GDP to GTP.
- This is a substrate level phosphorylation.
- GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

Succinyl-CoA Synthetase

- breaking succinyl-COA

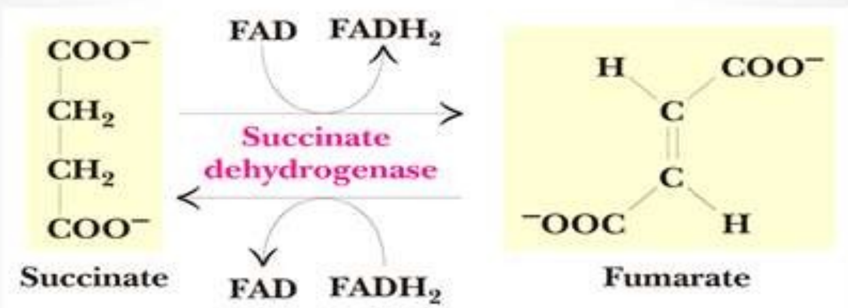


This interaction represents a step in the TCA phosphorylation only on the level of the foundation.
GTP formation is not oxidative phosphorylation.

Step: 8 Conversion of succinate to fumarate

- **Succinate is oxidized by succinate dehydrogenase to fumarate.**
- **This reaction results in the production of $FADH_2$.**
- **Step: 9 Formation of malate: The enzyme fumarase catalyses the conversion of fumarate to malate with the addition of H_2O.**

oxidation succinate.



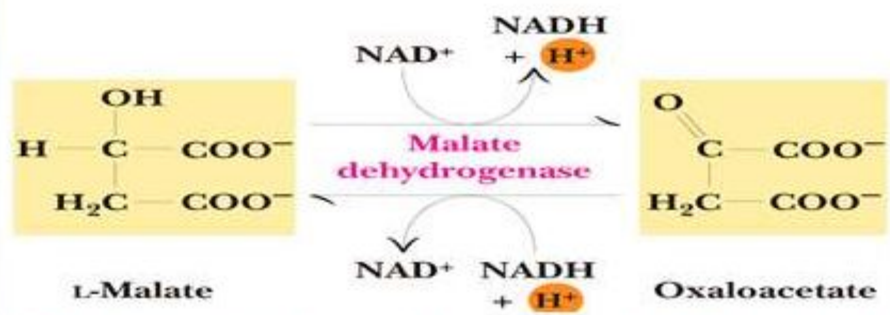
- The use of the electronic future of FAD instead of NAD
- ✓ Because the reductive power of compound succinate is not sufficient for the reduction of NAD.

Step:10 Conversion of malate to oxaloacetate

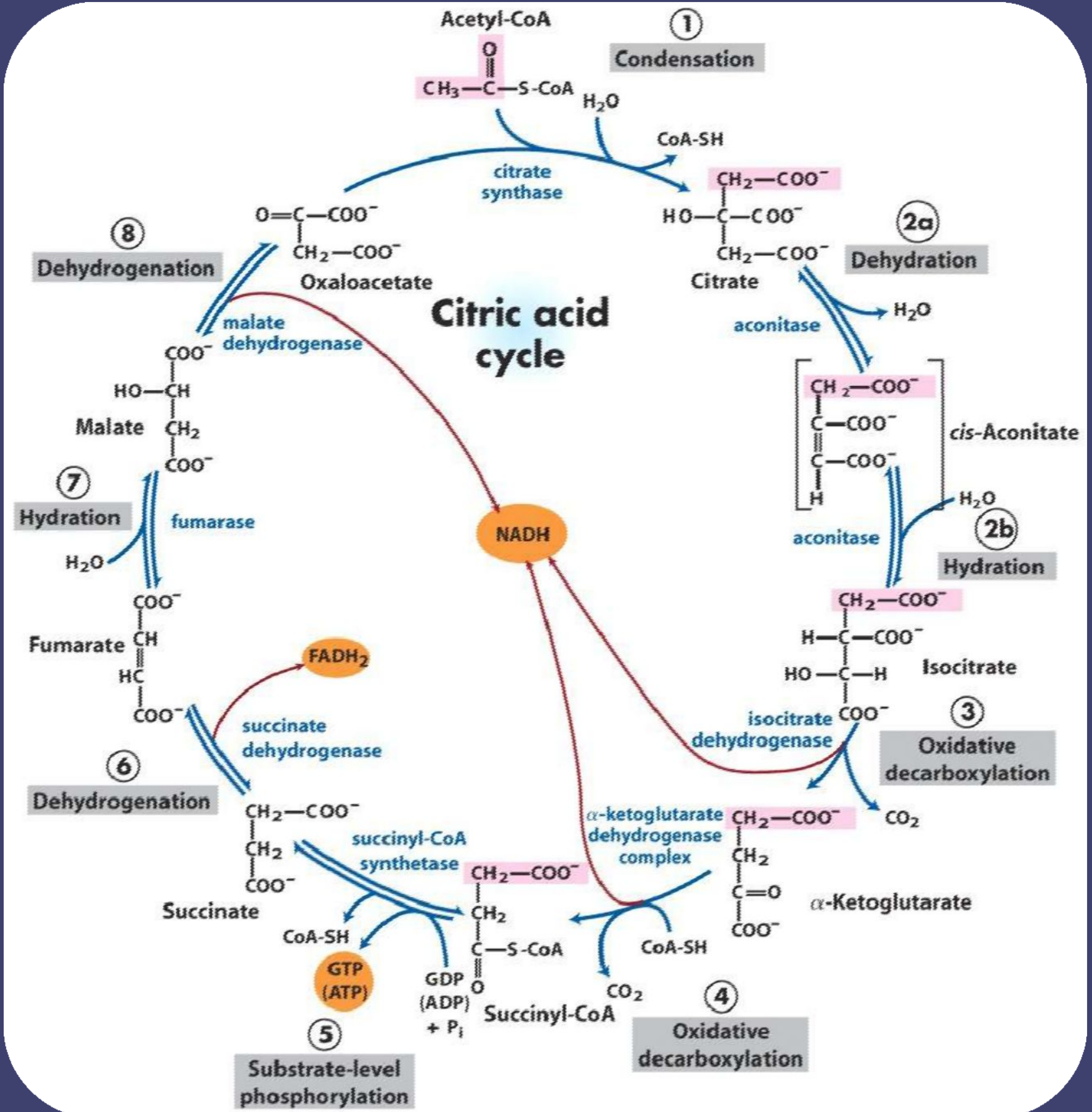
- **Malate is then oxidized to oxaloacetate by malate dehydrogenase.**
- **The third & final synthesis of NADH occurs at this stage.**
- **The oxaloacetate is regenerated which can combine with another molecule of acetyl CoA & continue the cycle.**

Malate Dehydrogenase

Oxidation of malate



–This reaction generates a three of the partial NADH cycle



Acetyl-CoA
1 Condensation
H_2O
CoA-SH
citrate synthase
Citrate
2a Dehydration
H_2O
aconitase
cis-Aconitate
H_2O
aconitase
2b Hydration
Isocitrate
isocitrate dehydrogenase
3 Oxidative decarboxylation
CO_2
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
CoA-SH
CO_2
4 Oxidative decarboxylation
Succinyl-CoA
GDP (ADP) + P_i
GTP (ATP)
CoA-SH
5 Substrate-level phosphorylation
succinyl-CoA synthetase
Succinate
6 Dehydrogenation
succinate dehydrogenase
$FADH_2$
Fumarate
H_2O
fumarase
7 Hydration
Malate
malate dehydrogenase
8 Dehydrogenation
Oxaloacetate
NADH
Citric acid cycle

- TCA cycle is an open cycle
- Operates only under aerobic conditions
- This is the Final common pathway of oxidative metabolism
- Two carbon dioxide molecules are released as a waste product of respiration

Stoichiometry of the TCA cycle

Reactants		Products
Acetyl CoA	→	$2\ CO_2$ + CoA-SH
Oxaloacetate		Oxaloacetate
FAD		$FADH_2$
$3NAD^+$		3 NADH + H^+
GDP + Pi		GTP

Energetics : 2 Acetyl CoA from 2 Pyruvate

Acetyl-CoA + 3 NAD^+ + [FAD] + GDP + Pi $\longleftrightarrow$ CoASH + 2 H_2O 3 NADH+3 H^+ +[$FADH_2$] + GTP + + 2 CO_2

- 1NADH+H^+ = 3
- 1$FADH_2$ = 2
- 1GTP = 1 ATP

In the cytoplasm		
Glycolysis:	2 ATP	⟶ 2 ATP
In the mitochondria		
From glycolysis:	2 NADH ⟶ 6 ATP	⟶ 6 ATP*
From respiration:		
Pyruvic acid ⟶ acetyl CoA:	1 NADH ⟶ 3 ATP	(× 2) ⟶ 6 ATP
Krebs cycle:	1 ATP 3 NADH ⟶ 9 ATP 1 $FADH_2$ ⟶ 2 ATP	(× 2) ⟶ 24 ATP
Total:		38 ATP

ATP Generation steps

Step No	Reactions	Co-enzyme	ATPs (old-calculation)	ATPs (new calculation)
3	Isocitrate → alpha keto glutarate	NADH	3	2.5
4	Alpha keto glutarate → succinyl CoA	NADH	3	2.5
5	Succinyl CoA→Succinate	GTP	1	1
6	Succinate → Fumarate	$FADH_2$	2	1.5
8	Malate → Oxalo acetate	NADH	3	2.5
		Total	12	10

Biomedical importance

- Bioenergetics is the study of the energy changes accompanying biochemical reactions. Biologic systems are essentially isothermic and use chemical energy to power living processes.
- Animal obtains suitable fuel from its food to provide the energy for metabolism

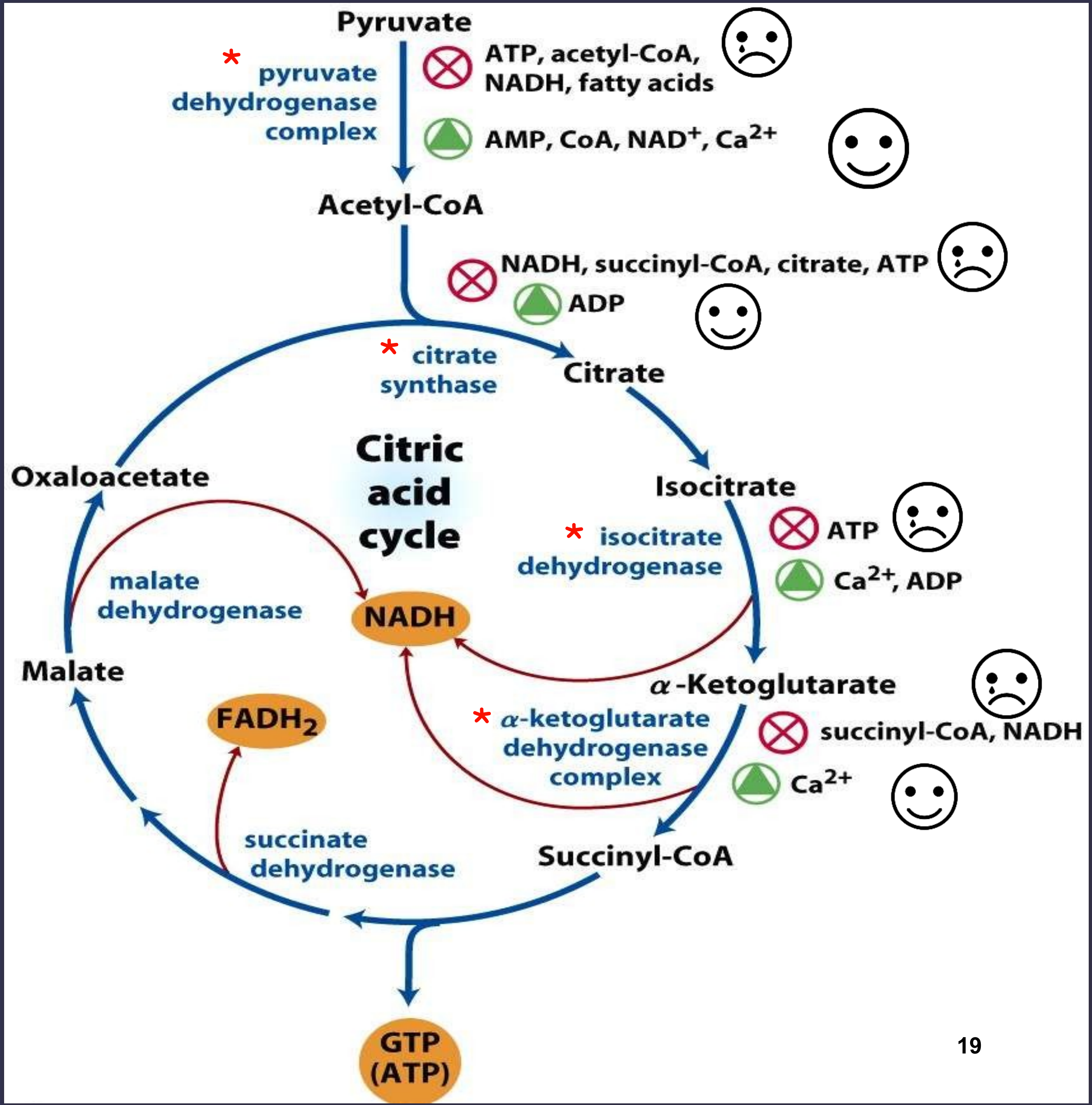
Biomedical importance

- **Thyroid hormones control the rate of energy release and disease results when they malfunction.**
- **Excess storage of surplus energy causes obesity.**

- This cycle is a series of chemical intermediates.
- Each step is catalyzed by a specific enzyme.

Regulation of TCA cycle

- Indicator molecules of higher energy state i.e. ATP, NADH, citrate, Acetyl CoA – inhibit TCA cycle
- Indicator molecules of low energy state i.e. ADP, AMP, NAD^+ – stimulate TCA cycle



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 inhibitedby 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.

Inhibitors of TCA cycle

- **Aconitase** – is inhibited by fluoroacetate (non-competitive inhibition)

- **α-ketoglutarate dehydrogenase** is inhibited by Arsenite (non-competitive inhibition)

- **Succinate dehydrogenase** is inhibited by Malonate (competitive inhibition)

METABOLIC DEFECTS

- Extremely rare

1. Defect in PDH

    - Lactic acidosis

    - Neurological disorders (brain, spine and the nerves

2. Defect In Pyruvate carboxylase

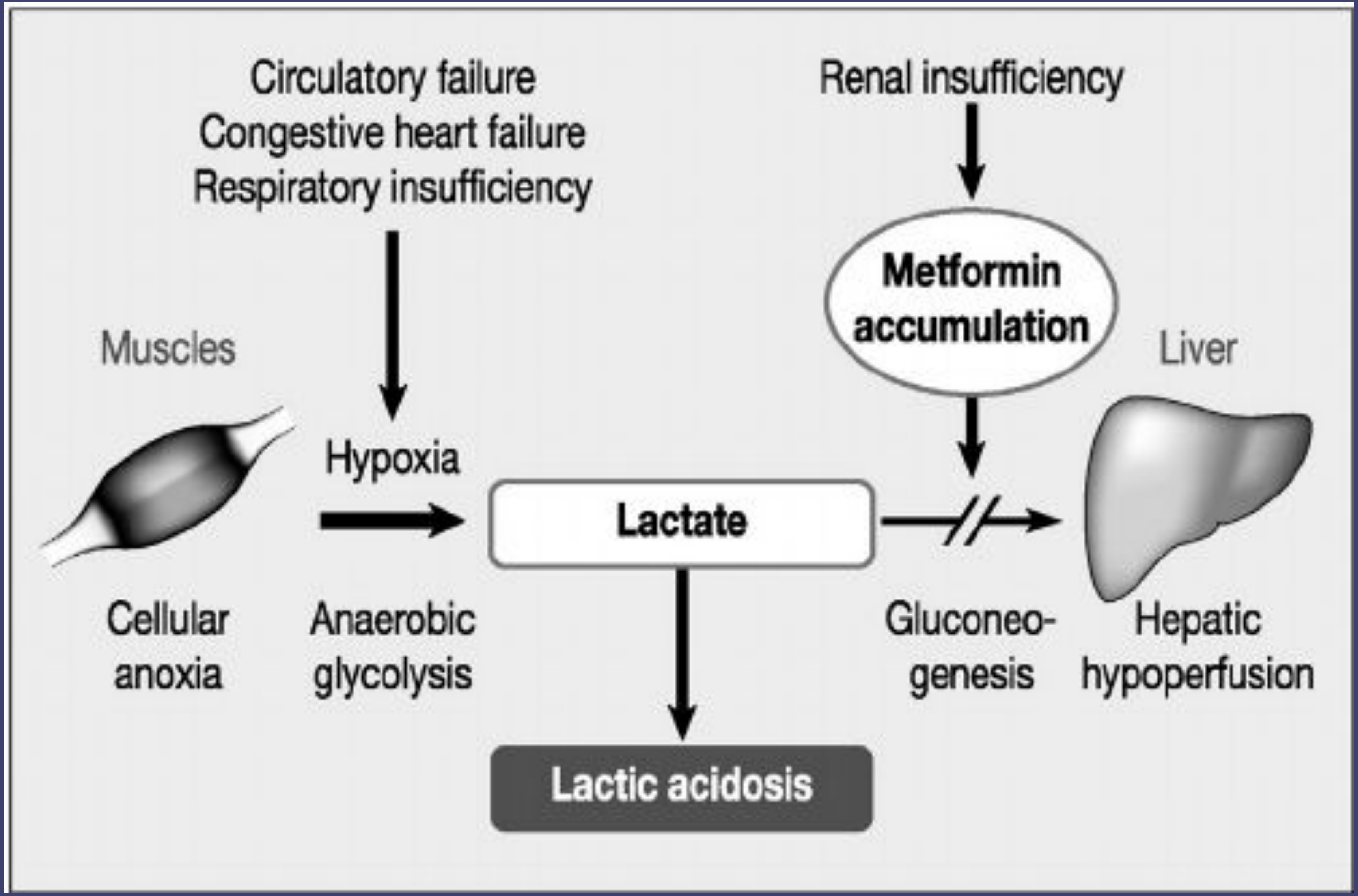
    - Oxaloacetate

    - Hyperammonemia

    - Lactic acidosis

    - Hyperalaninemia.

Lactic acidosis



WHY TCA IS CALLED AMPHIBOLIC?

It plays both catabolic and anabolic role.

***Catabolic role**:

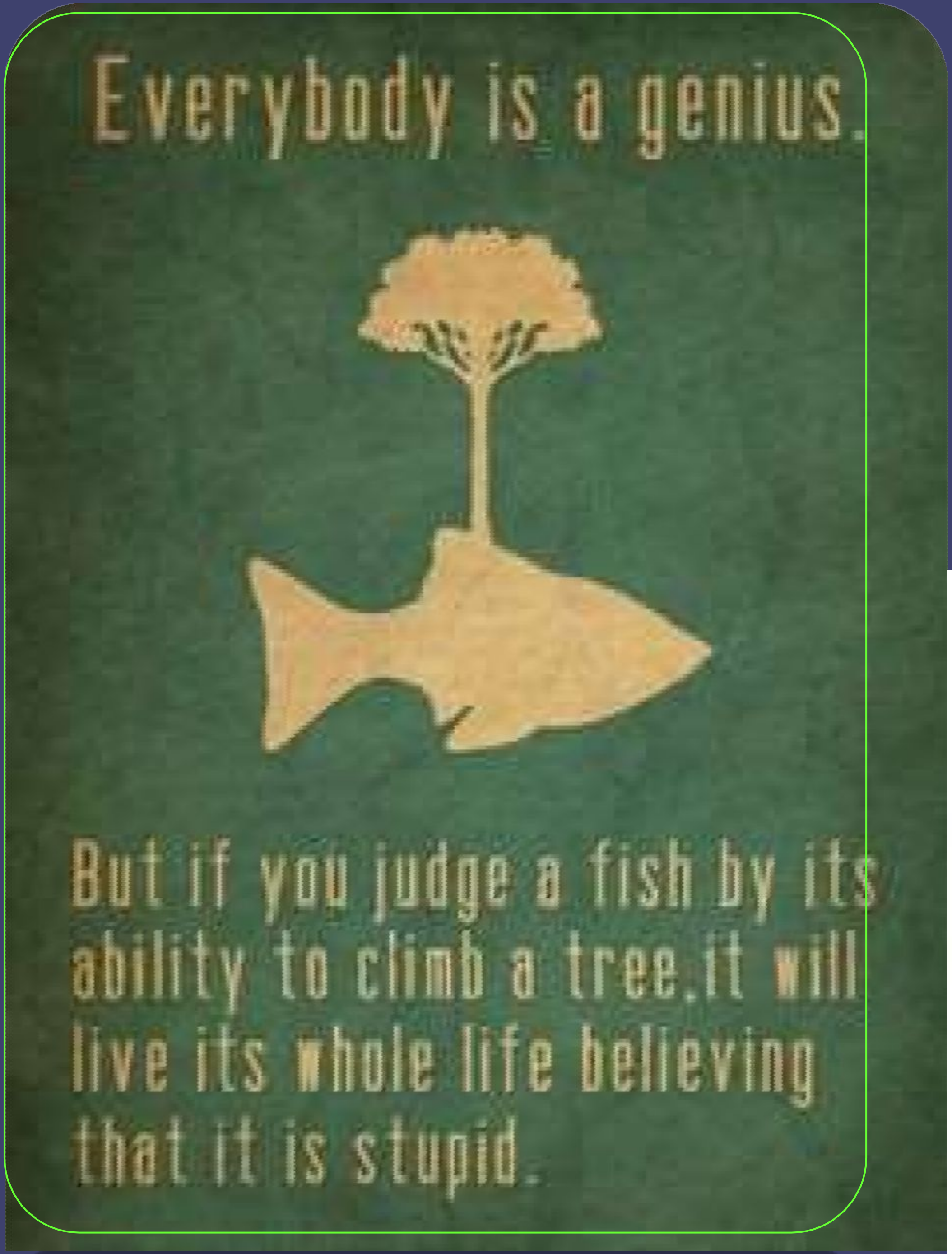
Acetyl CoA is oxidized to **CO_2, H_2O** giving out energy.

***Anabolic role**:

Intermediates of TCA cycle plays a role in synthesis like heme formation, FA synthesis, Cholesterol, Steroid synthesis.

SUMMARY

- Pyruvate is converted to acetyl-CoA by the action of pyruvate dehydrogenase complex, a huge enzyme complex.
- Acetyl-CoA is converted to 2 CO_2 via the eight-step citric acid cycle, generating three NADH, one $FADH_2$, and one ATP (by substrate-level phophorylation).


Everybody is a genius.
But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid.

THANK YOU