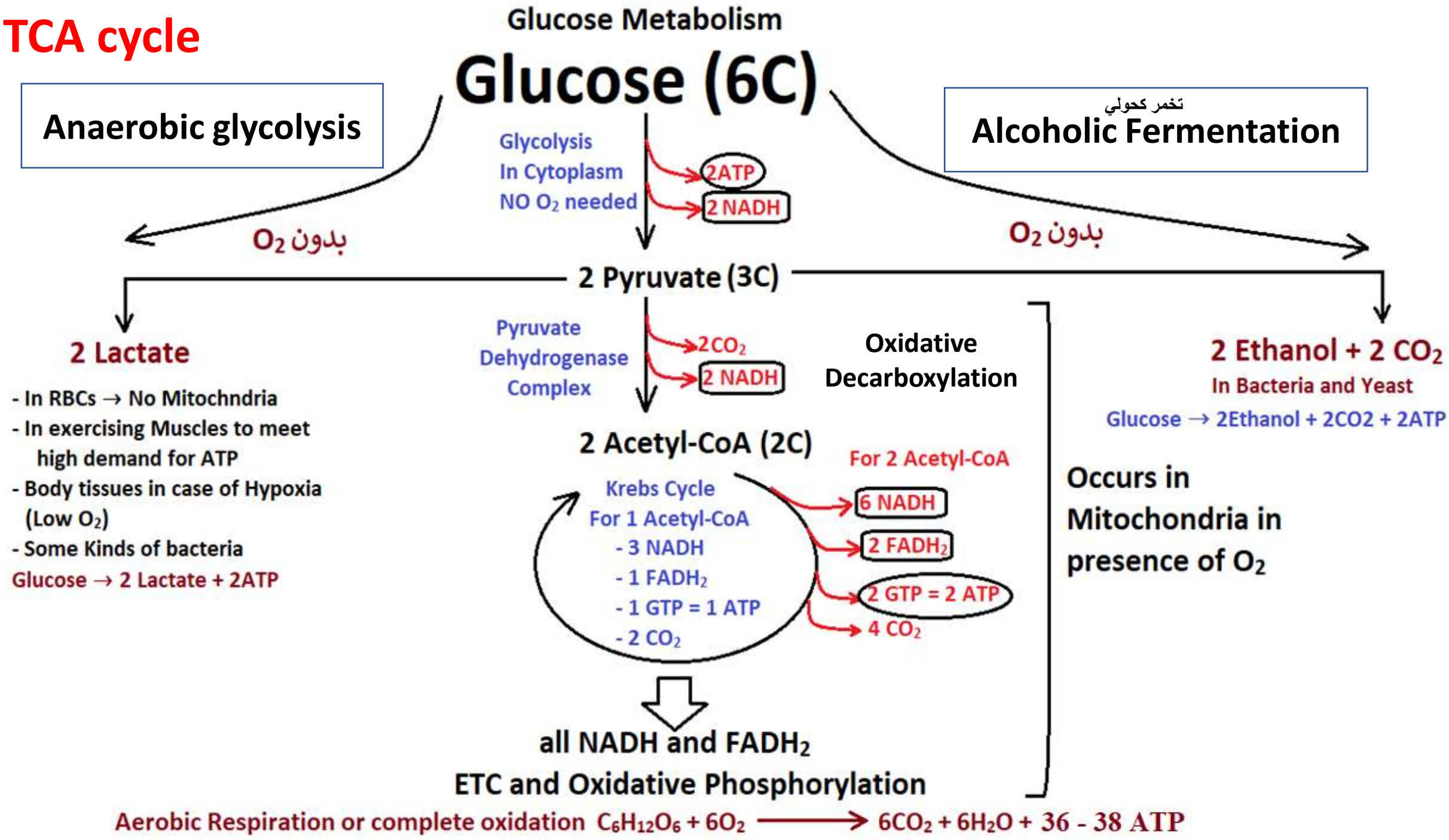
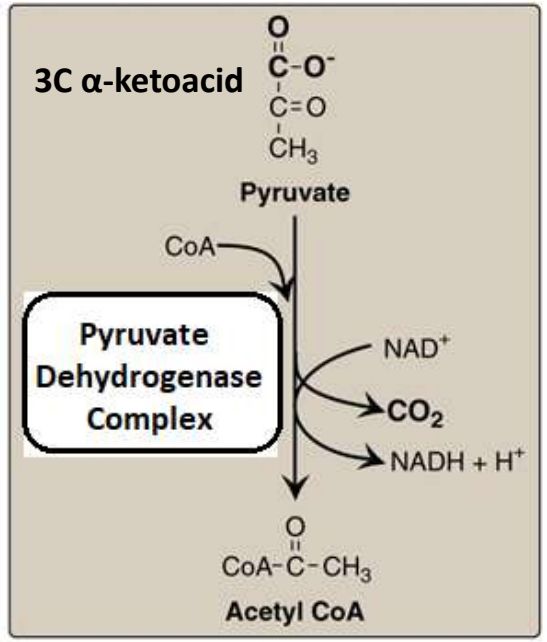
TCA cycle


Glucose Metabolism
Glucose (6C)
Anaerobic glycolysis
Alcoholic Fermentation
تخمر كحولي
Glycolysis
In Cytoplasm
NO O2 needed
2ATP
2 NADH
بدون O2
بدون O2
2 Pyruvate (3C)
2 Lactate
- In RBCs → No Mitochndria
- In exercising Muscles to meet high demand for ATP
- Body tissues in case of Hypoxia (Low O2)
- Some Kinds of bacteria
Glucose → 2 Lactate + 2ATP
Pyruvate Dehydrogenase Complex
2CO2
2 NADH
Oxidative Decarboxylation
2 Acetyl-CoA (2C)
Krebs Cycle
For 1 Acetyl-CoA
- 3 NADH
- 1 FADH2
- 1 GTP = 1 ATP
- 2 CO2
For 2 Acetyl-CoA
6 NADH
2 FADH2
2 GTP = 2 ATP
4 CO2
all NADH and FADH2
ETC and Oxidative Phosphorylation
2 Ethanol + 2 CO2
In Bacteria and Yeast
Glucose → 2Ethanol + 2CO2 + 2ATP
Occurs in Mitochondria in presence of O2
Aerobic Respiration or complete oxidation C6H12O6 + 6O2 → 6CO2 + 6H2O + 36 - 38 ATP

First Pyruvate is transported from the cytosol to mitochondrial Matrix via the **pyruvate mitochondrial carrier in the inner mitochondrial membrane**



Then, Conversion of Pyruvate to Acetyl-CoA

In this step:

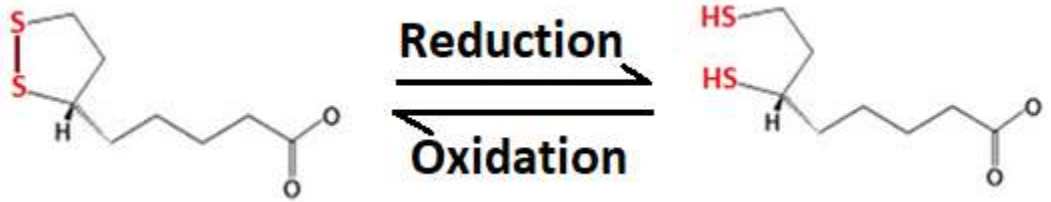
1. Decarboxylation of Pyruvate forming 2C compound called Hydroxyethyl
2. Oxidation of Hydroxyethyl forming Acetate (Acetyl) (NAD^+ reduced to NADH)
3. Binding of Acetyl with CoA by ***thioester bond*** "high energy bond"

The Enzyme that catalyze this step called **Pyruvate Dehydrogenase Complex (PDH)**

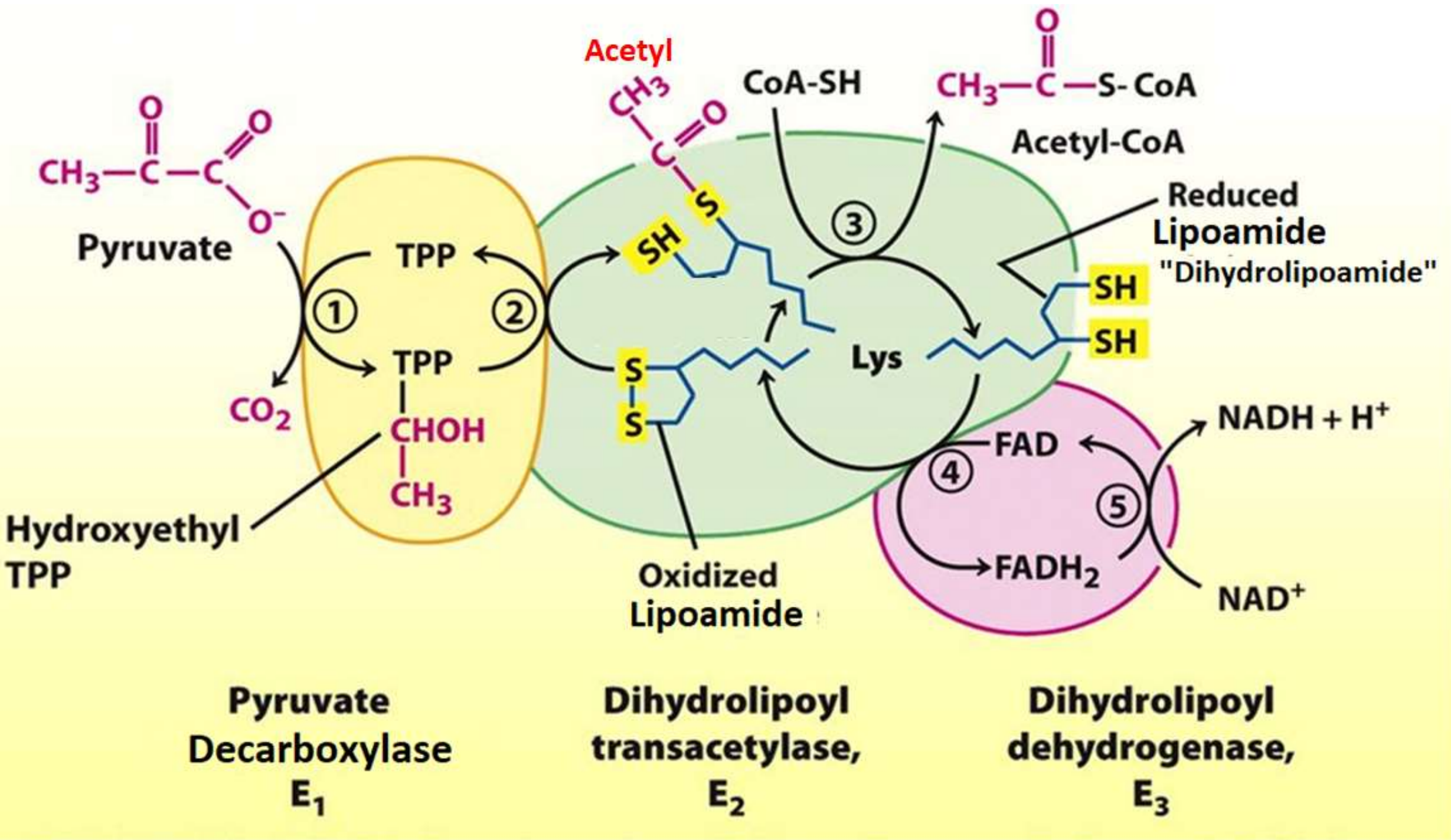
This Complex consist of 3 Enzymes

1. ***E1 "Pyruvate Decarboxylase"*** → require Thiamine Pyrophosphate "TPP" (Vitamin B1) as Prosthetic group

2. ***E2 "Dihydrolipoyl-Transacetylase"*** → requires Lipoic acid as prosthetic group and CoASH as Cosubstrate (Lipoic acid is bound covalently by amide bond to Lysine in the active site forming lipoamide or Lipolysine)

Oxidized form  **Reduced form "Dihydrolipoamid"**

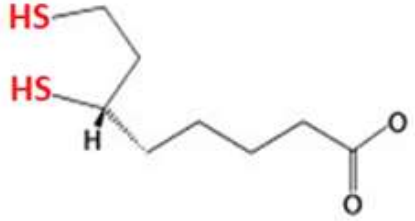
3. ***E3 "Dihydrolipoyl-Dehydrogenase"*** → requires FAD as prosthetic group and NAD^+ as Cosubstrate


Acetyl
CoA-SH
Acetyl-CoA
Reduced
Lipoamide
"Dihydrolipoamide"
Pyruvate
TPP
TPP
CHOH
CO_2
Hydroxyethyl
TPP
SH
S
S
Lys
SH
SH
FAD
$FADH_2$
$NADH + H^+$
NAD^+
Oxidized
Lipoamide
Pyruvate
Decarboxylase
E_1
Dihydrolipoyl
transacetylase,
E_2
Dihydrolipoyl
dehydrogenase,
E_3

In Words:

1. CO_2 is removed from pyruvate by **E1** which require TPP forming HydroxyEthyl-TPP
2. HydroxyEthyl (aldehyde) is oxidized to acetyl (carboxyl) and liopic acid is reduced and bind to the Acetyl by thioester bond
3. CoASH bind to the acetyl forming acetyl-CoA

By the end of step 3 lipoic acid is reduced and it must be regenerated to it's oxidized form in order for this complex to work again



(step 2 and 3 by **E2** which need Lipoic acid and CoASH)

4. Oxidation of lipoic acid and reduction of FAD to $FADH_2$
5. $FADH_2$ is reoxidized to FAD when the electrons are transferred to NAD^+ forming NADH

(Step 4 and 5 by **E3** which require FAD and NAD^+)

This complex use TPP (B1), Lipoic acid, CoASH (B5), FAD (B2) NAD^+ (B3)

The coenzymes contained within the complex "prosthetic groups" TPP, Lipoic acid, FAD

- This step is **<u>Irreversible</u>** step in human "all decarboxylation reactions are Irreversible"

Control of Pyruvate Dehydrogenase Complex

Covalent Control:

Pyruvate Dehydrogenase has 2 forms:

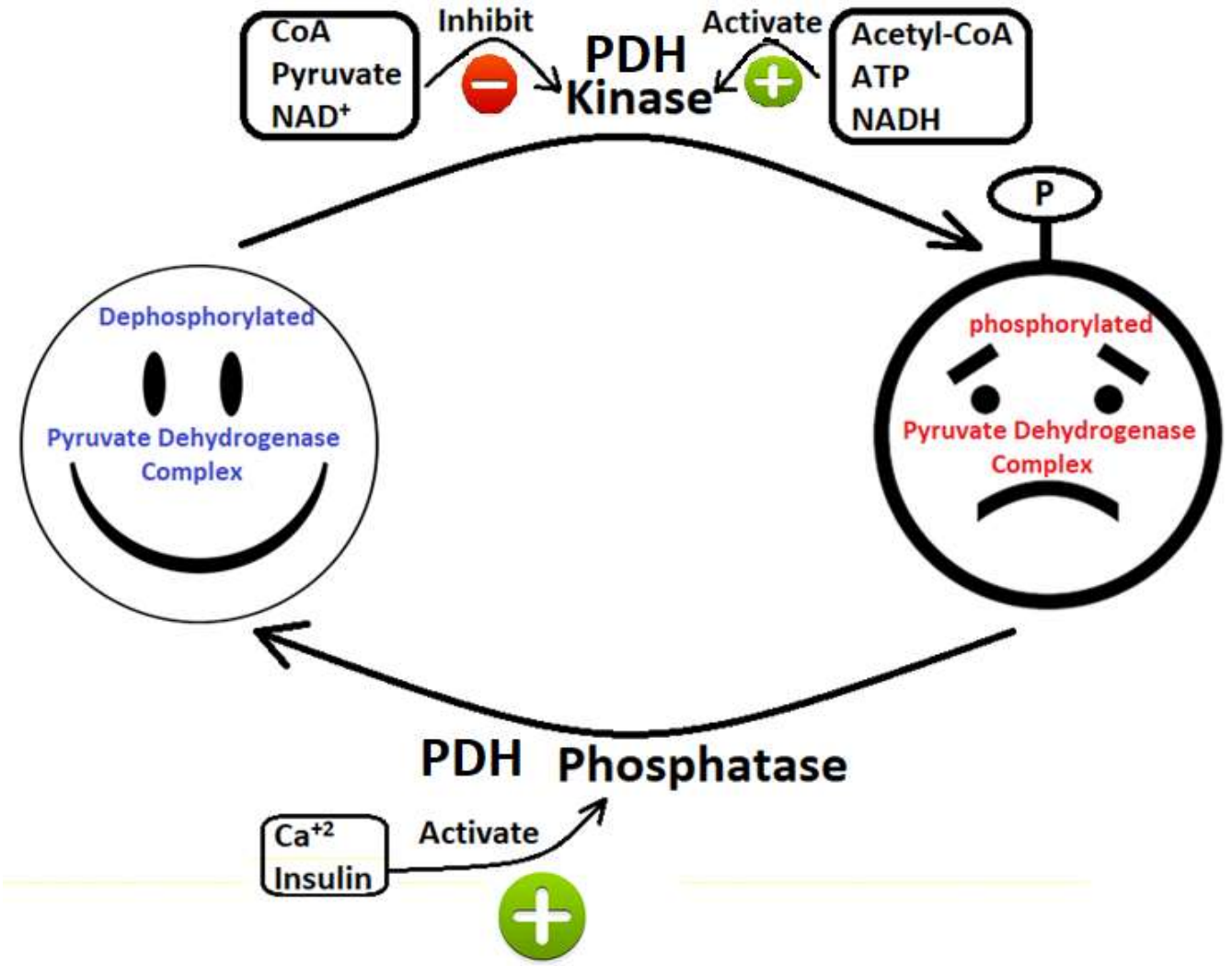
- Dephosphorylated (Active)
- Phosphorylated (Inactive)

- ***Pyruvate Dehydrogenase (PDH) Kinase*** inhibit pyruvate dehydrogenase by adding phosphate group
- ***Pyruvate Dehydrogenase (PDH) Phosphatase*** activate Pyruvate dehydrogenase by removing phosphate

High Acetyl-CoA, ATP, NADH allosterically activate PDH Kinase→ add phosphate to pyruvate dehydrogenase → inhibition

High CoA, Pyruvate, NAD^+ allosterically Inhibit PDH Kinase → No phosphate added → Activation

Ca^{+2} and Insulin activate ***PDH Phosphatase*** which remove phosphate → activation

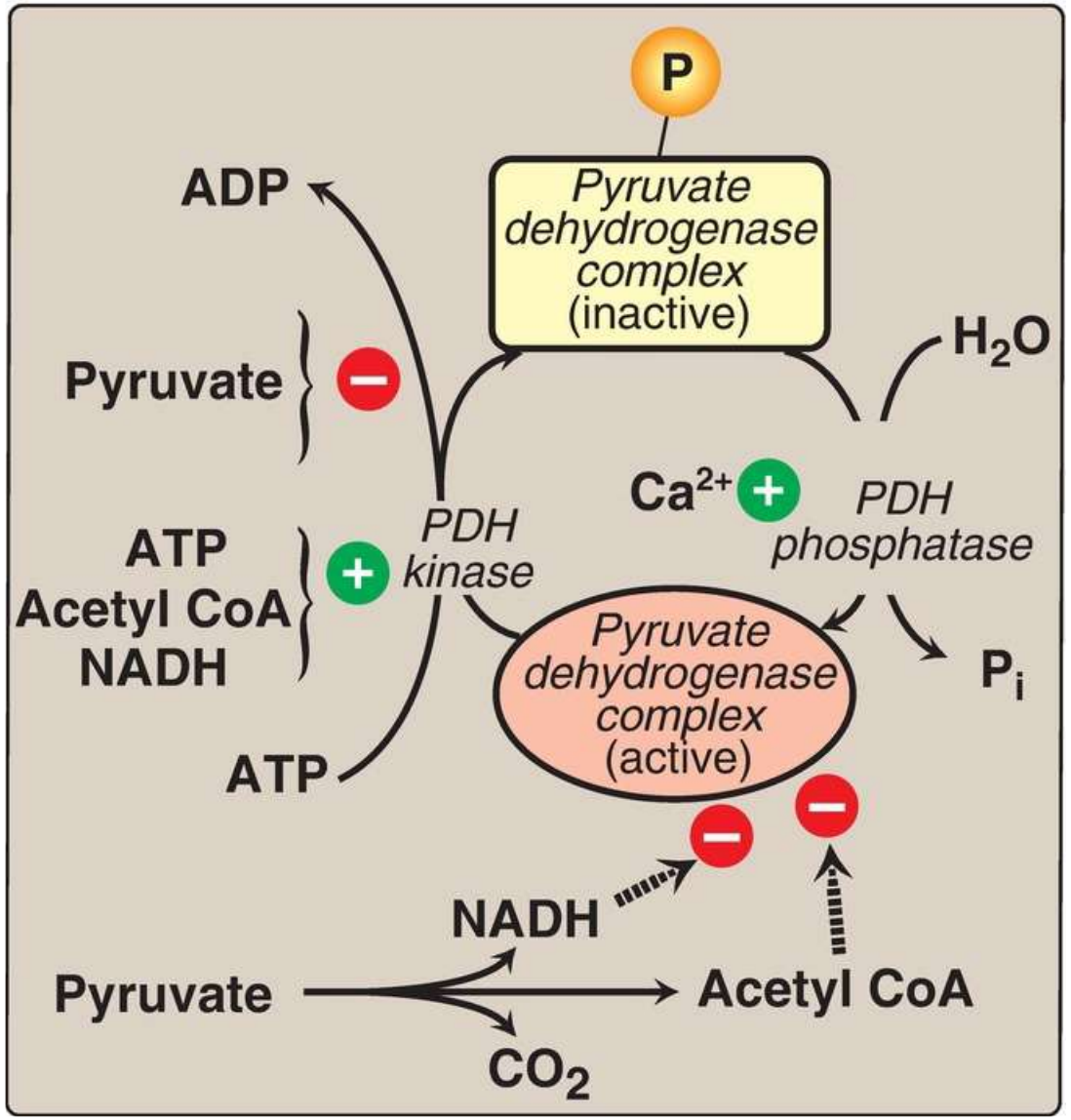


Increase Ca^{+2} indicate active cell → need energy → activate PDH complex by activating phosphatase

- High NADH/NAD+, acetyl CoA/CoA, or ATP/ADP ratio promotes phosphorylation, thus inhibition of Pyruvate dehydrogenase complex

- Acetyl-CoA and NADH “Products” also allosterically Inhibit Pyruvate dehydrogenase complex, so they inhibit the enzyme directly and indirectly by activating protein kinase

لا جديد فقط صورة الكتاب



In your body there are other 2 complexes exactly resemble PDH

α-ketoglutarate Dehydrogenase complex catalyze step 4 in TCA cycle

branched chains α-ketoacid Dehydrogenase complex

both require **TPP, Lipoic acid, CoA, FAD and NAD^+**

Any problem in this step will leads to:

- Low energy production affect mainly the central nervous system
- Accumulation of pyruvate → overproduction of Lactate → Lactic acidosis

Examples

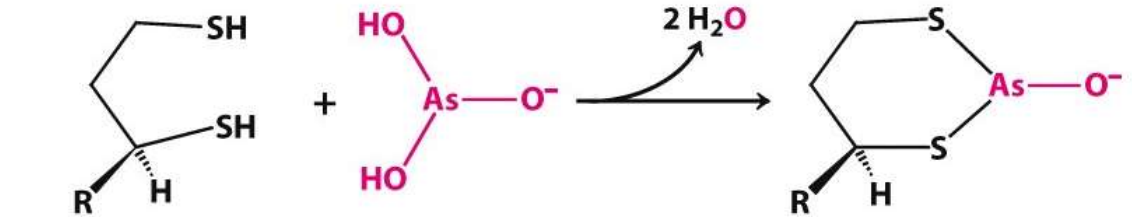
1. **Deficiency of Thiamine (B1)** "Beri-Beri disease and Wernicke-Korsakoff syndrome"

2. **Genetic deficiency of E1 component** of the PDHC, rare but it's the most common genetic deficiency that cause congenital lactic acidosis (X-linked dominant) Central Nervous system is mostly affected

- If E1 deficiency is partial (partial loss of the enzyme activity) the patient may respond to Thiamine supplement

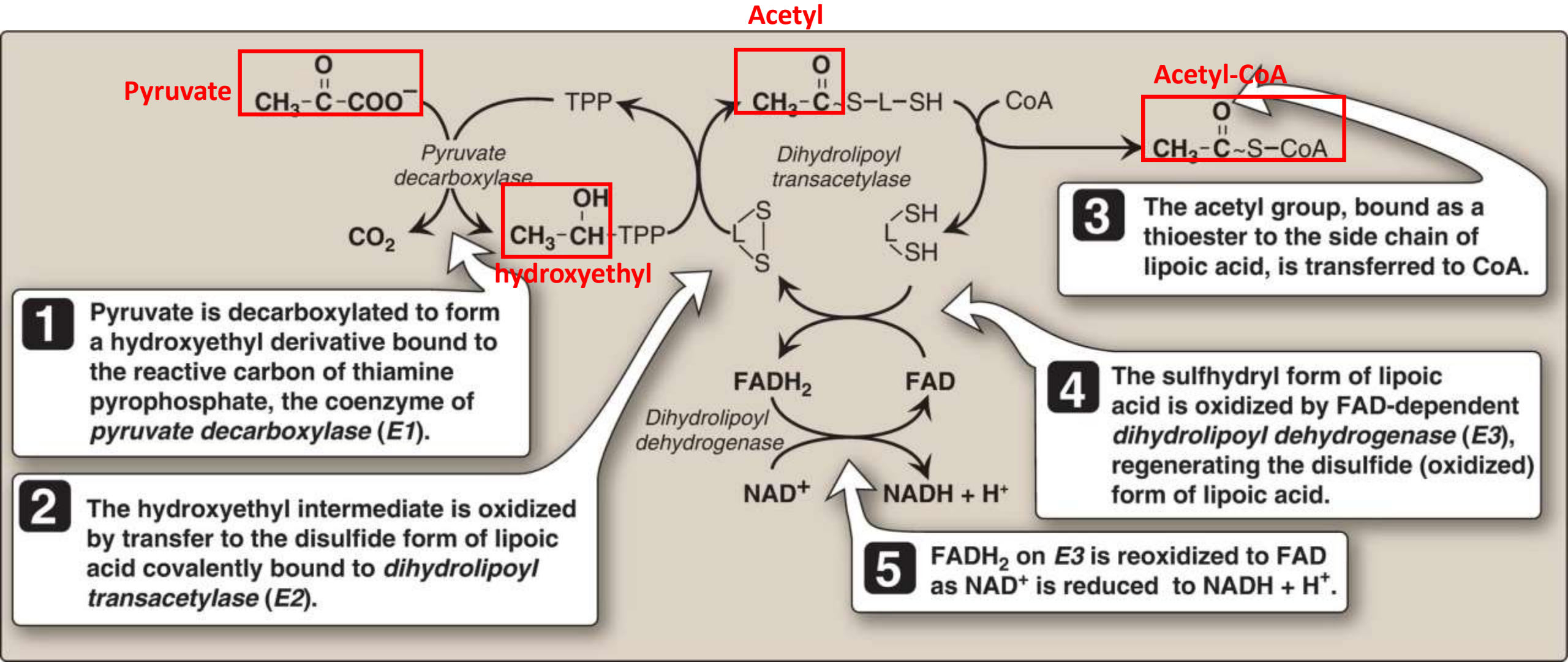
3. Trivalent Arsenic "Arsenite" (AsO_3^-)

- toxic and fatal
- it binds covalently to Lipoic acid, thus inhibiting E2, this will inhibit conversion of Pyruvate to acetyl-CoA and step 4 of TCA cycle
- Central Nervous system is mostly affected
-Low energy production + Lactic acidosis

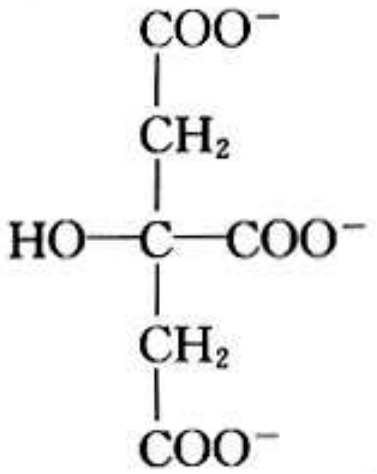


Dihydrolipoamide from pyruvate dehydrogenase Complex **Arsenite** **Arsenite chelate on enzyme**

لا جديد فقط صورة الكتاب لعملية تحويل ال Pyruvate الى Acetyl-CoA راجع الخطوات



Krebs Cycle , Citric acid Cycle, TCA Cycle (Tri-Carboxylic Acid cycle)



- 8 steps Cyclic Pathway occurs in the ***mitochondrial Matrix***
- Scientist : Hans Krebs
- First Compound: Citric Acid (Citrate)
- Citric acid → contain 3 carboxyls (Tri-Carboxylic Acid)

- **Krebs cycle is the Third stage of energy production**

. 1. Ingestion and Digestion → 2. Production of Acetyl CoA → 3. Krebs Cycle and Oxidative phosphorylation

- **Aim** of Krebs Cycle : Extract electrons (Oxidation) from Acetyl-CoA and give them to NAD^+ and FAD producing NADH and $FADH_2$ "Reduced Dinucleotides (Coenzymes)"

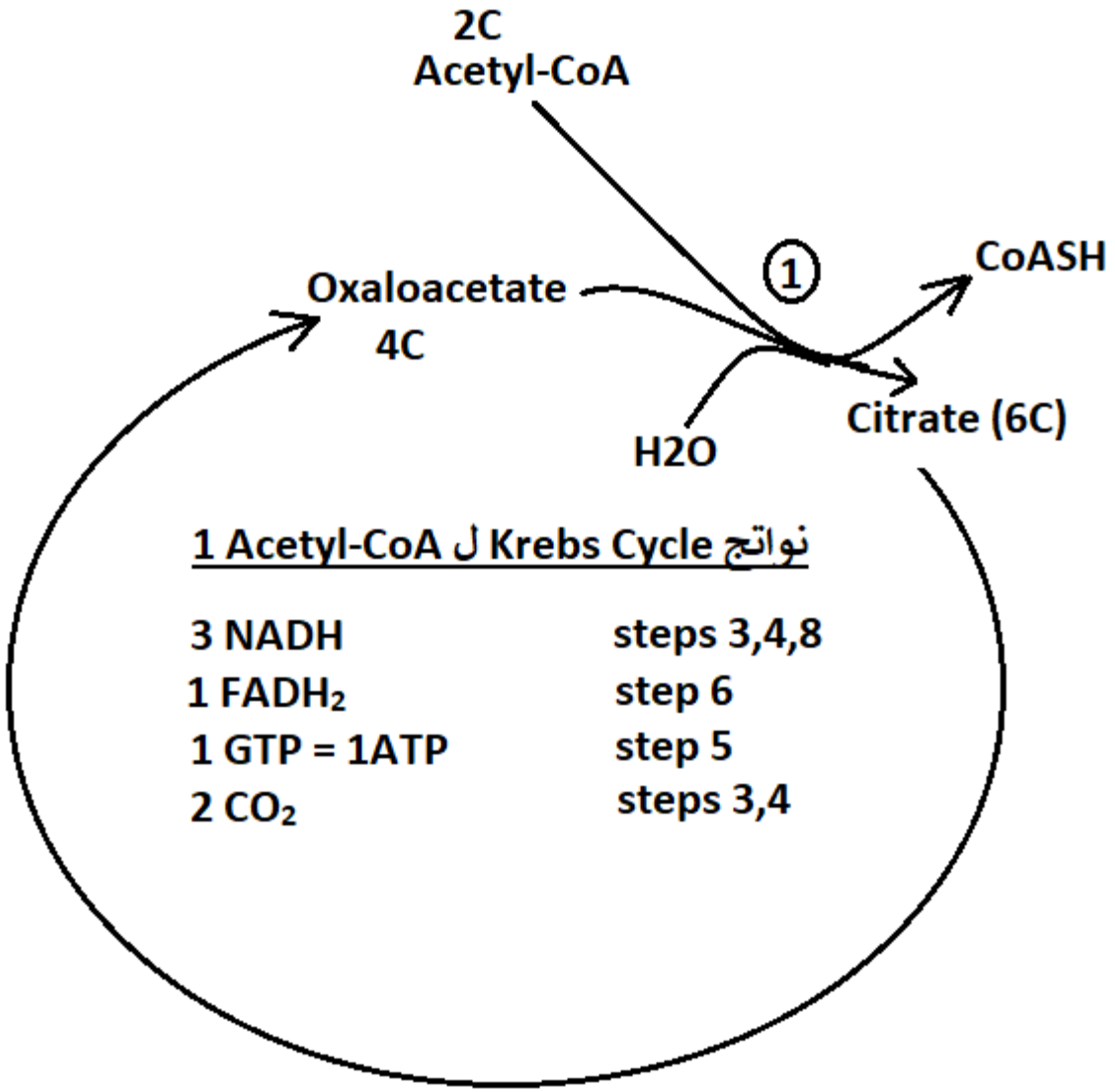
- Sources of Acetyl-CoA that enter Krebs Cycle
- Carbohydrate
- Fatty acids
- Amino-acids "Ketogenic amino acids"

TCA cycle final pathway of the oxidative catabolism of carbohydrates, amino acids, and fatty acids, where their carbon skeletons being converted to carbon dioxide (CO_2)

Overall

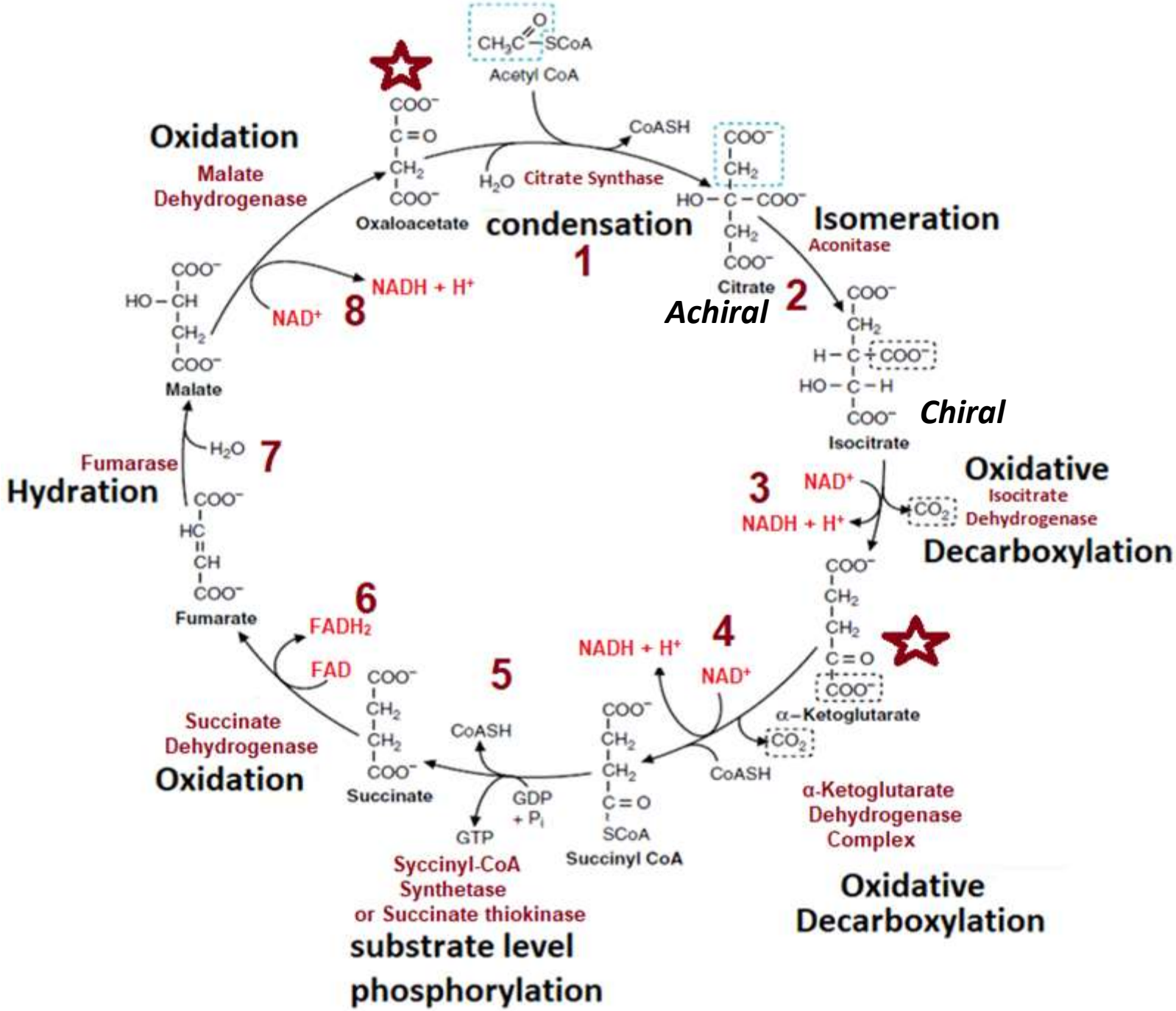
- **4 oxidation steps (3,4,6,8)**
- **Step 5 produce GTP by substrate level phosphorylation**
- **Steps 3,4 remove CO_2 (Decarboxylation)**

So, steps 3,4 called <u>Oxidative Decarboxylation</u>



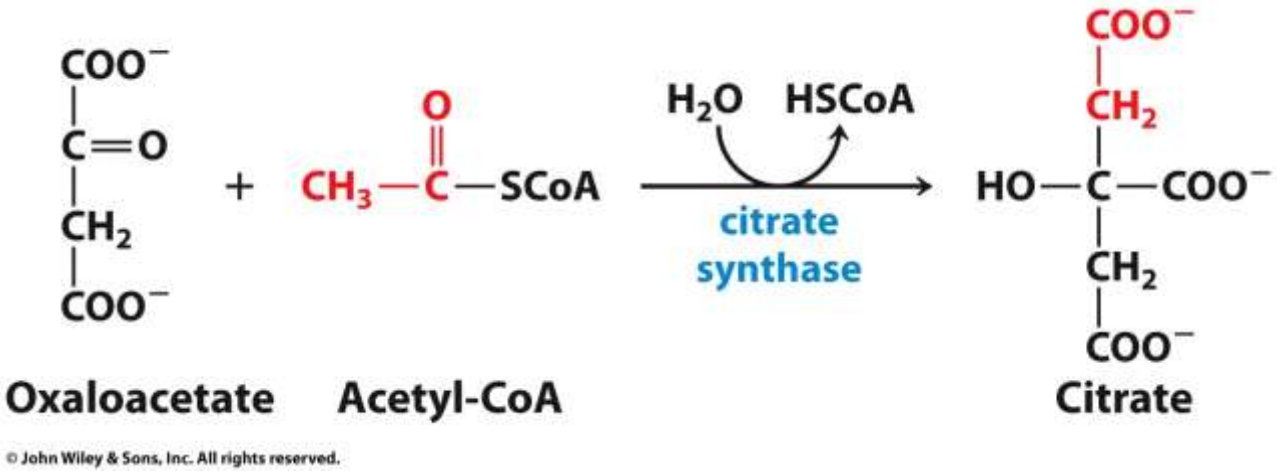
- All Enzymes of Krebs Cycle found in Mitochondrial Matrix **Except** the Enzyme that catalyze Step 6 (found in the inner mitochondrial membrane)

- **steps 1, 3, 4 have highly negative ΔG (Irreversible, Committed, Control steps)**
- **Step 3 is the slowest Step (most important, Rate-limiting step)**


Acetyl CoA
CoASH
Oxaloacetate
Citrate Synthase
condensation
1
Citrate
Isomeration
Aconitase
Achiral
2
Isocitrate
Chiral
Oxidation
Malate
Dehydrogenase
NADH + H+
NAD+
8
Malate
Fumarase
Hydration
7
Fumarate
3
NAD+
NADH + H+
Oxidative
Isocitrate
Dehydrogenase
Decarboxylation
6
FADH2
FAD
Succinate
Dehydrogenase
Oxidation
5
CoASH
GDP
+ Pi
GTP
Succinate
Syccinyl-CoA
Synthetase
or Succinate thiokinase
substrate level
phosphorylation
Succinyl CoA
4
NADH + H+
NAD+
CoASH
α–Ketoglutarate
α-Ketoglutarate
Dehydrogenase
Complex
Oxidative
Decarboxylation

Steps in Details

Step 1 "Condensation"

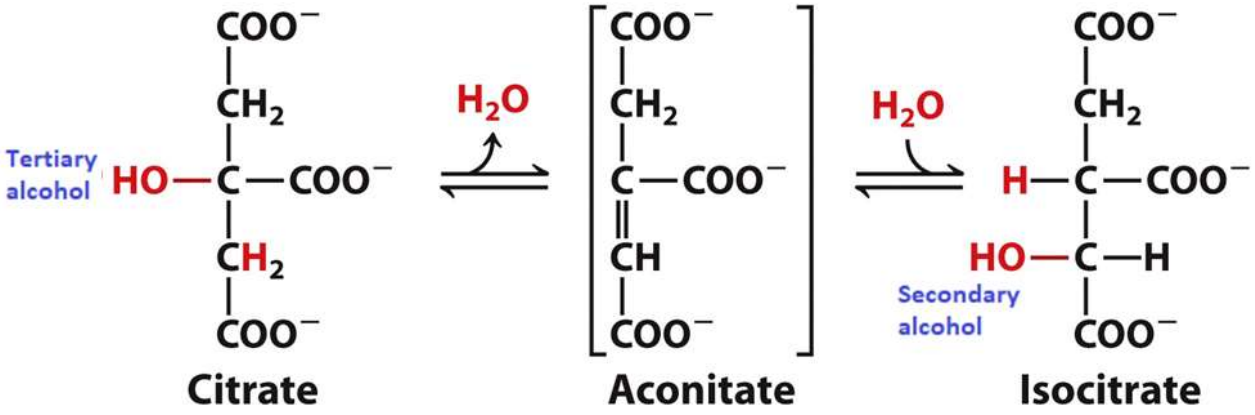


Condensation of Acetate to Oxaloacetate require energy; this energy comes from breaking CoA (thioester bond) making this step highly Exergonic and Irreversible

Citrate synthase Activated by ADP, inhibited by ATP, NADH, Citrate and succinyl-CoA

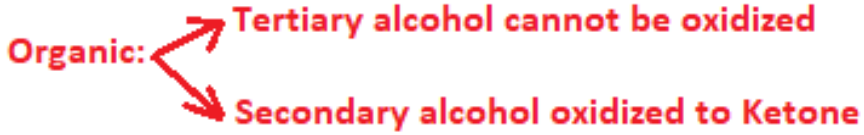
Step 2 "Isomeration"

By Aconitase/ Aconitate Hydratase



There is Intermediate in this step called ***Aconitate***

The aim of this step is to convert 3° alcohol of Citrate to 2° alcohol in Isocitrate in order to be oxidized

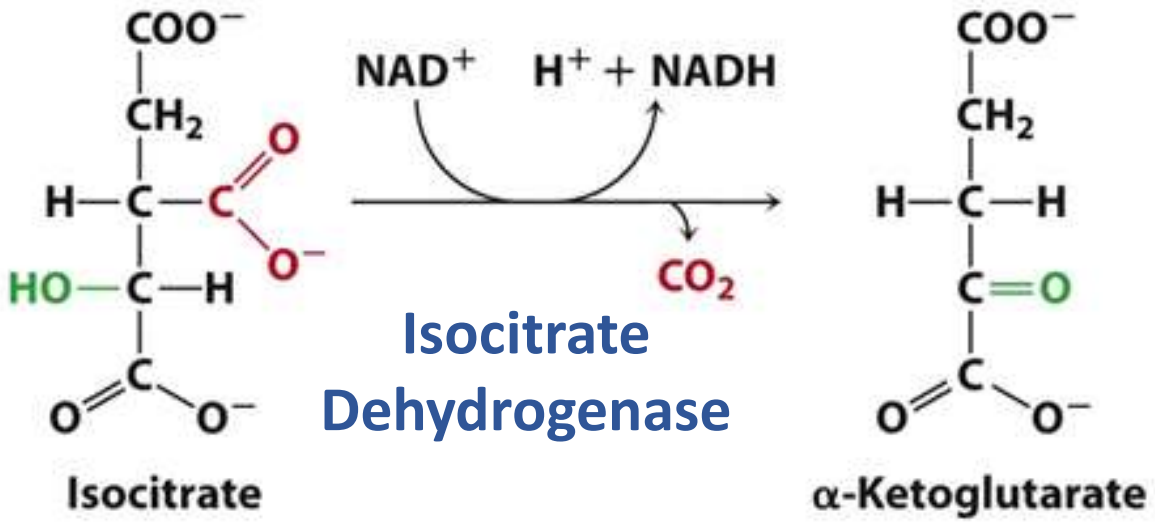


Aconitase an iron-sulfur protein

Aconitase is inhibited by Fluroacetate

plant toxin that is used as a pesticide. Fluoroacetate is converted to fluoroacetyl CoA that condenses with OAA to form ***fluorocitrate***, a potent inhibitor of aconitase

Step 3 "Oxidative Decarboxylation"

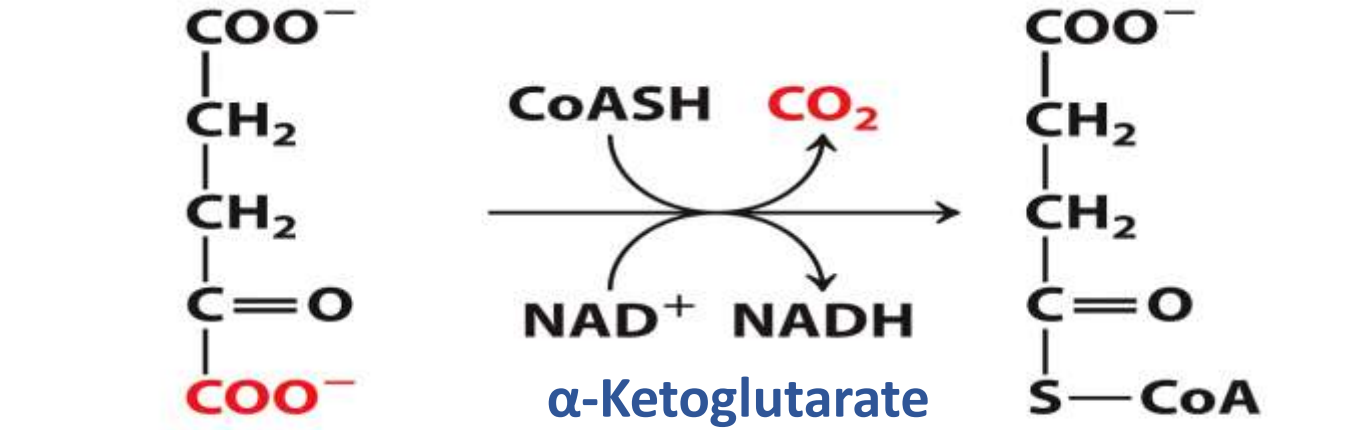


The Most important Step, Slowest Step, Rate-limiting Step

Irreversible

Allosterically activated by ADP and Ca^{+2} and inhibited by ATP and NADH.

Step 4 "Oxidative Decarboxylation"



Decarboxylation + Oxidation + binding of CoA

Irreversible

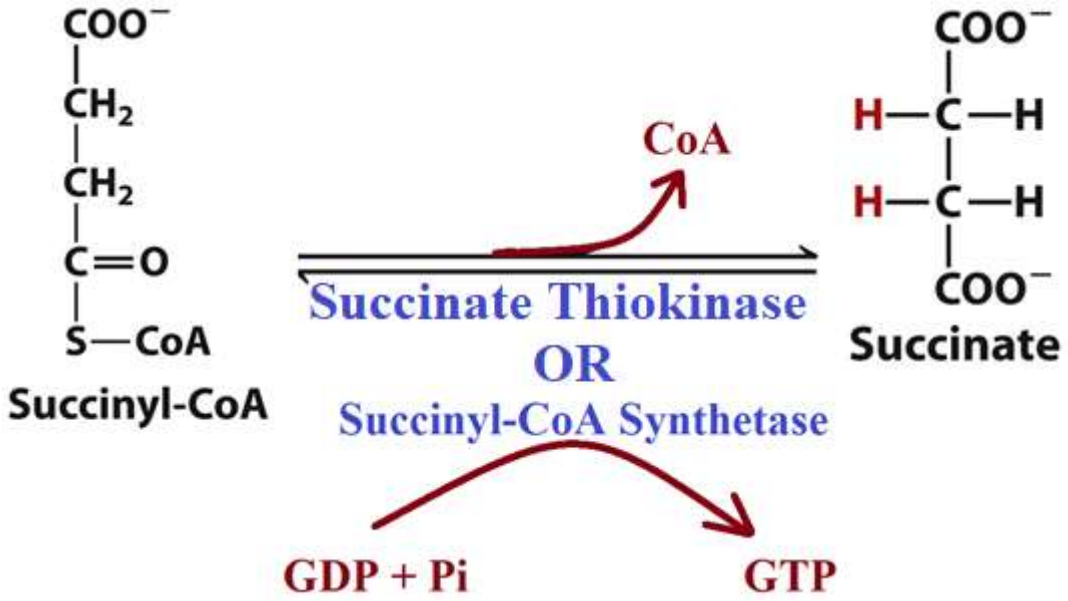
The complex here exactly similar to pyruvate dehydrogenase complex requires

TPP, Lipoic acid, CoA, FAD and NAD^+

Affected by Arsenite

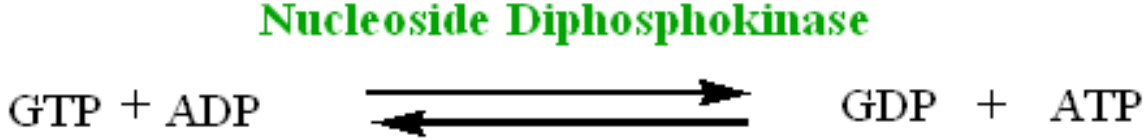
inhibited by its product ATP, GTP, NADH and Succinyl Co-A and activated by Ca^{+2}.

Step 5 “Substrate Level Phosphorylation” produce GTP

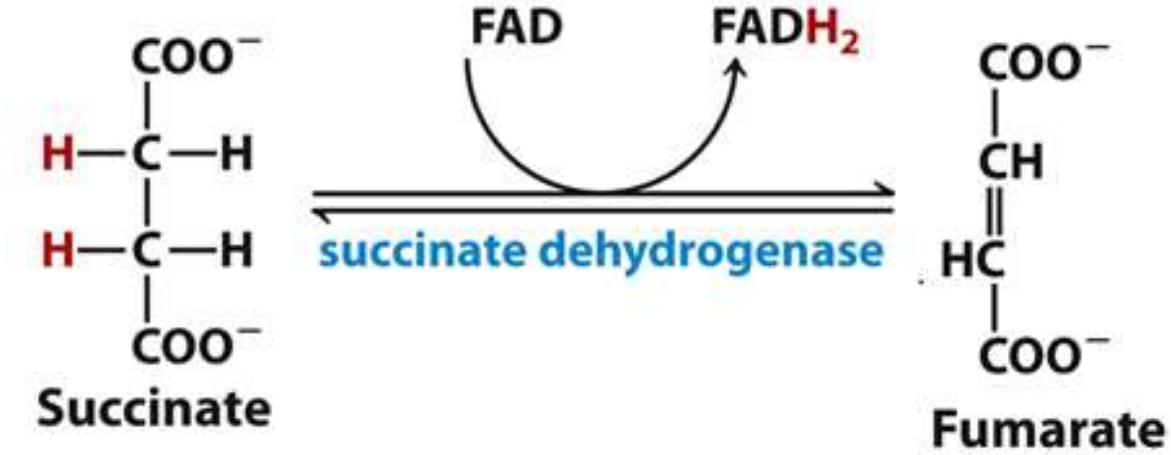


Breaking the thioester bond of CoA release Energy
This energy used to synthesize GTP

GTP and ATP are interconvertible by the nucleoside diphosphate kinase



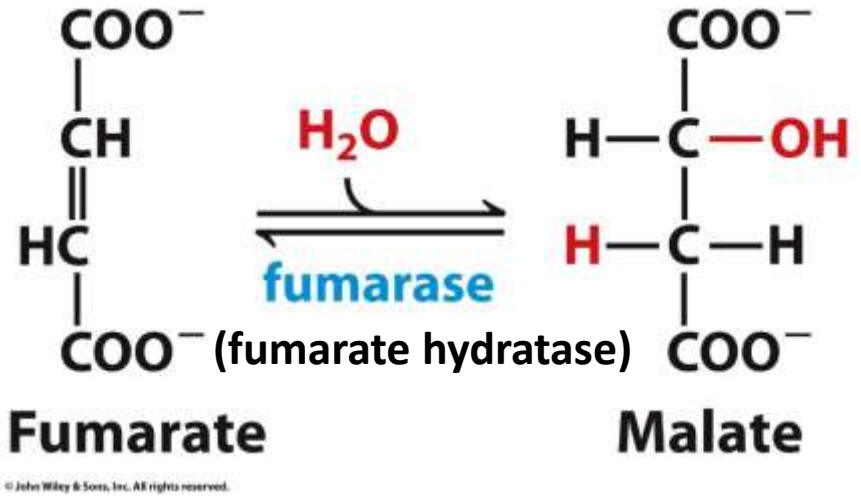
Step 6 “Oxidation”



The enzyme that catalyze this step
(Succinate Dehydrogenase)
Embedded in the inner Mitochondrial Enzyme
Its Complex II of ETC

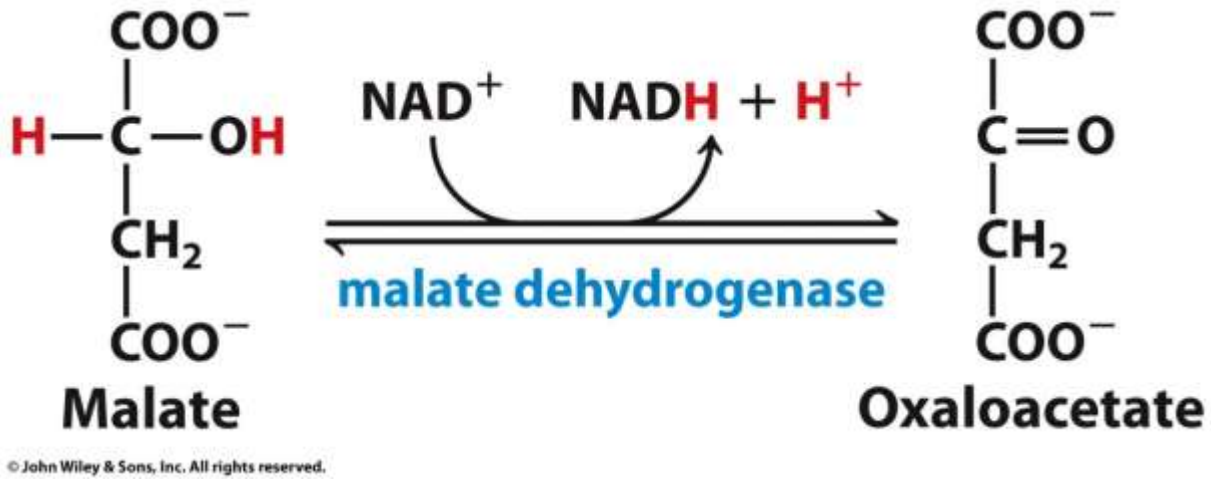
Malonate is a competitive inhibitor

Step 7 “Hydration” اضافة ماء



TCA Cycle is an aerobic pathway it’s part of the Aerobic Respiration (uses oxygen “O_2”) as final electron acceptor from NADH and $FADH_2$

Step 8 “Oxidation”



ENERGY PRODUCED BY THE CYCLE

Four pairs of electrons (8 electrons) are transferred during one turn of the TCA cycle: three pairs reducing $3NAD^+$ to 3NADH and one pair reducing FAD to $FADH_2$.

Oxidation of one NADH by the ETC leads to formation of 3ATP.

Oxidation of $FADH_2$ by the ETC leads to formation of 2ATP

ATP produced by complete oxidation of 1 Acetyl-CoA

Energy-producing reaction	Number of ATP produced
$3\ NADH \longrightarrow 3\ NAD^+$	9
$FADH_2 \longrightarrow FAD$	2
$GDP + P_i \longrightarrow GTP$	1
	12 ATP/acetyl CoA oxidized

Complete oxidation of 1 Pyruvate yields:

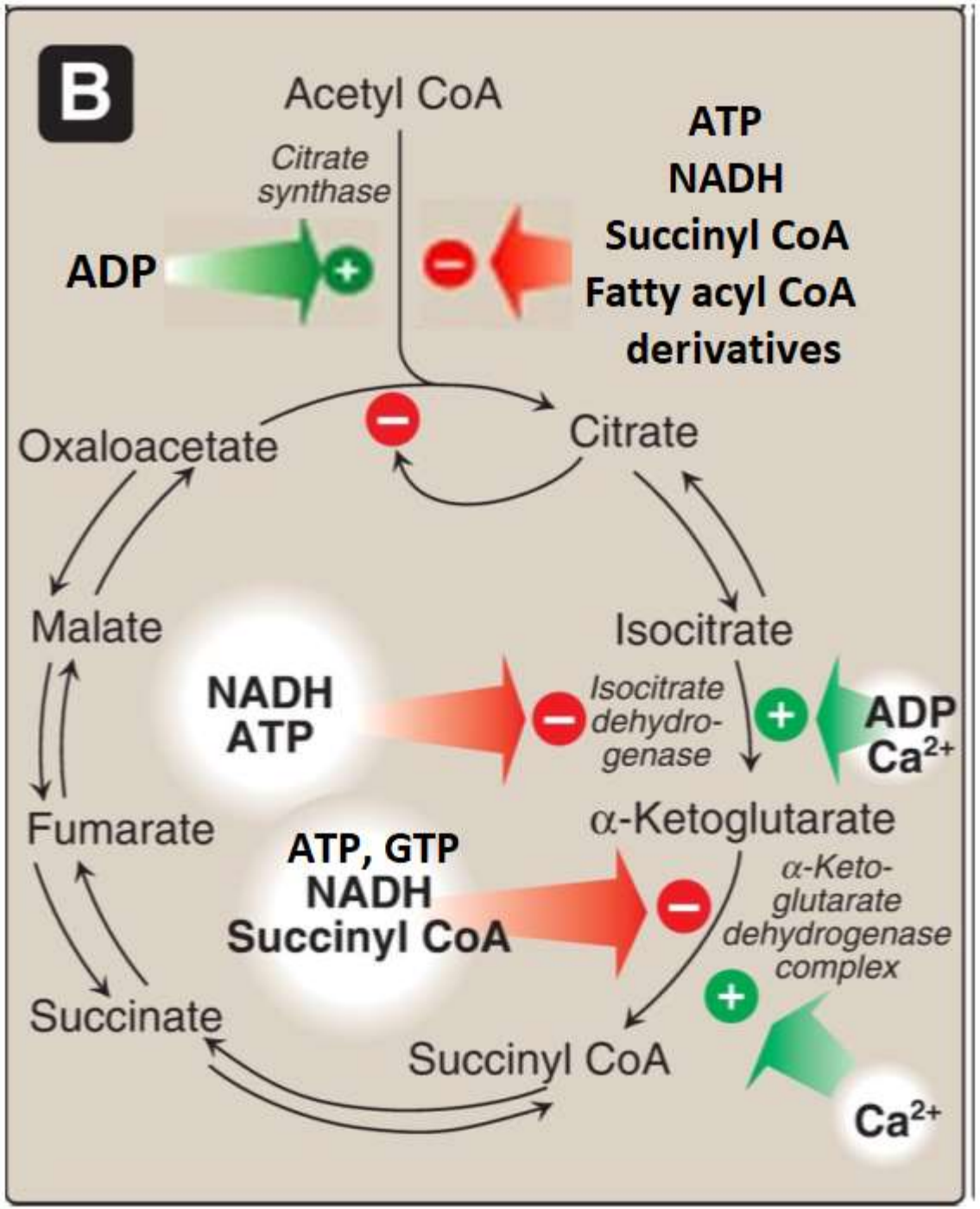
4 NADH (1NADH by PDH and 3 in TCA cycle) x 3ATP = 12ATP

$1FADH_2$ x 2ATP = 2ATP

1GTP = 1ATP

Total = 12 + 2 + 1 = 15 ATP

Summary of TCA cycle Control



- Intermediate of Krebs cycle used as precursors for synthesis of many compounds

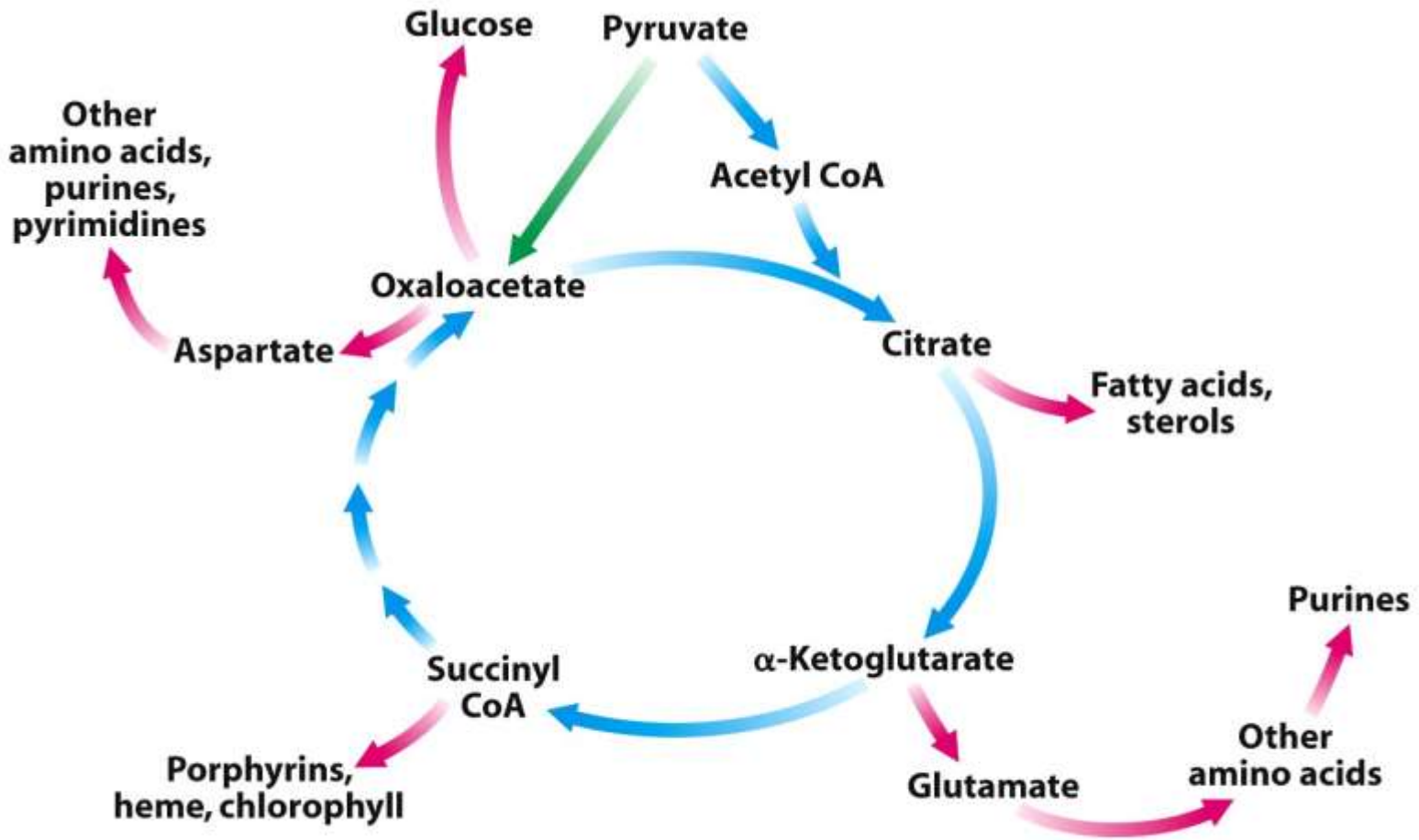


Figure 17.20
Biochemistry, Seventh Edition

TCA cycle is **Amphibolic,** means it's important for both Catabolism and Anabolism

Q: Can you synthesize Glucose from Citrate or other TCA cycle intermediates?

The cycle plays a very important role in synthesis of many compounds, so it is both catabolic and anabolic (**amphibolic**). The most important anabolic functions of the cycle are as follows:

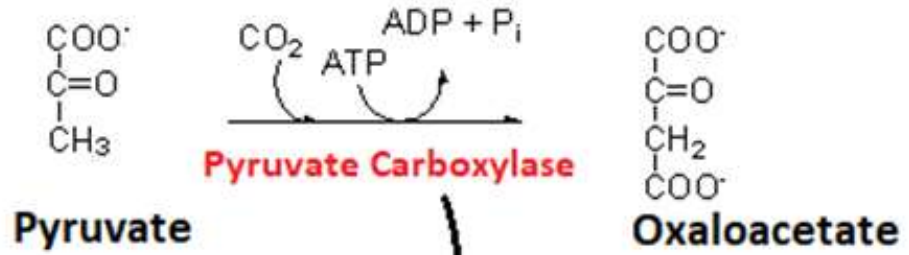
1- Citrate: Citrates formed in the mitochondria go to the cytoplasm (citrate shuttle). In the cytosol it gives oxaloacetate and acetyl- CoA by ATP-citrate lyase. Acetyl- CoA in the cytosol is used for synthesis of fatty acids and cholesterol.
2- α- ketoglutarate: By transamination, it is converted to glutamate, which has many important functions.
3- Succinyl- CoA: It is used for heme synthesis, oxidation of ketone bodies (ketolysis) and detoxification.
4- Oxaloacetate: By transamination, it is converted to aspartate. Oxaloacetate in the cytosol is converted to phosphoenol pyruvate (PEP), which is converted to glucose (gluconeogenesis).
5- Malate: It gives pyruvate by malic enzyme in the cytosol.

Anaplerotic reactions:

Reactions that fill up (Replenish) تعوض Krebs cycle intermediates when they become low

The reverse of the previous slide

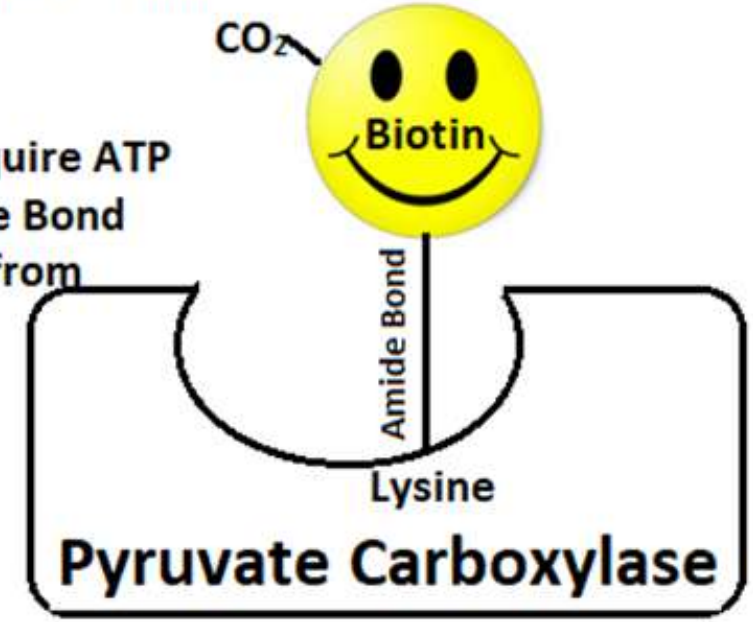
The most important one



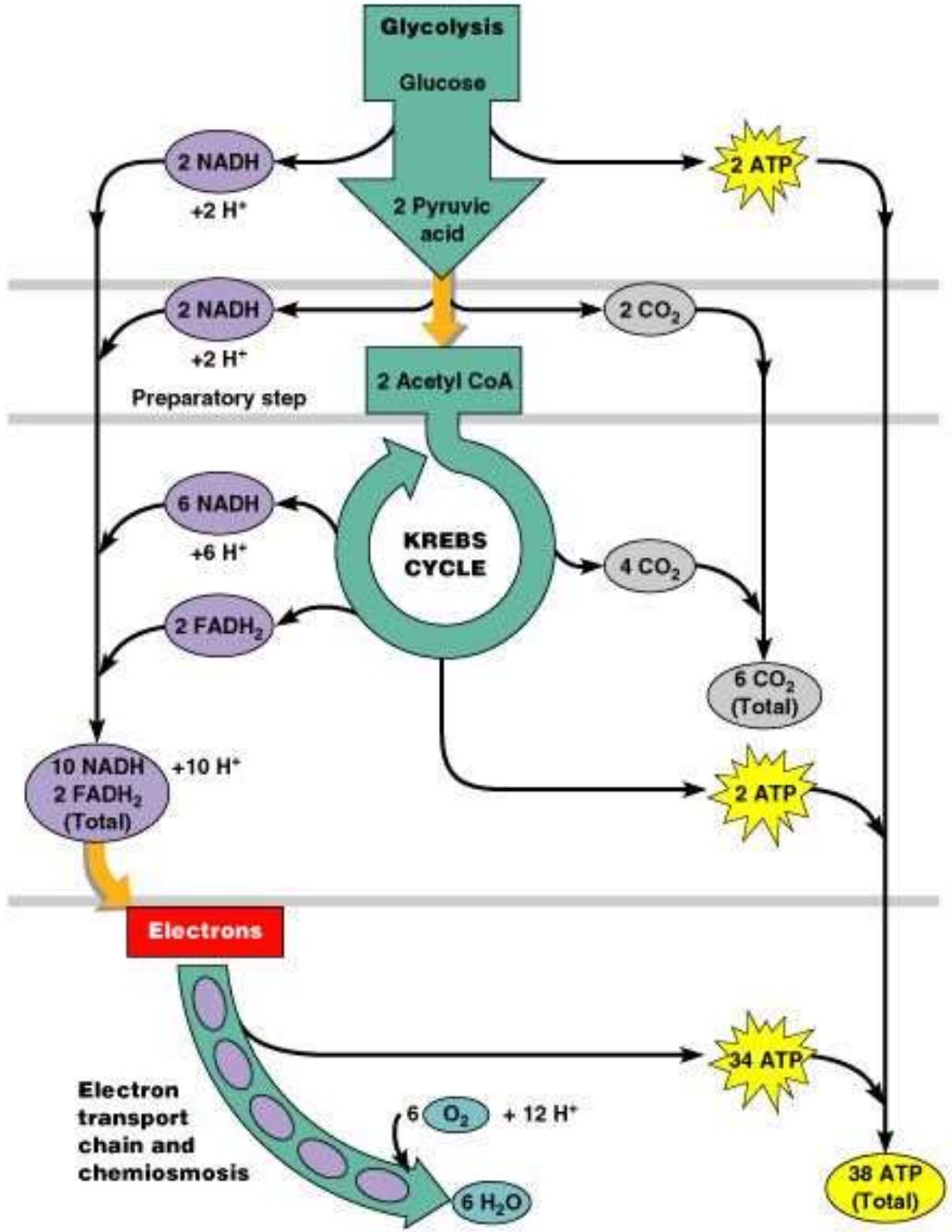
(B7)

Requir biotin as Coenzyme

biotin: CO_2 Carrier



- Addition of CO_2 to Biotin Require ATP
- CO_2 bound to biotin by Amide Bond
- CO_2 bound to biotin derived from Bicarbonate (HCO_3^-)



حاول تتذكر وين مروا معك بالمادة

Pyruvate Kinase

Pyruvate Decarboxylate

Pyruvate Dehydrogenase

Pyruvate Carboxylase