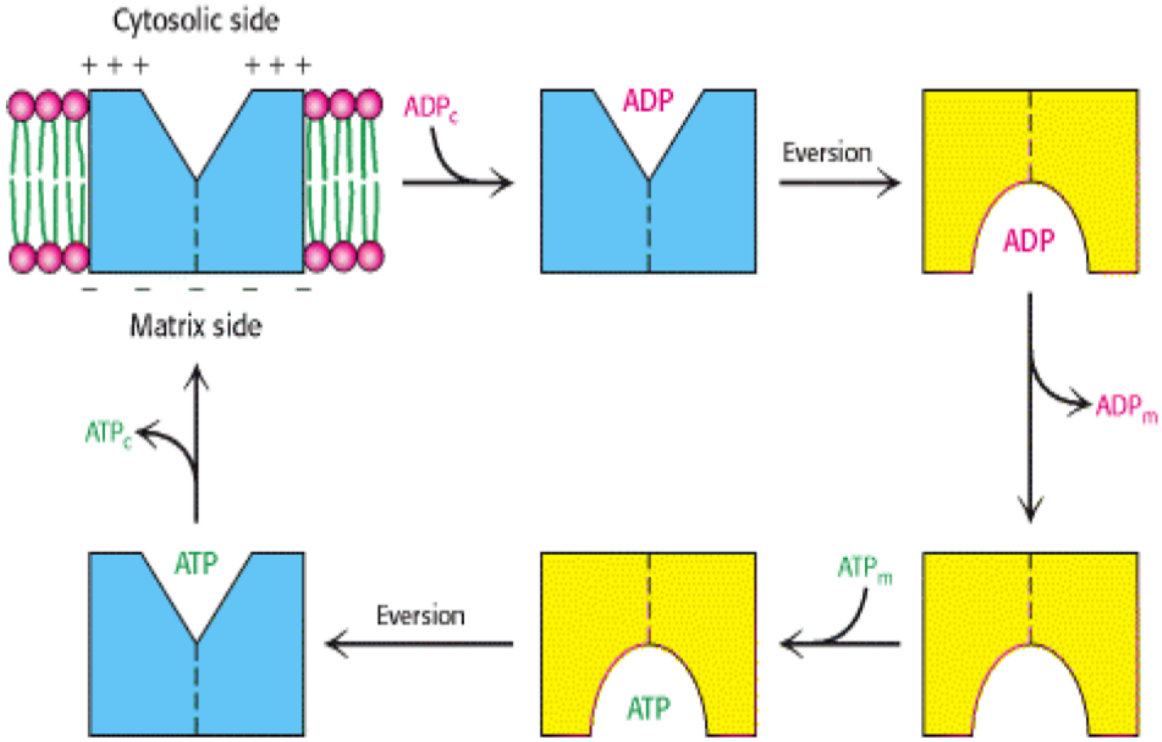
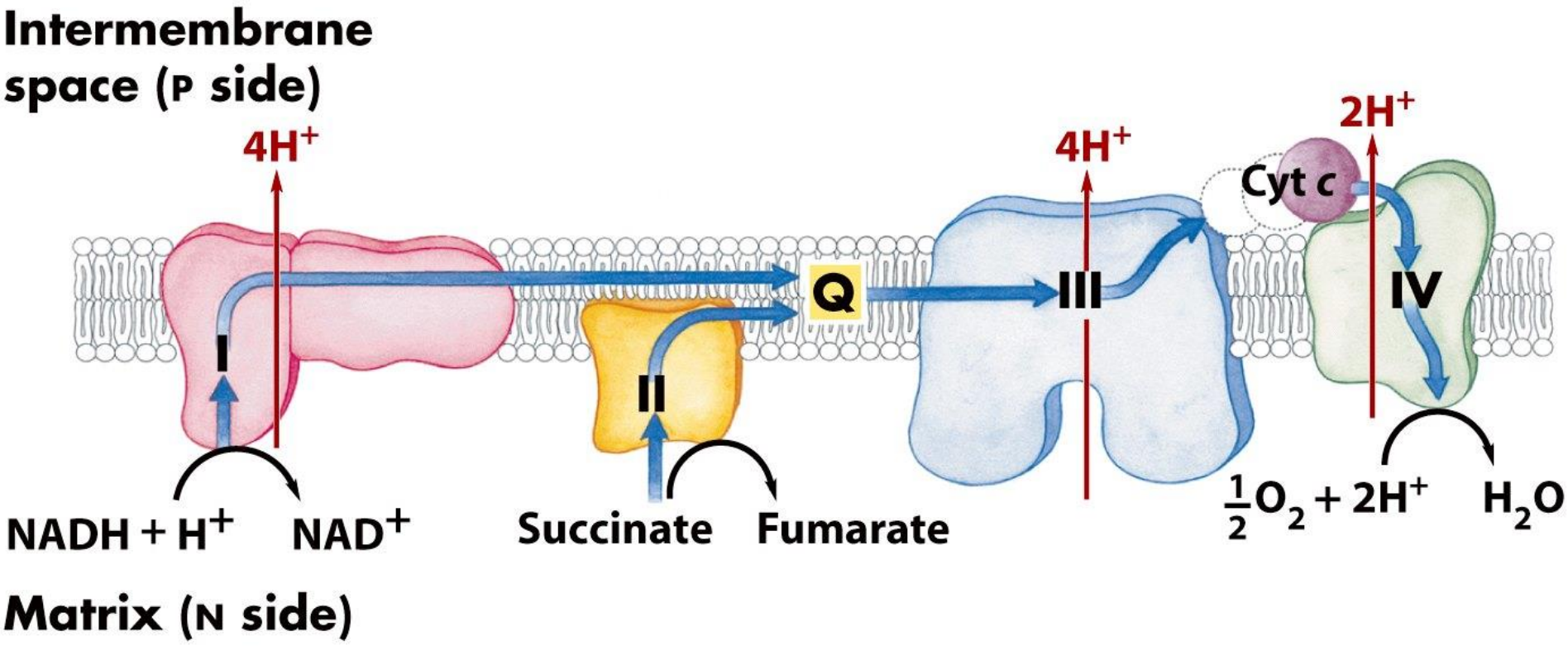
Transport of ATP/ADP across mitochondria

- **ATP-ADP Translocase**
- **The flows of ATP and ADP are coupled (ADP enters only if ATP exits**
- **Highly abundant (14% of IMM proteins)**



- **Contains a single nucleotide-binding site (alternates)**
- **Similar affinity to ATP and ADP**
- **Endergonic (25% of ETC)**
- **Inhibition leads to subsequent inhibition of cellular respiration**

Pumping of Protons by complexes



- For every 2 electrons passing:
- $4H^+$ (complex I); $0H^+$ (complex II); $4H^+$ (complex III), $2H^+$ (complex IV)

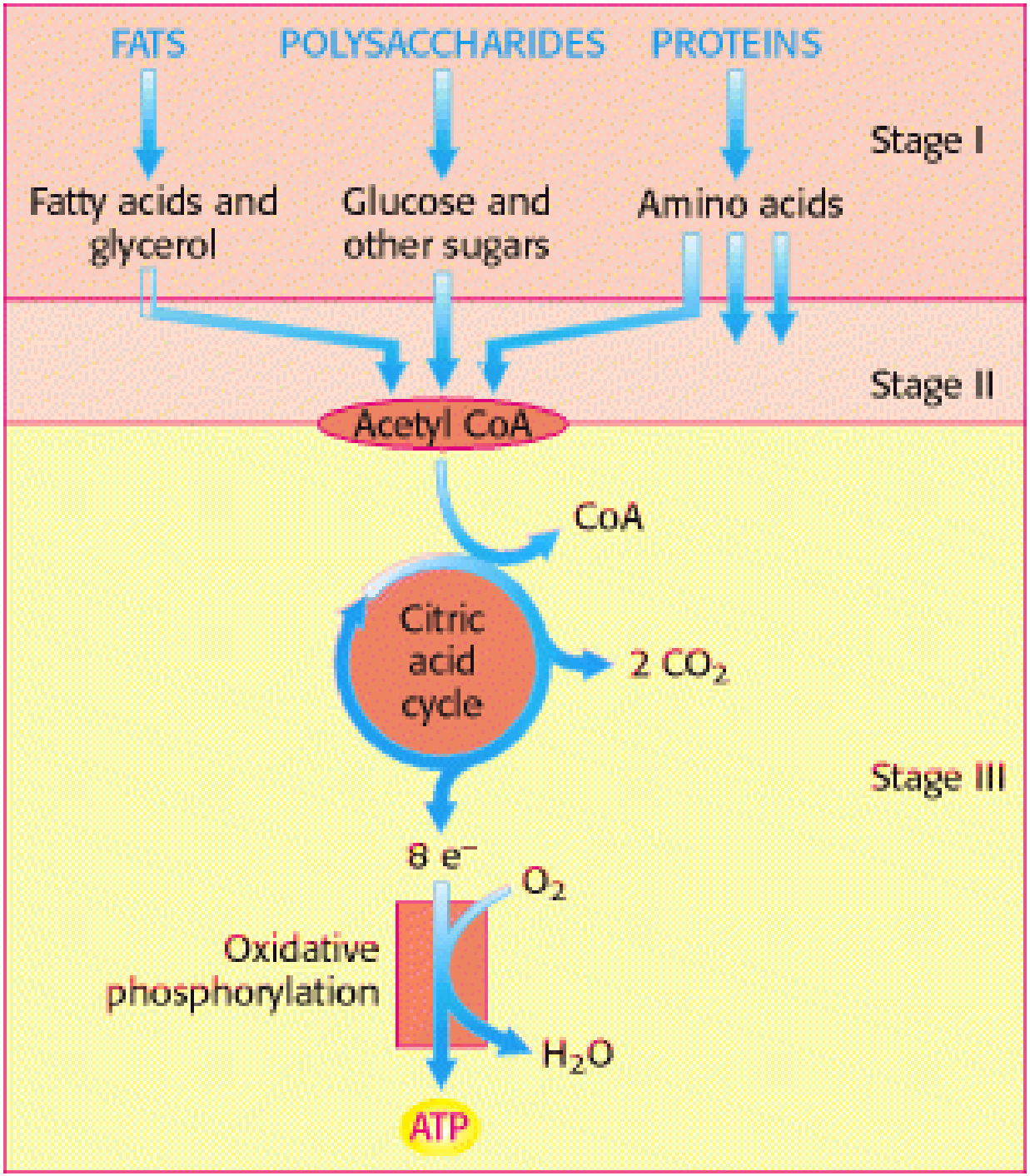
The citric acid cycle

also known as

Tricarboxylic acid cycle TCA

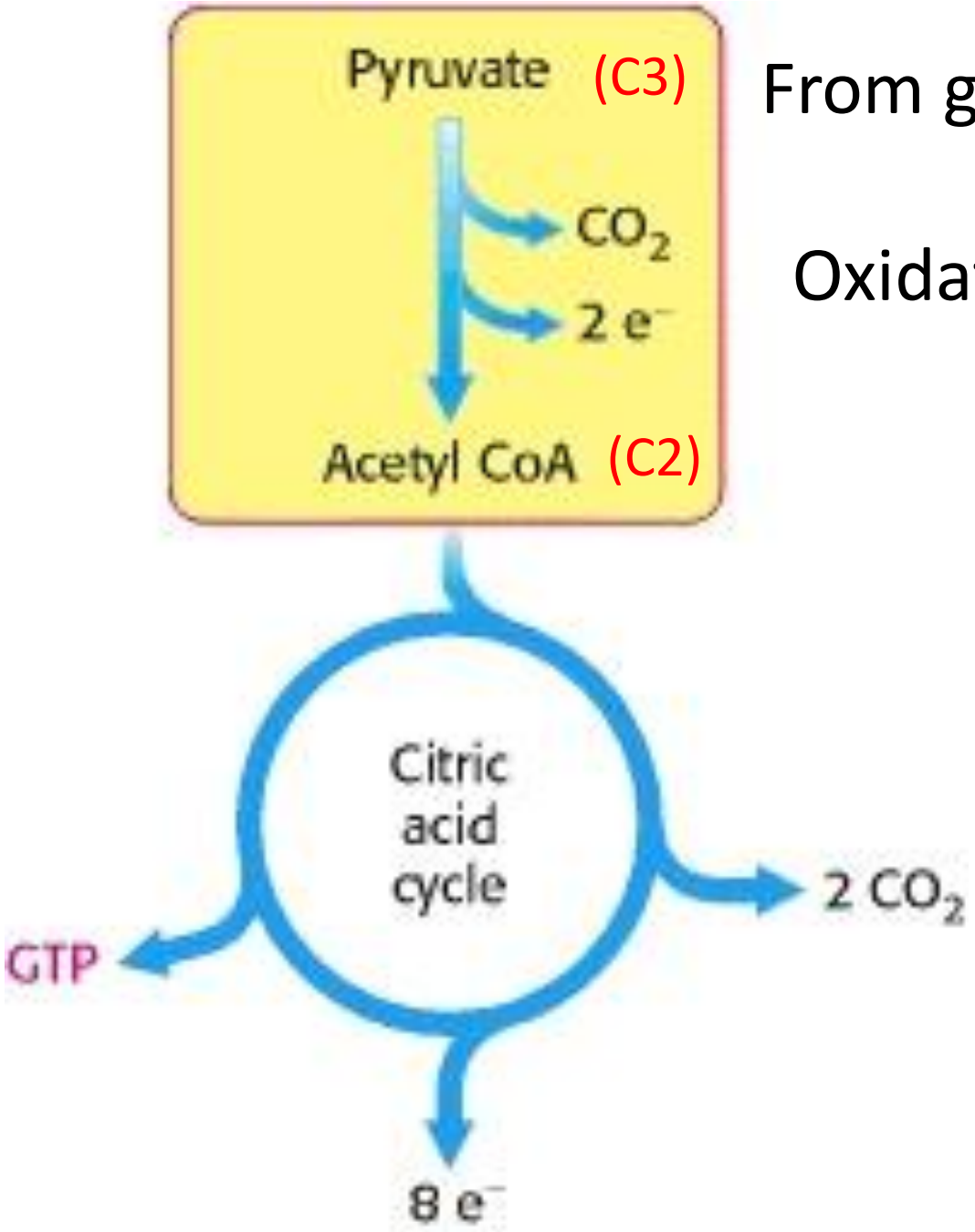
Krebs cycle

Lippincott chapter 9



Krebs cycle is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids convert their carbon skeletons to CO_2.

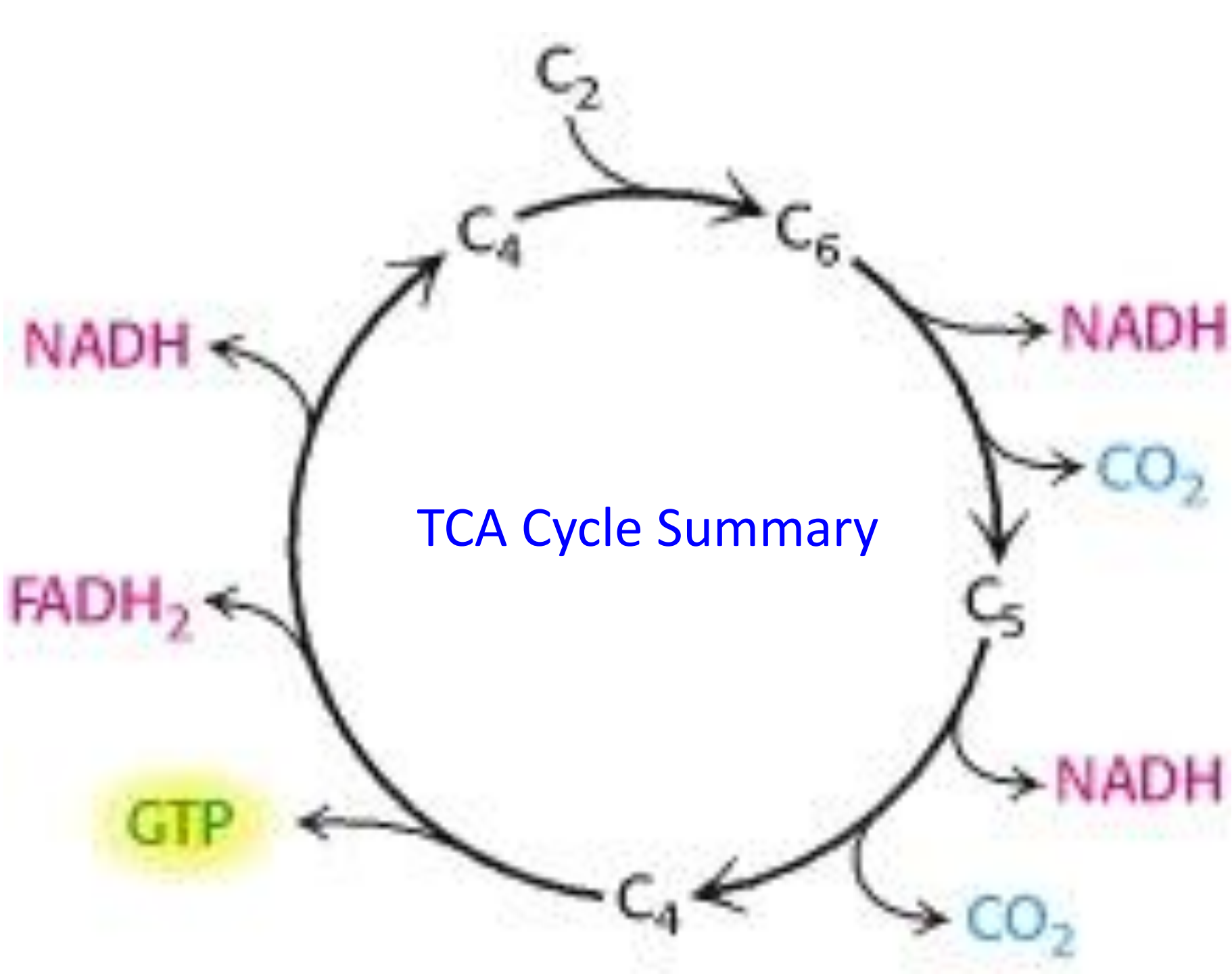
Most of the body's catabolic pathways converge on the TCA cycle



From glycolysis to TCA Cycle

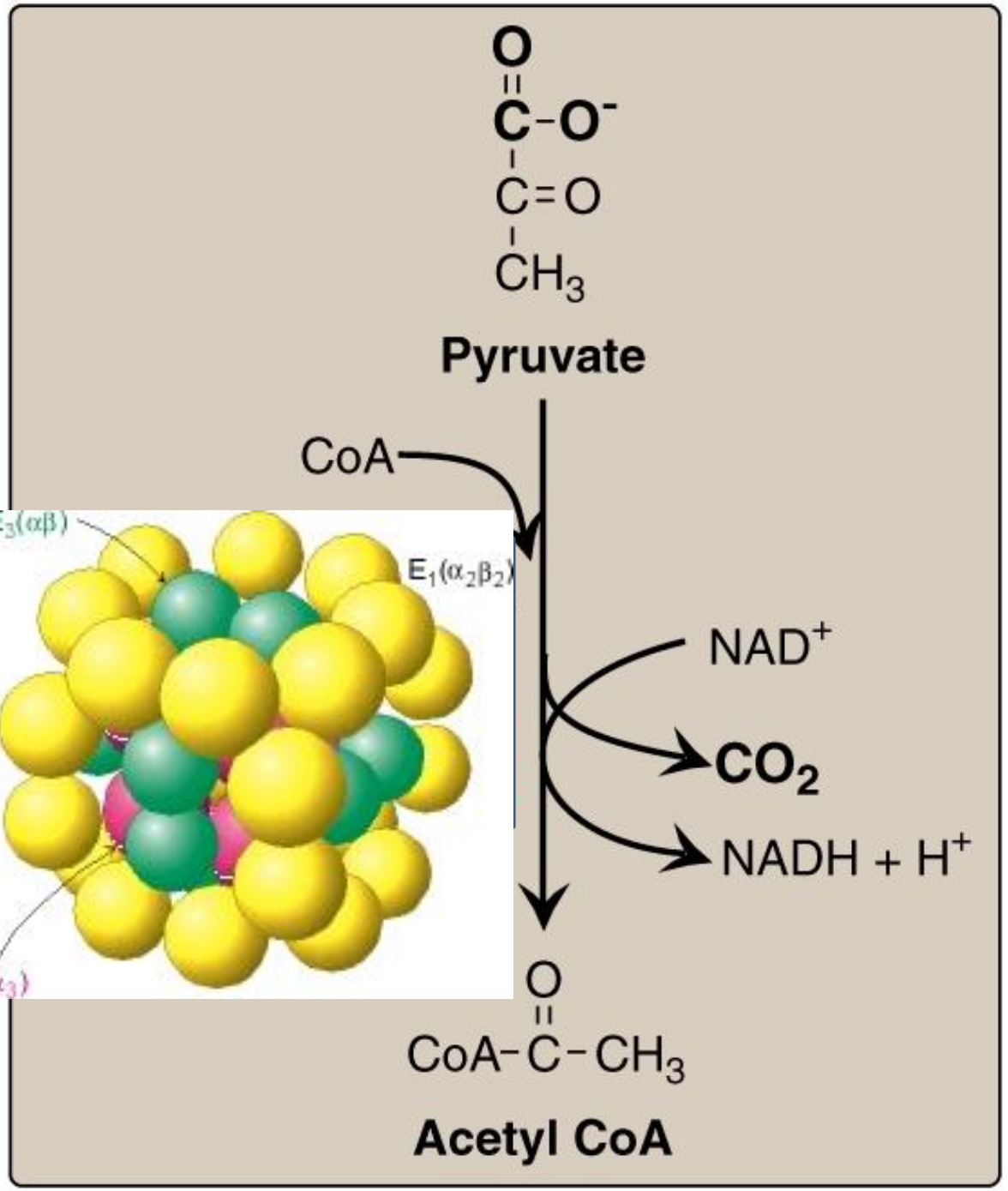
Oxidative decarboxylation of pyruvate

In the mitochondrial matrix, pyruvate undergoes oxidative decarboxylation to form acetyl CoA.


TCA Cycle Summary
C_2
C_4
C_6
NADH
CO_2
C_5
NADH
CO_2
C_4
GTP
$FADH_2$
NADH

From Pyruvate to Acetyl Co-A

Pyruvate dehydrogenase complex (a multienzyme complex)

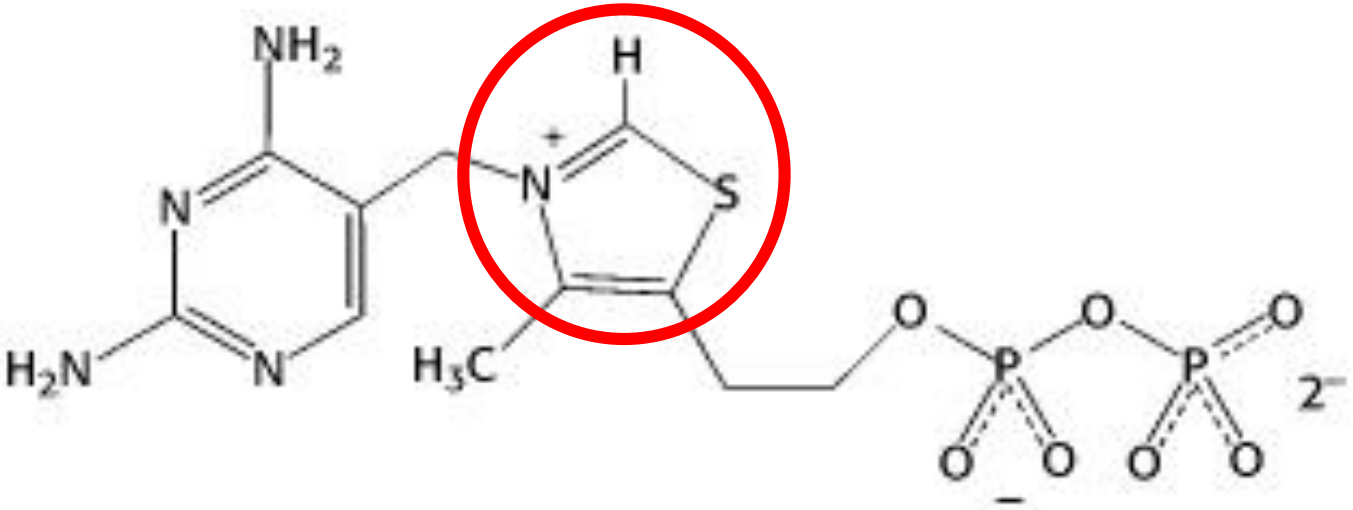


The Formation of Acetyl CoA from Pyruvate

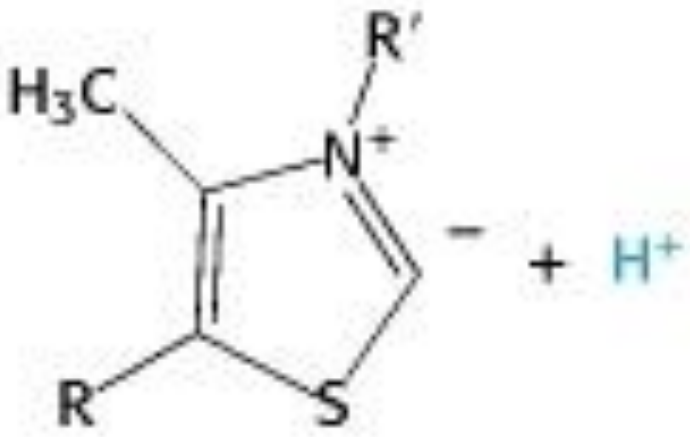
- Irreversible reaction
- Pyruvate Dehydrogenase complex: large, complex of three kinds of enzymes
- Five coenzymes:
  - thiamine pyrophosphate (TPP),
  - lipoic acid,
  - FAD
  - CoA
  - NAD^+.
- Two additional enzymes regulate its activity

Pyruvate Decarboxylation Steps

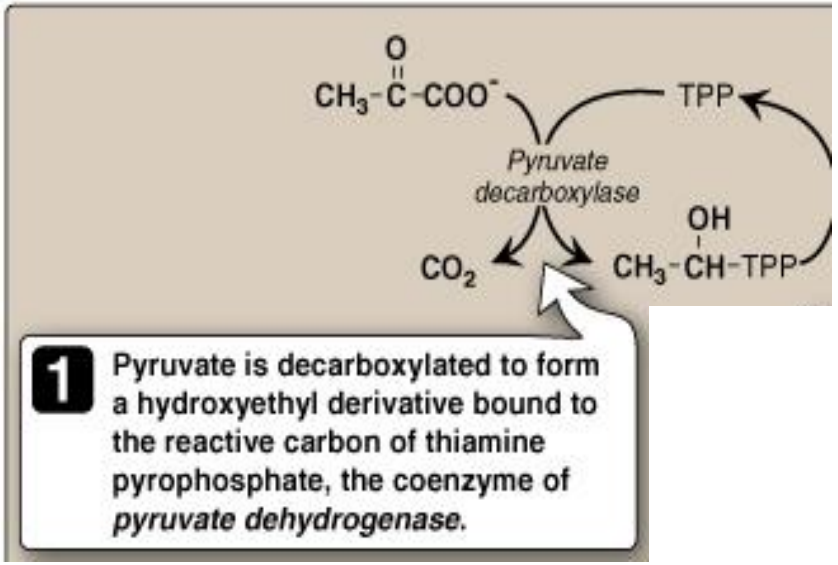
Step 1: Decarboxylation



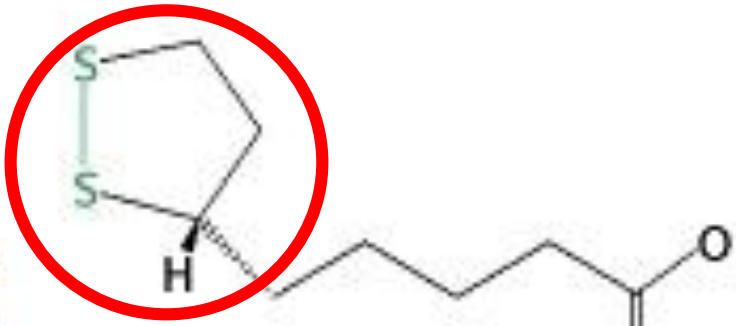
Thiamine pyrophosphate (TPP)



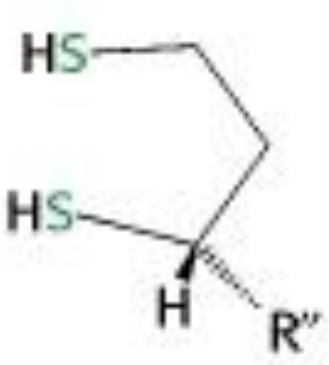
Carbanion of TPP



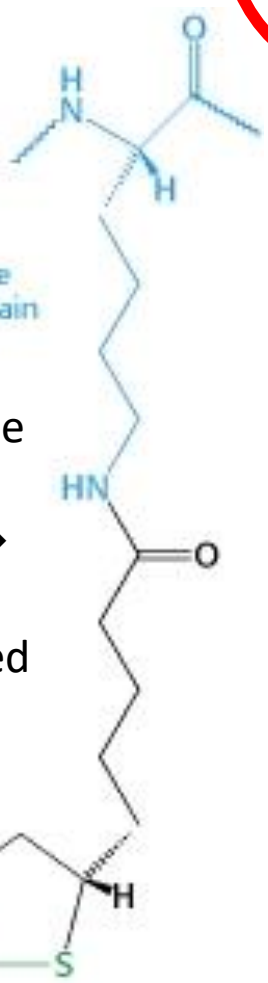
Step 2: Oxidation



Lipoic acid

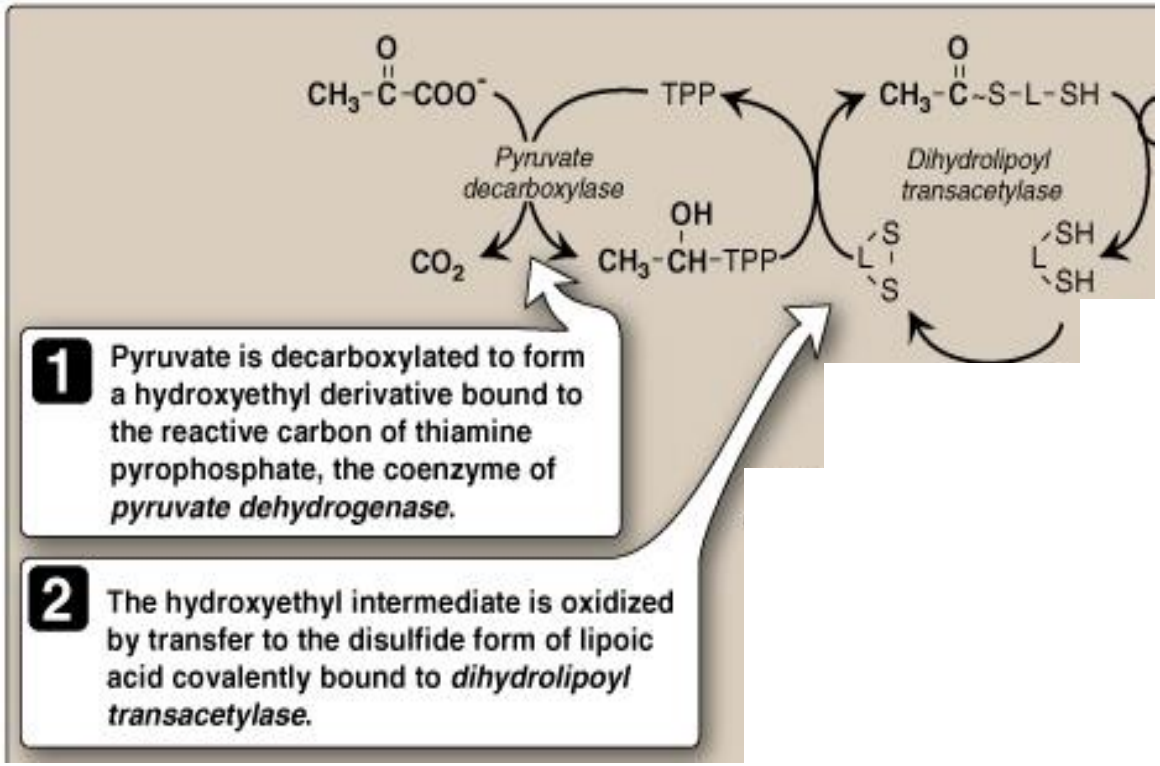


Dihydrolipoamide

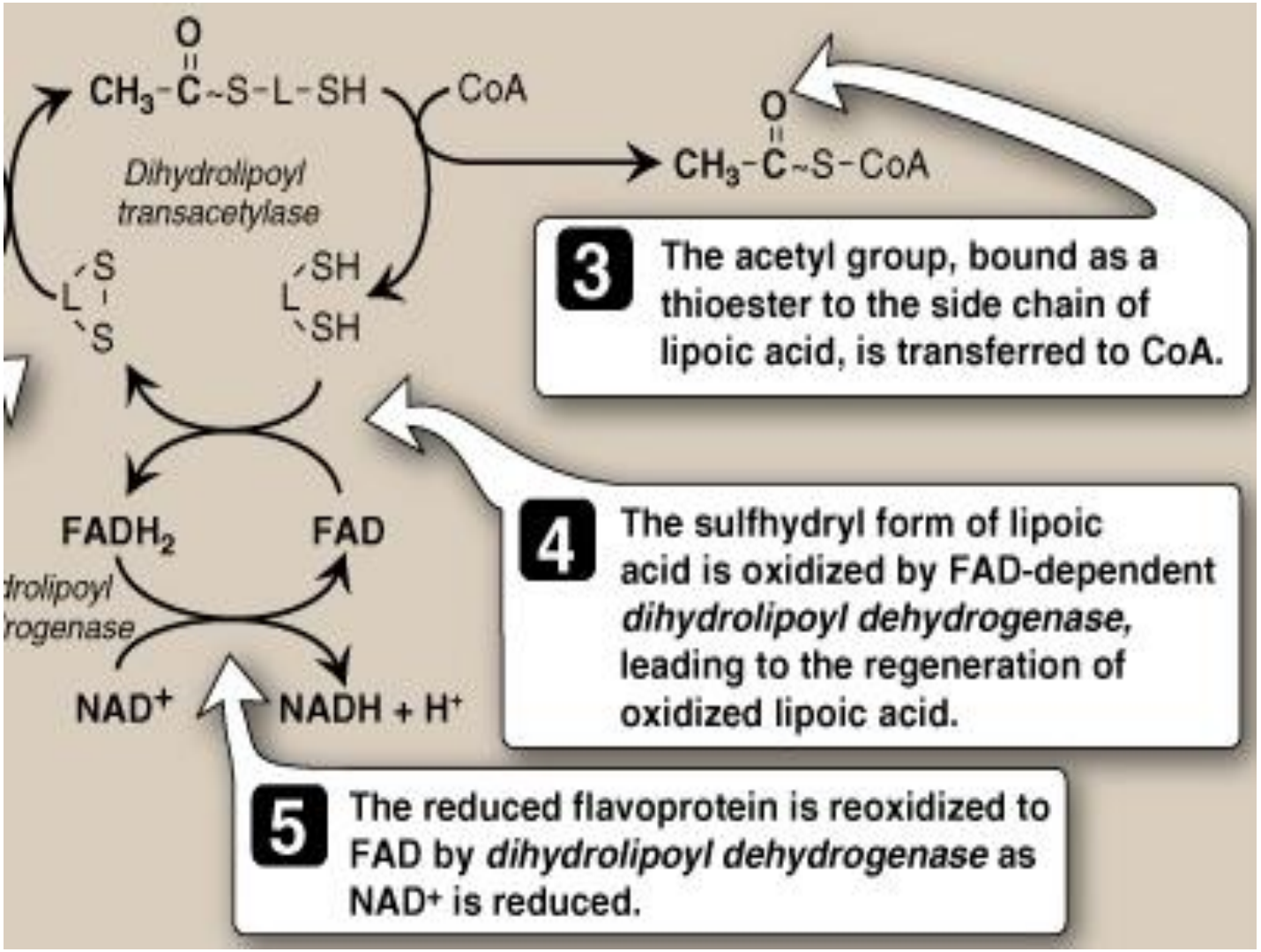


Lipoamide

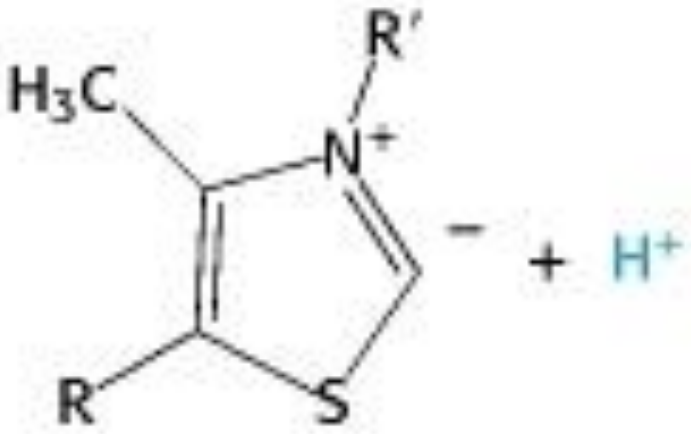
Lipoic acid is attached to the enzyme by amide bond➔ lipoamide
Can be oxidized or reduced



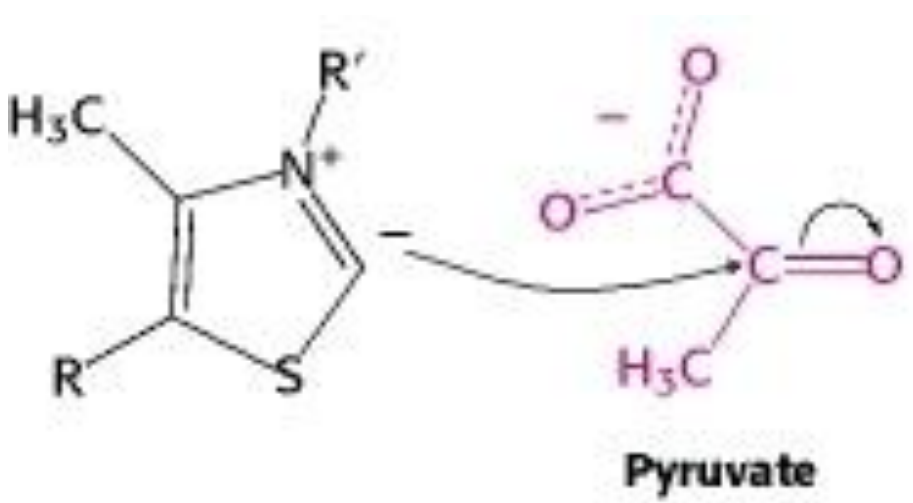
Step 3: Transfer of the resultant acetyl group to CoA Oxidation



Conversion of pyruvate into acetyl CoA consists of three steps: decarboxylation, oxidation, and transfer of the resultant acetyl group to CoA.

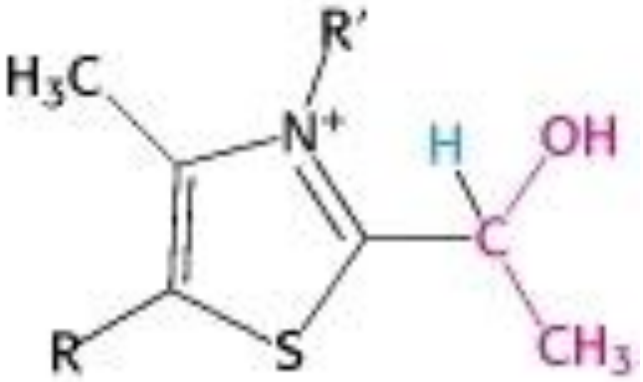


Carbanion of TPP

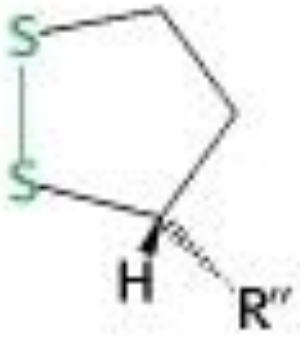


Pyruvate

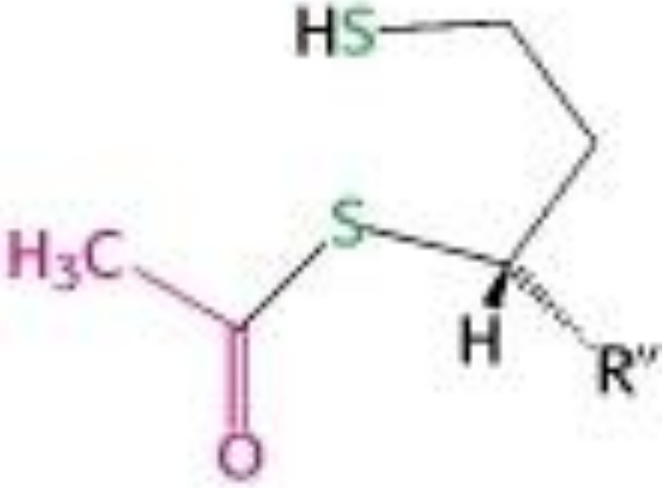
The mechanism of the pyruvate dehydrogenase reaction is more complex than is suggested by its relatively simple stoichiometry.



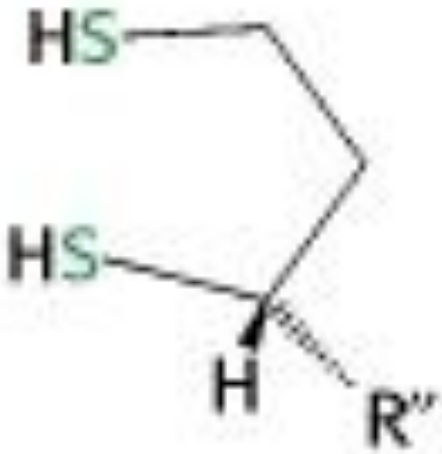
Hydroxyethyl-TPP



Lipoamide



Acetyllipoamide



Dihydrolipoamide

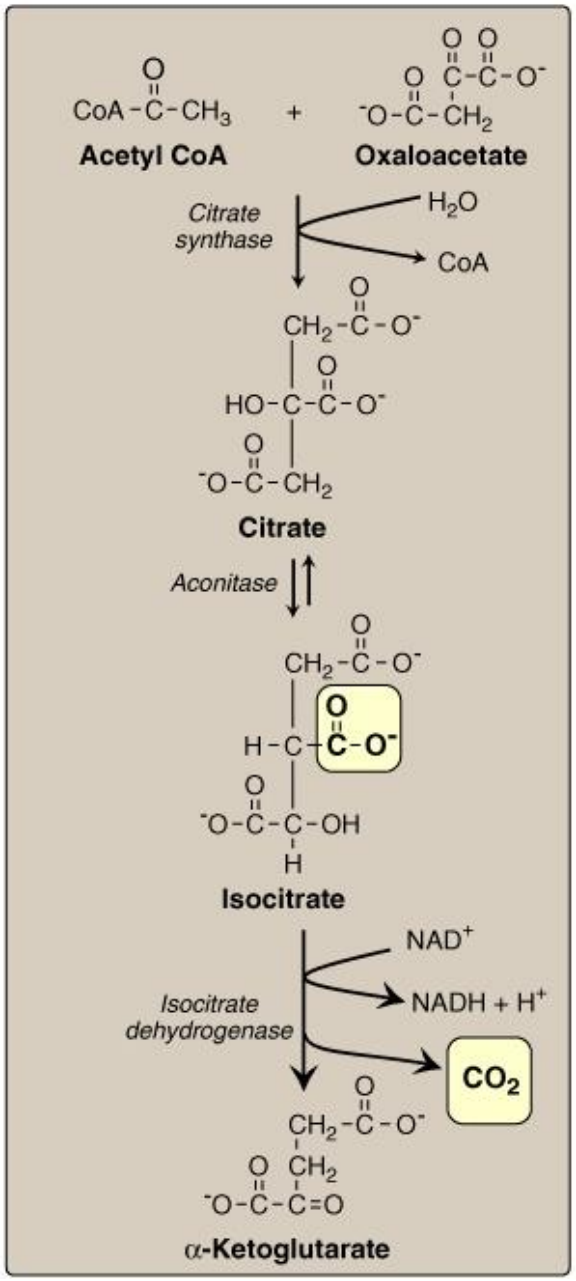
Clinical Hint: Pyruvate dehydrogenase deficiency

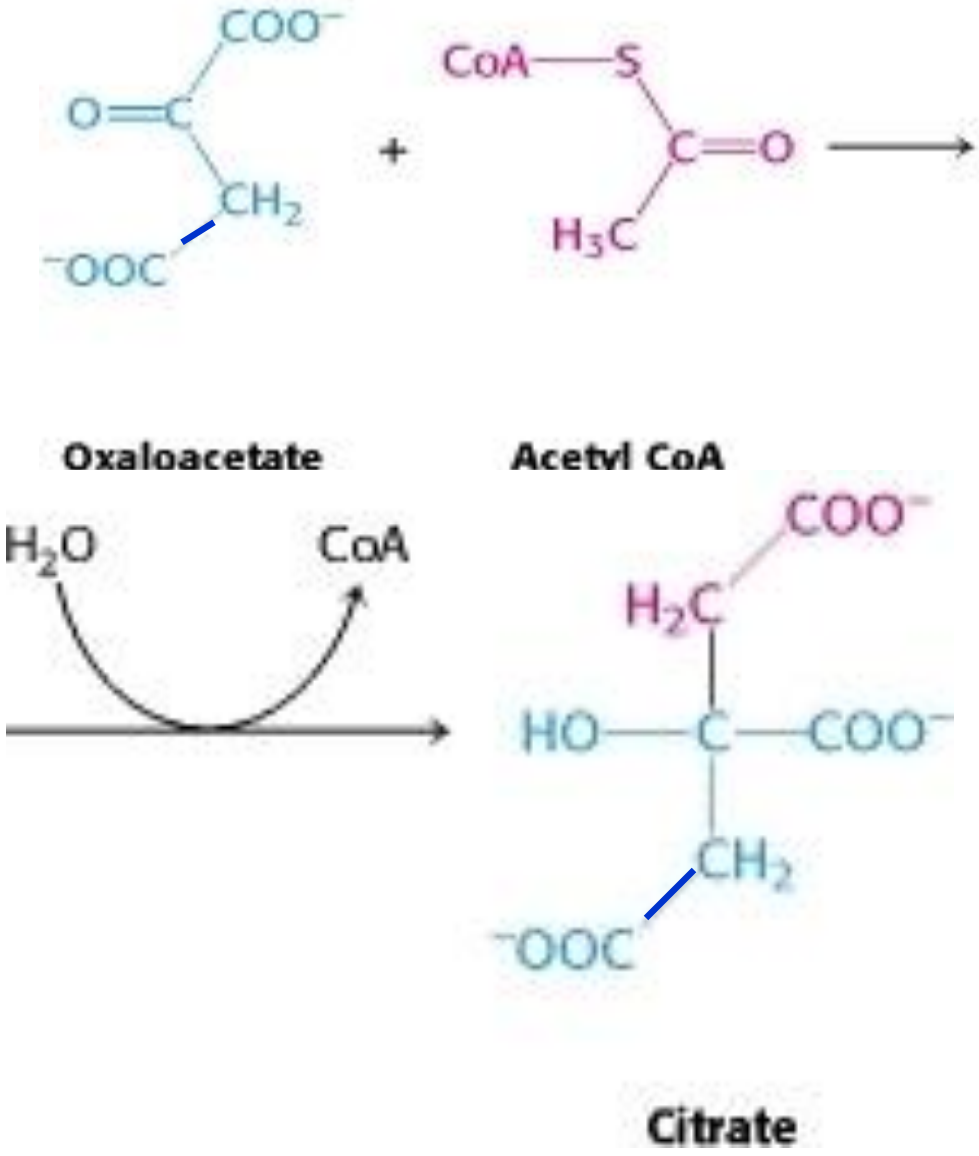
- E1 deficiency is rare but is the most common biochemical cause of congenital lactic acidosis.
- E1 deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactic acid via lactate dehydrogenase.
- E1 deficiency leads to brain problems because it relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis.
- Symptoms: neurodegeneration, muscle spasticity and, in the neonatal onset form, early death.
- The E1 defect is X-linked dominant.
- No proven treatment for PDH deficiency but dietary restriction of carbohydrate and supplementation with TPP may reduce symptoms in select patients.

Clinical Hint: Arsenic poisoning

- Arsenic poisoning due to inhibition of enzymes that require lipoic acid as a coenzyme such as E2 of the PDH complex .
- Arsenite (the trivalent form of arsenic) forms a stable complex with the –SH groups of lipoic acid, making that compound unavailable to serve as a coenzyme.
- When arsenite binds to lipoic acid in the PDH complex , pyruvate and lactate accumulate.
- Arsenic poisoning particularly affects brain, causing neurologic disturbances and death.

TCA Cycle



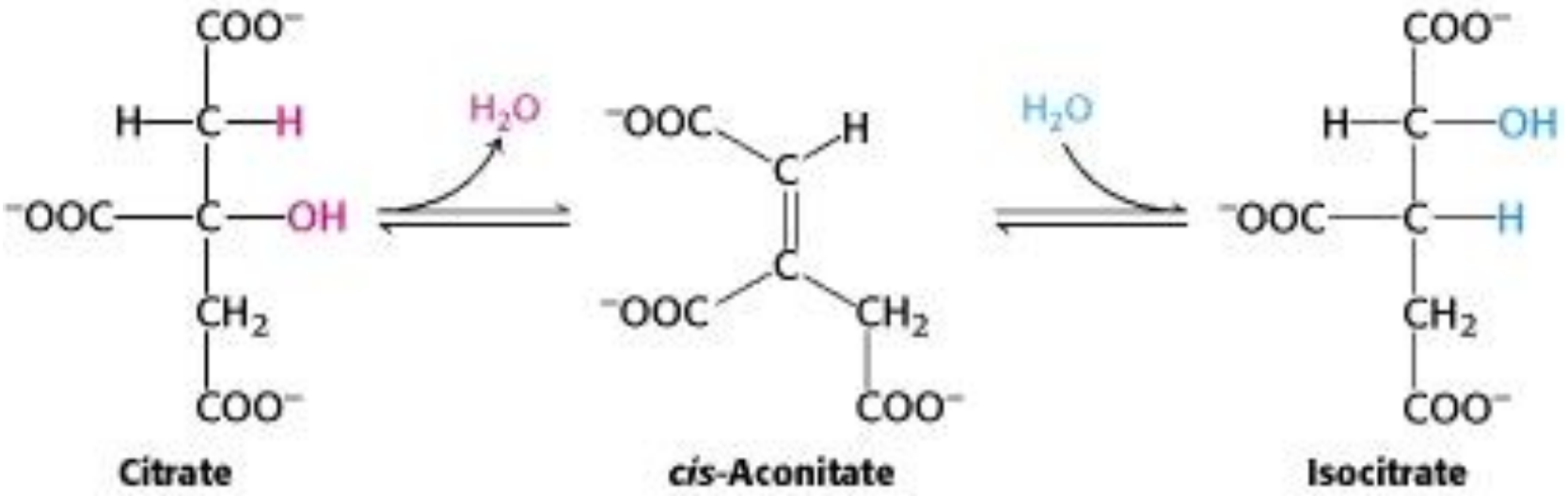


Wasteful hydrolysis of acetyl CoA is prevented.

Citrate Is Isomerized into Isocitrate

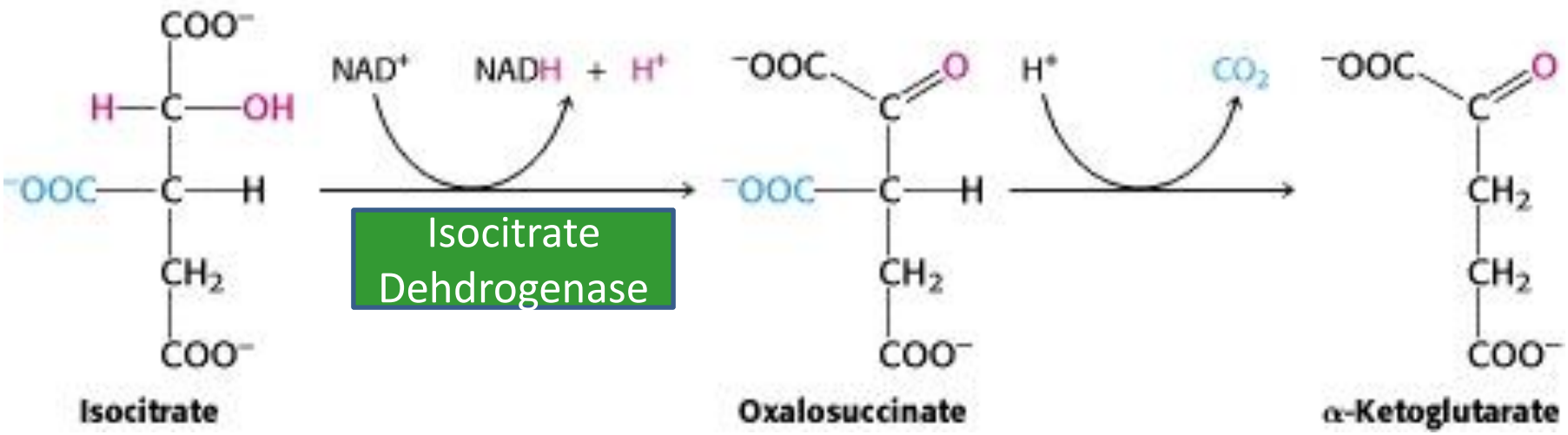
Dehydration step followed by a hydration step.

Catalyzed by aconitase



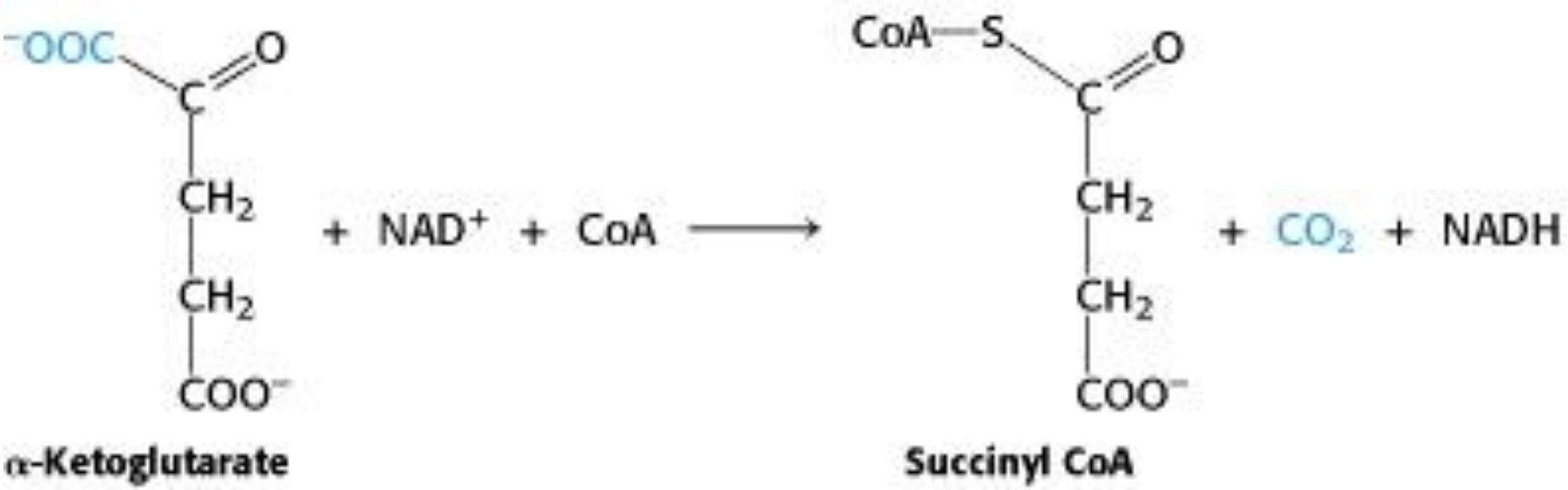
Hint: Aconitase is inhibited by fluoroacetate, a compound that is used as a rat poison.

Oxidation and decarboxylation of isocitrate



- ✓ Irreversible and one of the rate-limiting steps of the TCA cycle.
- ✓ The enzyme is allosterically activated by ADP (a low-energy signal) and Ca2+, and is inhibited by ATP and NADH, whose levels are elevated when the cell has abundant energy stores.

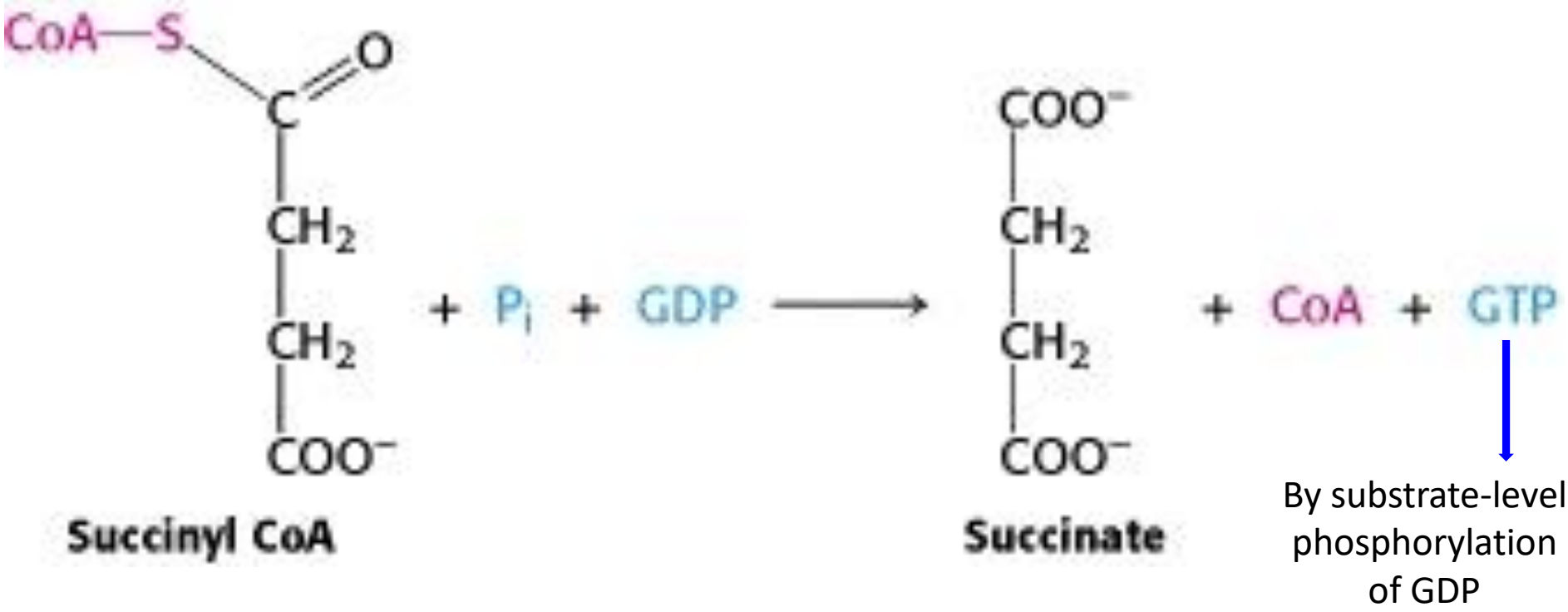
Oxidative decarboxylation of α-ketoglutarate



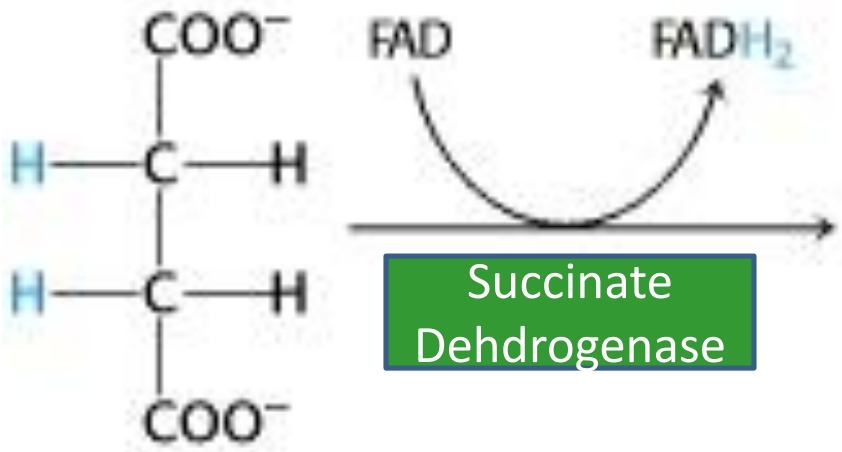
α-Ketoglutarate

Succinyl CoA

Cleavage of the high-energy thioester bond of succinyl CoA

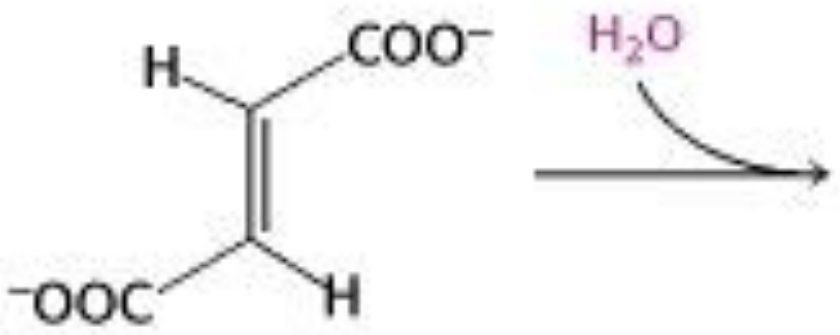


Oxidation of succinate



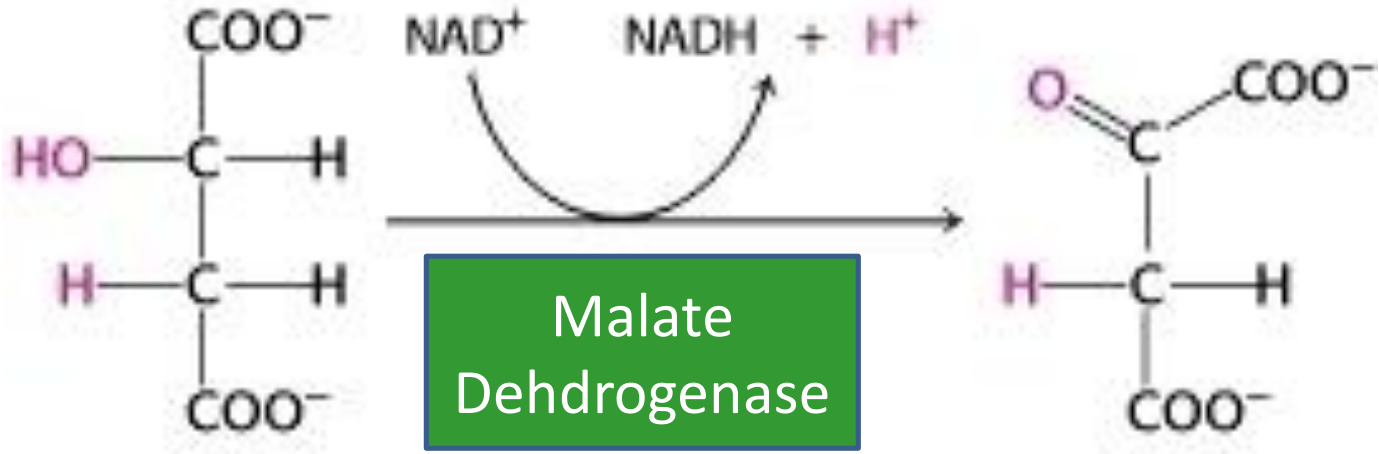
Succinate

Hydration of fumarate to malate



Fumarate

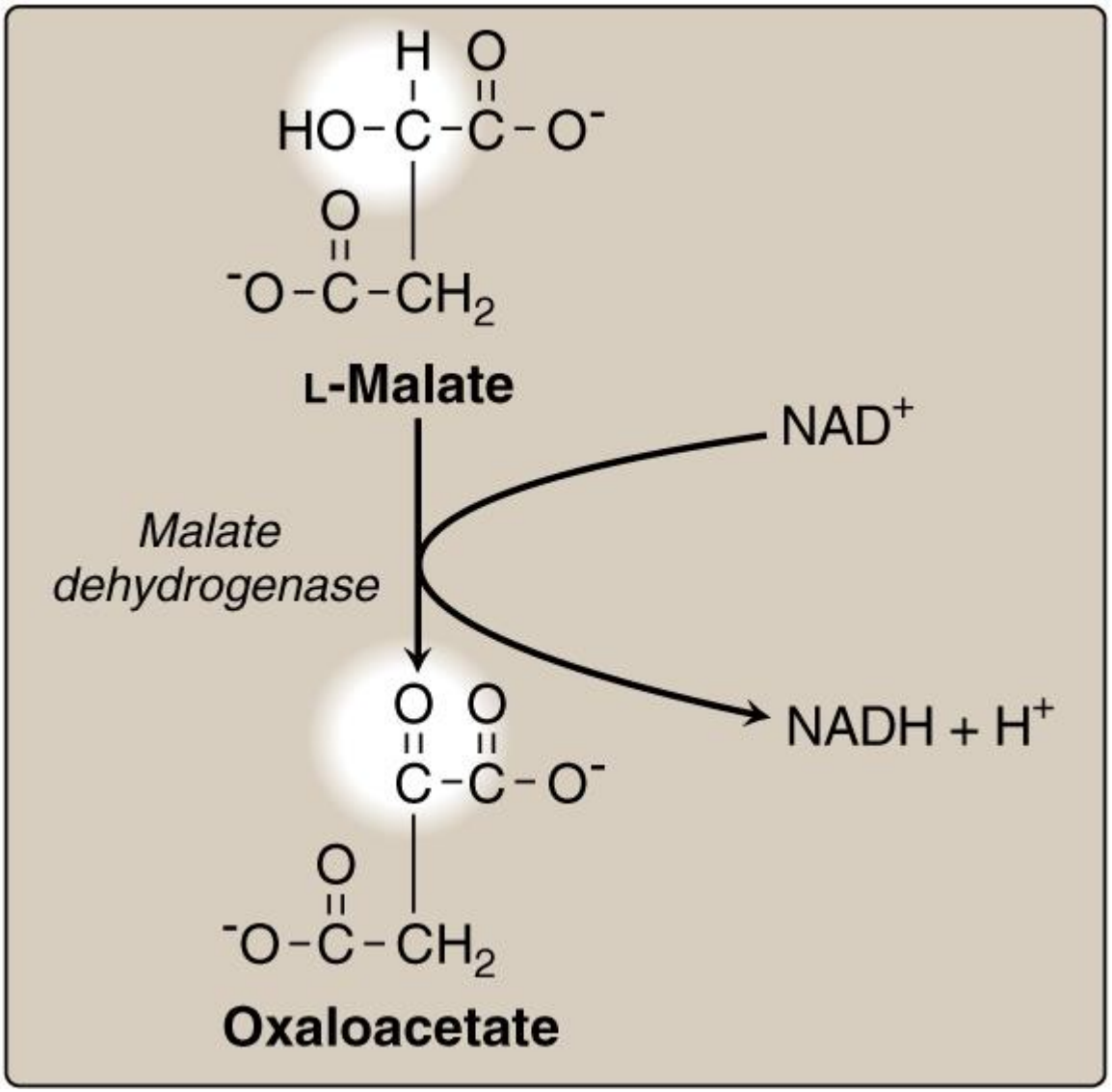
Oxidation of malate



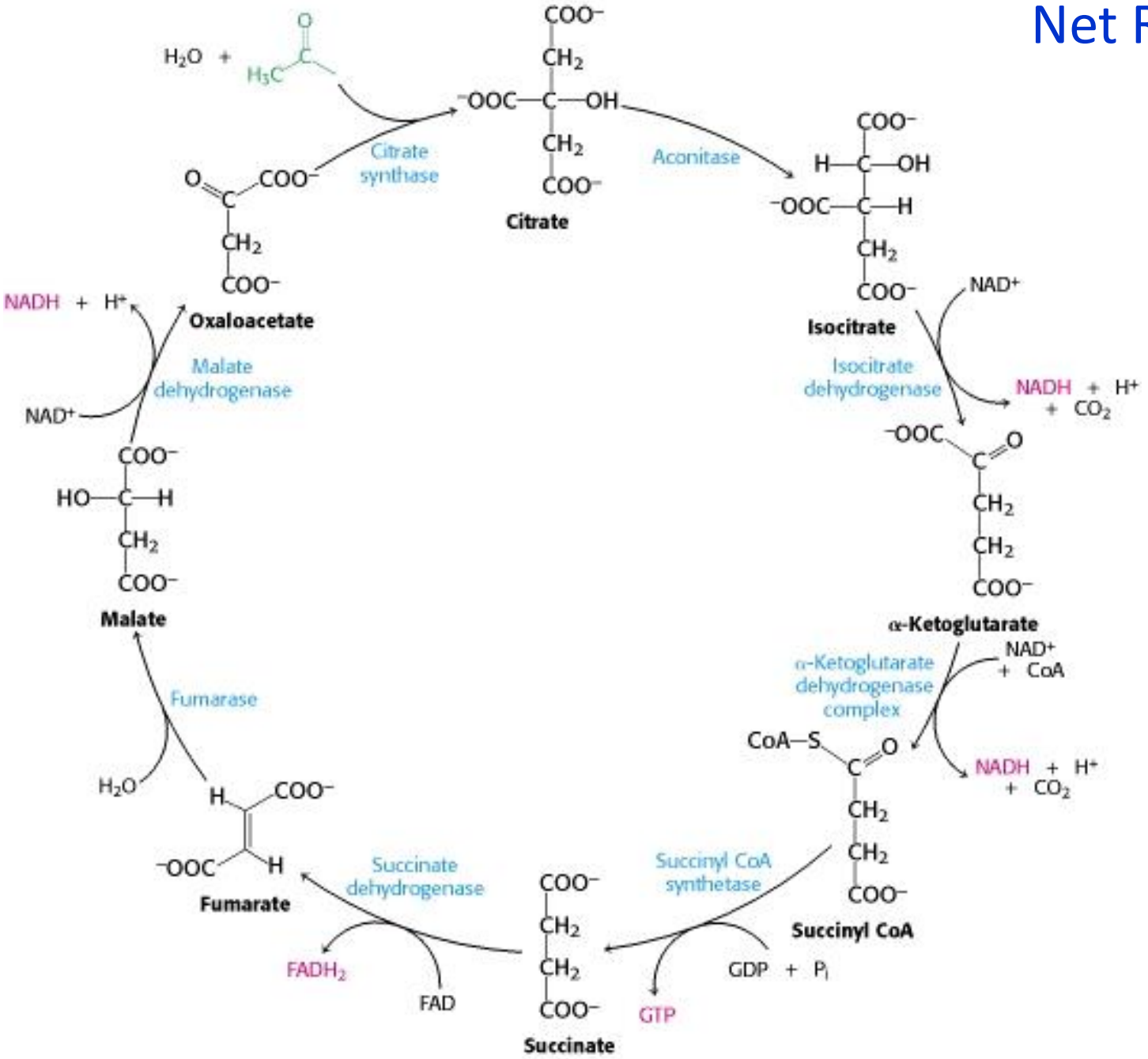
Malate

Oxaloacetate

Oxidation of malate



Net Reaction?



CIA Sends Soldiers From My Office

Citrate

Isocitrate

Alfa Keto Glutarate

Succinyl CoA

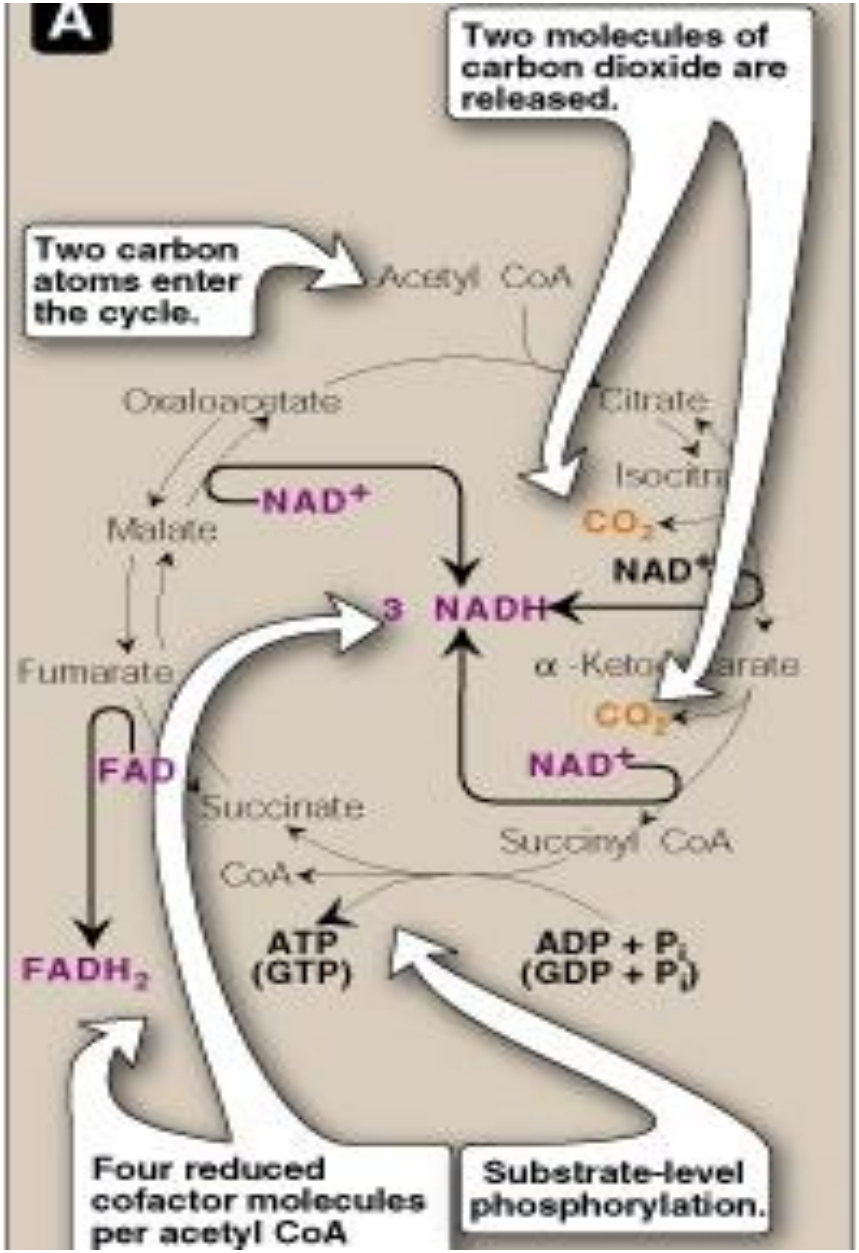
Succinate

Fumarate

Malate

Oxaloacetate

Net Reaction

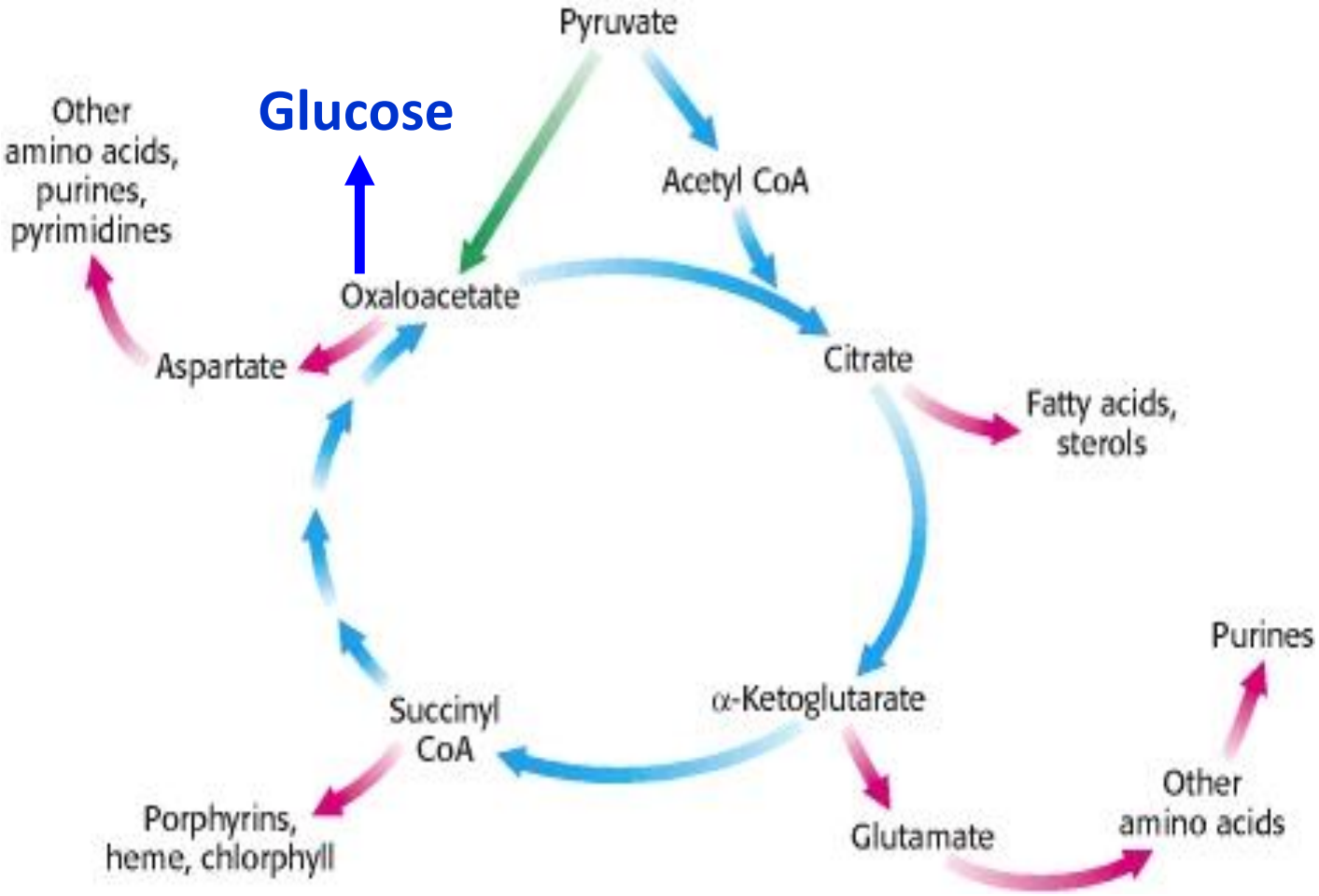


Energy producing reaction	Number of ATP produced
3 NADH ⟶ 3 NAD^+	9
$FADH_2$ ⟶ FAD	2
GDP + P_i ⟶ GTP	1
	12 ATP/acetyl CoA oxidized

Figure 9.7
Number of ATP molecules produced from the oxidation of one molecule of acetyl CoA (using both substrate-level and oxidative phosphorylation).

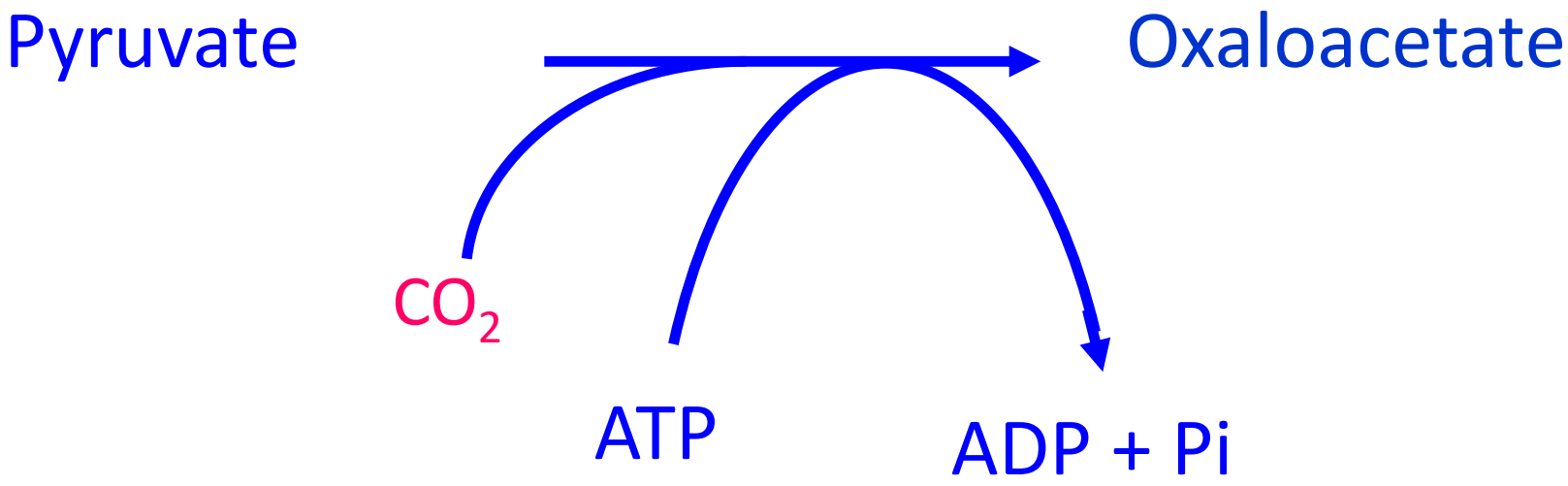
$$\text{Acetyl CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2\,H_2O \longrightarrow 2\,CO_2 + 3\,NADH + FADH_2 + GTP + 2\,H^+ + CoA$$

The Citric Acid Cycle Is a Source of Biosynthetic Precursors



Intermediates must be replenished if any are drawn off for biosyntheses.

Carboxylation of Pyruvate produces Oxaloacetate



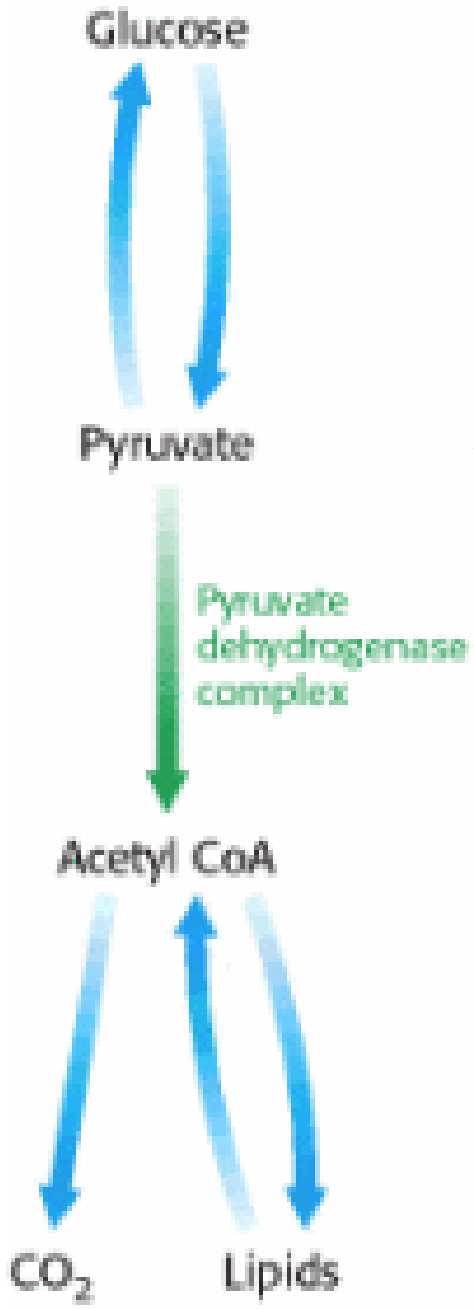
Pyruvate Carboxylase

Biotin-Containing Enzyme

Example of an anaplerotic reaction (“fill up”)

Regulation of Citric Acid Cycle

- It is the final common pathway for the aerobic oxidation of fuel molecules
- It is an important source of building blocks for a number of biomolecules.
- Entry into the cycle and the rate of the cycle itself are controlled at several stages.



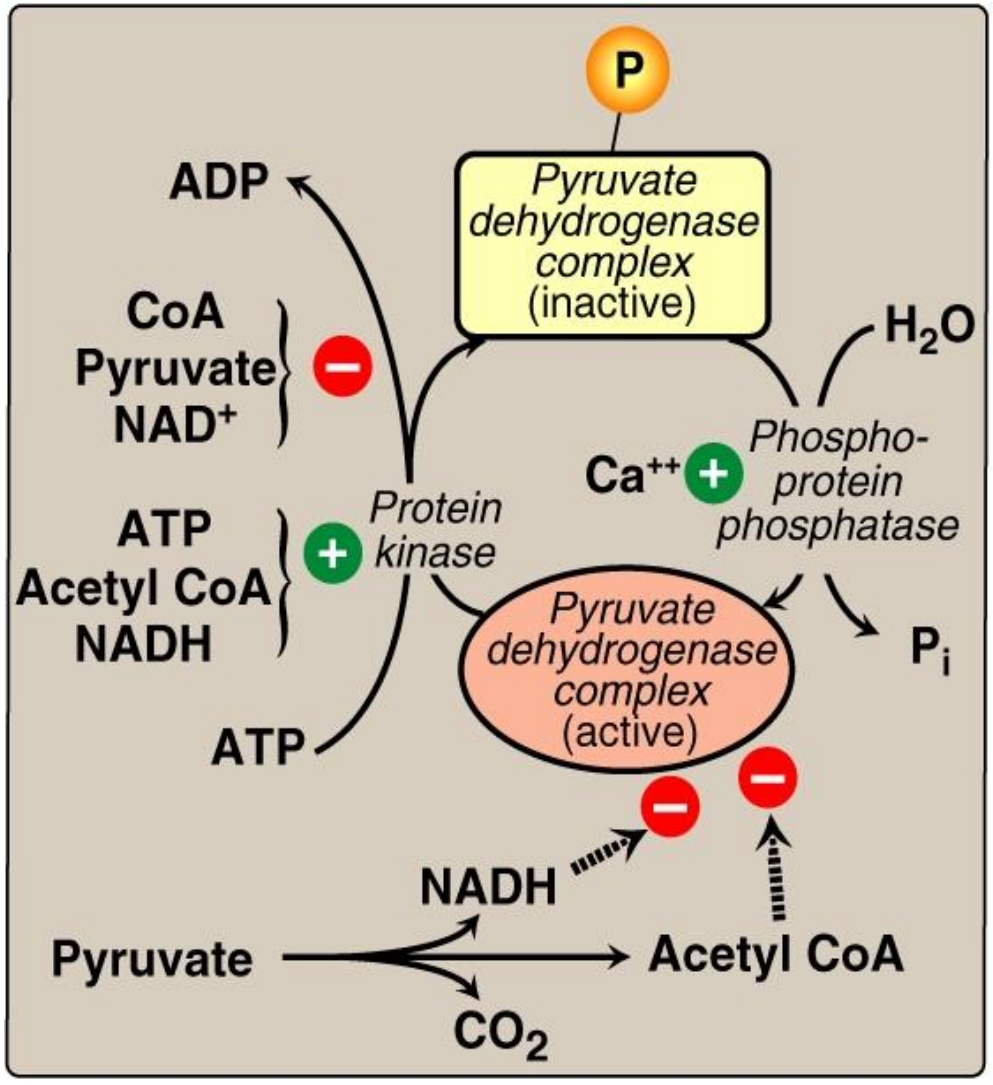
glucose can be formed from pyruvate.

However, the formation of acetyl CoA from pyruvate is an irreversible step in animals.

They are unable to convert acetyl CoA back into glucose

Fat can be produced from glucose,
But fat can not be converted to glucose

PDH Kinase (Protein Kinase) and PDH phosphatase: Two regulatory enzymes, Part of PDH complex



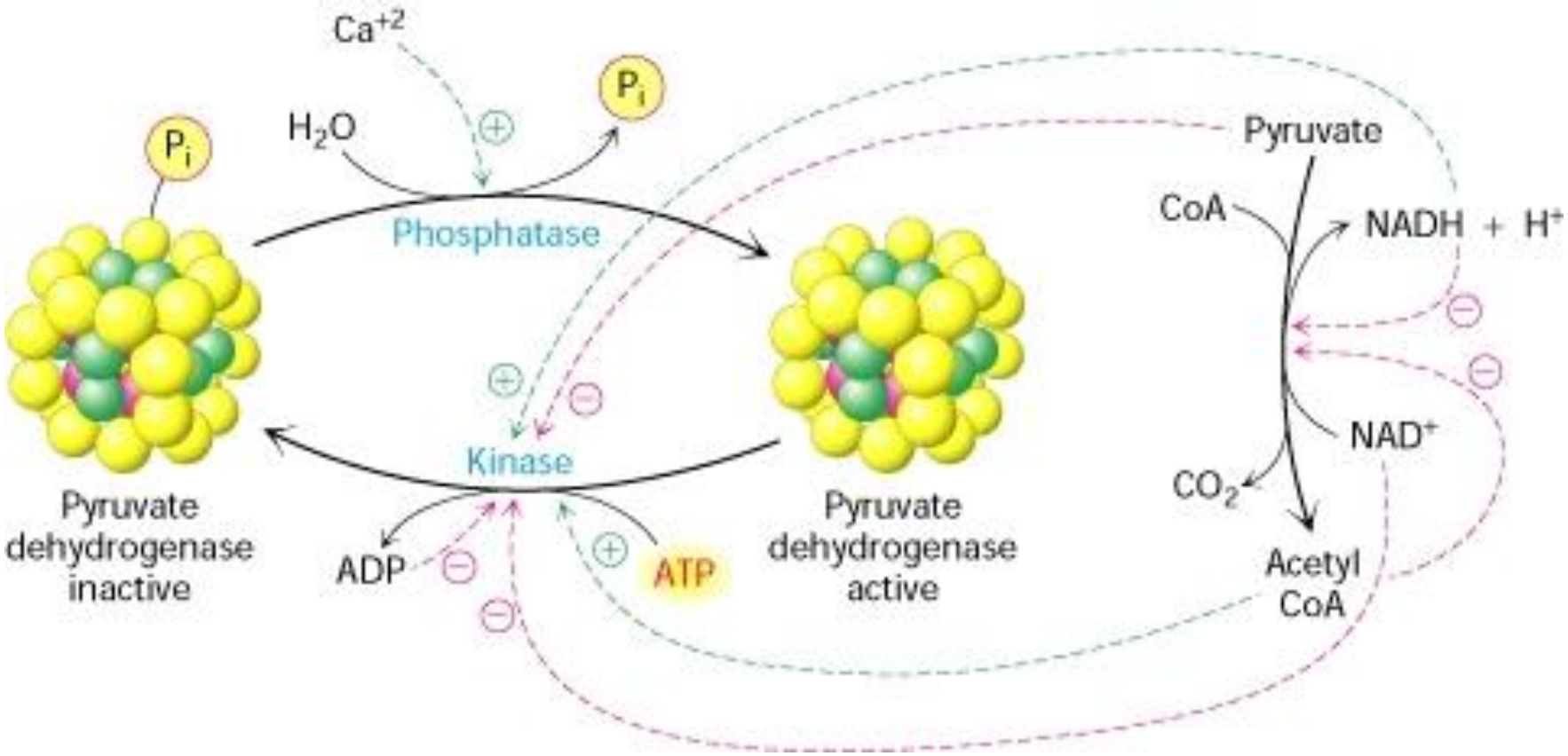
- Allosteric regulation and reversible phosphorylation
- High concentrations of reaction products inhibit the complex
- Increasing the NADH/NAD+, acetyl CoA/CoA, or ATP/ADP ratio promotes phosphorylation
- Pyruvate as well as ADP activate the dehydrogenase by inhibiting the kinase.
- The rise in mitochondrial $Ca2^{+}$ activates the pyruvate dehydrogenase complex by stimulating the phosphatase.
- Insulin also accelerates the conversion of pyruvate into acetyl CoA by stimulating the phosphatase

Phosphatase Deficiency.

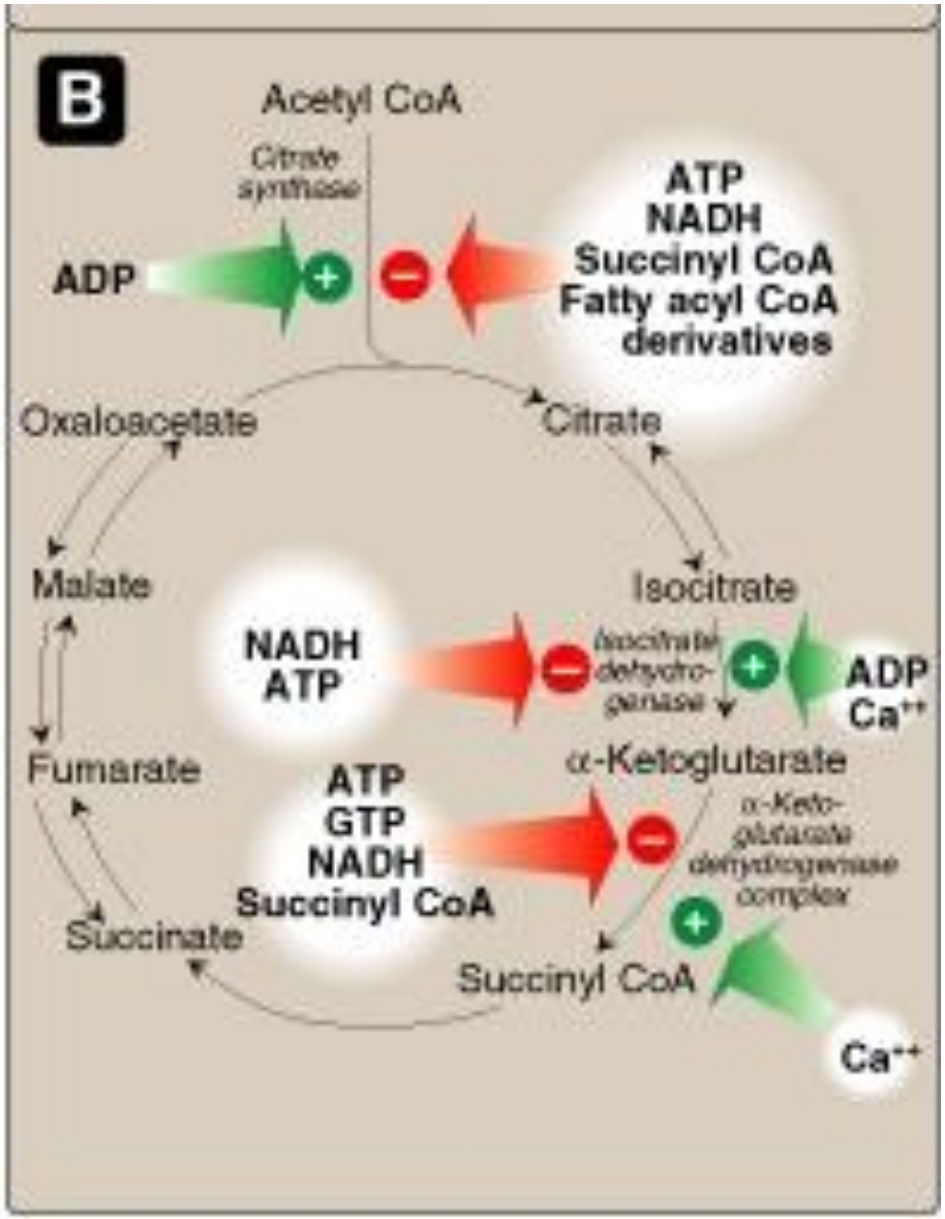
Pyruvate dehydrogenase is always phosphorylated. and thus inactive,

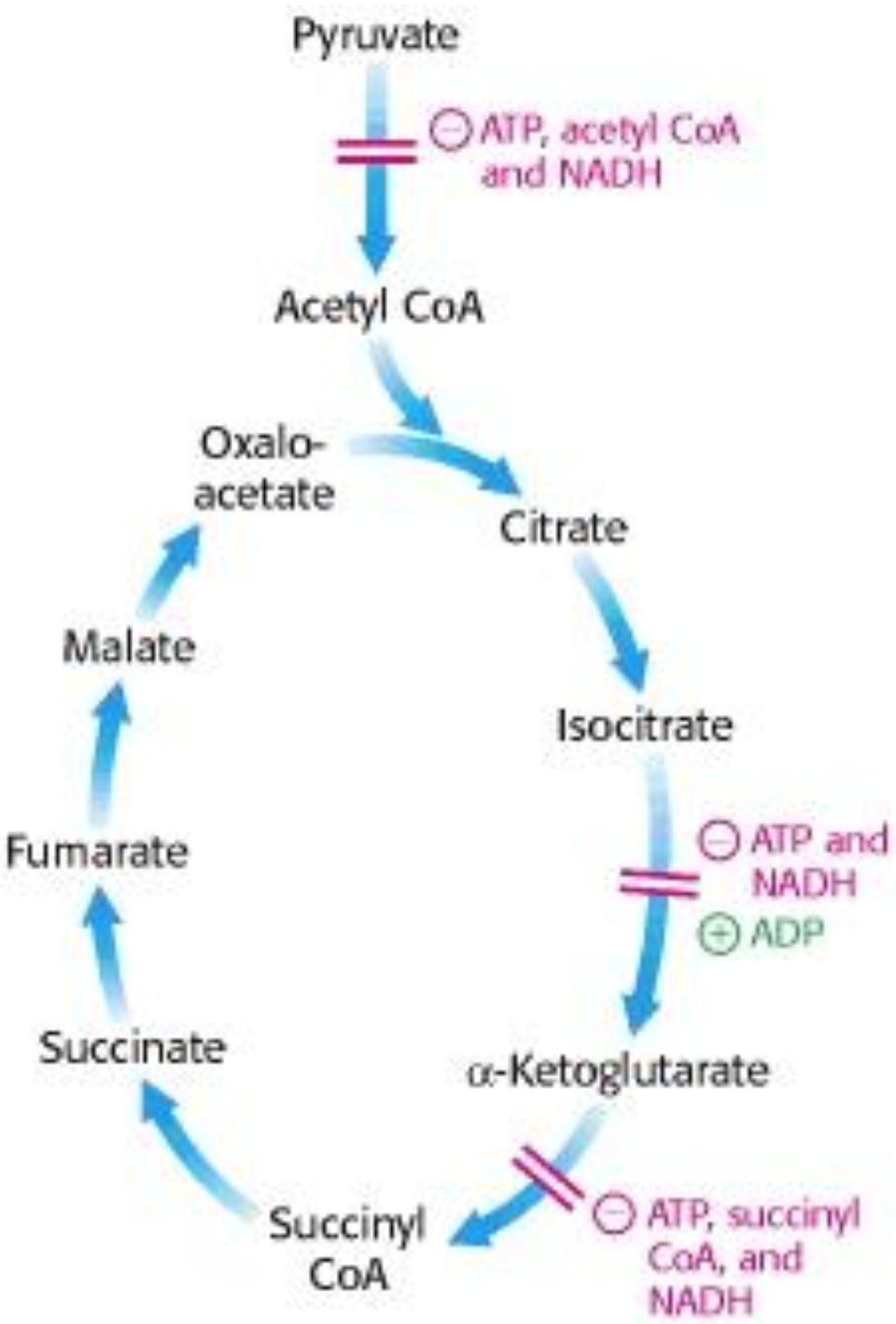
glucose is processed to lactic acid. ➔ lactic acidosis

➔ Malfunctioning of many tissues, most notably the central nervous system


Ca+2
Pi
H2O
Pi
Phosphatase
Pyruvate
CoA
NADH + H+
Kinase
NAD+
CO2
Pyruvate dehydrogenase inactive
ADP
ATP
Pyruvate dehydrogenase active
Acetyl CoA

Regulation of Citric acid Cycle





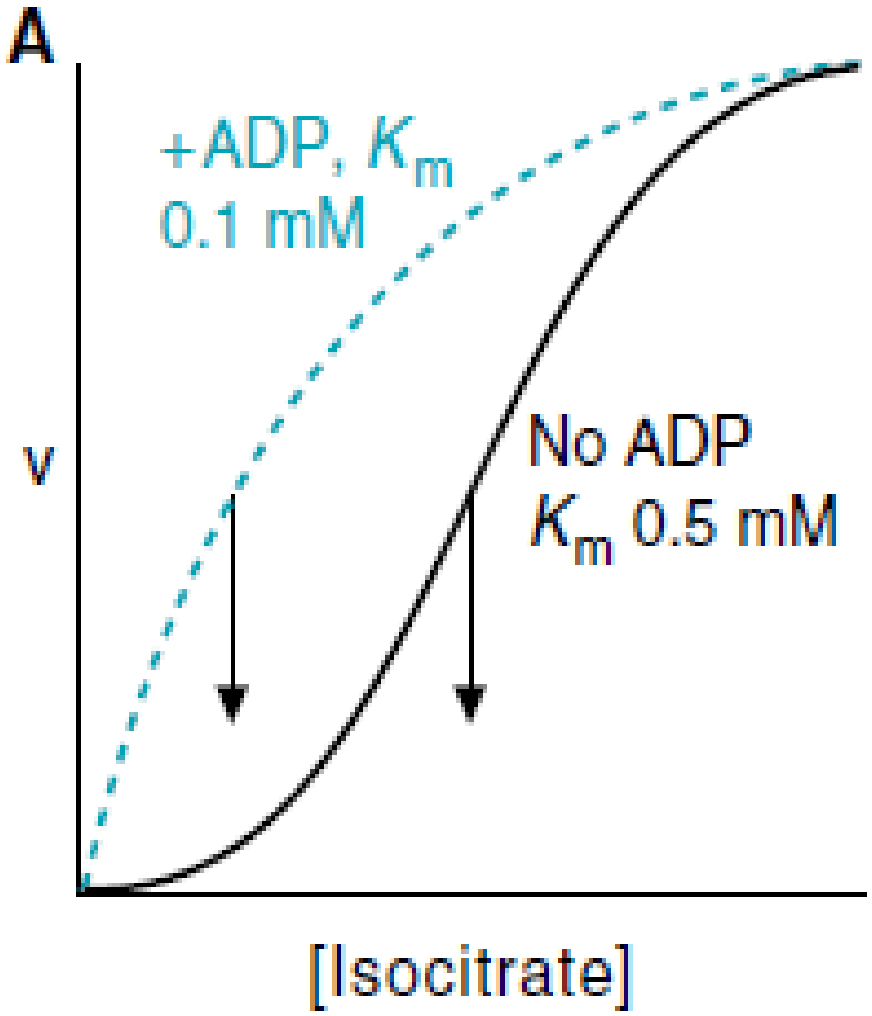
TCA Regulation

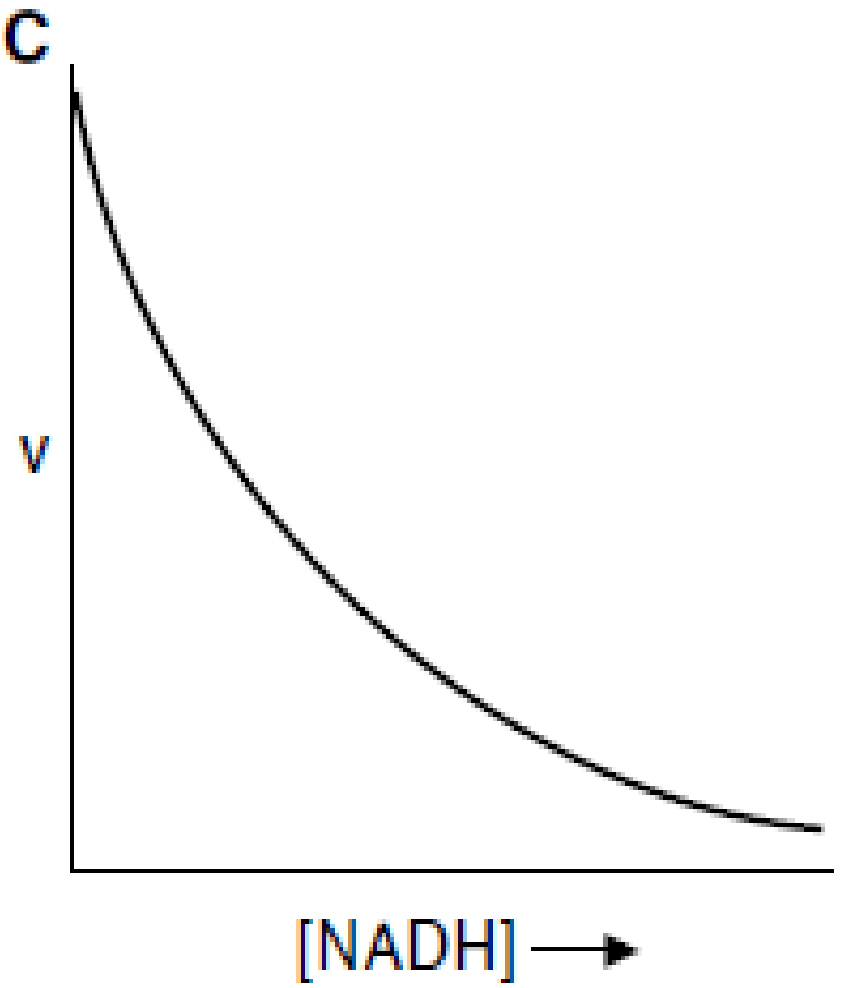
The citric acid cycle is regulated primarily by [ATP] and [NADH]. The key control points are isocitrate dehydrogenase α-ketoglutarate dehydrogenase.

Isocitrate dehydrogenase (IDH)is allosterically stimulated by ADP. Inhibited by NADH (IDH) through displacing NAD^+.
ATP is inhibitory too.

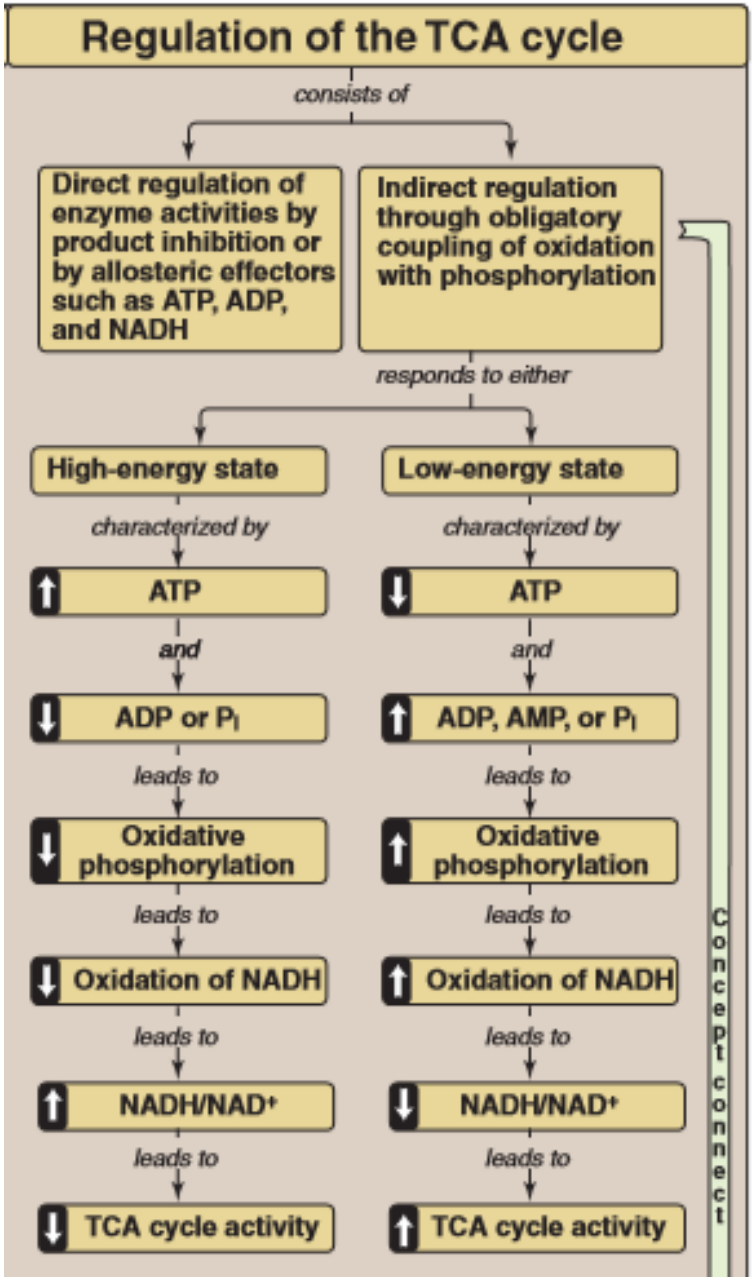
α-Ketoglutarate dehydrogenase is inhibited by:
succinyl CoA and NADH,
high energy charge ATP/ADP.

The effect of ADP and NADH on the activity of isocitrate Dehydrogenase





Regulation of the TCA Cycle



Application: Dietary deficiency of thiamine (vitamin B1)

- A serious health problem in communities where rice is the major food.
- In alcoholics who are severely malnourished
- Characterized by neurologic and cardiac symptoms
- Thiamine pyrophosphate is cofactor of: pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and transketolase.
- ↑↑pyruvate and α-ketoglutarate in the blood.
- transketolase activity of red cells, easily measured, is reliable diagnostic indicator of the disease.

Application: Mercury or Arsenite (AsO33-) Poisoning.

- Both elements have a high affinity for neighboring sulfhydryls, (dihydrolipoyl groups)
- The binding of mercury or arsenite to the dihydrolipoyl groups inhibits the complex

  ➔ central nervous system pathologies.

Treatment for these poisons is the administration of sulfhydryl reagents with adjacent sulfhydryl groups