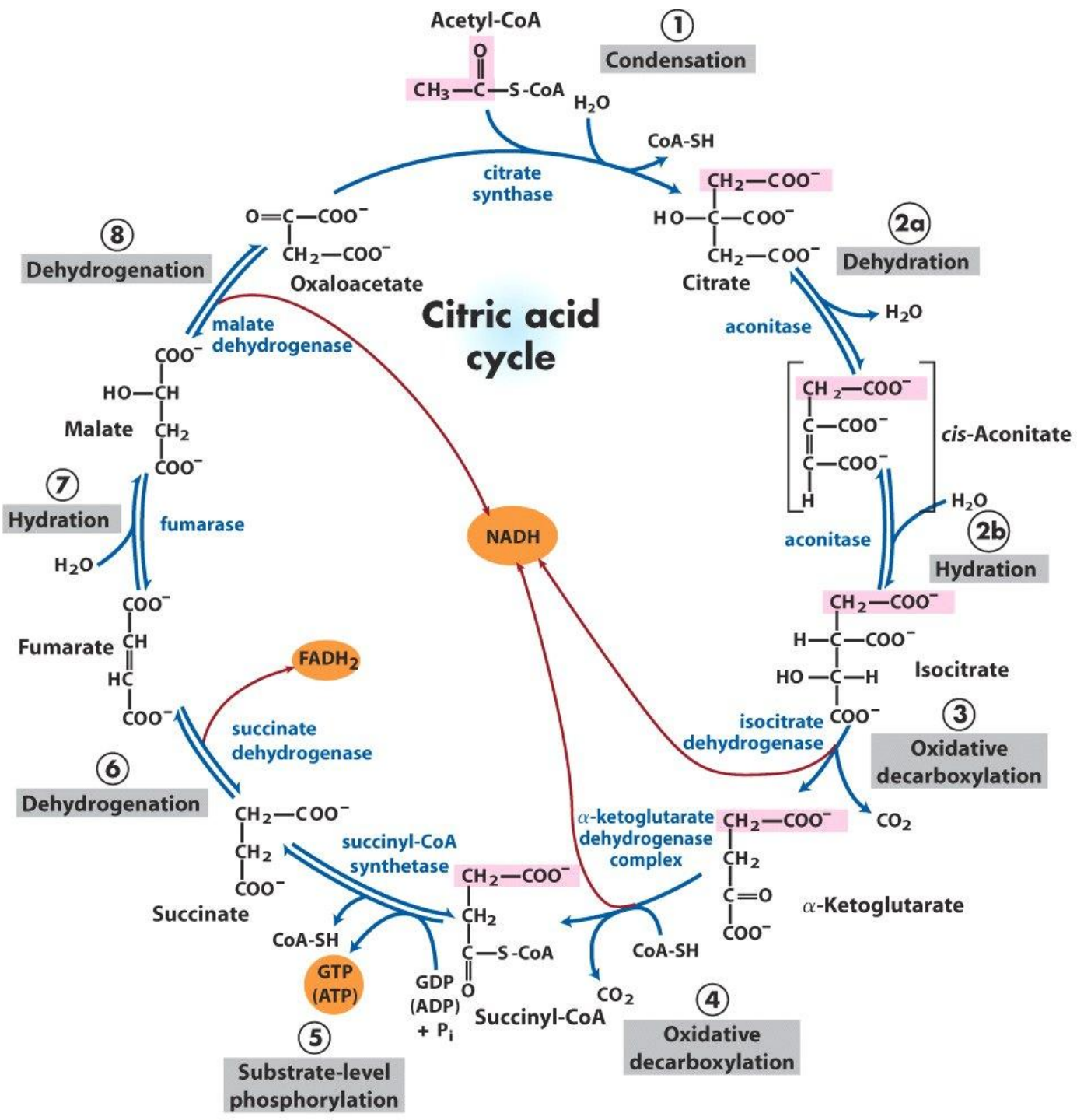
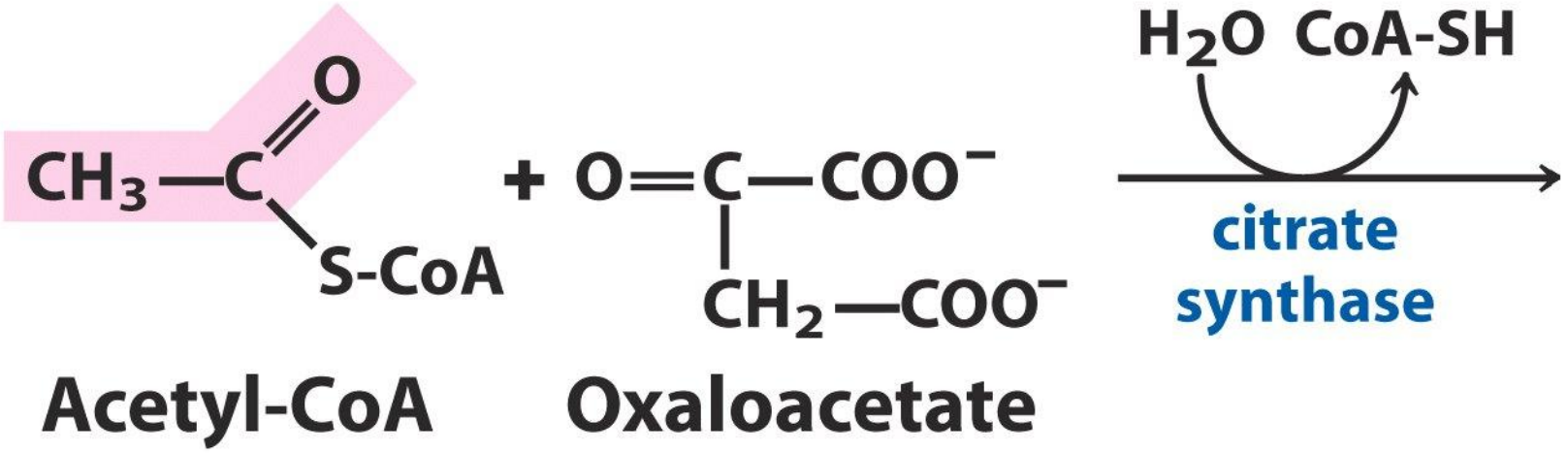
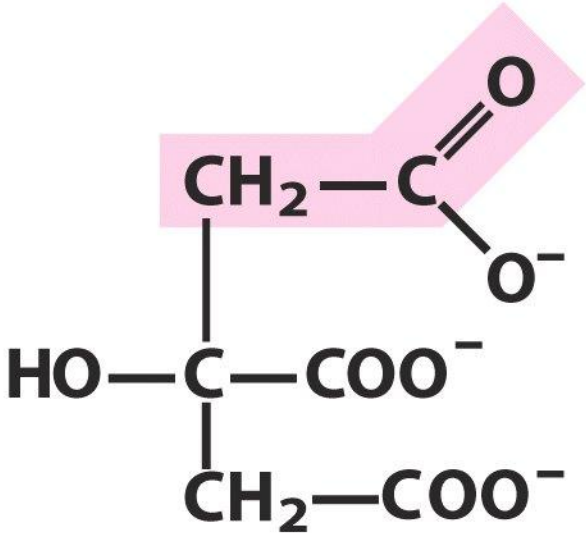
Anaplerotic reaction
Glyoxylate cycle

Dr.Sulieman Al-Khalil


Citric acid cycle
Acetyl-CoA
1
Condensation
citrate synthase
CoA-SH
H2O
Citrate
2a
Dehydration
aconitase
H2O
cis-Aconitate
aconitase
H2O
2b
Hydration
Isocitrate
isocitrate dehydrogenase
NADH
CO2
3
Oxidative decarboxylation
α-ketoglutarate dehydrogenase complex
α-Ketoglutarate
CoA-SH
CO2
4
Oxidative decarboxylation
Succinyl-CoA
GDP (ADP) + Pi
GTP (ATP)
5
Substrate-level phosphorylation
CoA-SH
succinyl-CoA synthetase
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
Fumarate
7
Hydration
fumarase
H2O
Malate
malate dehydrogenase
8
Dehydrogenation
Oxaloacetate

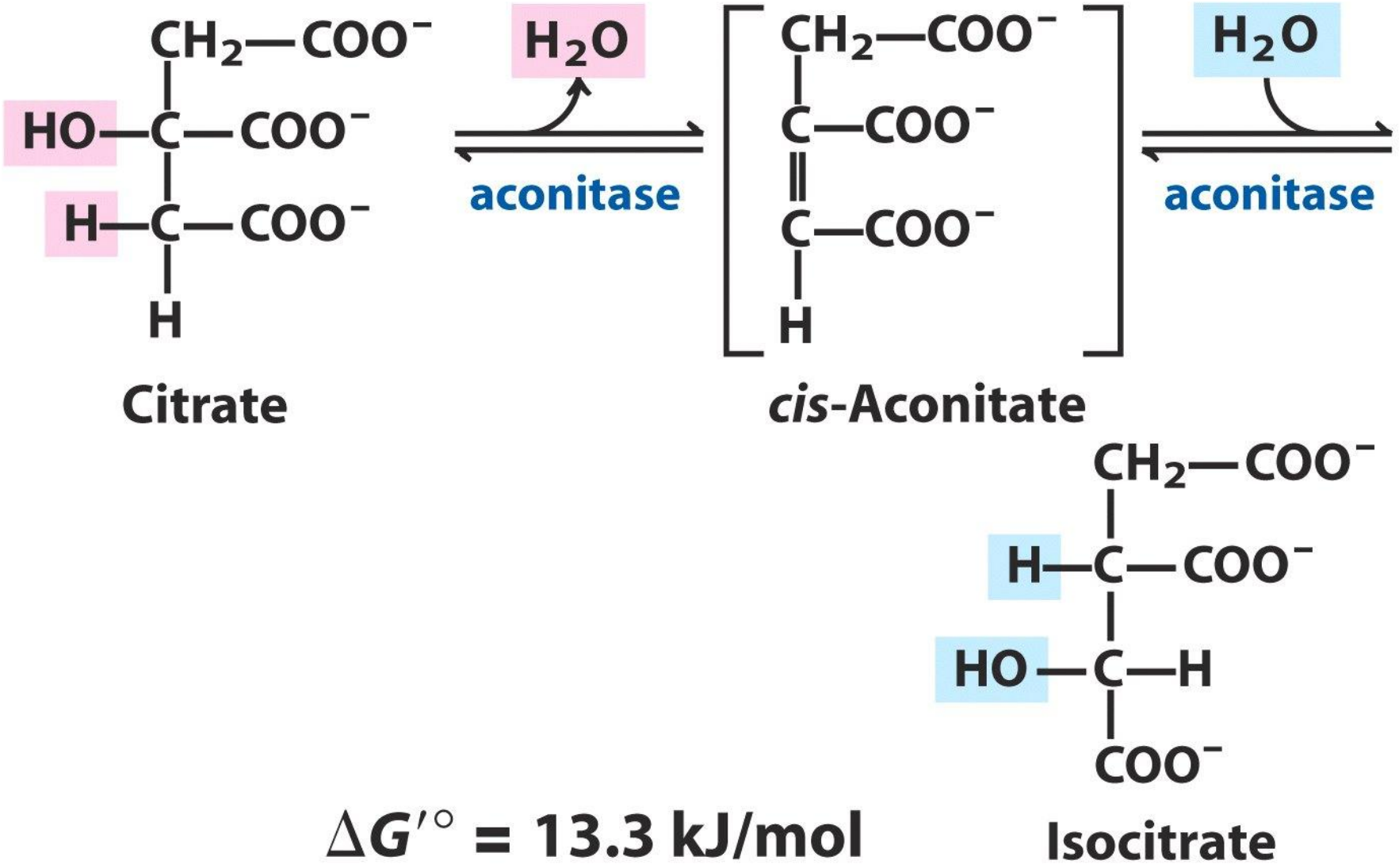
Step 1: Formation of Citrate



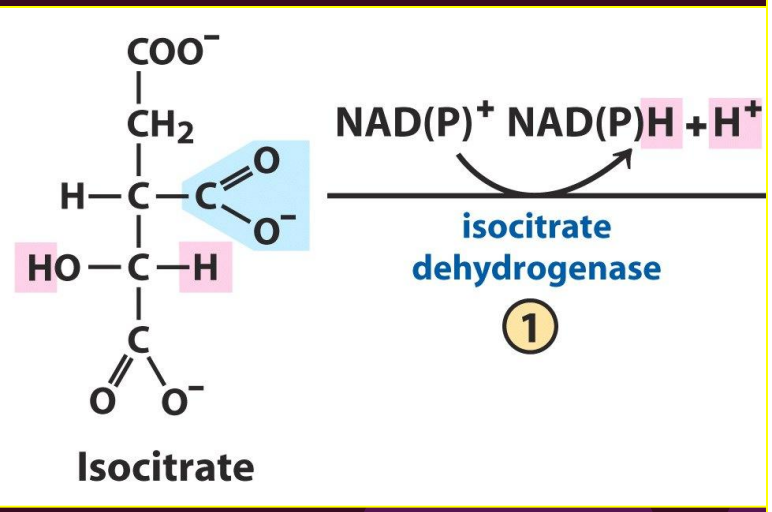


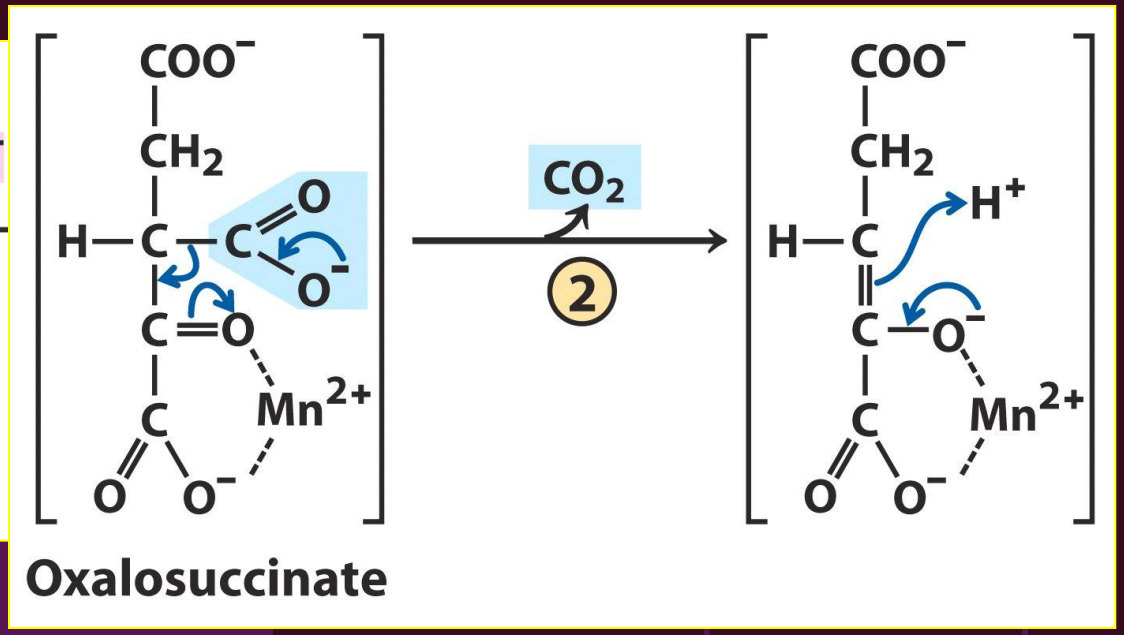
$\Delta G'^{\circ} = -32.2$ kJ/mol

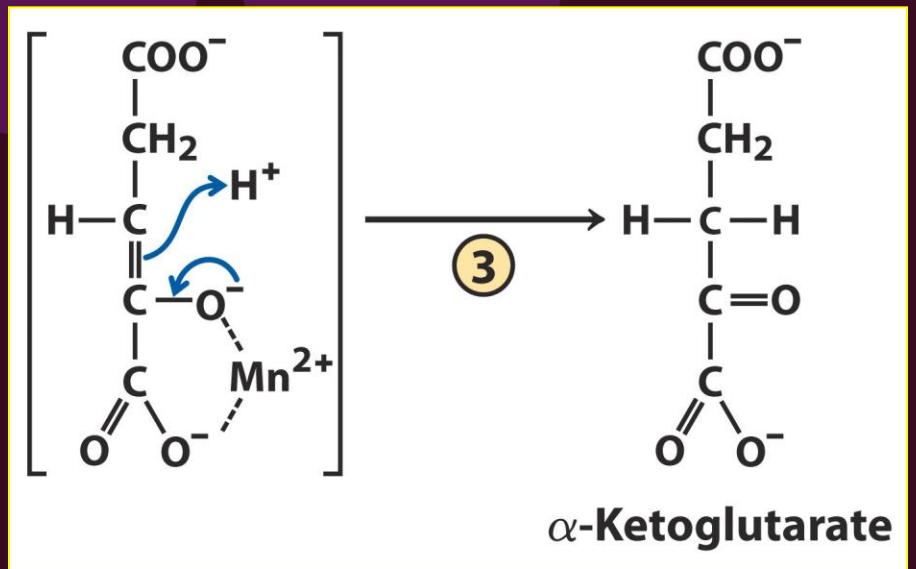
Step 2: Formation of Isocitrate



Step 3: Oxidation to a-Ketoglutarate and CO_2

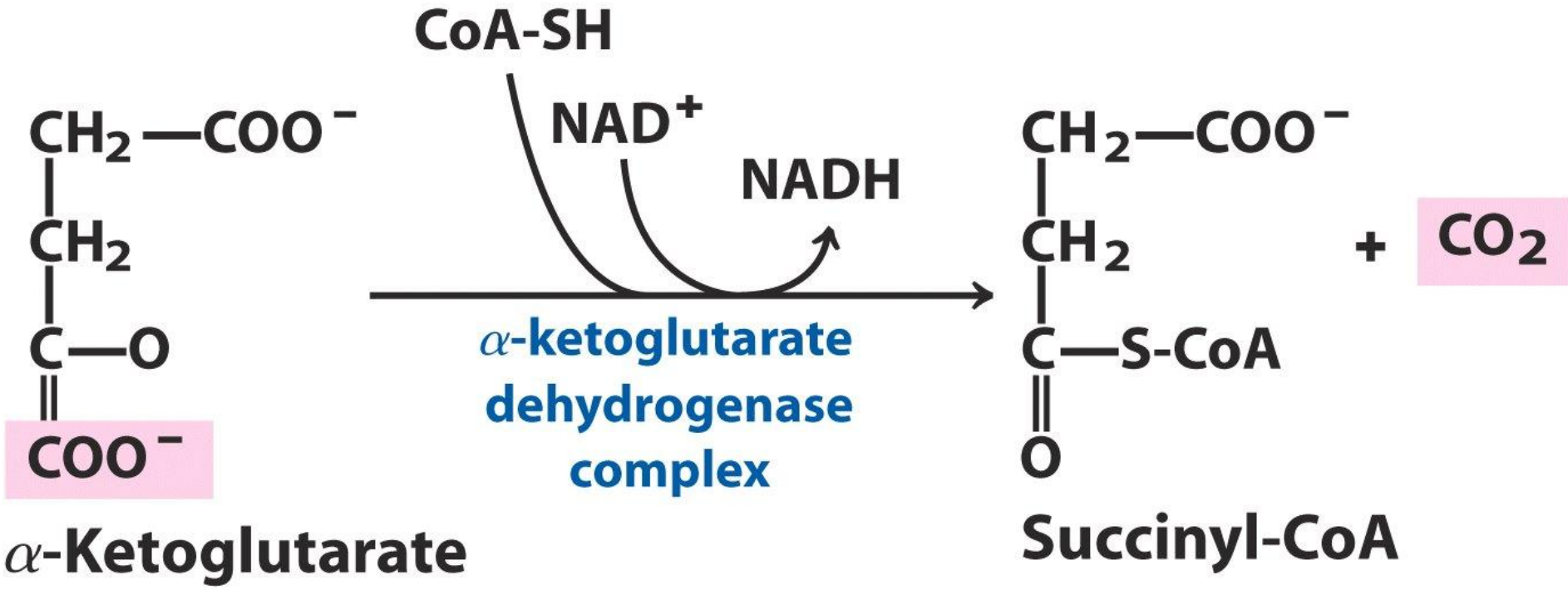






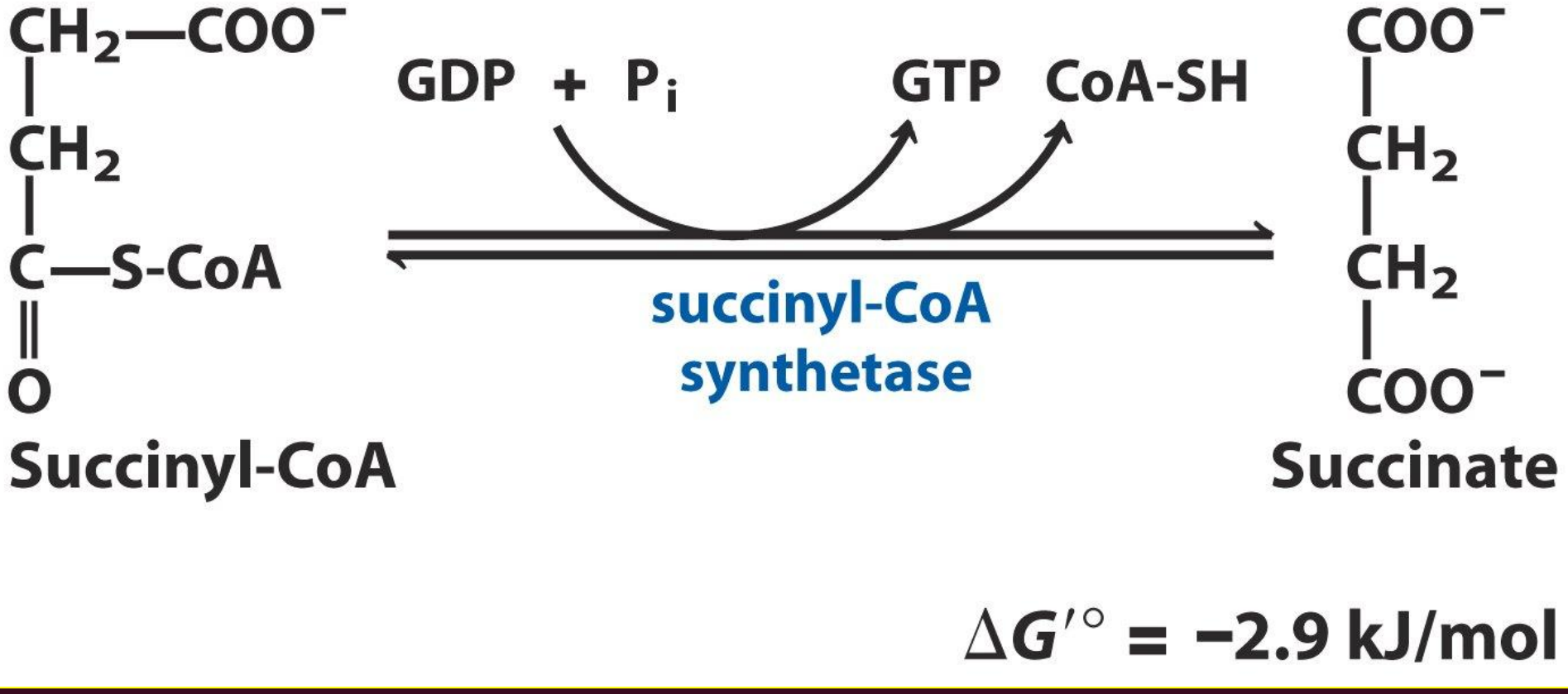
$\Delta G'^{\circ} = -20.9$ kJ/mol

Step 4: Oxidation to Succinyl-CoA and CO_2

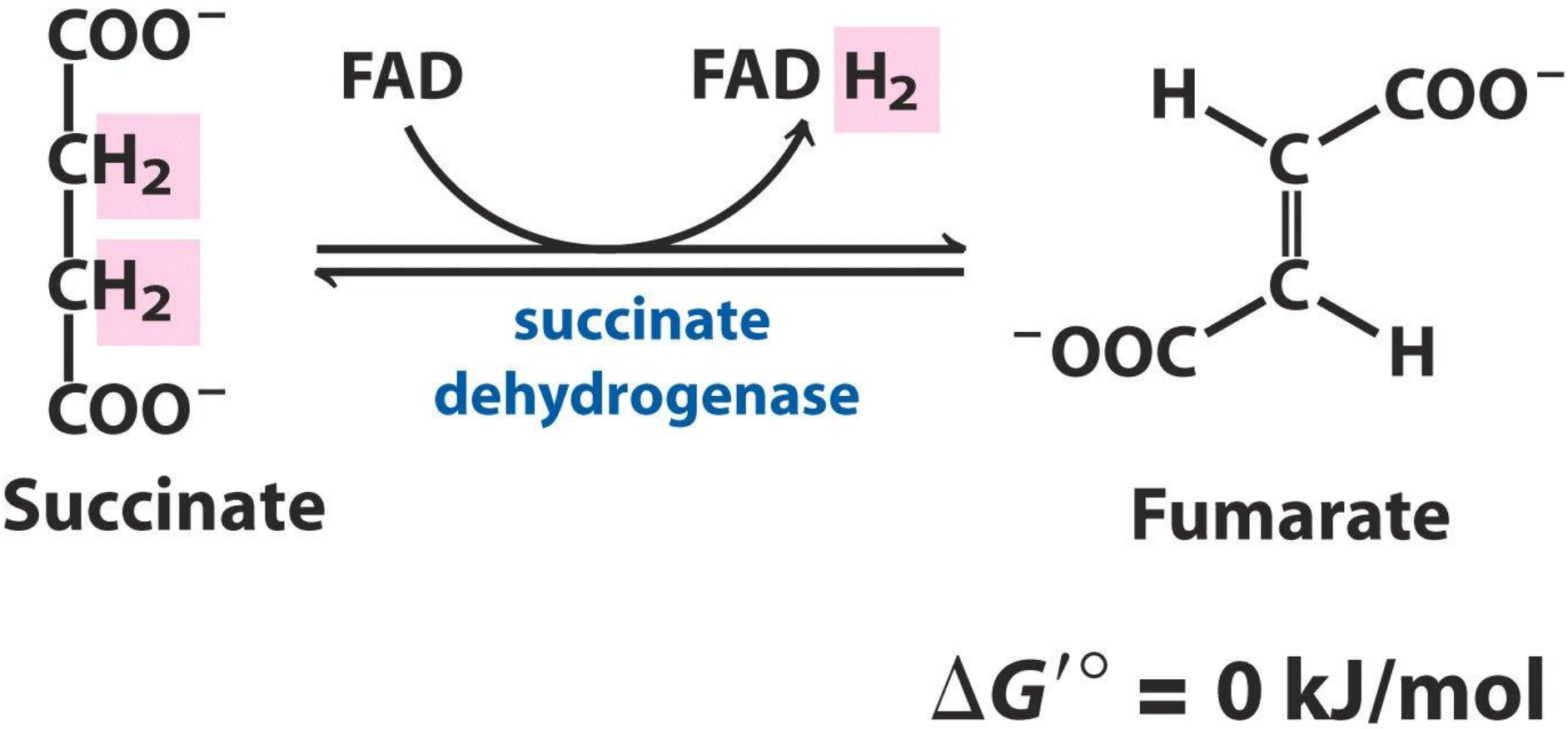


$\Delta G'^{\circ} = -33.5$ kJ/mol

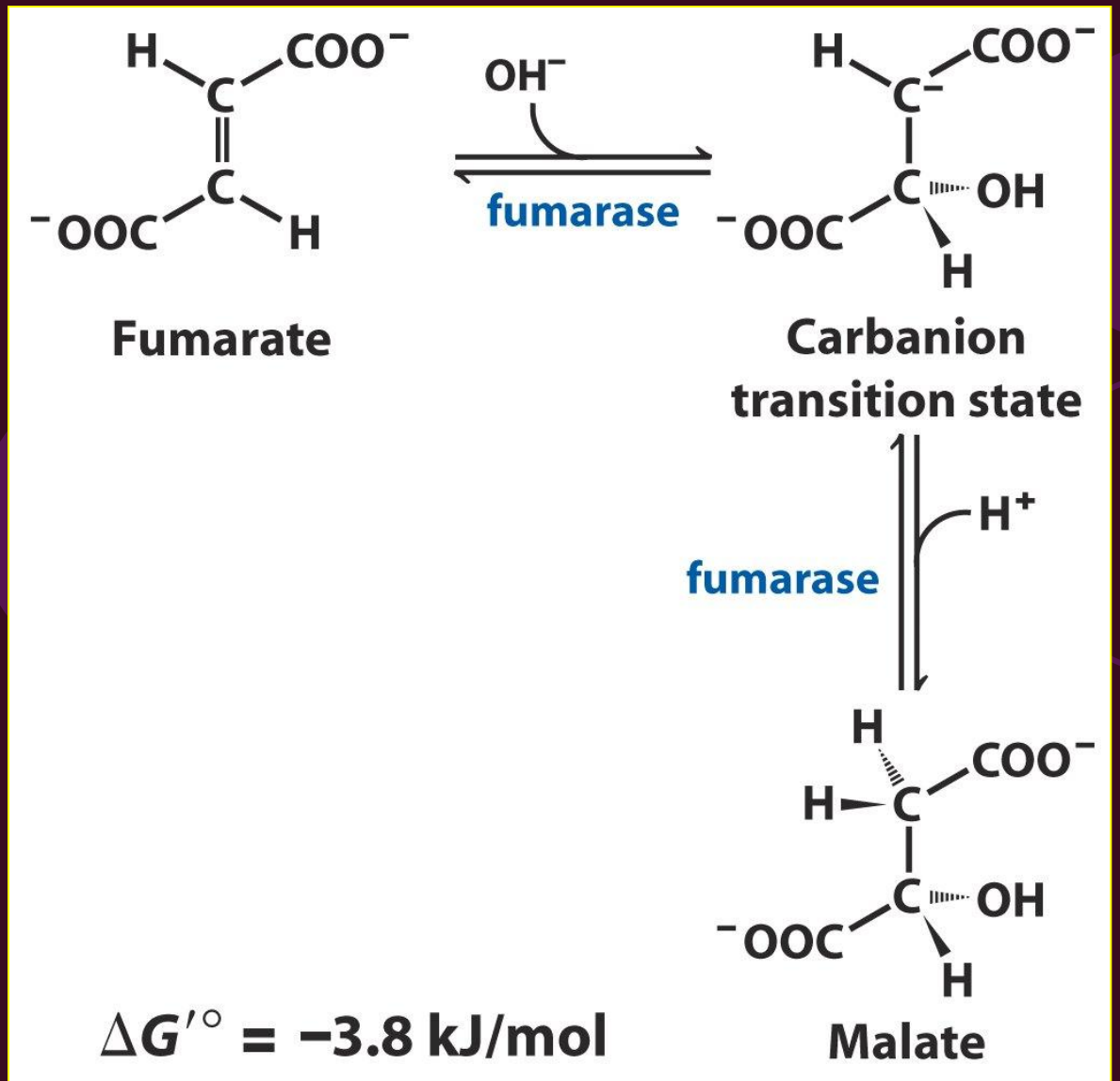
Step 5: Conversion to Succinate



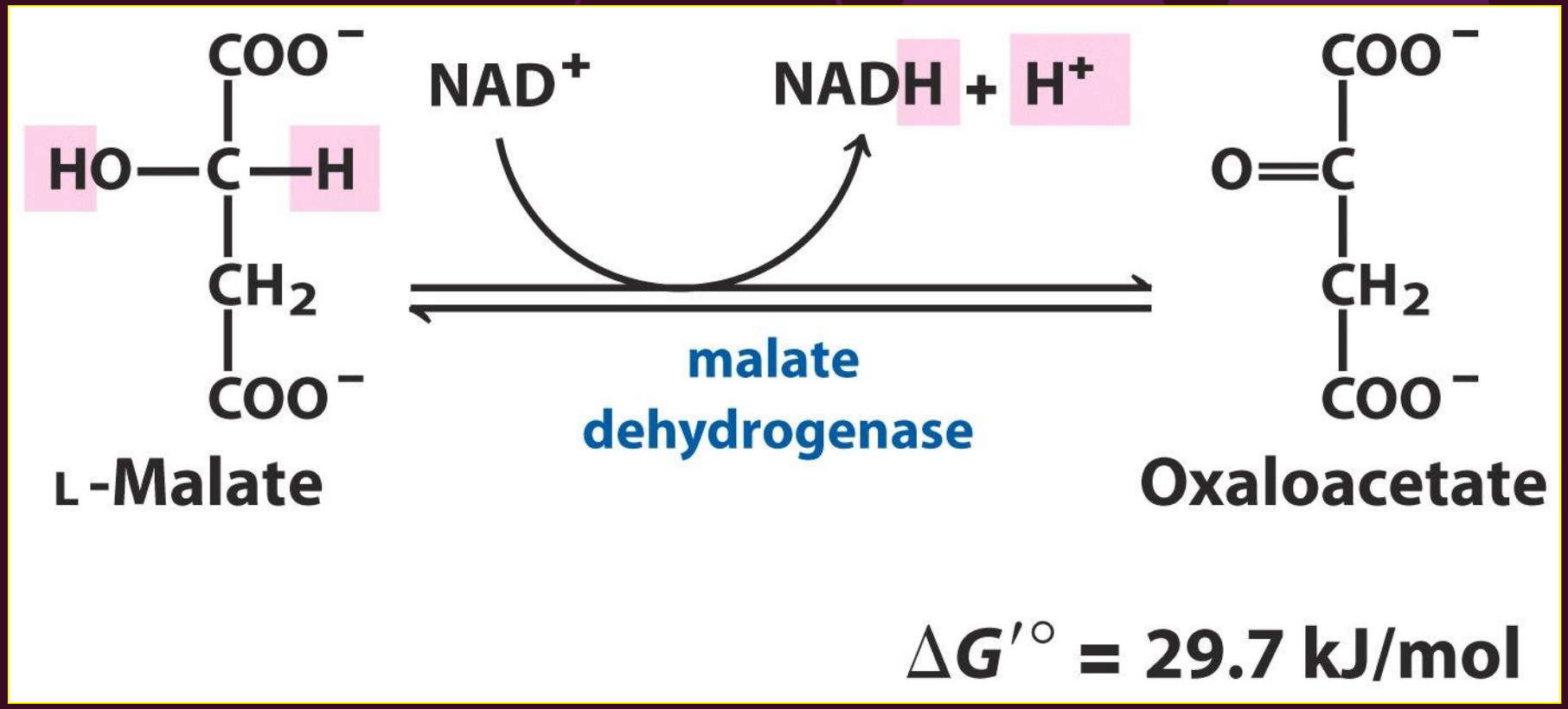
Step 6: Oxidation to Fumarate



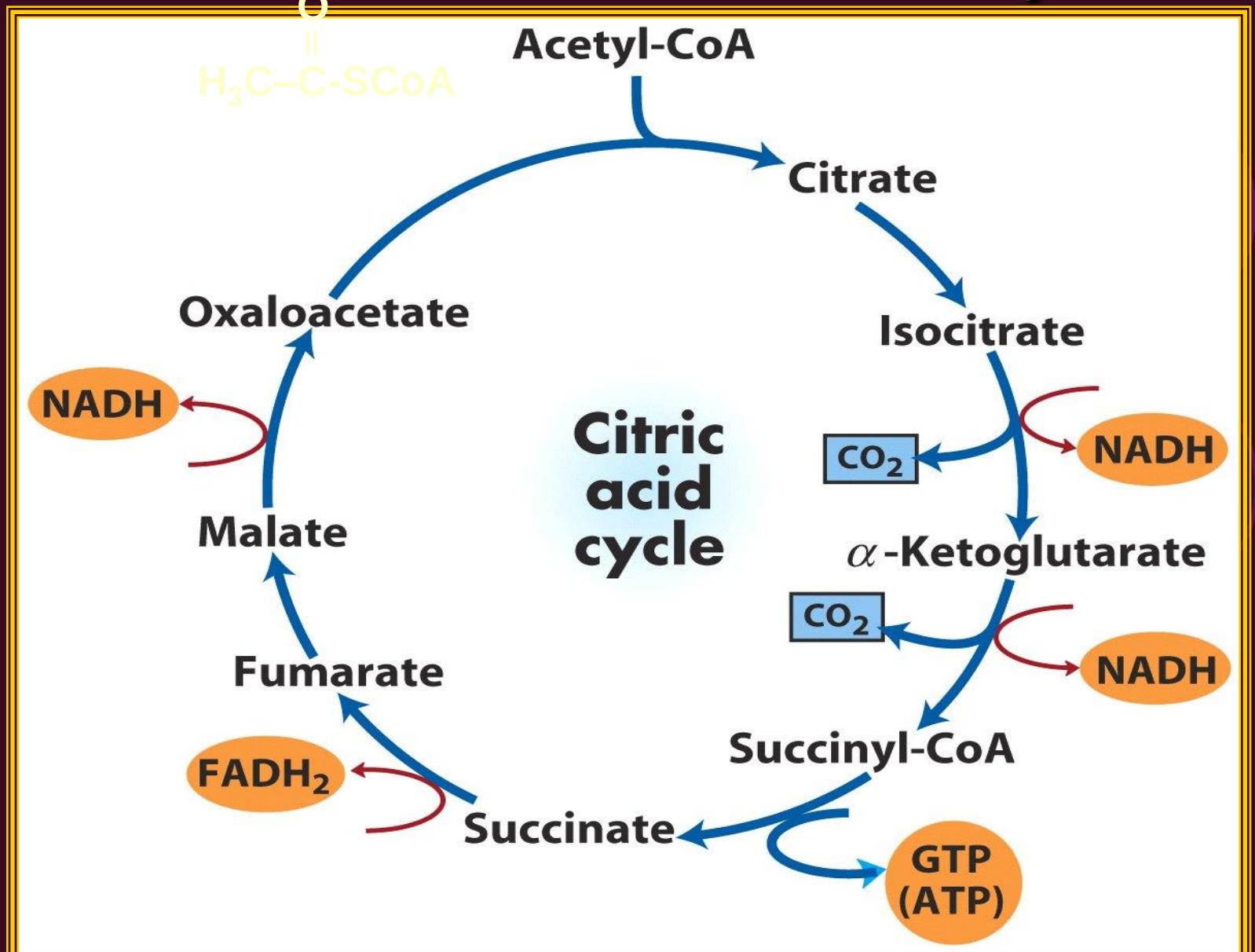
Step 7: Hydration to Malate



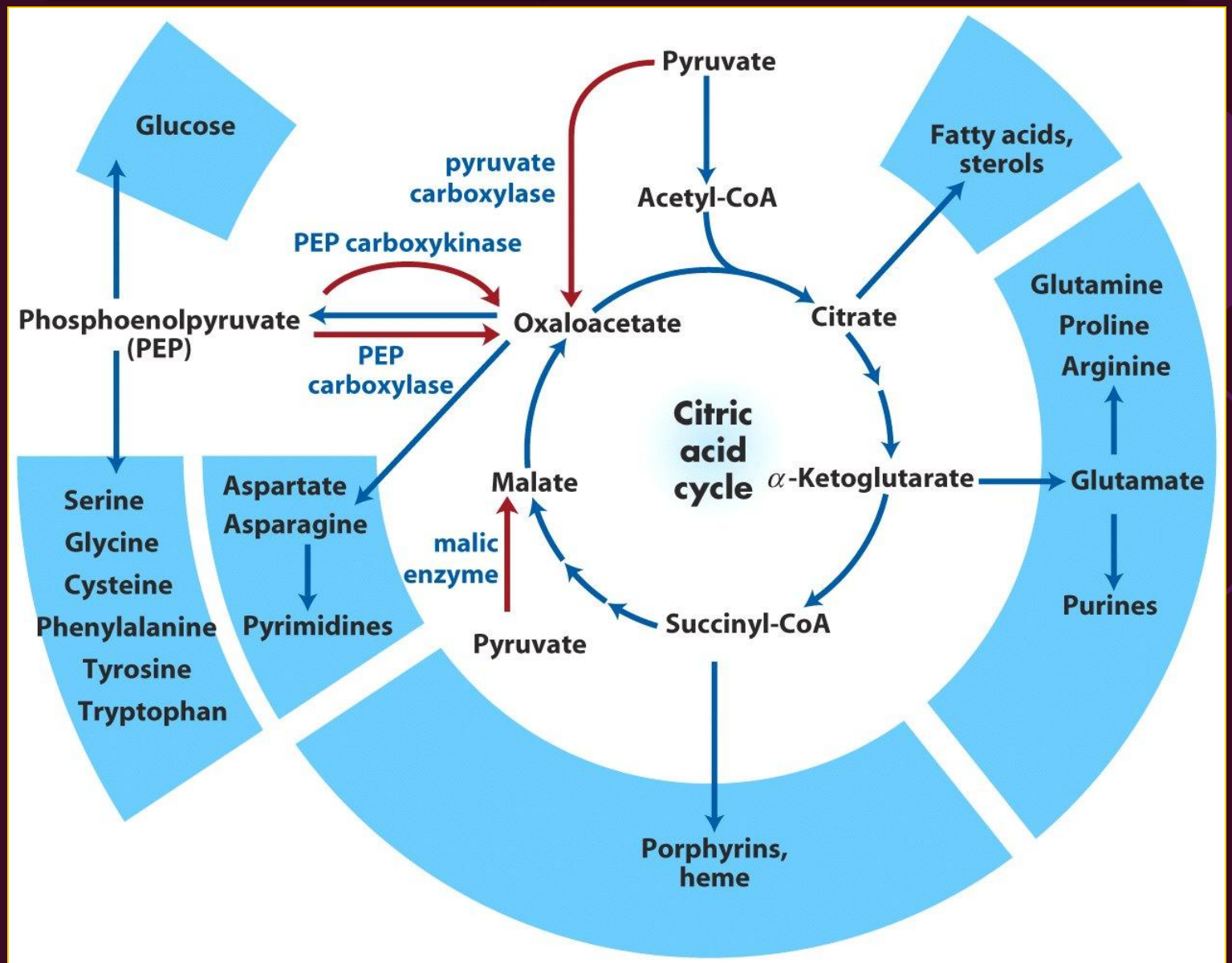
Step 8: Oxidation to Oxaloacetate



Products of the Citric Acid Cycle



Citric Acid Cycle in Anabolism



1. Anaplerotic reactions

- In addition to its role in catabolism, the citric acid cycle is important in anabolic metabolism by providing precursors for many biosynthetic pathways.
- These pathways include the synthesis of amino acids aspartate and glutamate from a-ketoglutarate and oxaloacetate, as well as other amino acids, purines and pyrimidines, heme, and electron carriers (cytochromes) .

Important Anaplerotic (Replenishing) Reactions

TABLE 16-2 Anaplerotic Reactions

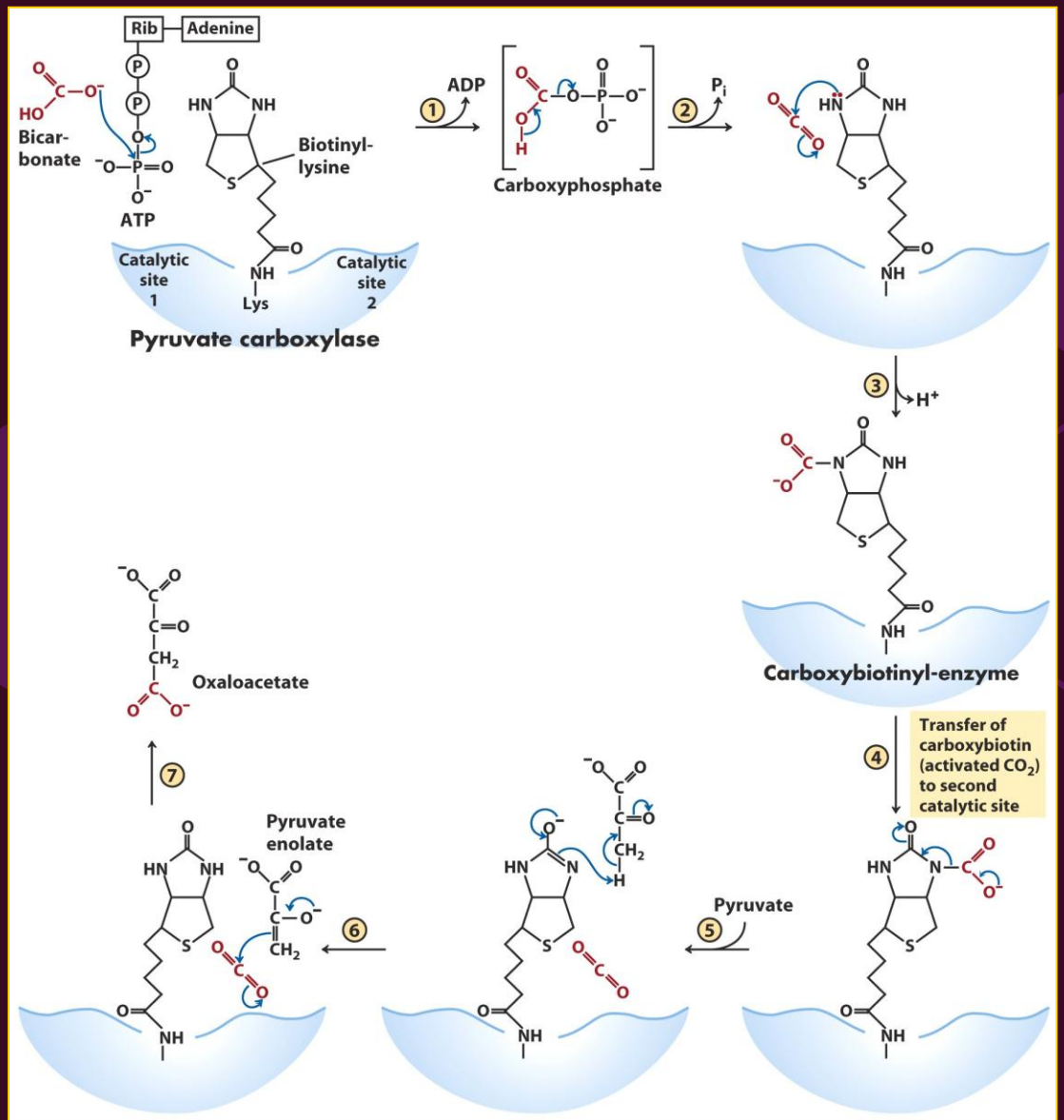
Reaction	*Tissue(s)/organism(s)*
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and prokaryotes

- As intermediates of the citric acid cycle are removed to serve in these biosynthetic pathways, they are replenished by reactions called **anaplerotic reactions.**
- All the anaplerotic reactions convert either pyruvate or phosphoenolpyruvate to oxaloacetate or malate.
- Pyruvate + HCO_3^- + ATP → oxaloacetate + ADP + Pi
- Catalyzed by **pyruvate carboxylase**
- This is the most important anaplerotic reaction in mammalian liver and kidney.

- It is activated when the citric acid cycle is low in oxaloacetate or any other intermediate.
- Pyruvate carboxylase is inactive in the absence of acetyl-CoA.
- When acetyl-CoA is present at sufficiently high concentration it activates the enzyme, leading to production of more oxaloacetate which can condense with the acetyl-CoA and allow it to enter the citric acid cycle.

- Pyruvate uses a prosthetic group called **biotin** .
- Biotin can be thought of as a carrier of activated CO2.
- The group is activated in a reaction that transfers a phosphoryl group from ATP, first to bicarbonate (HCO3–), and then to water as a carboxyl group from bicarbonate becomes attached to the biotin.
- The carboxyl group is then activated for transfer to pyruvate to form oxaloacetate.

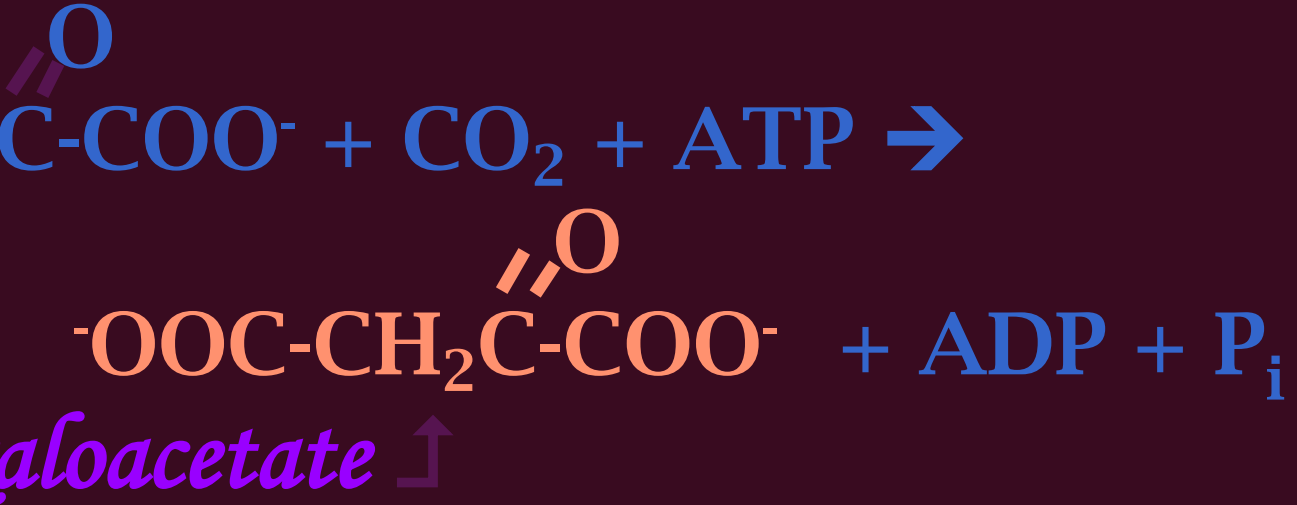
Role of Biotin in the Pyruvate Carboxylase Reaction



- Phosphoenolpyruvate + CO_2 + GDP → oxaloacetate + GTP
- Catalyzed by **PEP carboxykinase**
- Important in muscle, converts phosphoenolpyruvate to oxaloacetate instead of to pyruvate (as in glycolysis).

Anaplerotic reactions

a- pyruvate carboxylase - replaces **oxaloacetate**- most important, especially in liver and kidney.



Note: same rxn in gluconeogenesis

b- malic enzyme - replaces **malate**

-- pyruvate + CO_2 + NADPH → **malate** + $NADP^+$

c- from amino acids

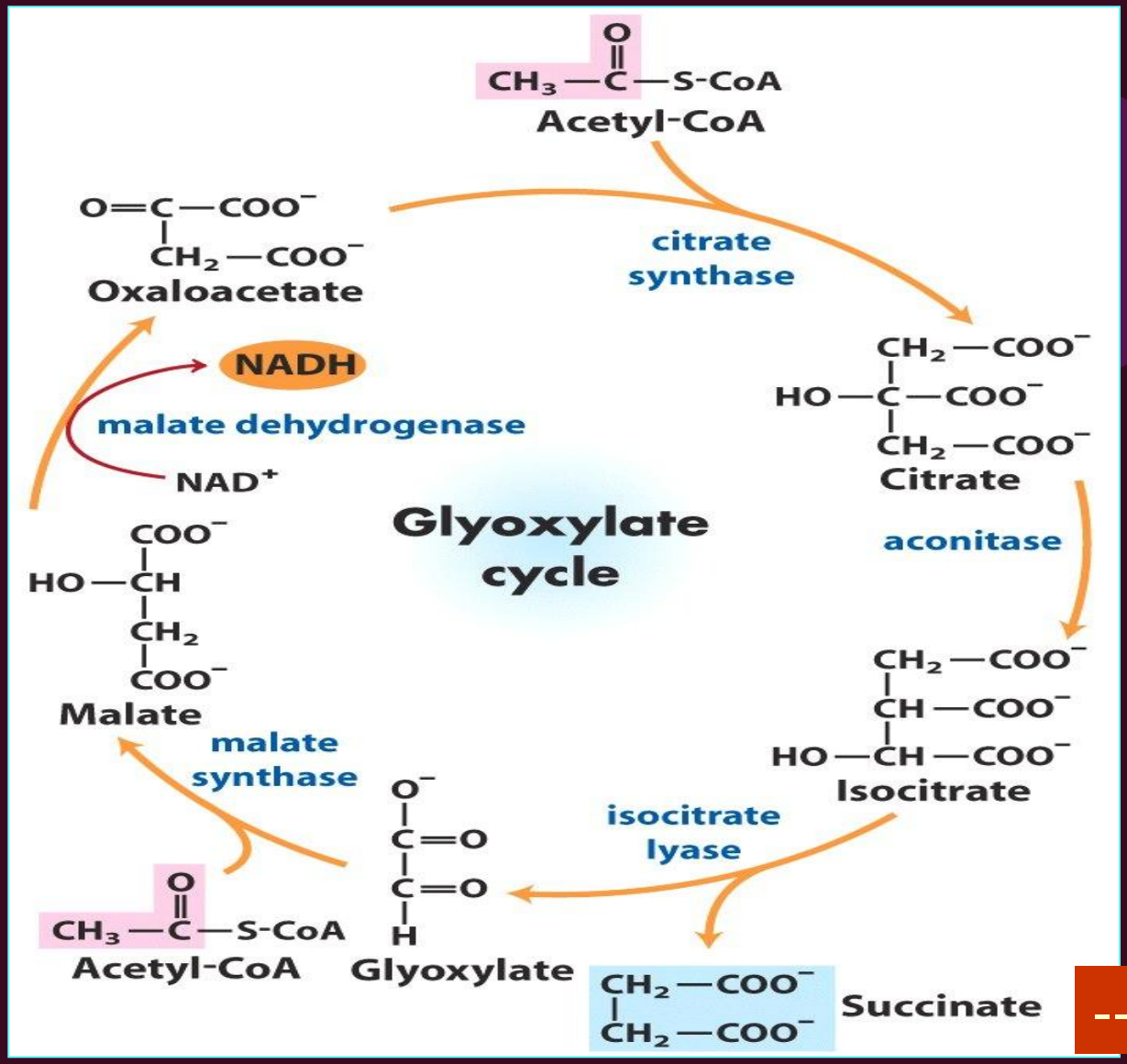
① reversals of **transaminations**

-- restores **oxaloacetate** or **α-ketoglutarate** with abundant asp or glu

② **glutamate dehydrogenase**

glu + $NAD(P)^+$ → **α-ketoglutarate** + $NAD(P)H + NH_4^+$

The Glyoxylate Cycle



--> gluconeogenesis

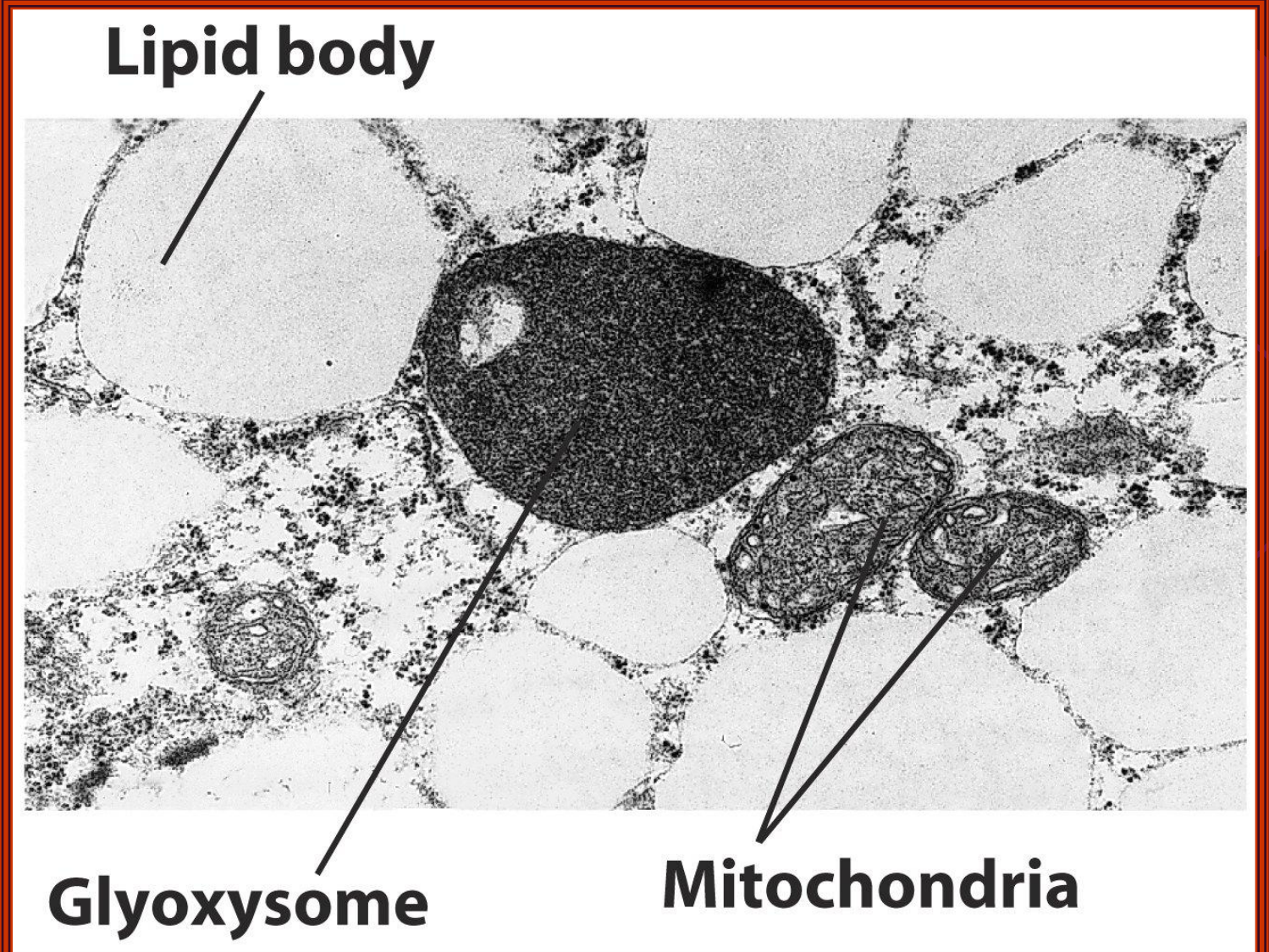
2. The glyoxylate cycle

- Vertebrates cannot convert fatty acids or the acetate derived from them into carbohydrates. However, in many organisms the glyoxylate cycle serves to convert acetate to carbohydrate :
- 2 Acetyl-CoA + NAD+ + 2H2O → succinate + 2CoA + NADH + H+
- Acetyl-CoA first condenses with oxaloacetate to form citrate, then citrate is converted to isocitrate, as in the citric acid cycle.

- Then, however, isocitrate is cleaved to give succinate and glyoxylate in a reaction catalyzed by **isocitrate lyase**.
- The glyoxylate condenses with a second molecule of acetyl-CoA to form malate, which is converted to oxaloacetate to continue in the cycle.
- The succinate can also be converted into a second molecule of oxaloacetate, which can be converted to phosphoenolpyruvate by PEP carboxykinase, the reverse reaction of the anaplerotic reaction, and then be converted to glucose by gluconeogenesis.

- In plants, the enzymes that perform the glyoxylate cycle are sequestered away from those that perform the citric acid cycle and held in **glyoxysomes .**
- Glyoxysomes are only present in certain cell types, such as in the lipid-rich seeds.
- Glyoxysomes also contain all the enzymes needed for degradation of fatty acids, allowing fatty acids present in seeds to be converted to succinate, then exported to mitochondria where it can be converted to malate, then to the cytoplasm for conversion to glucose via gluconeogenesis

Glyoxylate Cycle Takes Place in Glyoxysomes



The Glyoxylate Cycle

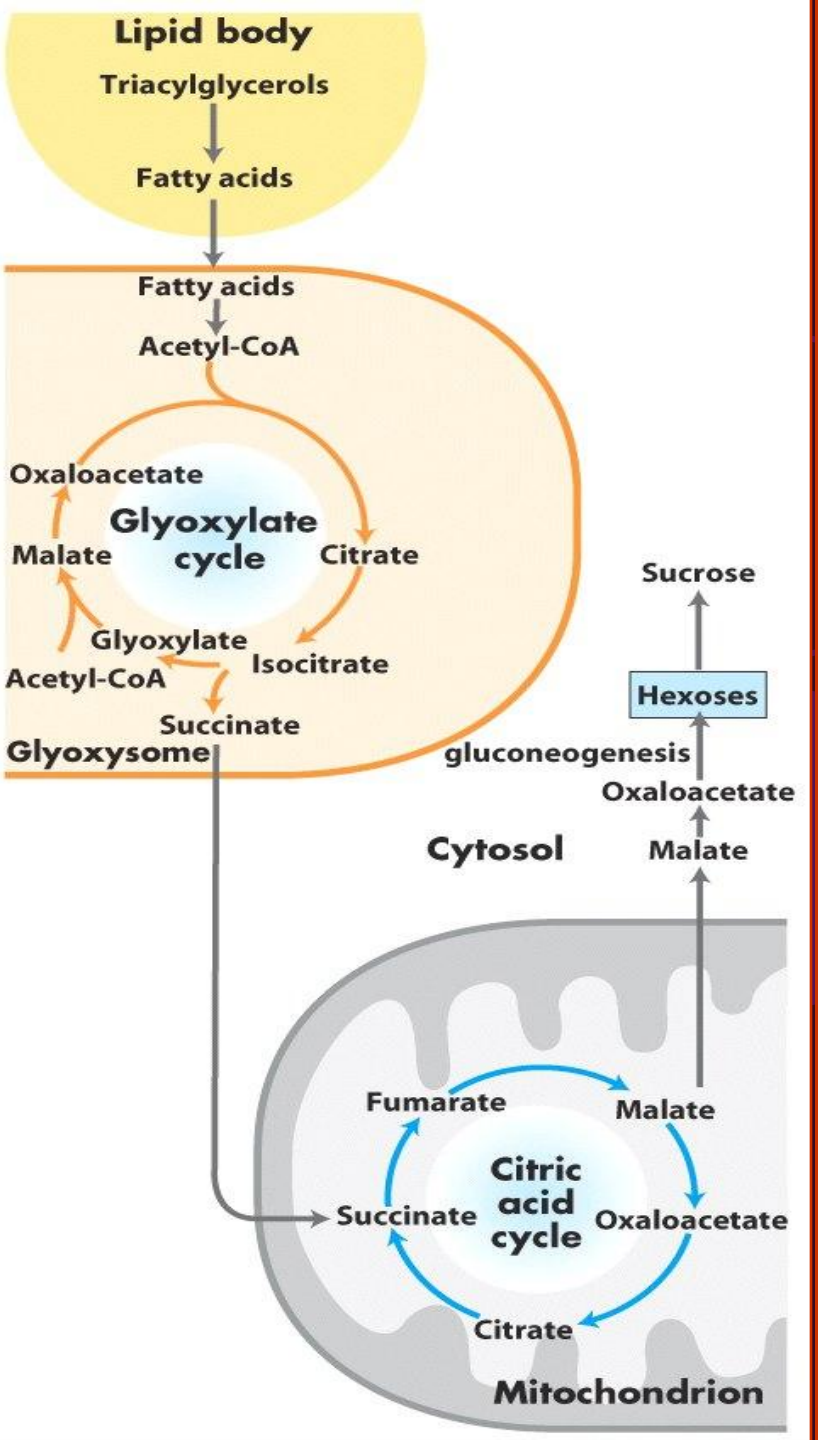
2 Acetyl-CoA + NAD^+ + $2H_2O$ -->
succinate + 2CoA + NADH + H^+

Compare with Citric Acid Cycle

Acetyl-CoA + $3NAD^+$ + FAD + GDP + P_i + $2H_2O$ -->
$2CO_2$ + CoA + 3NADH + $3H^+$ + $FADH_2$ + GTP

3. Coordinated regulation of the citric acid and glyoxylate cycles

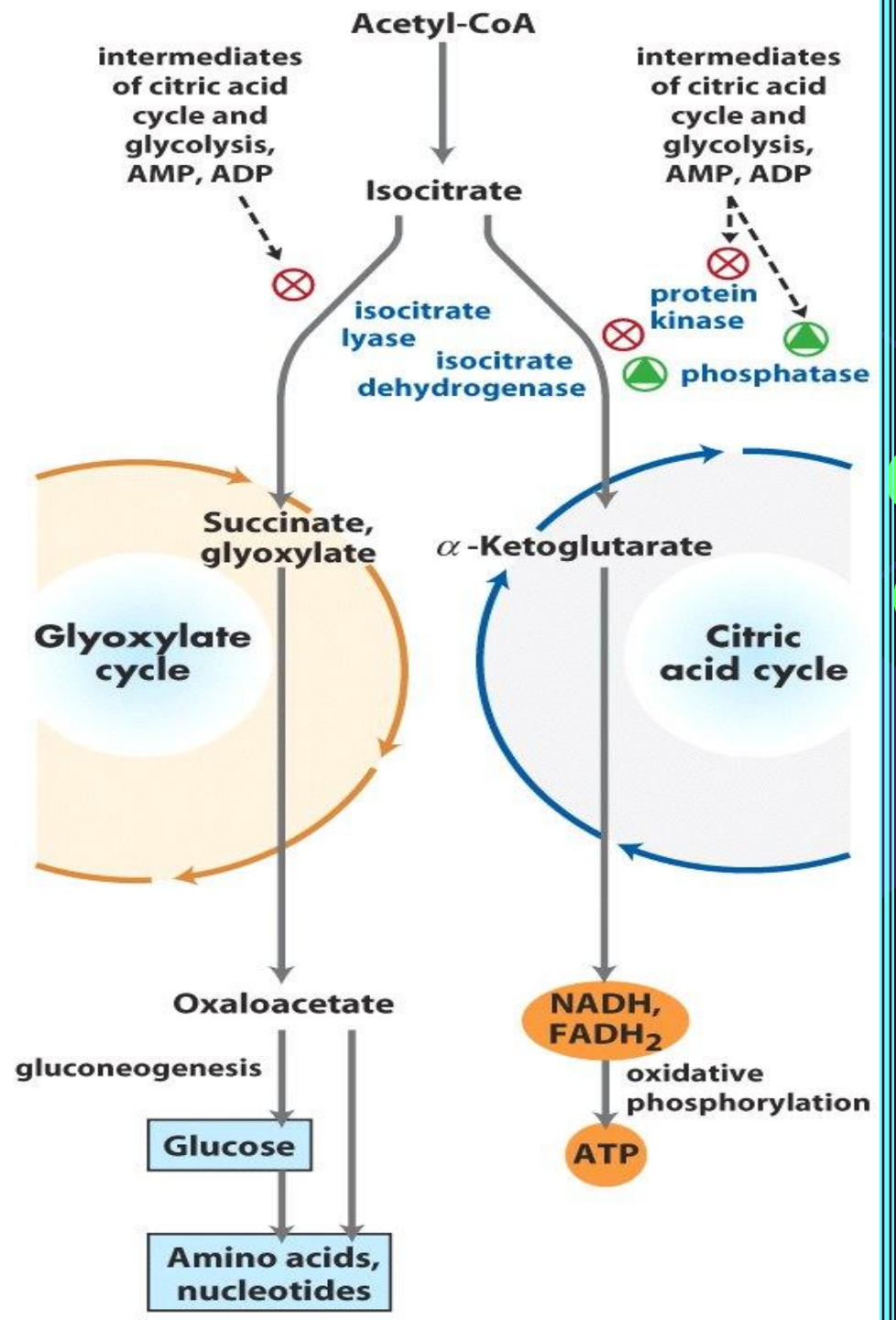
- These cycles share common intermediates and therefore must be coordinately regulated.
- In plants an important element of this regulation is that the enzymes for the two cycles are in separate compartments: the glyoxylate cycle is done in glyoxysomes and the citric acid cycle is done in the mitochondria.



Connections Between Glyoxylate Cycle and Citric Acid Cycle

- On the other hand, bacteria can also do these reactions and all enzymes are in the same compartment; the cytoplasm. *E. coli* can use these reactions to grow on acetate as their sole source of carbon and energy!
- Isocitrate is a critical intermediate because it can enter either pathway .
- It's not surprising that this is an important point of regulation.
- Isocitrate dehydrogenase (which sends isocitrate onward in the citric acid cycle) is inhibited by phosphorylation.

- Intermediates of the citric acid cycle and glycolysis and indicators of reduced energy supply all activate isocitrate dehydrogenase by stimulating the phosphatase that dephosphorylates isocitrate dehydrogenase, as well as by inhibiting its kinase.
- The same intermediates that activate isocitrate dehydrogenase also inhibit isocitrate lyase, so that they turn on the citric acid cycle and turn off the glyoxylate cycle



Isocitrate: An Intermediate Common to the Glyoxylate and Citric Acid Cycles

Key Points

1. Anaplerotic reactions are used to maintain adequate supplies of citric acid cycle intermediates.

2. The glyoxylate cycle (not present in vertebrates) allows some organisms to convert acetate, the product of fatty acid metabolism, into carbohydrate.

3. The glyoxylate cycle is an alternative pathway for citric acid cycle intermediates, and these two cycles are coordinately regulated.