HNS 103: BIOCHEMISTRY

LECTURE 3: KREB CYCLE (TCA cycle)

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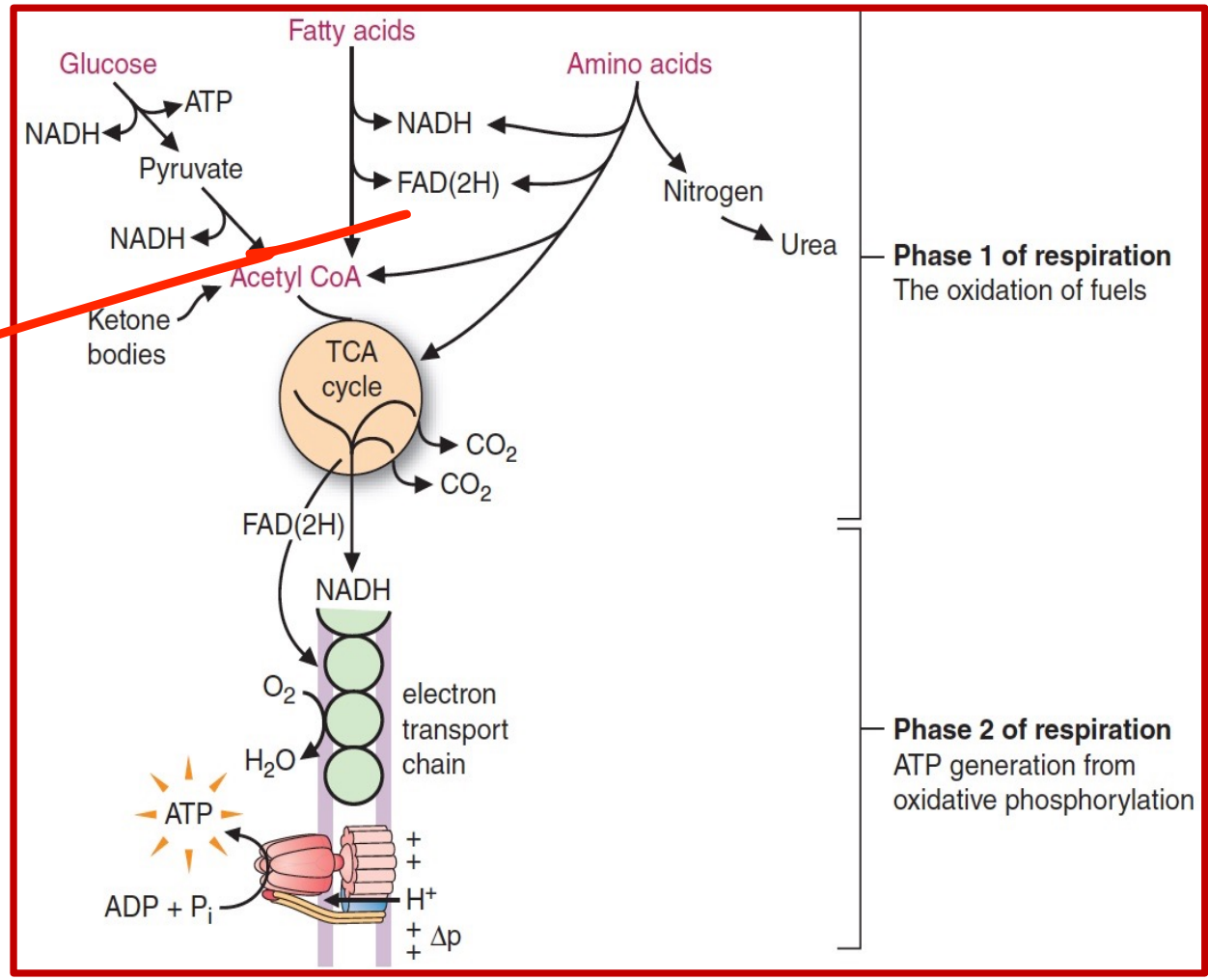
Course Outline

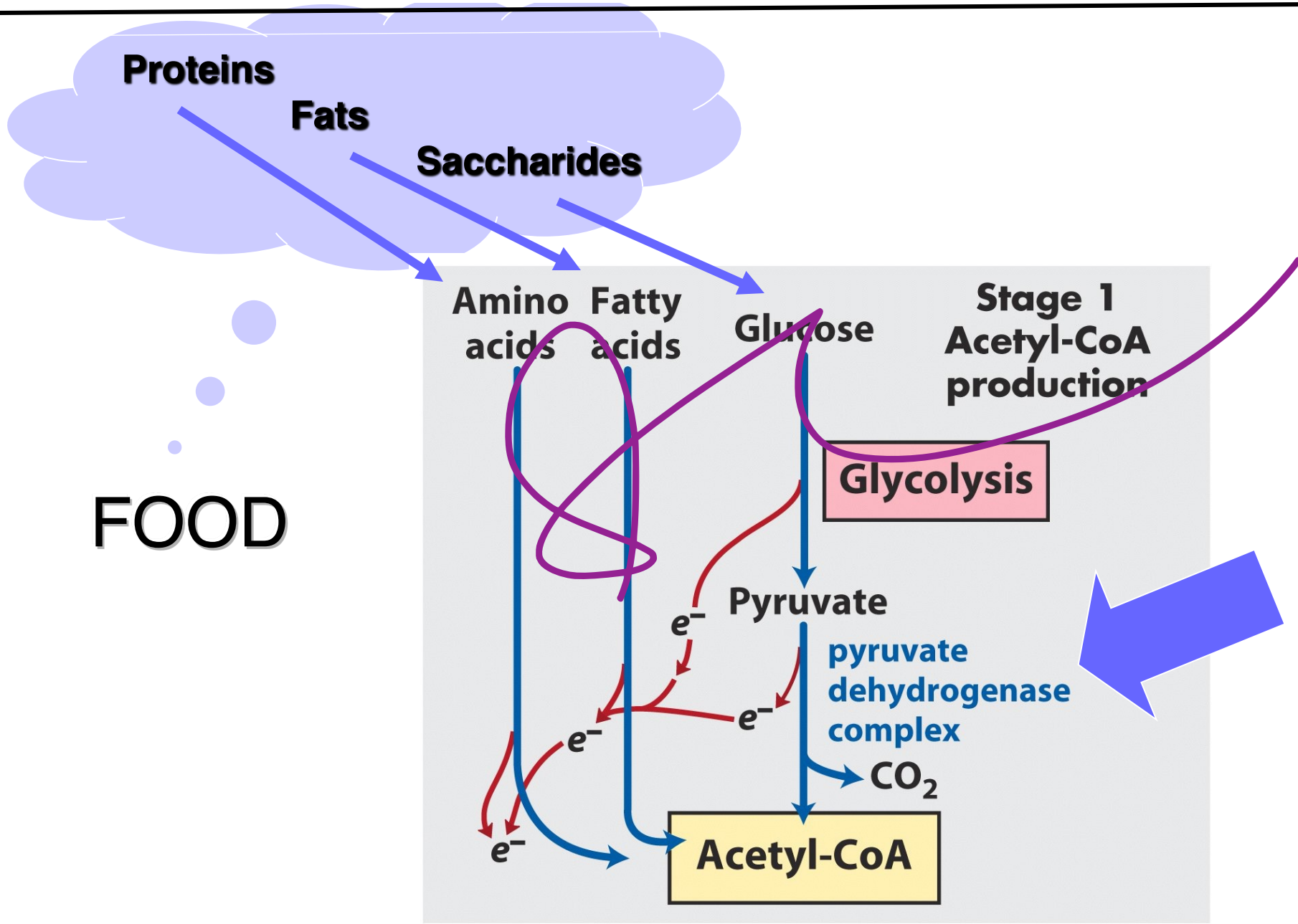
WEEK	DATE	TOPIC
1	22.02	BIOENERGETICS: standard free energy in chemical reactions, exergonic and endergonic reactions; Standard free energy of hydrolysis of ATP, Enzymatic transfer of phosphate groups to ATP; Properties of. ATP and high energy phosphate compounds
2	01.03	**CARBOHYDRATE METABOLISM:** Carbohydrate digestion & mobilization; Glycolysis and Its regulations, Substrate Level Phosphorylation; pyruvate oxidation.
3	08.03	**KREBS CYCLE:** Krebs cycle and regulation; Anaploretic reactions; ~~phosphogluconate pathway.~~
4	15.03	**MITOCHODRIAL STRUCTURE & FUNCTION:** Electron Transfer Chain; Oxidative Phosphorylation; Mechanisms of ATP generation; Uncouplers; inhibitors of ATP generation
5	22.03	**DISACCHARIDE METABOLISM:** Phosphogluconate pathway; Glycogen metabolism; Glycogenolysis and gluconeogenesis; Regulation of glycogen metabolism; Covalent modification; cAMP and hormonal regulation; Glycogen storage disease
	29.03	• CAT I

TCA cycle : An Introduction

- The TCA cycle, also known as the citric acid cycle or the Krebs cycle, is the **major energy producing pathway in the body**. This cycle occurs in mitochondria.
- Foodstuffs feed into the cycle as acetyl-CoA which is **oxidized to CO_2 + H_2O** inorder to generate energy.
- The cycle also serves in the **synthesis of fatty acids, amino acids, and glucose.**
- The cycle starts with the 4-carbon compound oxaloacetate, adds two carbons from acety-CoA, loses two carbons as CO2, and regenerates the 4-carbon compound oxaloacetate.
- Electrons are transferred by the cycle to **NAD^+ and FAD.**
- As the electrons are subsequently passed to O_2 by the electron transport chain, ATP is generated by the process of **oxidative phosphorylation.**
- ATP is also generated from GTP, produced in one reaction of the cycle by **substrate-level phosphorylation.**

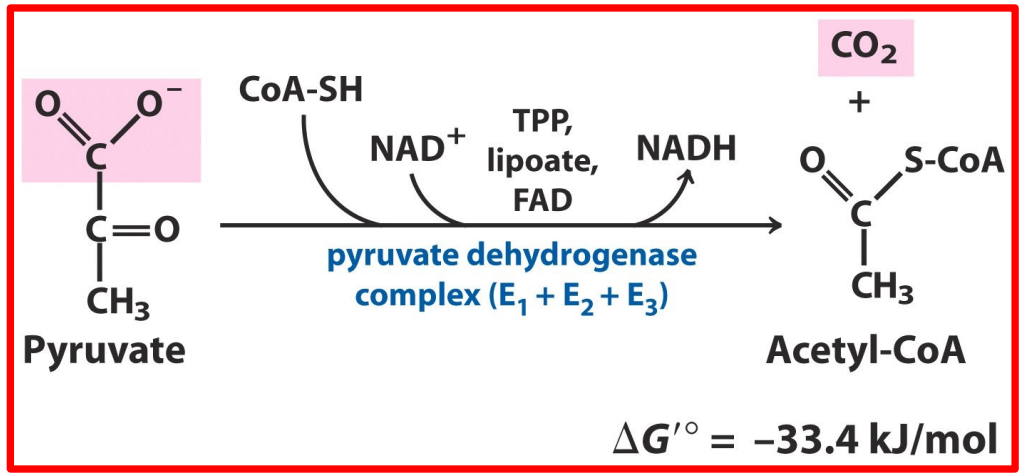


TCA cycle : Conversion of pyruvate to Acetyl-CoA



Conversion of pyruvate to Acetyl-CoA

In order for carbons from glucose to enter the TCA cycle, glucose is first converted to pyruvate by glycolysis, then pyruvate forms acetyl-CoA a reaction catalysed by pyruvate dehydrogenase complex.



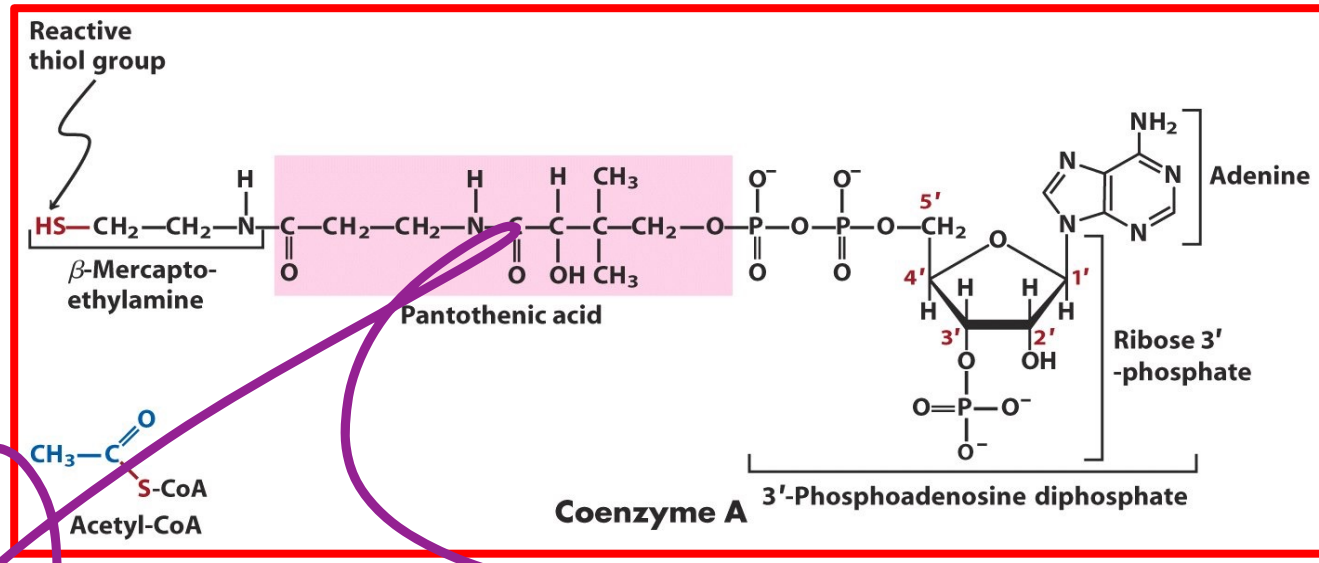
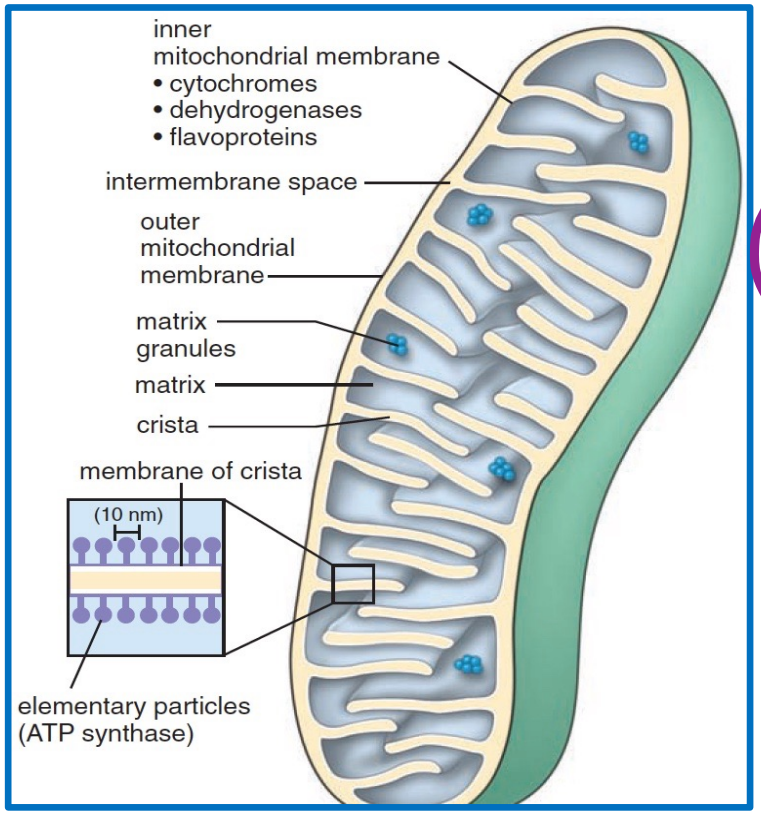


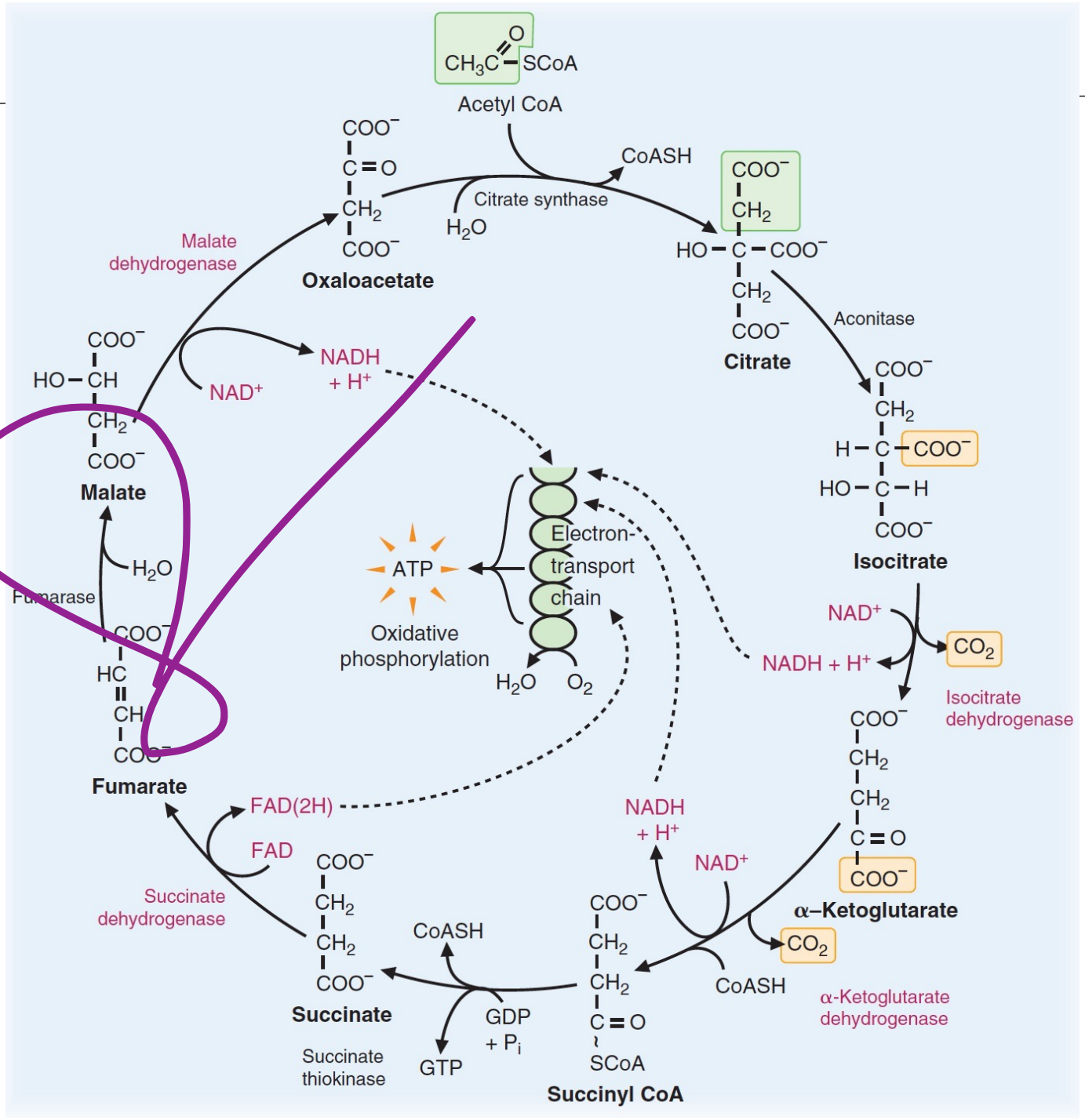
TABLE 17.1 Pyruvate dehydrogenase complex of *E. coli*

Enzyme	Abbreviation	Number of chains	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	24	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	24	Lipoamide	Transfer of the acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	12	FAD	Regeneration of the oxidized form of lipoamide

TCA CYCLE

- All the enzymes of the TCA cycle are in the **mitochondrial matrix** except **succinate dehydrogenase,** which is in the inner mitochondrial membrane.





The oxidation–reduction enzymes and coenzymes are shown in red. The entry of the two carbons of acetyl-CoA into the TCA cycle is indicated with the green box. The carbons released as CO_2 are shown with yellow boxes.

TCA CYCLE

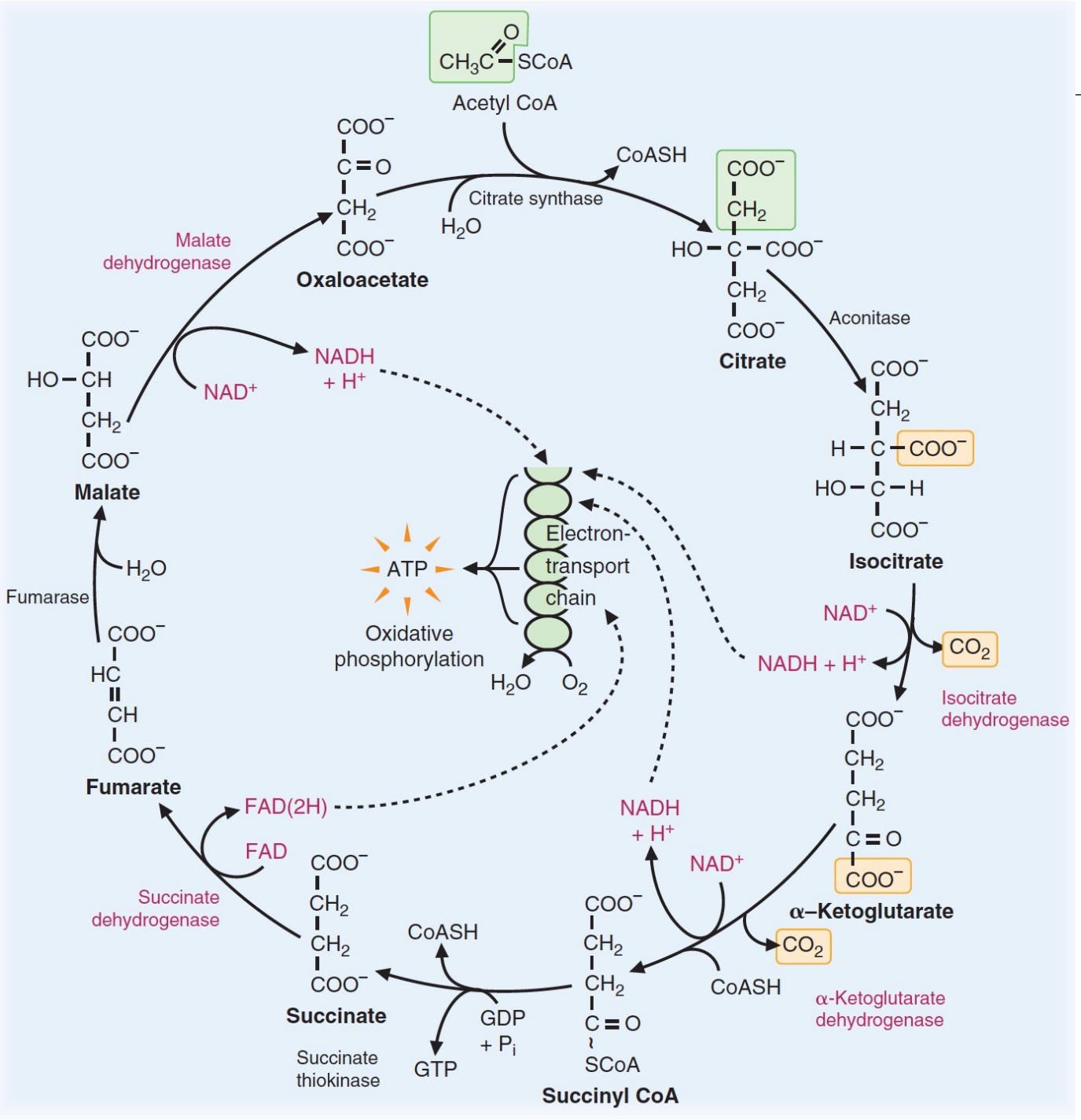
Step 1. Oxidation of the carbons of acetyl-CoA to carbon dioxide requires capturing eight electrons from the molecule.

Acetyl-CoA and **oxaloacetate** condense, forming citrate.

- Enzyme: **citrate synthase.**
- Cleavage of the high-energy thioester bond in acetyl-CoA provides the energy for this condensation.
- Citrate (the product) is an inhibitor of this reaction.

Step 2: Citrate is isomerized to isocitrate by a rearrangement of the molecule.

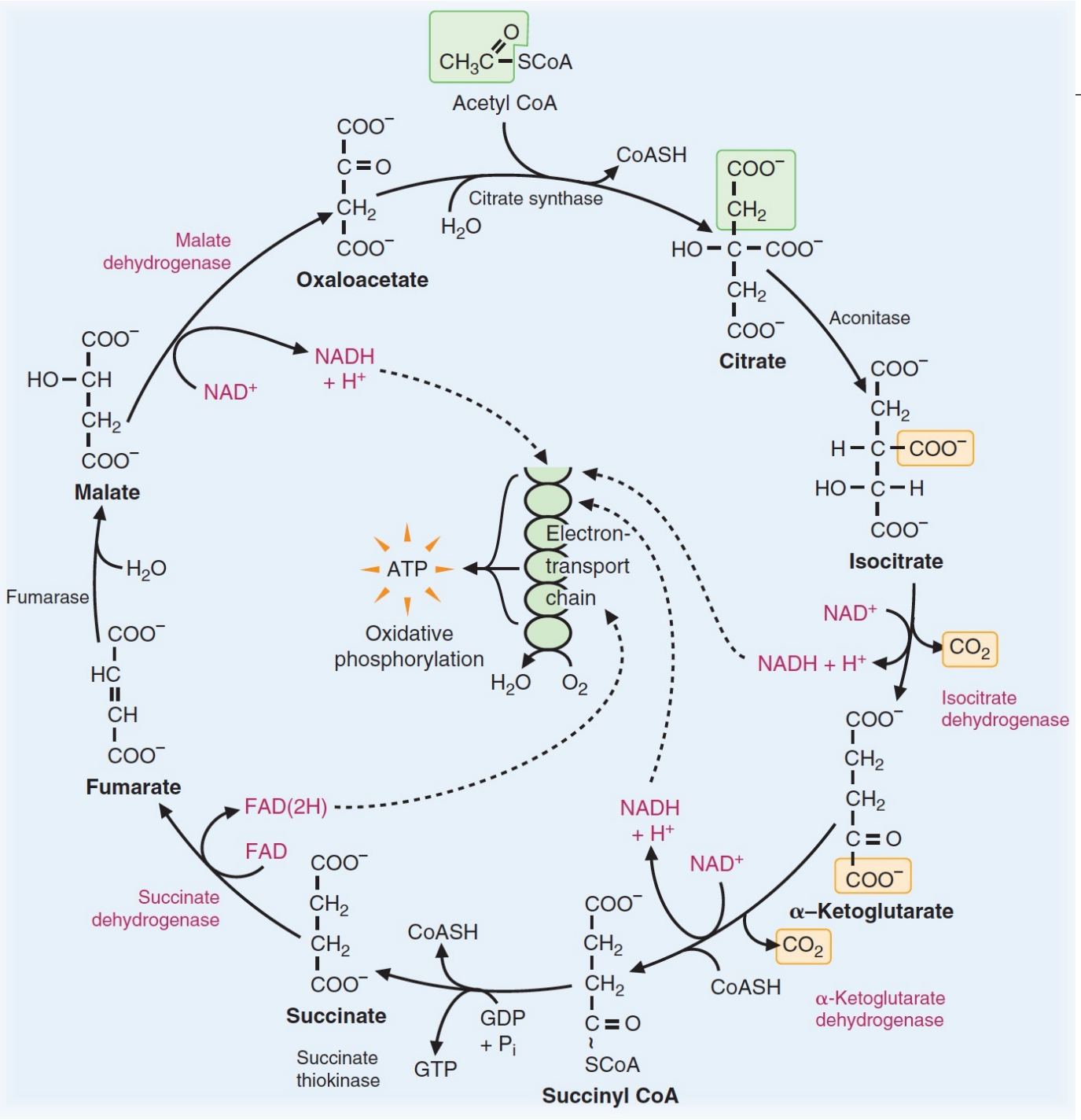
- Enzyme: **aconitase.**
- Aconitate serves as an enzyme-bound intermediate.
- Under physiological conditions, this is an unfavourable reaction, favouring citrate formation.



TCA CYCLE

Step 3. **Isocitrate** is oxidized to α-ketoglutarate, in a two-step reaction in which there is first an oxidation, and then a decarboxylation. CO_2 is produced, and the electrons are passed to NAD^+ to form **NADH and H^+**. This step **captures two of the eight electrons** present in the carbons of acetyl-CoA.

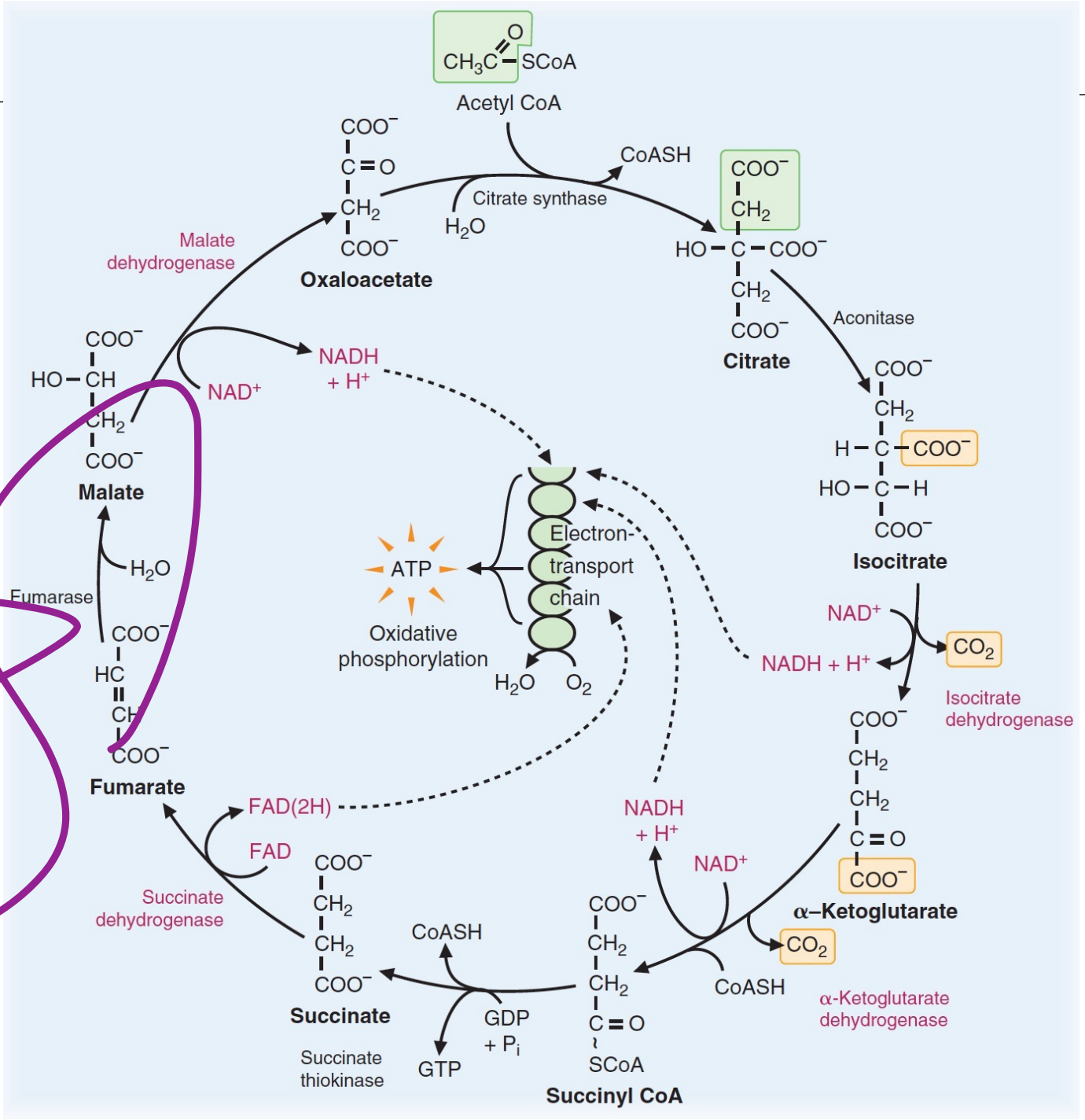
- Enzyme: **isocitrate dehydrogenase.**
- This key regulatory enzyme of the TCA cycle is allosterically activated by ADP and inhibited by NADH.



TCA CYCLE

Step 4: **α - Ketoglutarate** is converted to succinyl-CoA in an oxidative decarboxylation reaction, mechanistically the same as the pyruvate dehydrogenase reaction. CO_2 is released, and succinyl-CoA, **NADH, and H^+** are produced. This step captures another two electrons from the carbons of acetyl-CoA.

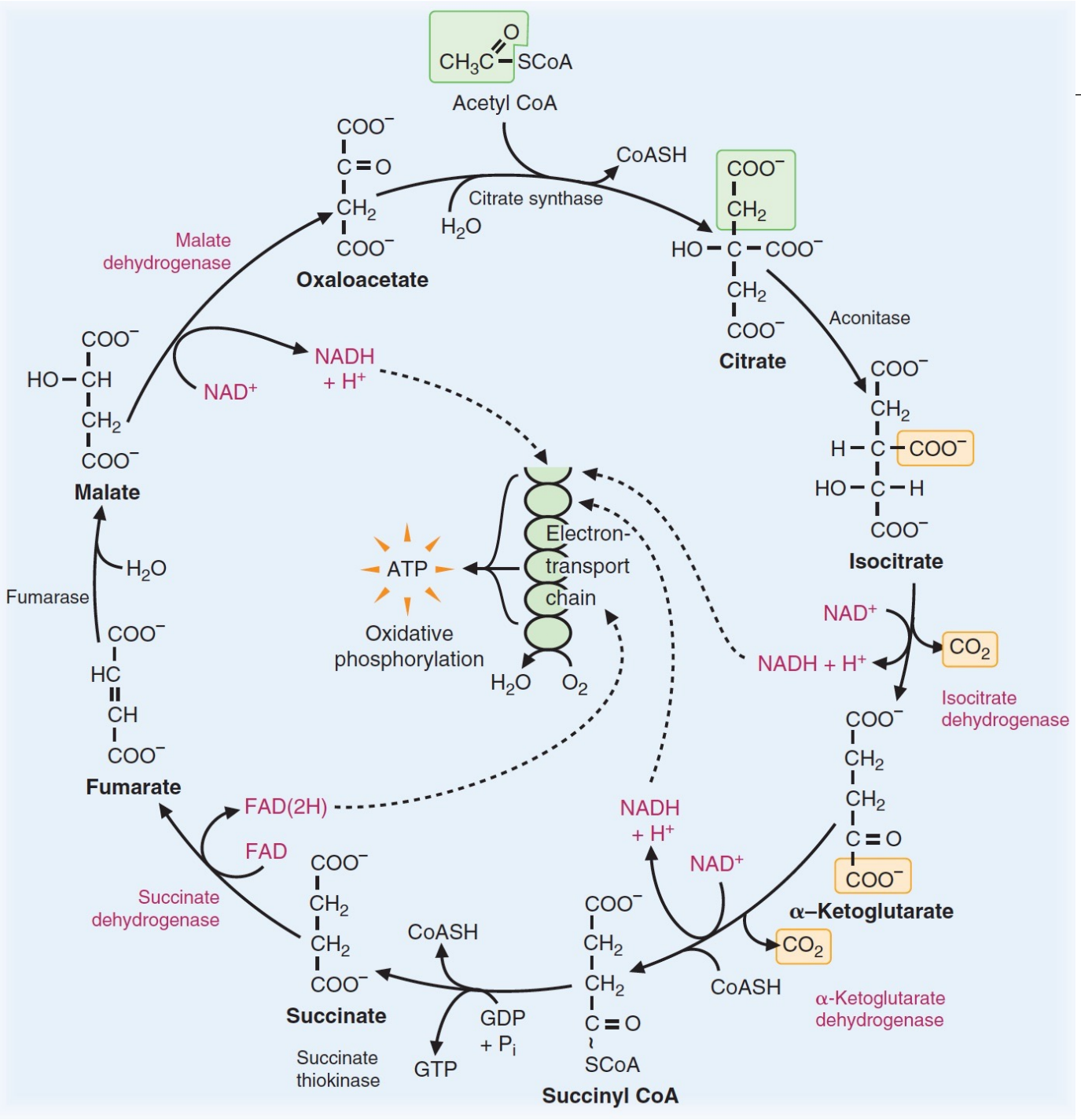
- Enzyme: **`α -ketoglutarate dehydrogenase.**
- This enzyme requires five cofactors: Thiamine pyrophosphate, lipoic acid, CoASH, FAD and NAD^+.



TCA CYCLE

Step 5. **Succinyl-CoA** is cleaved to succinate. Cleavage of the high-energy thioester bond of succinyl-CoA provides energy for the **substrate-level phosphorylation of GDP to GTP**. Since this does not involve the electron transport chain, it is not an oxidative phosphorylation; however, if electron flow were to stop, this step would also be blocked.

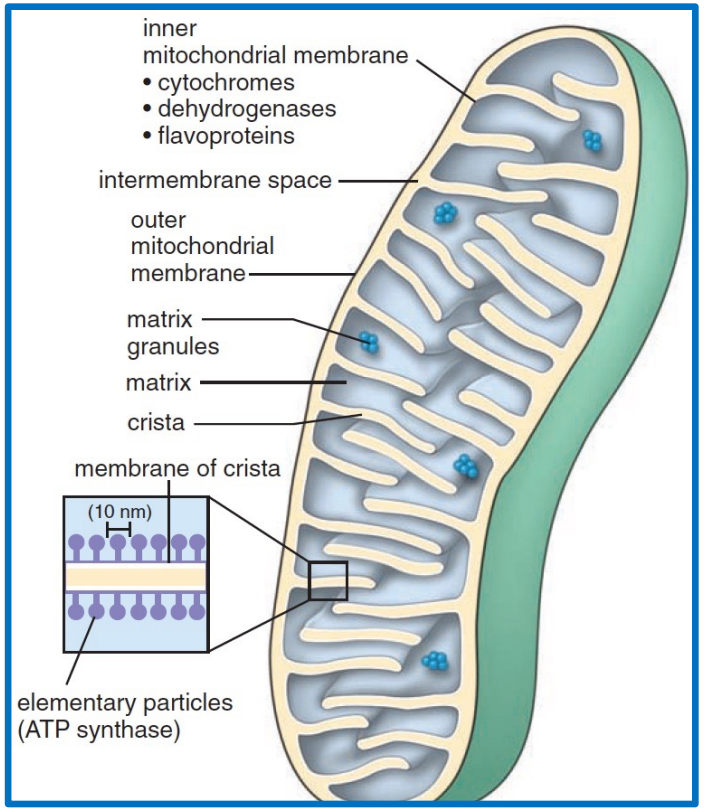
- Enzyme: **succinate thiokinase** or succinyl-CoA synthetase.

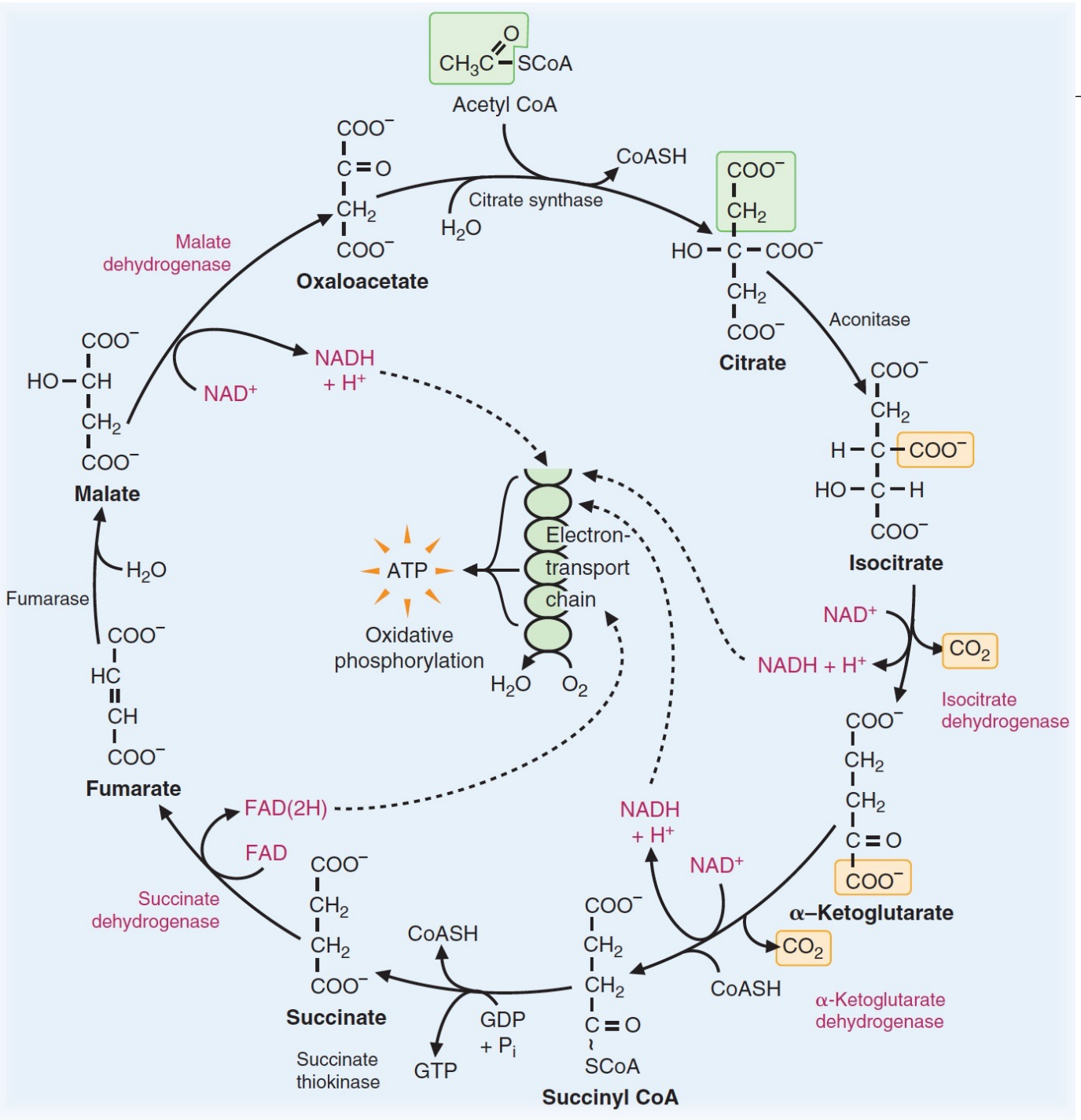


TCA CYCLE

Step 6: Succinate is oxidized to fumarate. Succinate transfers two hydrogens together with their electrons to **FAD, which forms FADH2.** After this step, six of the eight electrons from the carbons in acetyl-CoA have been captured.

- Enzyme: **succinate dehydrogenase.**
- This enzyme is present in the inner mitochondrial membrane. The other enzymes of the cycle are in the matrix.

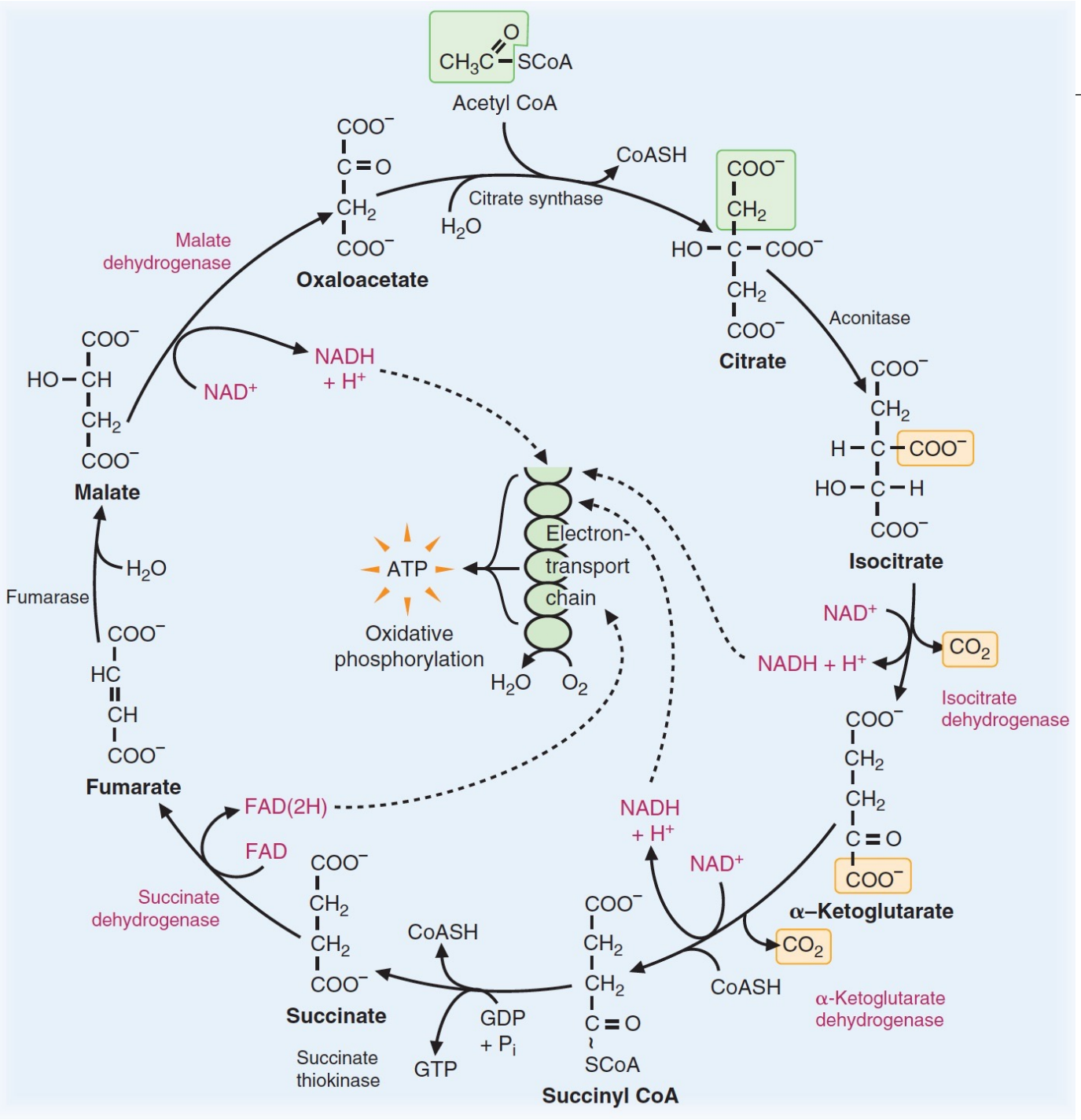




TCA CYCLE

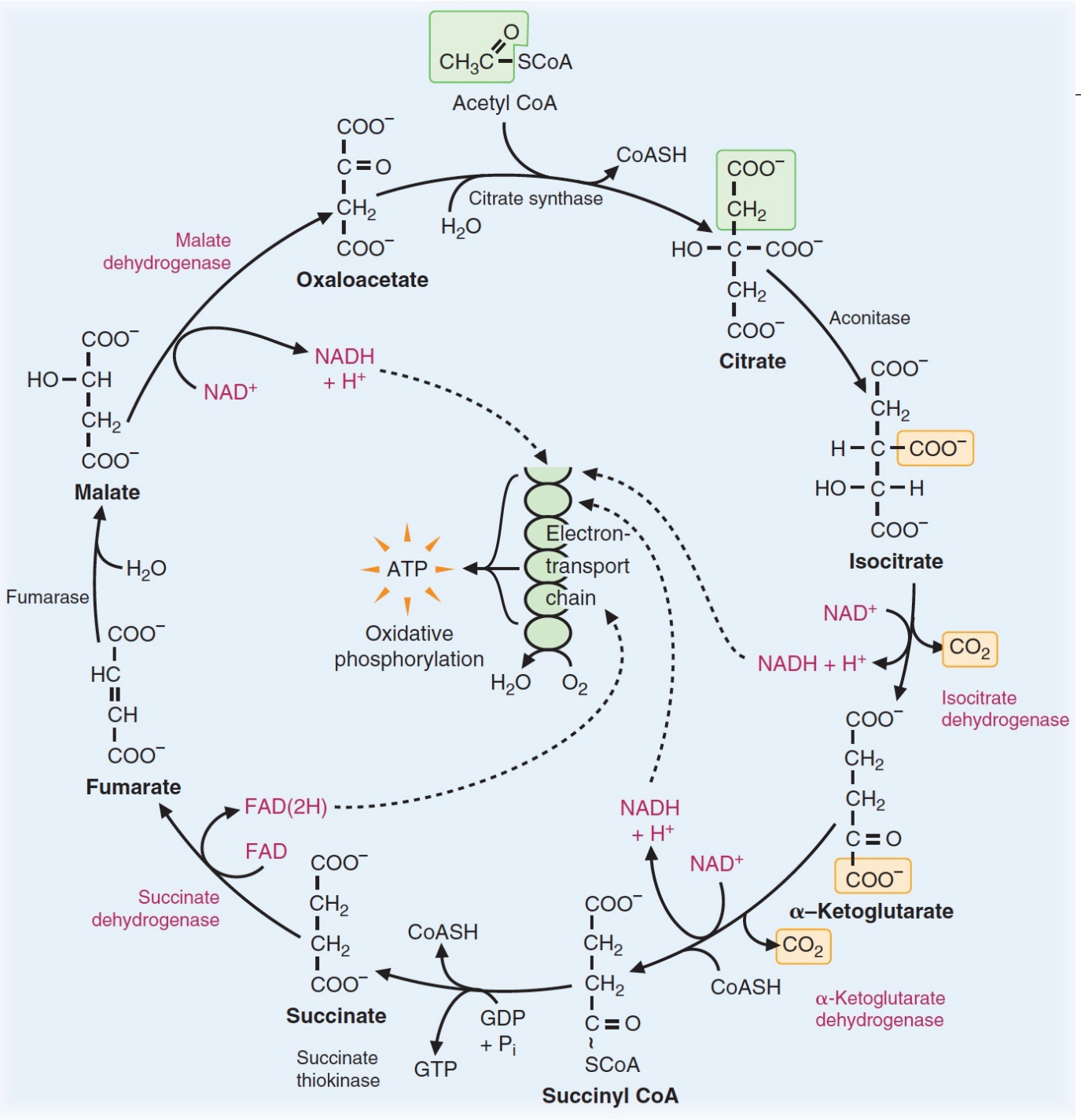
Step 7: **Fumarate** is converted to malate by the addition of water across the double bond.

- Enzyme: **fumarase.**



Step 8: **Malate** is oxidized, regenerating oxaloacetate and thus completing the cycle. Two hydrogens along with their electrons are passed to NAD^+, producing **NADH and H^+**, and **finishing the capture of the eight electrons from the carbons of acetyl-CoA.**

- Enzyme: **malate dehydrogenase.**

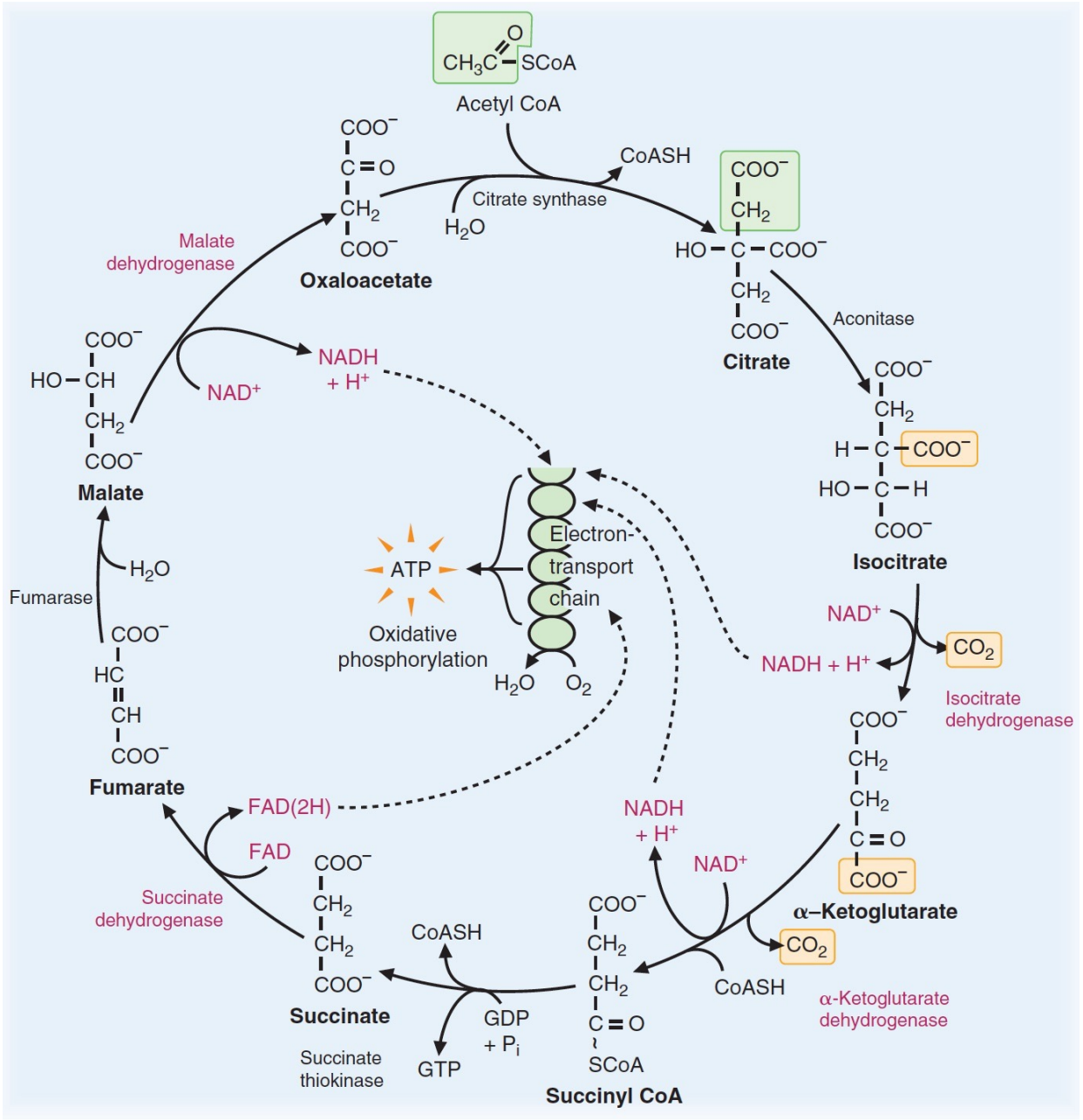


Energy production by the TCA cycle

1. The NADH and $FADH_2$ (produced by the cycle) donate electrons to the electron transport chain. For each mole of **NADH, approximately 2.5 moles of ATP** are generated, and for each mole of **$FADH_2$, approximately 1.5 moles of ATP** are generated by the passage of these electrons to O_2 (oxidative phosphorylation). In addition, GTP is produced when succinyl-CoA is cleaved. GTP produces ATP:

$$(GTP + ADP \rightleftharpoons ATP + GDP)$$

2. The **total energy** generated by one round of the cycle, starting with 1 mole of acetyl-CoA, is approximately **10 moles of ATP.**



Regulation of the TCA cycle

The TCA cycle is regulated by the **cell's need for energy** in the form of ATP. The TCA cycle acts in concert with the electron transport chain and the ATP synthase in the inner mitochondrial membrane to produce ATP:

i. When the cell has limited amounts of adenine nucleotides (ATP, ADP, and AMP).

ii. When ATP is utilized, ADP and inorganic phosphate (Pi) are produced.

iii. When **ADP levels are high** relative to ATP. i.e, when the cell needs energy ➔ the reactions of the electron transport chain are accelerated. NADH is rapidly oxidized; consequently, the TCA cycle speeds up.

iv. When the concentration of ATP is high i.e, when the cell has an adequate energy supply ➔ the electron transport chain slows down, NADH builds up, and consequently the **TCA cycle is inhibited.**

- ❖ NADH allosterically inhibits isocitrate dehydrogenase (step 3). Isocitrate accumulates, and because the aconitase equilibrium favors citrate, the concentration of citrate rises. Citrate inhibits citrate synthase (step 1), the first enzyme of the cycle.
- ❖ High NADH (and low NAD^+) levels also affect the reactions of the cycle that generate NADH, resulting in a slowing down of the cycle by mass action.

*** Oxaloacetate is converted to malate when NADH is high and, therefore, less substrate (OAA) is available for the citrate synthase reaction.

Regulation of the pyruvate dehydrogenase

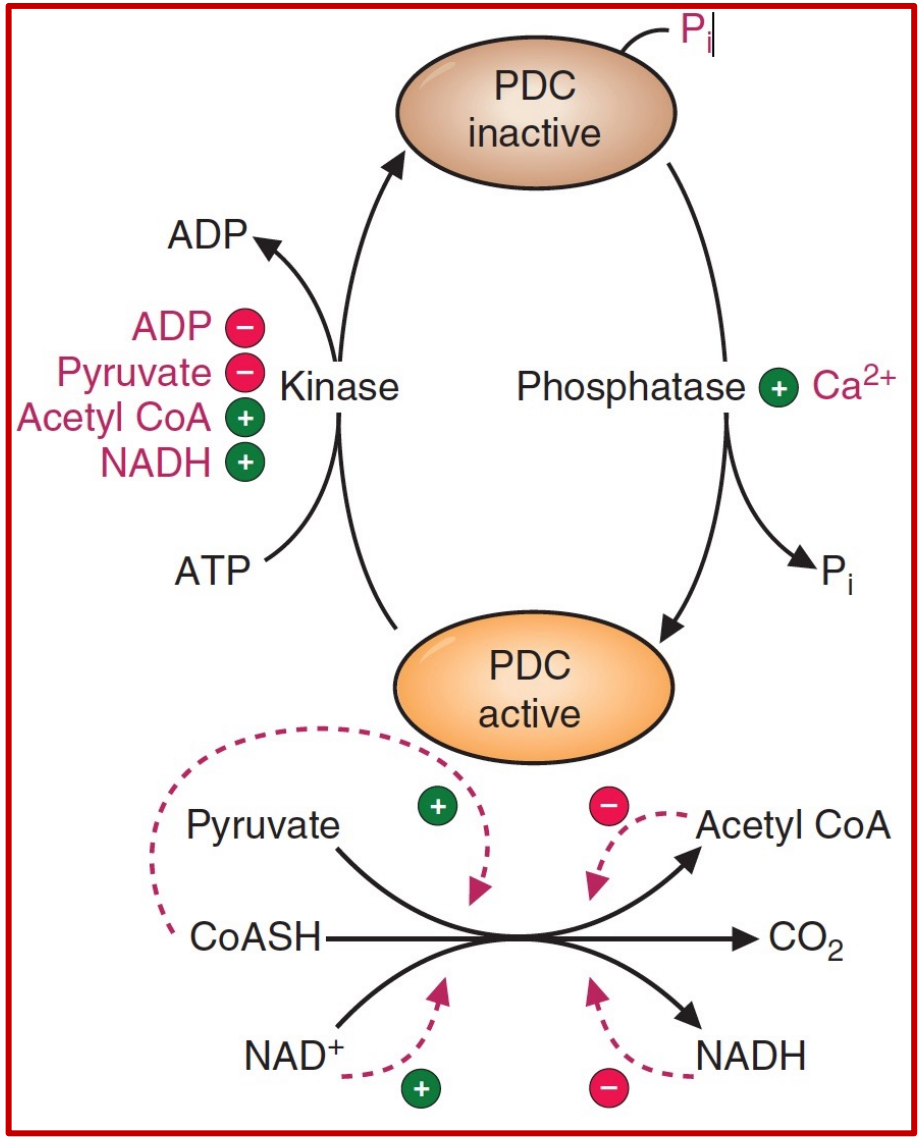
a. Pyruvate dehydrogenase exists in a **phosphorylated (inactive) form and a dephosphorylated (active) form.**

a. A kinase associated with the multienzyme complex **phosphorylates the pyruvate decarboxylase** subunit, inactivating the pyruvate dehydrogenase complex.

The products of the pyruvate dehydrogenase reaction, **acetyl-CoA and NADH, activate the kinase**, and the substrates, **CoASH and NAD+, inactivate the kinase**. The kinase is also inactivated by ADP.

b. A phosphatase dephosphorylates and activates the pyruvate dehydrogenase complex.

c. When the **concentration of substrates is high, the dehydrogenase is active, and pyruvate is converted to acetyl-CoA.** When the concentration of products is high, the dehydrogenase is relatively inactive



Vitamins required for reactions of the TCA cycle

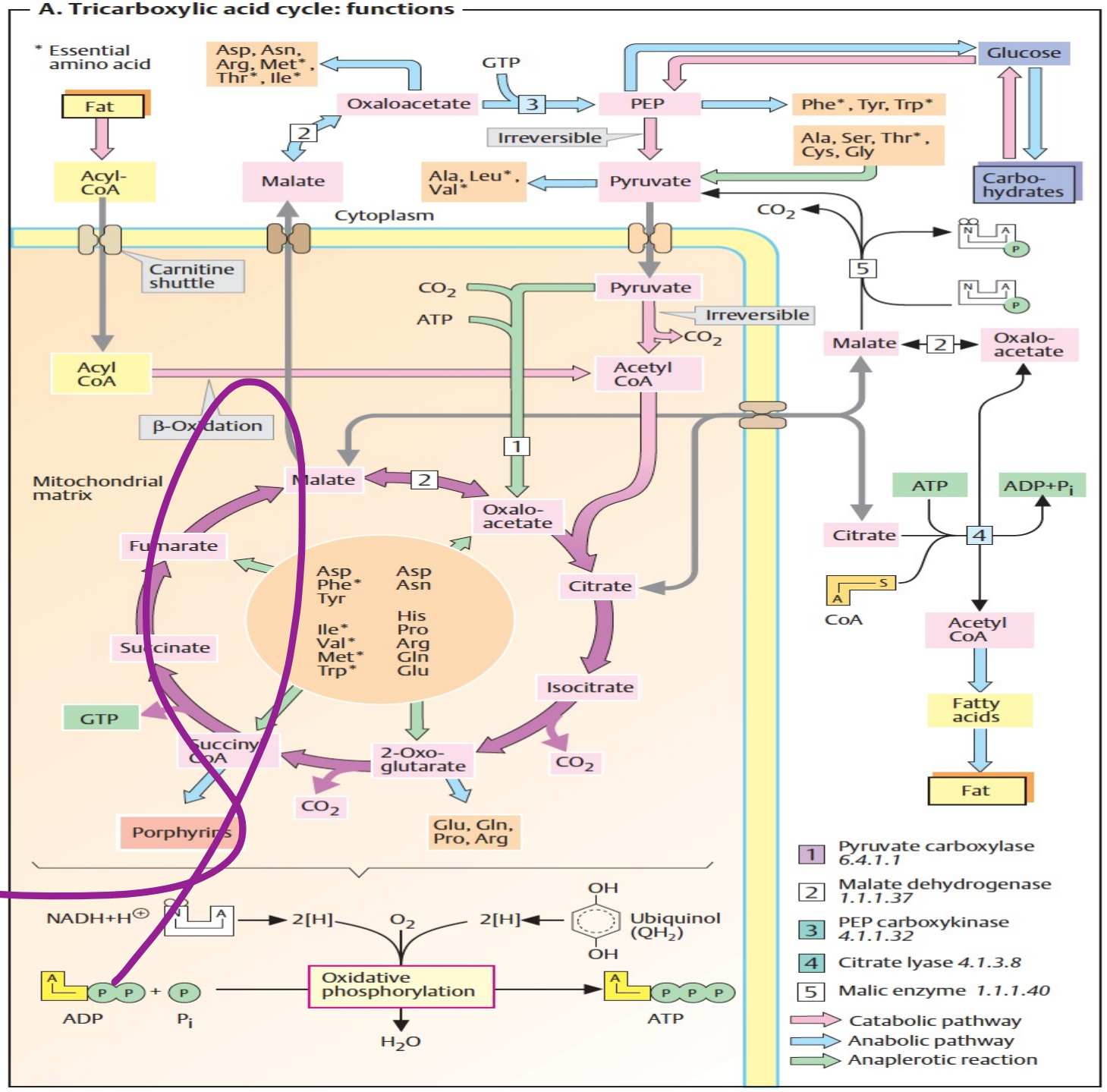
1. **Niacin (vitamin B3)** is used for the synthesis of the nicotinamide portion of NAD, which is used in the isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase reactions.

2. Riboflavin (vitamin B2) is used for the synthesis of FAD, which is the cofactor for succinate dehydrogenase. FAD is also required by α-ketoglutarate dehydrogenase.

3. α-Ketoglutarate dehydrogenase, a multienzyme complex, contains lipoic acid and four other cofactors that are synthesized from vitamins.

   a. **Thiamine (vitamin B1)** is used for the synthesis of thiamine pyrophosphate.
   b. **Pantothenate (vitamin B5)** for CoASH.
   c. **Riboflavin (vitamin B2)** for FAD.
   d. **Niacin (vitamin B3)** for NAD^+.

Synthetic functions of the TCA cycle



Synthetic functions of the TCA cycle

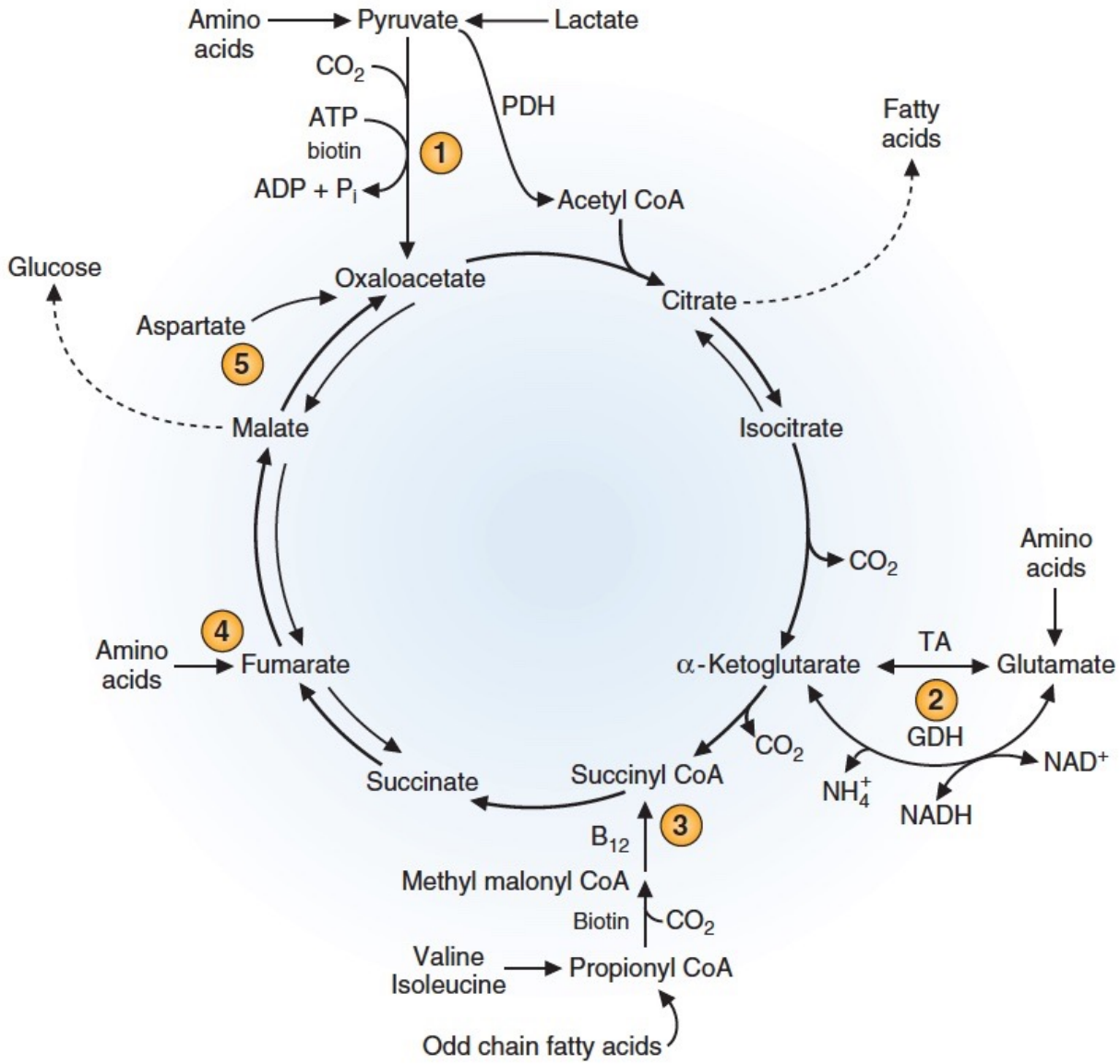
Anaplerotic reactions replenish intermediates of the TCA cycle as they are removed for the synthesis of glucose, fatty acids, amino acids, or other compounds.

a. A key anaplerotic reaction is catalyzed by pyruvate carboxylase, which carboxylates pyruvate, forming **oxaloacetate.**

- Pyruvate carboxylase requires biotin, a cofactor that is commonly involved in CO2- fixation reactions.
- Pyruvate carboxylase, found in liver, brain, and adipose tissue (but not in muscle), is activated by acetyl-CoA.

b. Amino acids produce intermediates of the TCA cycle through anaplerotic reactions.

- Glutamate is converted to α-ketoglutarate.
- Amino acids that form glutamate include glutamine, proline, arginine, and histidine.
- Aspartate is transaminated to form oxaloacetate. Asparagine can produce aspartate.
- Valine, isoleucine, methionine, and threonine produce propionyl-CoA, which is converted to methylmalonyl-CoA and subsequently to succinyl-CoA, an intermediate of the TCA cycle.
- Phenylalanine, tyrosine, and aspartate form fumarate.



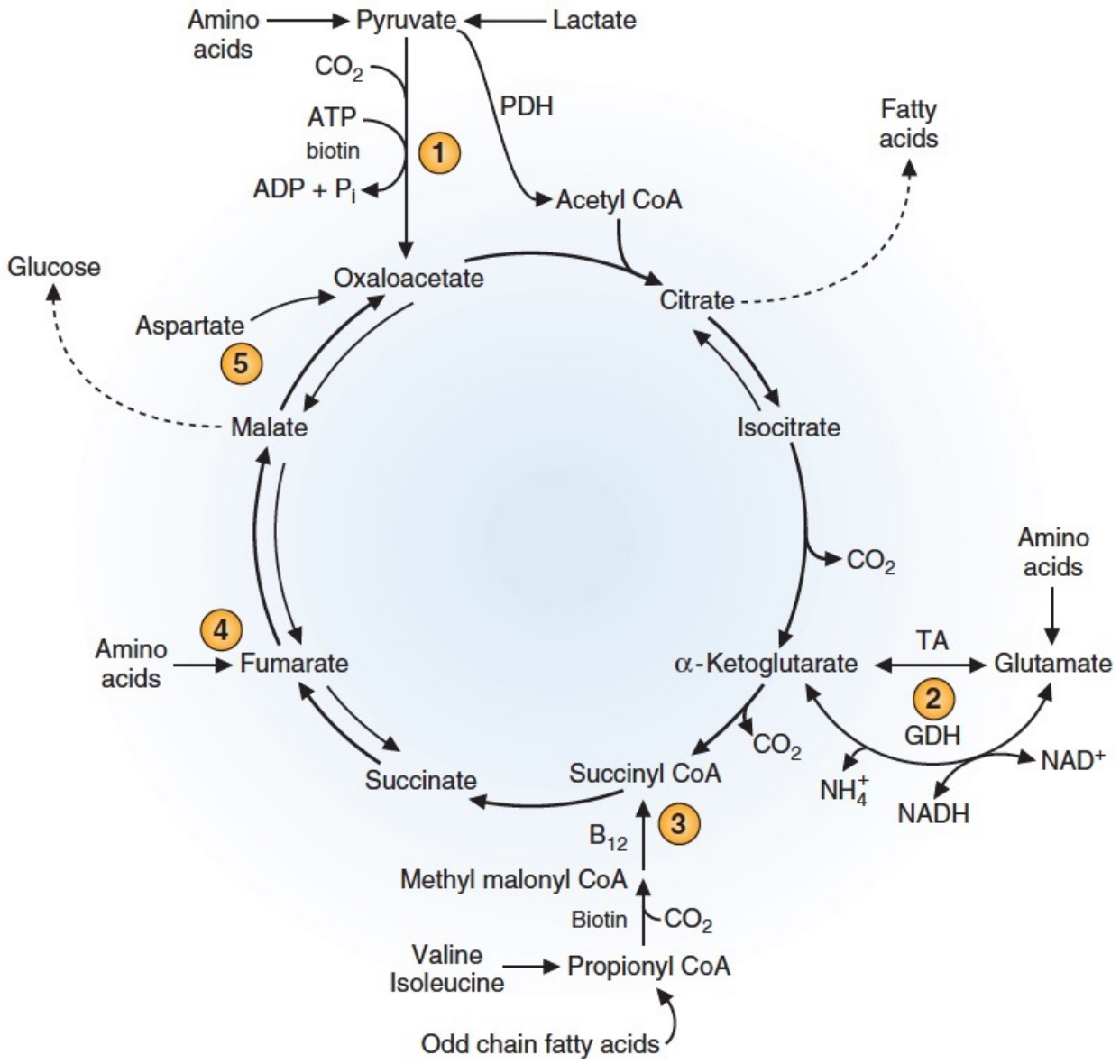
Synthetic functions of the TCA cycle

Synthesis of glucose

a. The synthesis of glucose occurs by the pathway of gluconeogenesis, which involves intermediates of the TCA cycle.

b. As glucose is synthesized, malate or oxaloacetate is removed from the TCA cycle and replenished by anaplerotic reactions.

- Pyruvate, produced from lactate or alanine, is converted by pyruvate carboxylase to oxaloacetate, which forms malate.
- Various amino acids that supply carbon for gluconeogenesis are converted to intermediates of the TCA cycle, which form malate and, thus, glucose.

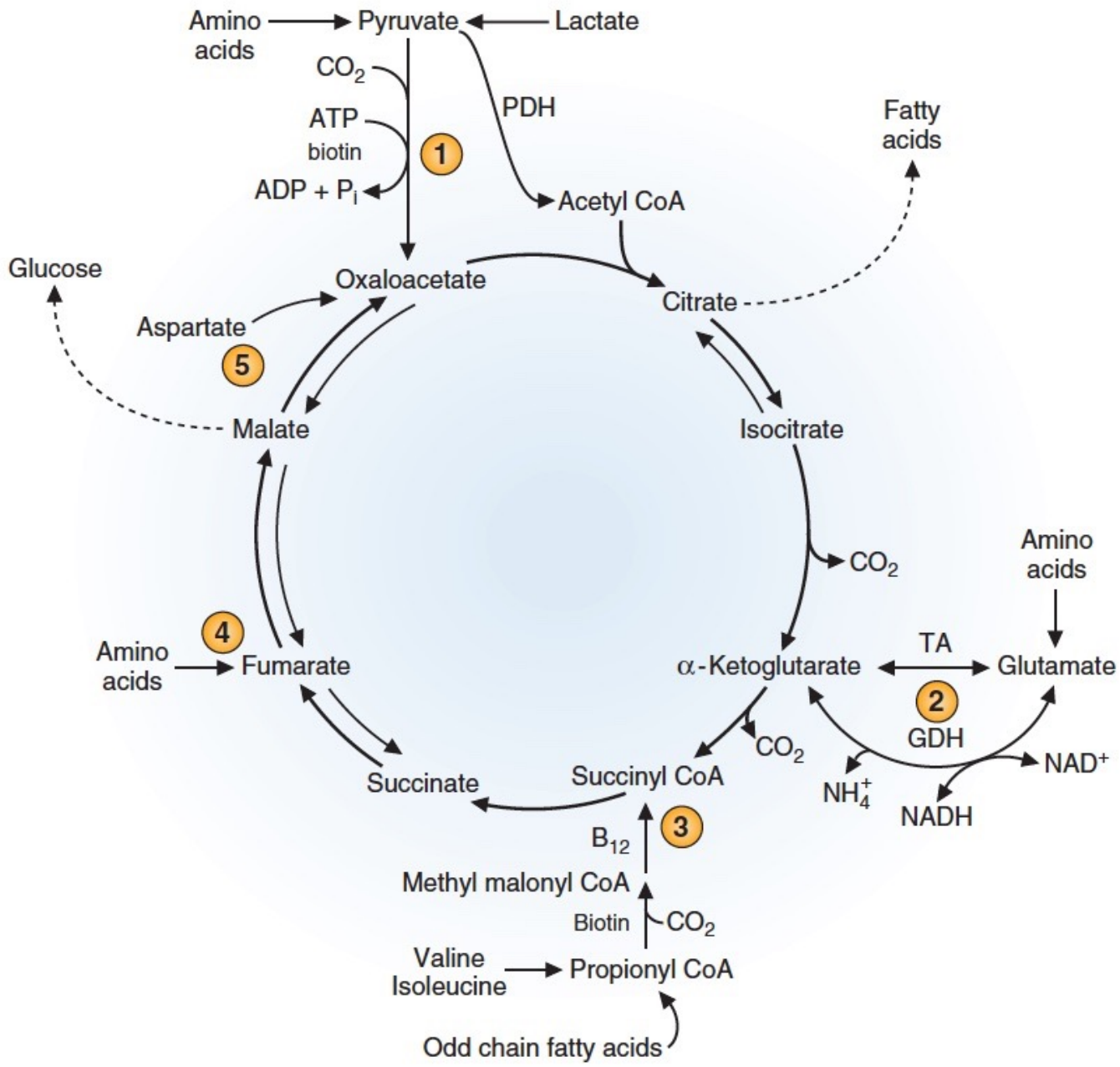


Synthetic functions of the TCA cycle

Synthesis of fatty acids

a. The pathway for fatty acid synthesis from glucose includes reactions of the TCA cycle.

- From glucose, pyruvate is produced and converted to oxaloacetate (by pyruvate carboxylase) and to acetyl-CoA (by pyruvate dehydrogenase).
- Oxaloacetate and acetyl-CoA condense to form citrate, which is used for fatty acid synthesis.
- Pyruvate carboxylase catalyzes the anaplerotic reaction that replenishes the TCA cycle intermediates.



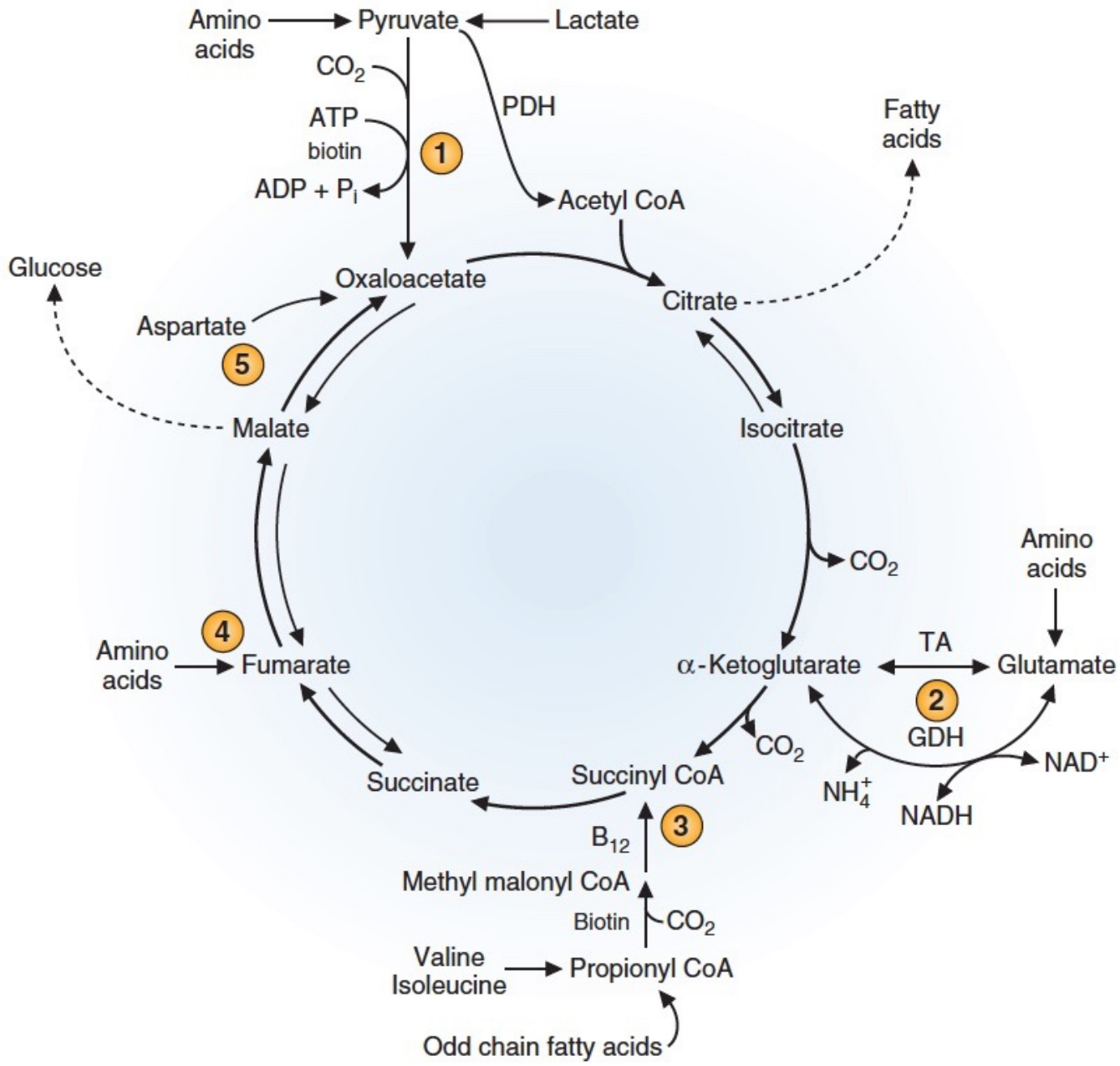
Synthetic functions of the TCA cycle

Synthesis of amino acids

a. Synthesis of amino acids from glucose involves intermediates of the TCA cycle.

- Glucose is converted to pyruvate, which forms oxaloacetate, which by transamination forms aspartate and, subsequently, asparagine.
- Glucose is converted to pyruvate, which forms both oxaloacetate and acetyl-CoA, which condense, forming citrate. Citrate forms isocitrate and then α-ketoglutarate, from which glutamate, glutamine, proline, and arginine are produced.

Interconversion of amino acids involves intermediates of the TCA cycle. For example, the carbons of glutamate can feed into the TCA cycle at the α-ketoglutarate level and traverse the cycle, forming oxaloacetate, which may be transaminated to aspartate.



Questions & Answers