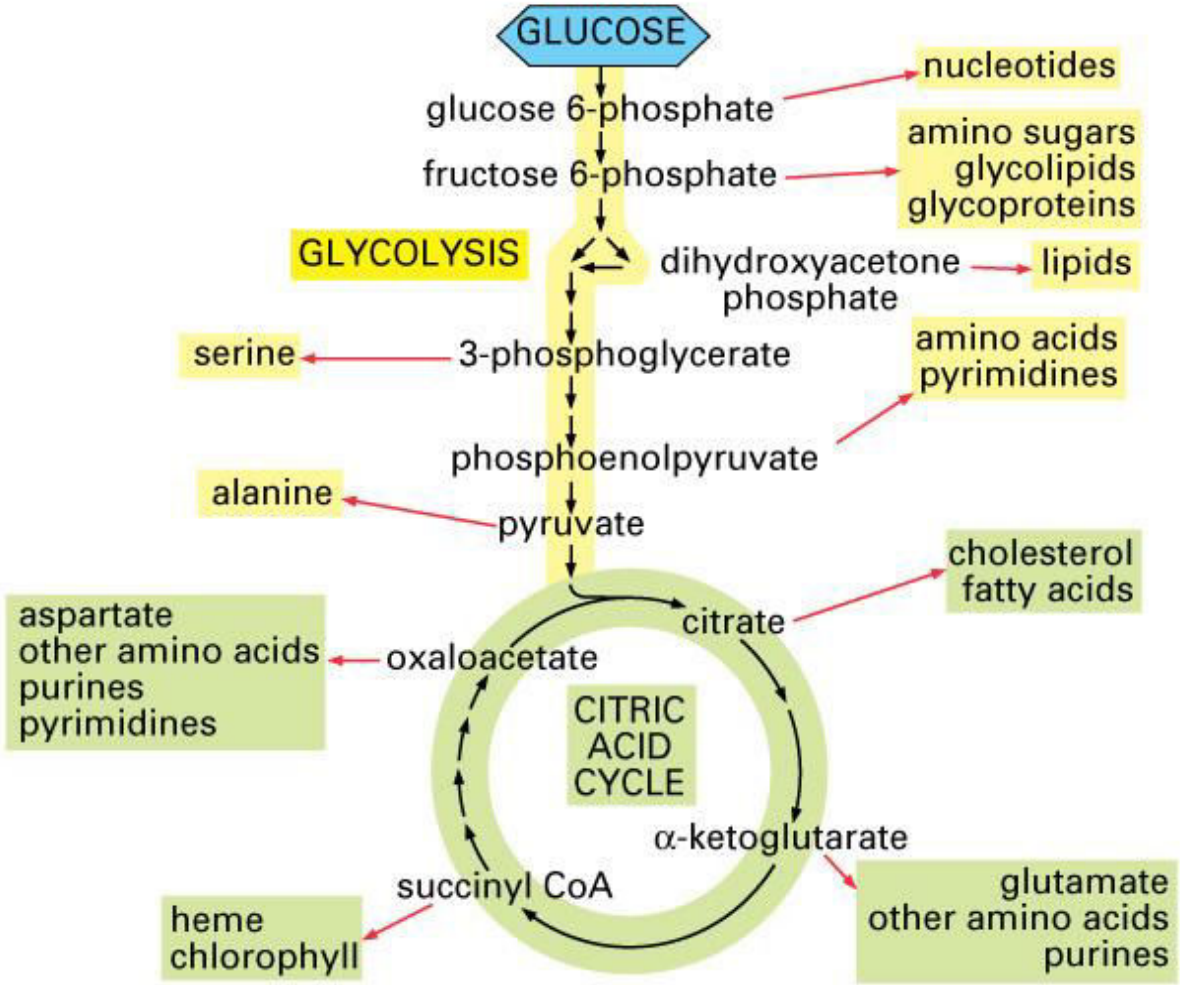
# Tricarboxylic Acid Cycle

BIOCHEMISTRY

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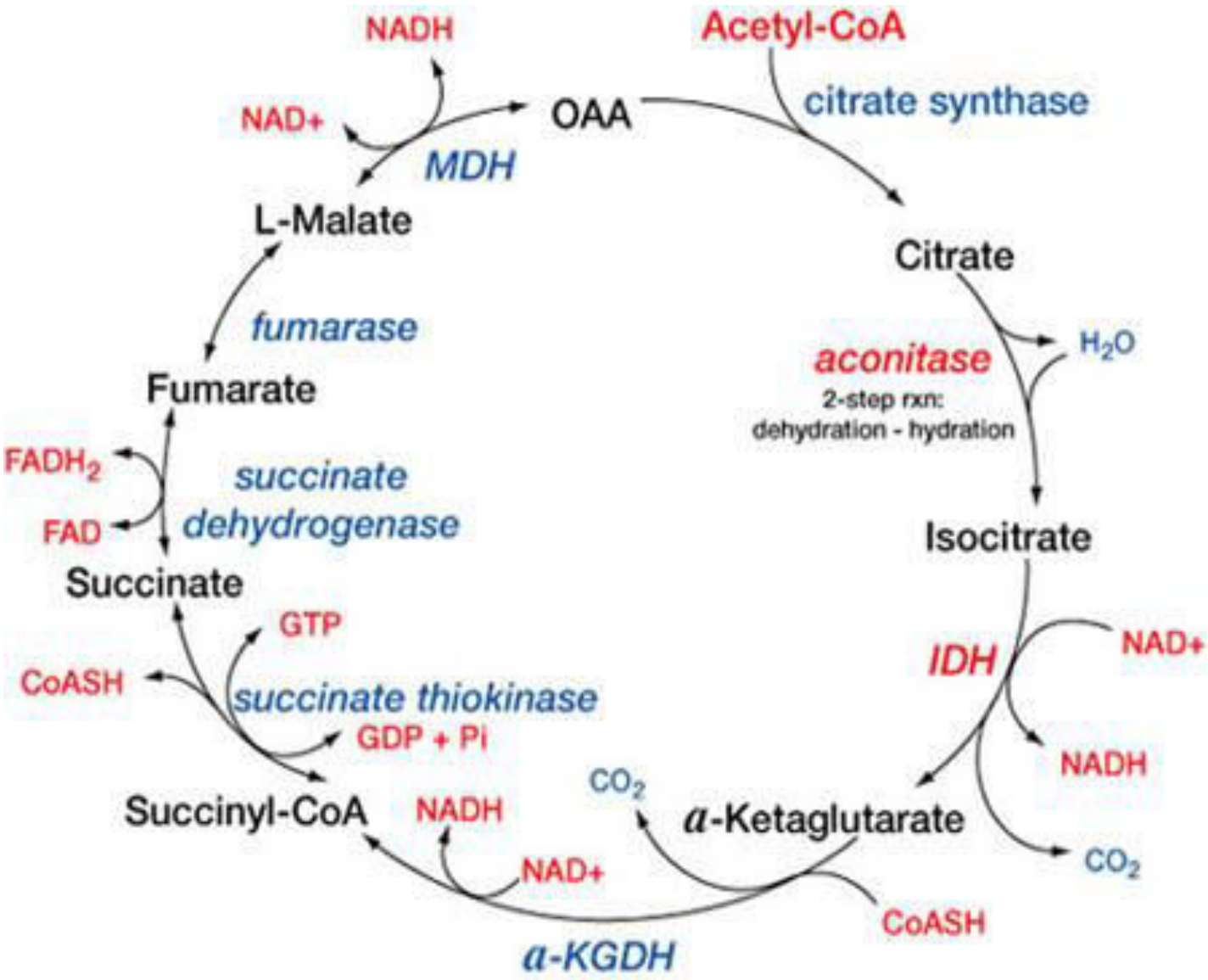
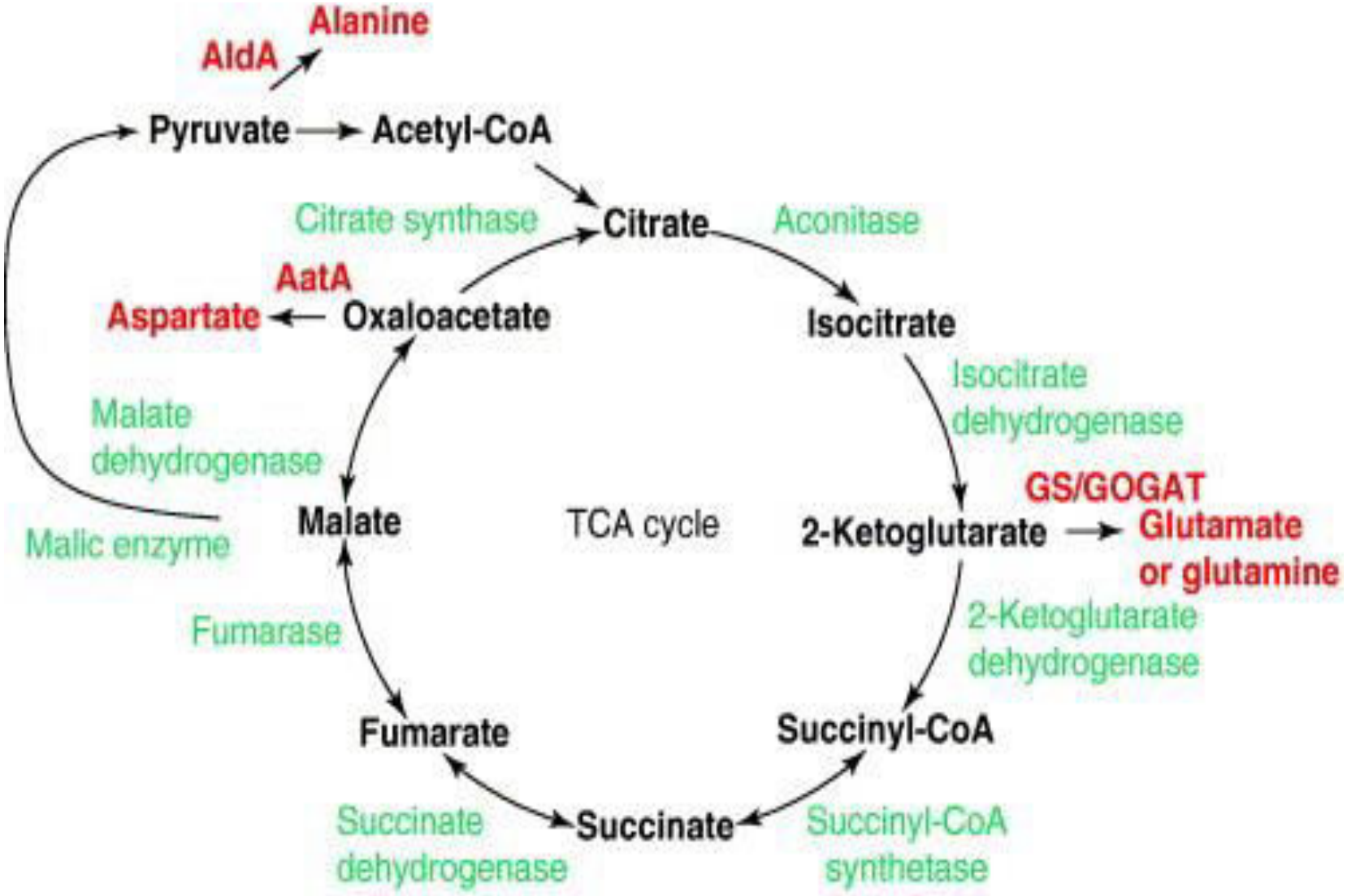




- also called the Krebs cycle or the citric acid cycle
- It is the final pathway where the oxidative metabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to $CO_2$

- The cycle occurs totally in the mitochondria and is, therefore, in close proximity to the reactions of electron transport
- The TCA cycle is an aerobic pathway, because $O_2$ is required as the final electron acceptor

- Reactions such as the catabolism of some amino acids generate intermediates of the cycle and are called anaplerotic reactions

- The citric acid cycle also participates in a number of important synthetic reactions. For example, the cycle functions in the formation of glucose from the carbon skeletons of some amino acids
- and it provides building blocks for the synthesis of some amino acids and heme

# Oxidative decarboxylation of pyruvate

- Pyruvate, the endproduct of aerobic glycolysis, must be transported into the mitochondrion before it can enter the TCA cycle.

- This is accomplished by a specific pyruvate transporter that helps pyruvate cross the inner mitochondrial membrane

- Once in the matrix, pyruvate is converted to acetyl CoA by **the pyruvate dehydrogenase complex**, which is a multienzyme complex

# Component enzymes

- The pyruvate dehydrogenase complex is a multimolecular aggregate of **three enzymes**, **pyruvate dehydrogenase** ($E_1$, also called a decarboxylase), **dihydrolipoyl transacetylase** ($E_2$), and **dihydrolipoyl dehydrogenase** ($E_3$).

- the complex also contains two tightly bound regulatory enzymes, pyruvate dehydrogenase **kinase** and pyruvate dehydrogenase **phosphatase**.

# Coenzymes:

- The pyruvate dehydrogenase complex contains **five coenzymes**
- $E_1$ requires **thiamine pyrophosphate**, $E_2$ requires **lipoic acid** and **CoA**, and $E_3$ requires **FAD and $NAD^+$.**

# Regulation of the pyruvate dehydrogenase complex

- The two regulatory enzymes that are part of the complex alternately activate and inactivate $E_1$.

- The cyclic AMP-independent PDH **kinase phosphorylates and, thereby, inhibits $E_1$** whereas **PDH phosphatase activates $E_1$**

- The kinase is allosterically activated by ATP, acetyl CoA, and NADH.

- Therefore, in the presence of these high-energy signals, the pyruvate dehydrogenase complex is turned off.

# Pyruvate dehydrogenase deficiency

- A deficiency in the $E_1$ component of the pyruvate dehydrogenase complex is the most common biochemical cause of **congenital lactic acidosis.**

- This enzyme deficiency results in an inability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactic acid via lactate dehydrogenase

- This causes particular problems for the brain, which relies on the TCA cycle for most of its energy, and is particularly sensitive to acidosis.
- The defect is classified as X-linked dominant.
- There is no proven treatment for pyruvate dehydrogenase complex deficiency

- Leigh syndrome (subacute necrotizing encephalomyelopathy) - mutations in PDH complex, electron transport chain, ATP synthase.
- Nuclear and mt DNA can be affected

- arsenic poisoning- this particularly affects the brain, causing neurologic disturbances and death

# Synthesis of citrate from acetyl CoA and oxaloacetate

- The condensation of acetyl CoA and oxaloacetate to form **citrate** is catalyzed by **citrate synthase**

- Citrate is isomerized to **isocitrate** by **aconitase**

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

- **Isocitrate dehydrogenase** catalyzes the irreversible of isocitrate to **α-ketoglutarate**

- yielding the first of three **NADH molecules** produced by the cycle, and the **first release of $CO_2$**

- The enzyme is allosterically activated by ADP, a low-energy signal) and $Ca^{2+}$,

- and is inhibited by adenosine triphosphate (ATP) and NADH, whose levels are elevated when the cell has abundant energy stores.

- conversion of α-ketoglutarate to **succinyl CoA** is catalyzed by the **α-ketoglutarate dehydrogenase complex**, which consists of three enzymatic activities

- Succinate thiokinase (also called succinyl CoA synthetase) cleaves succinyl CoA to **succinate**

- This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP).

- Succinate is oxidized to **fumarate** by **succinate dehydrogenase,** producing the reduced coenzyme $FADH_2$

- Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane.

- Fumarate is hydrated to **malate** in a freely reversible reaction catalyzed by **fumarase**

- Malate is oxidized to **oxaloacetate** by **malate dehydrogenase**

- This reaction produces the third and final NADH of the cycle

# Regulation of the TCA Cycle

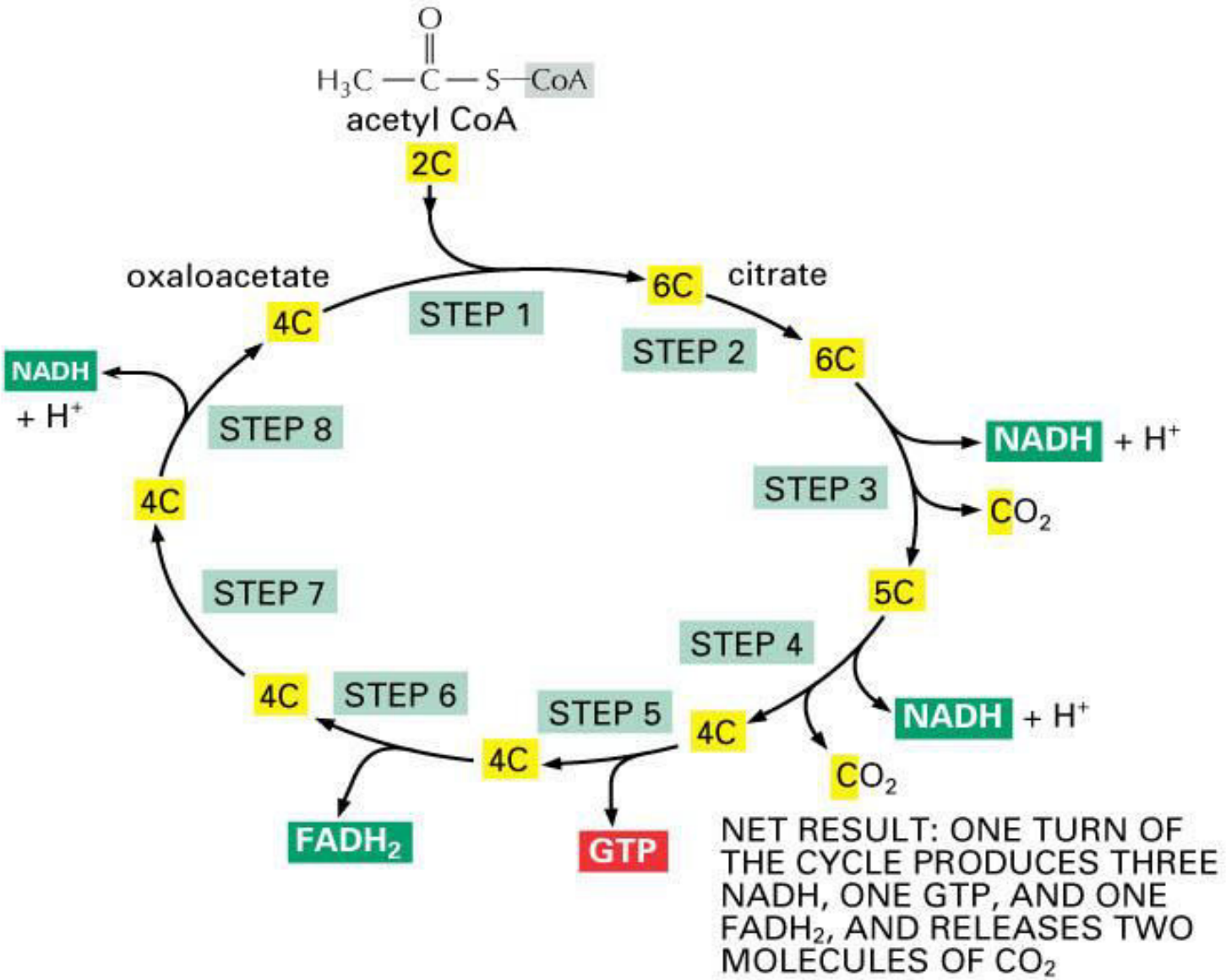
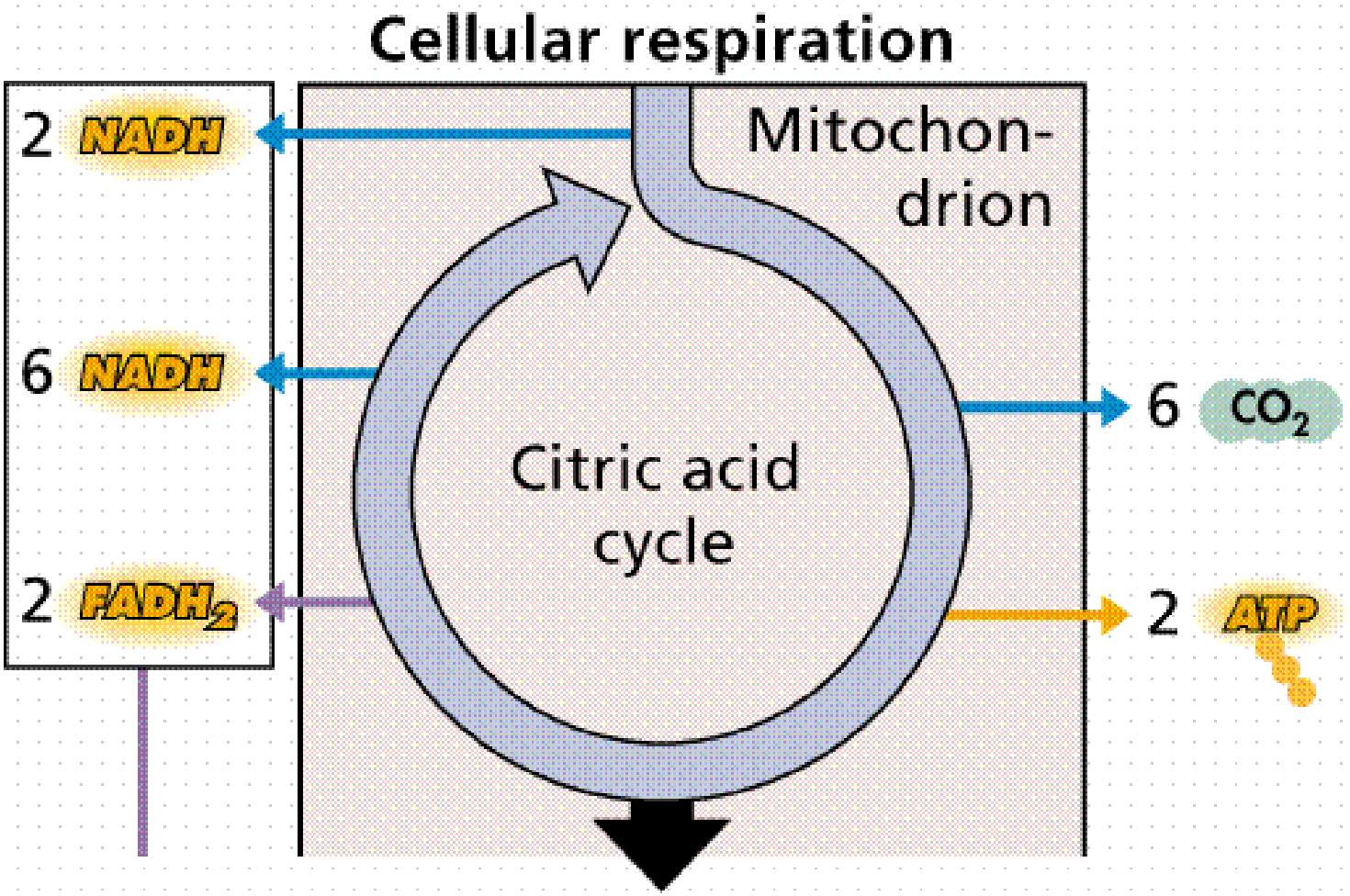



Figure 13-11 Essential Cell Biology, 2/e. (© 2004 Garland Science)



| 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 |

| | | | |
|---|---|---|---|
| **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 | (× 2) → 24 ATP |
| | 1 $FADH_2$ → | 2 ATP | |
| **Total:** | | | 38 ATP |

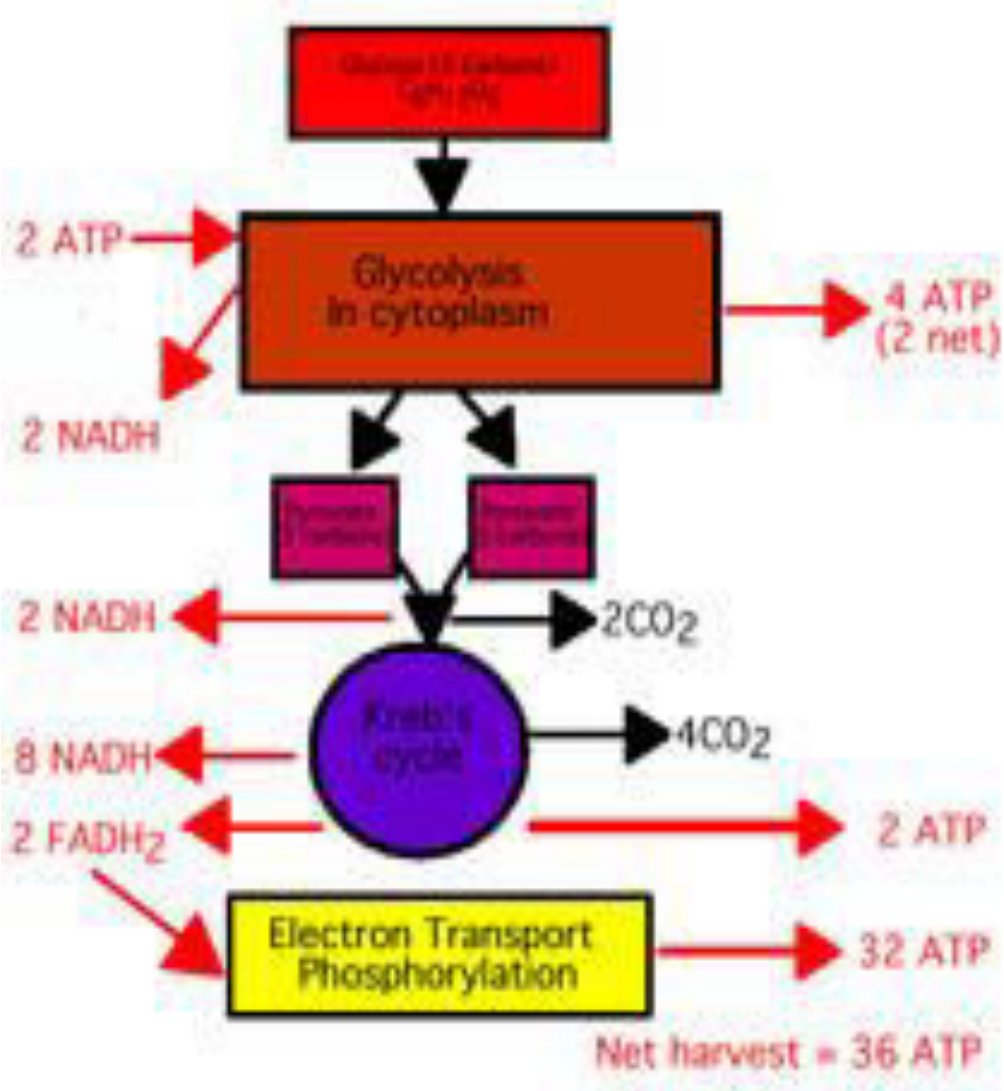
Oxidation of glucose yields:

| | |
|---|---|
| glycolysis: | 2 NADH |
| pyruvate dehydrogenase: | 2 NADH |
| citric acid cycle: | 6 NADH + 2 $FADH_2$ |

Oxidation of glucose yields ~30 ATP

Glycolysis = 2
Citric acid cycle = 2
] Substrate level phosphorylation

Ox. phos. = ~26


2 ATP
Glycolysis
in cytoplasm
4 ATP
(2 net)
2 NADH
2 NADH
$2CO_2$
Krebs cycle
8 NADH
$4CO_2$
$2 FADH_2$
2 ATP
Electron Transport
Phosphorylation
32 ATP
Net harvest = 36 ATP

- Through aerobic respiration, the glucose molecule is thoroughly broken down, and a great amount of energy is used to form ATP molecules. To calculate the net gain in ATP of aerobic respiration, we must return all the way back to the first stage which we discussed: **glycolysis.**

- Two molecules of ATP were required to begin the reaction of glycolysis, but four were produced as a result. Therefore, there was a **net gain of two ATP molecules**. Also, glycolysis resulted in the formation of **two molecules of NADH**, each of which provides the energy for the formation of **three molecules of ATP through the electron transport chain.**

- Therefore, the two NADH molecules produce **six ATP molecules total**. **So, the total number of ATP molecules formed from glycolysis is eight.** When each molecule of pyruvic acid is oxidized, one molecule of NADH is produced. This occurs twice, since **one glucose molecule splits into two molecules of pyruvic acid.**

- Therefore, two molecules of NADH are produced, each of which results in the formation of three molecules of ATP, for a total of six molecules of ATP.

- In the Krebs cycle, **two molecules of ATP, six of NADH, and two of $FADH_2$** are formed from the breakdown of one glucose molecule, since the Krebs cycle occurs twice for each glucose molecule.

- The six NADH molecules result in the production of eighteen ATP molecules, and the two molecules of $FADH_2$ produce four ATP molecules, for a total of 22. The total is therefore the two ATP molecules produced directly plus the 22 molecules formed through the electron transport chain, which equals 24..

- Adding together the 8 ATP molecules formed during glycolysis, the 6 from the oxidation of pyruvic acid, and the 24 from the Krebs cycle, we obtain a **final net total of 38 molecules of ATP formed for each molecule of glucose**