9: Tricarboxylic Acid Cycle and Pyruvate Dehydrogenase Complex

Cycle Overview

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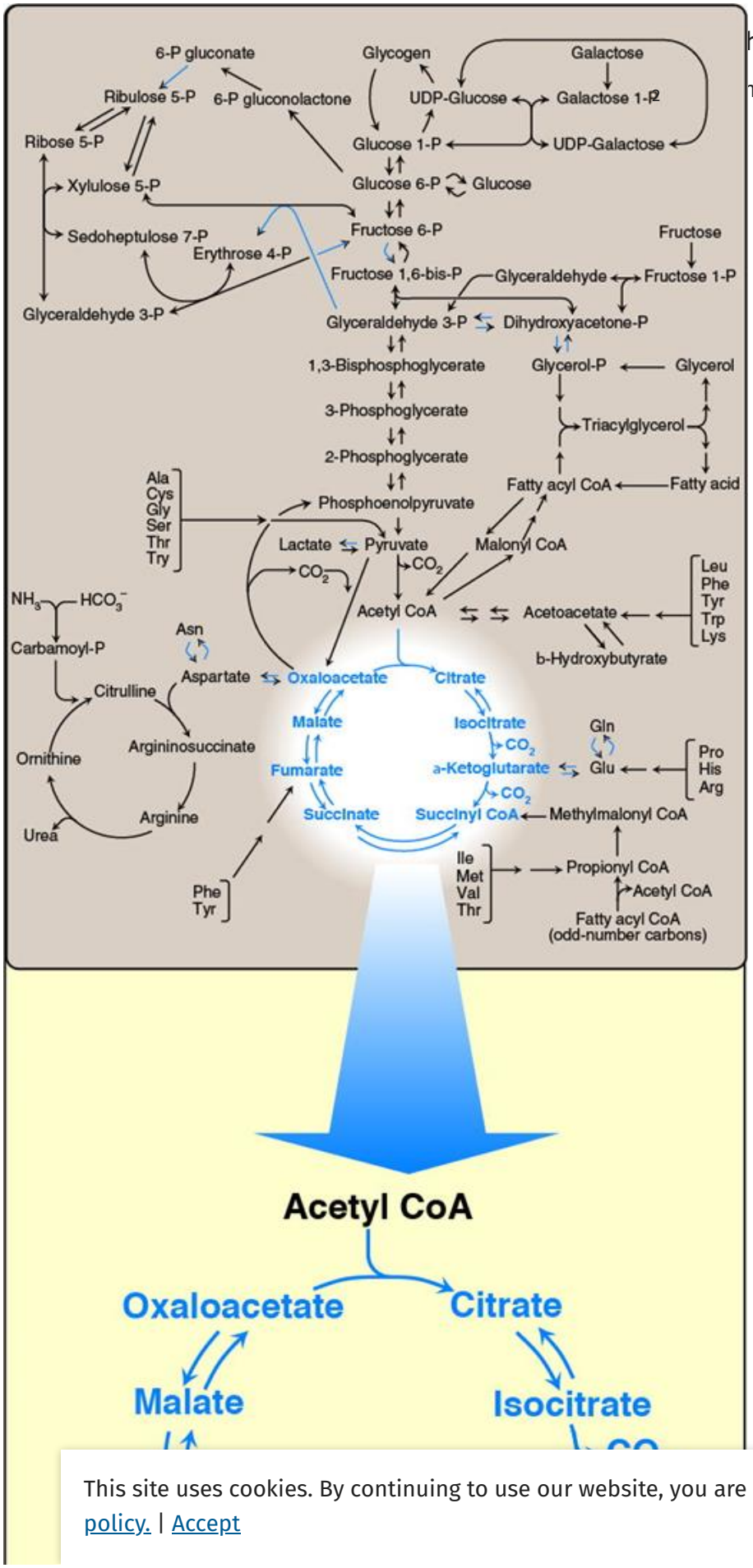
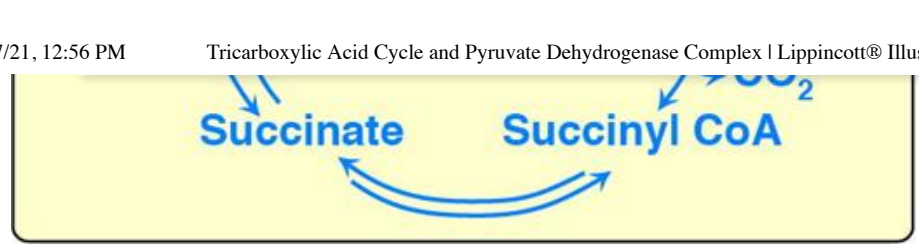
The **tricarboxylic acid cycle** (TCA cycle) can also be referred to as the citric acid cycle or the Krebs cycle, and plays several roles in metabolism. It is the final pathway where the oxidative catabolism of carbohydrates, amino acids, and fatty acids converge, their carbon skeletons being converted to carbon dioxide (CO_2), as shown in Figure 9.1. This oxidation provides energy for the production of the majority of ATP in most animals, including humans. Because the TCA cycle occurs totally in mitochondria, it is in close proximity to the electron transport chain ([ETC]), which oxidizes the reduced coenzymes nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) produced by the cycle. The TCA cycle is an aerobic pathway, because oxygen (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 (from the Greek for “filling up”) reactions. The TCA cycle also provides intermediates for a number of important anabolic reactions, such as glucose formation from the carbon skeletons of some amino acids and the synthesis of some amino acids (see Chapter 20 Section V) and heme (see Chapter 21 Section II B). Therefore, this cycle should not be viewed as a closed system but, instead, as an open one with compounds entering and leaving as required.

FIGURE 9.1



hways of energy metabolism.

n dioxide; CoA = coenzyme A.



Cycle Reactions

Listen

In the TCA cycle, oxaloacetate (OAA) is first condensed with an acetyl group from **acetyl coenzyme A** (CoA) and then is regenerated as the cycle is completed (see Fig. 9.1). Two carbons enter the cycle as acetyl CoA and two leave as CO_2. Therefore, the entry of one acetyl CoA into one round of the TCA cycle does not lead to the net production or consumption of intermediates.

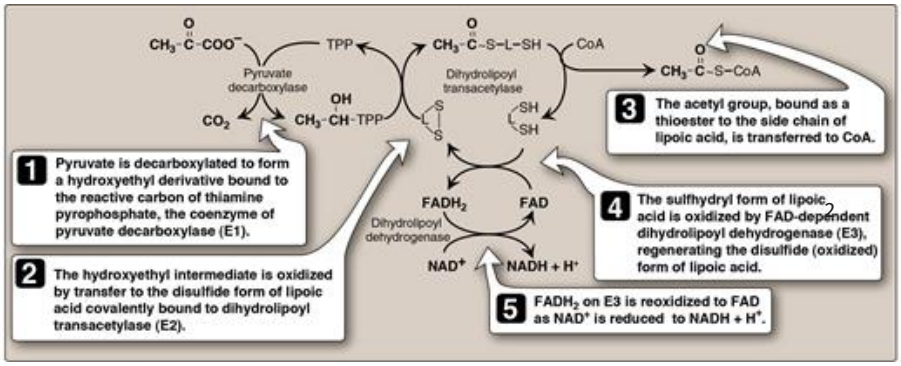
Acetyl CoA production

The major source of acetyl CoA for the TCA cycle is the oxidative decarboxylation of **pyruvate** by the multienzyme **pyruvate dehydrogenase complex** (PDH complex, or PDHC). However, the PDHC (described below) is not a component of the TCA cycle. Pyruvate, the end product of glycolysis, is transported from the cytosol into the mitochondrial matrix by the pyruvate mitochondrial carrier of the inner mitochondrial membrane. In the matrix, the PDHC converts pyruvate to acetyl CoA. (Note: Fatty acid oxidation is another source of acetyl CoA [see Chapter 16 Section IV].)

PDHC component enzymes

The PDHC is a protein aggregate of multiple copies of three enzymes, pyruvate decarboxylase ([E1] sometimes called PDH), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Each catalyzes a part of the overall reaction (Fig. 9.2). Their physical association links the reactions in proper sequence without the release of intermediates. In addition to the enzymes participating in the conversion of pyruvate to acetyl CoA, the PDHC also contains two regulatory enzymes, **pyruvate dehydrogenase kinase** (**PDH kinase**) and **pyruvate dehydrogenase phosphatase** (**PDH phosphatase**).

FIGURE 9.2



ogenase complex.

ved from vitamins. TPP is from ic acid.) CO_2 = carbon dioxide; TPP = NAD(H) = flavin and nicotinamide

Coen

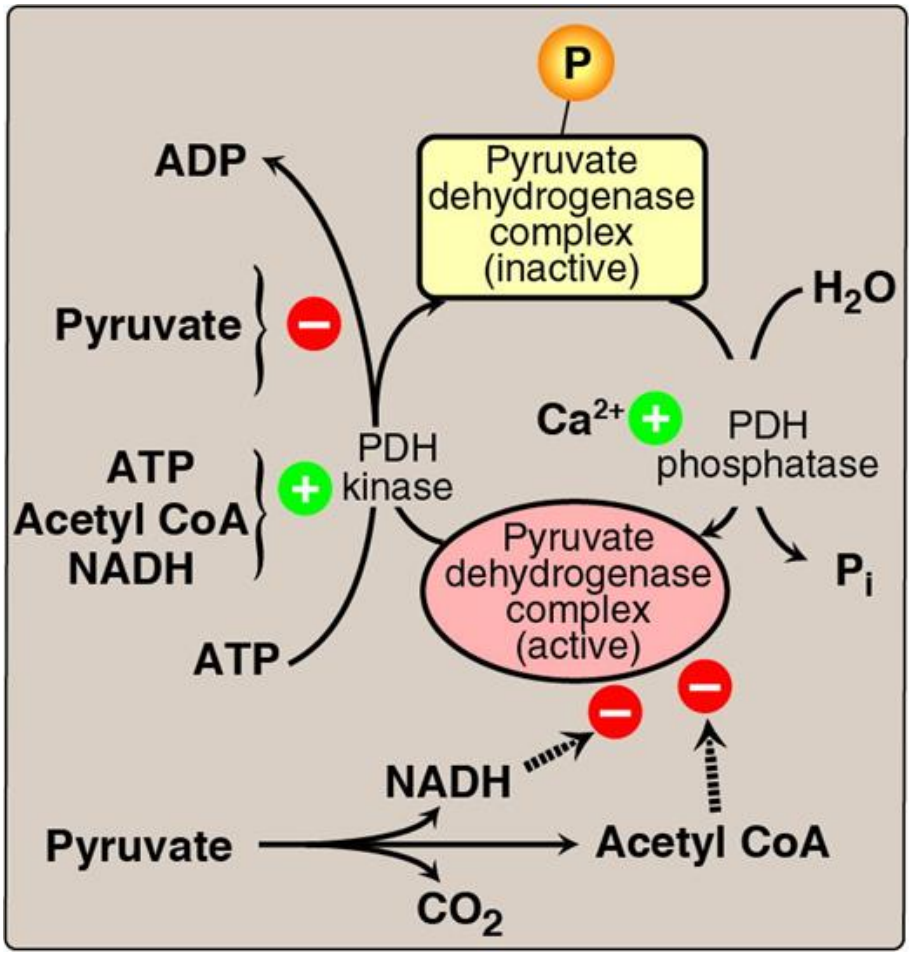
The PDHC contains five coenzymes that act as carriers or oxidants for the intermediates of the reactions shown in Figure 9.2. E1 requires **thiamine pyrophosphate** (TPP), E2 requires **lipoic acid** and CoA, and E3 requires **FAD** and **NAD^+**. (Note: TPP, lipoic acid, and FAD are tightly bound to the enzymes and function as coenzymes–prosthetic groups [see p. 58].)

Deficiencies of thiamine or niacin can cause serious central nervous system problems. This is because brain cells are unable to produce sufficient ATP via the TCA cycle if the PDHC is inactive. Wernicke–Korsakoff, an encephalopathy-psychosis syndrome due to thiamine deficiency, may be seen in persons with alcohol use disorder.

Regulation

Covalent modifications by the two regulatory enzymes of the PDHC alternately activate and inactivate E1. **PDH kinase** phosphorylates and inactivates E1, whereas PDH phosphatase dephosphorylates and activates E1 (Fig. 9.3). The kinase itself is allosterically activated by ATP, acetyl CoA, and NADH. Therefore, in the presence of these high-energy products, the PDHC is turned off. (Note: It is actually the rise in the ATP/ADP [adenosine diphosphate], NADH/NAD^+, or acetyl CoA/CoA ratios that affects enzymic activity.)

FIGURE 9.3



Pyruvate is a potent inhibitor of PDH kinase. Therefore, if pyruvate concentrations are elevated, E1 will be maximally active. Calcium (Ca^{2+}) is a strong activator of PDH phosphatase, stimulating E1 activity. This is particularly important in skeletal muscle, where Ca^{2+} release during contraction stimulates the PDHC and, thus, energy production. (Note: Although covalent regulation by the kinase and phosphatase is primary, the PDHC is also subject to product [NADH and acetyl CoA] inhibition.)

Deficiency

A deficiency of the α subunits of the tetrameric E1 component of the PDHC, although very rare, is the most common biochemical cause of **congenital lactic acidosis**. The deficiency results in a decreased ability to convert pyruvate to acetyl CoA, causing pyruvate to be shunted to lactate via lactate dehydrogenase (see p. 113). This creates particular problems for the brain, which relies on the TCA cycle for most of its energy and is particularly sensitive to acidosis. Symptoms are variable and include neurodegeneration, muscle spasticity, and, in the neonatal-onset form, early death. The gene for the α-subunit is located on the X chromosome. Inheritance of just one X chromosome with the mutation results in disease; the inheritance pattern is X-linked dominant, with both males and females affected. Although there is no proven treatment for PDHC deficiency, dietary restriction of carbohydrate and supplementation with thiamine may reduce symptoms in select patients.

Leigh syndrome (subacute necrotizing encephalomyelopathy) is a rare, progressive, neurodegenerative disorder caused by defects in mitochondrial ATP production, primarily as a result of mutations in genes that encode proteins of the PDHC, the ETC, or ATP synthase. Both nuclear and mitochondrial DNA can be affected.

Arsenic poisoning

As previously described (see p. 111), pentavalent arsenic (arsenate) can interfere with glycolysis at the glyceraldehyde 3-phosphate step, thereby decreasing ATP production. However, **arsenic poisoning** is due primarily to inhibition of enzyme complexes that require lipoic acid as a coenzyme, including PDH, α-ketoglutarate dehydrogenase (see E. below), and branched-chain α-keto acid dehydrogenase (see Chapter 20 Section III). Arsenite (the trivalent form of arsenic) forms a stable complex with the thiol (–SH) groups of lipoic acid, making that compound unavailable to serve as a coenzyme. When it binds to lipoic acid in the PDHC, pyruvate (and, consequently, lactate) accumulates. As with PDHC deficiency, this particularly affects the brain, causing neurologic disturbances and death.

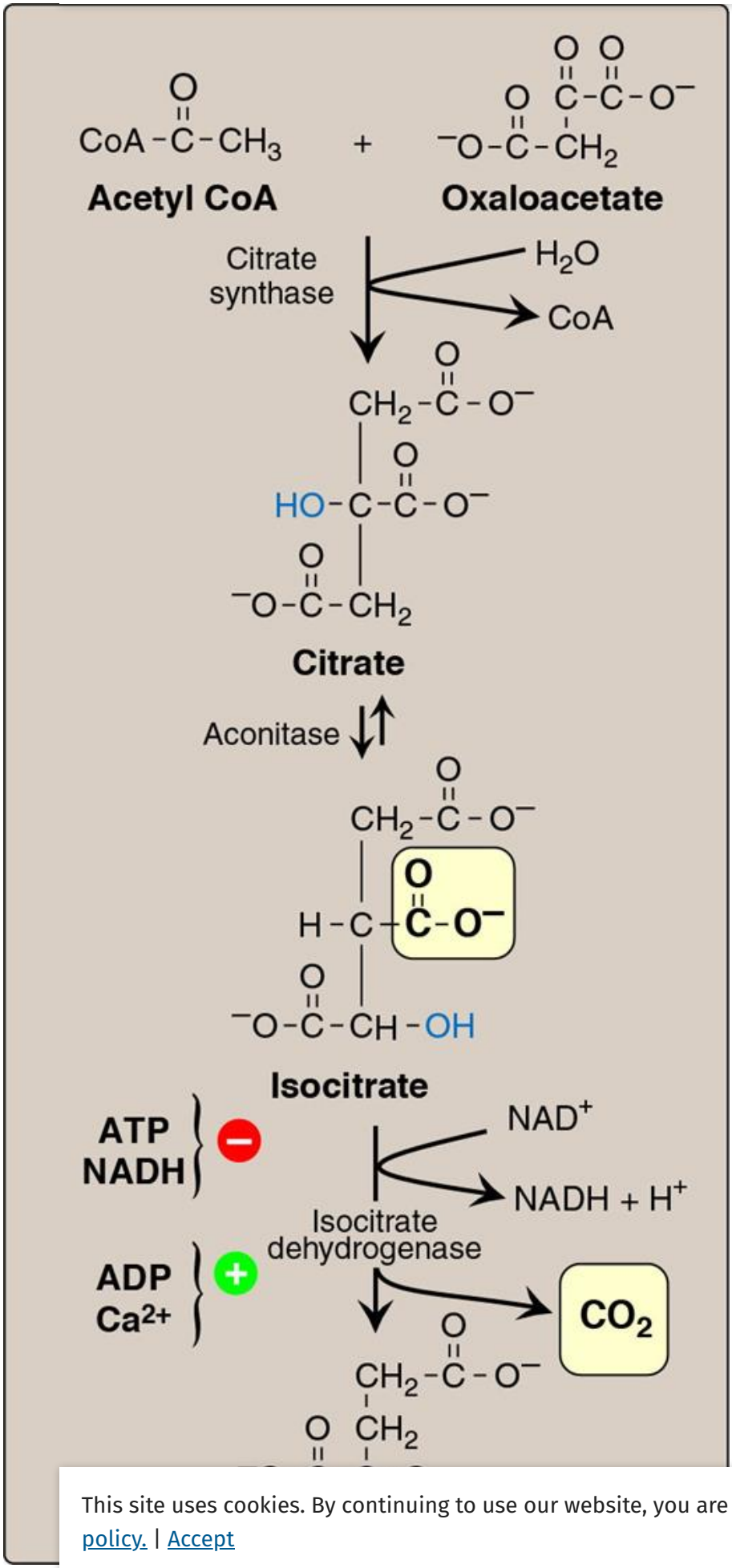
Citrate synthesis

The irreversible condensation of acetyl CoA and OAA to form citrate (a TCA) is catalyzed by citrate synthase, the initiating enzyme of the TCA cycle (Fig. 9.4). This aldol condensation has a highly negative change in standard free energy ([ΔG^0]), which strongly favors citrate formation. The enzyme is inhibited by citrate (product inhibition). Substrate availability is another means of regulation for citrate synthase. The binding of OAA greatly increases the enzyme's affinity for acetyl CoA. (Note: Citrate, in addition to being an intermediate in the TCA cycle, is a source of acetyl CoA for the cytosolic synthesis of fatty acids and cholesterol. Citrate also inhibits phosphofructokinase-1 [PFK-1], the rate-limiting enzyme of glycolysis, and activates acetyl CoA carboxylase [the rate-limiting enzyme of fatty acid synthesis, see Chapter 16 Section III].)

FIGURE 9.4

Formation of α-ketoglutarate from acetyl coenzyme A (CoA) and oxaloacetate.

NAD(H) = nicotinamide adenine dinucleotide; CO_2 = carbon dioxide.



Citrate isomerization

Citrate is isomerized to **isocitrate** through hydroxyl group migration catalyzed by **aconitase** (aconitate hydratase), an iron-sulfur protein (see Fig. 9.4). (Note: Aconitase is inhibited by fluoroacetate, a 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.)

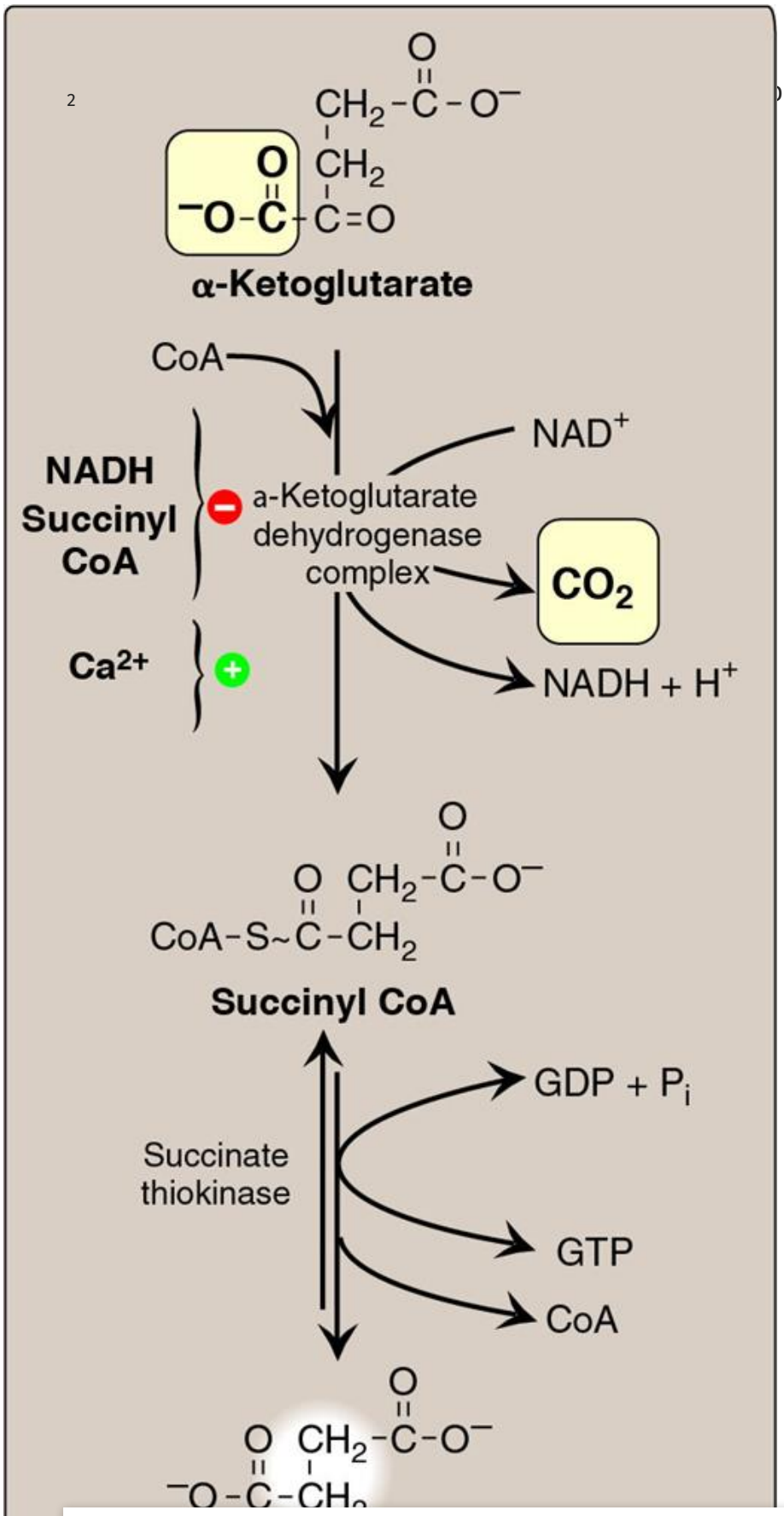
Oxidative decarboxylation of isocitrate

Isocitrate dehydrogenase catalyzes the irreversible oxidative decarboxylation of isocitrate to **α-ketoglutarate**, yielding the first of three NADH molecules produced by the cycle and the first release of CO_2 (see Fig. 9.4). This is one of the rate-limiting steps of the TCA cycle. The enzyme is allosterically activated by ADP (a low-energy signal) and Ca^{2+} and is inhibited by ATP and NADH, levels of which are elevated when the cell has abundant energy stores.

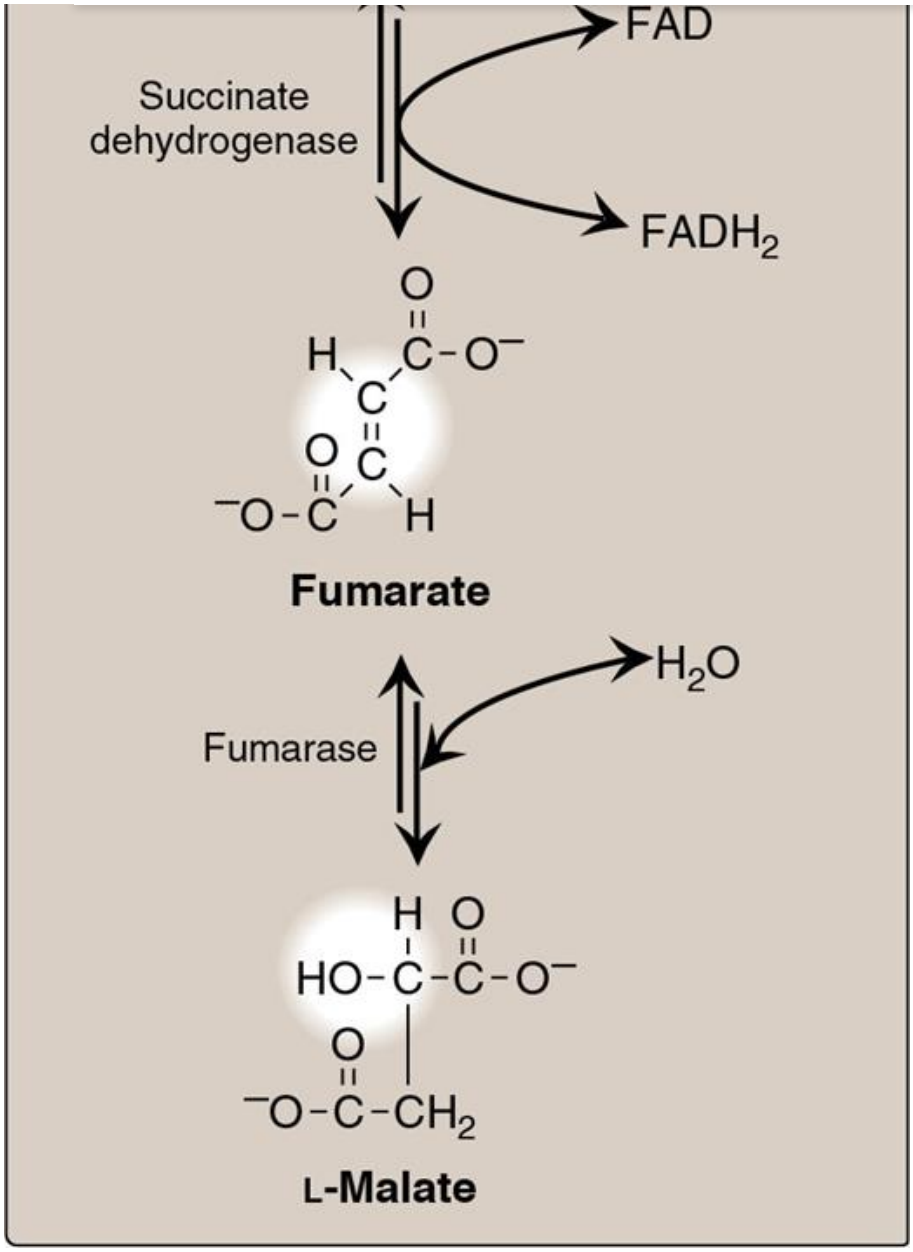
Oxidative decarboxylation of α-ketoglutarate

The irreversible conversion of **α-ketoglutarate** to **succinyl CoA** is catalyzed by the α-ketoglutarate dehydrogenase complex, a protein aggregate of multiple copies of three enzymes (Fig. 9.5). The mechanism of this oxidative decarboxylation is very similar to that used for the conversion of pyruvate to acetyl CoA by the PDHC. The reaction releases the second CO_2 and produces the second NADH of the cycle. The coenzymes required are TPP, lipoic acid, FAD, NAD^+, and CoA. Each functions as part of the catalytic mechanism in a way analogous to that described for the PDHC. The large negative ΔG^0 of the reaction favors formation of succinyl CoA, a high-energy thioester similar to acetyl CoA. The α-ketoglutarate dehydrogenase complex is inhibited by its products, NADH and succinyl CoA, and activated by Ca^{2+}. However, it is not regulated by phosphorylation/dephosphorylation reactions as described for the PDHC. (Note: α-Ketoglutarate is also produced by the oxidative deamination and transamination of the amino acid glutamate.)

FIGURE 9.5



2)P and GTP = guanosine di- and



Succinyl CoA cleavage

Succinate thiokinase (also called succinyl CoA synthetase, named for the reverse reaction) cleaves the high-energy thioester bond of succinyl CoA (see Fig. 9.5). This reaction is coupled to phosphorylation of guanosine diphosphate (GDP) to guanosine triphosphate (GTP). GTP and ATP are energetically interconvertible by the nucleoside diphosphate kinase reaction:

$$GTP + ADP \rightleftarrows GDP + ATP$$

The generation of GTP by succinate thiokinase is another example of **substrate-level** phosphorylation (see p. 112). (Note: Succinyl CoA is also produced from propionyl CoA derived from the metabolism of fatty acids with an odd number of carbon atoms and from the metabolism of several amino acids. It can be converted to pyruv

Succ

Succinate is oxidized to **fumarate** by succinate dehydrogenase, as its coenzyme FAD is reduced to $FADH_2$ (see Fig. 9.5). Succinate dehydrogenase is the only enzyme of the TCA cycle that is embedded in the inner mitochondrial membrane. As such, it functions as Complex II of the ETC (see p. 83). (Note: FAD, rather than NAD^+, is the electron acceptor because the reducing power of succinate is not sufficient to reduce NAD^+.)

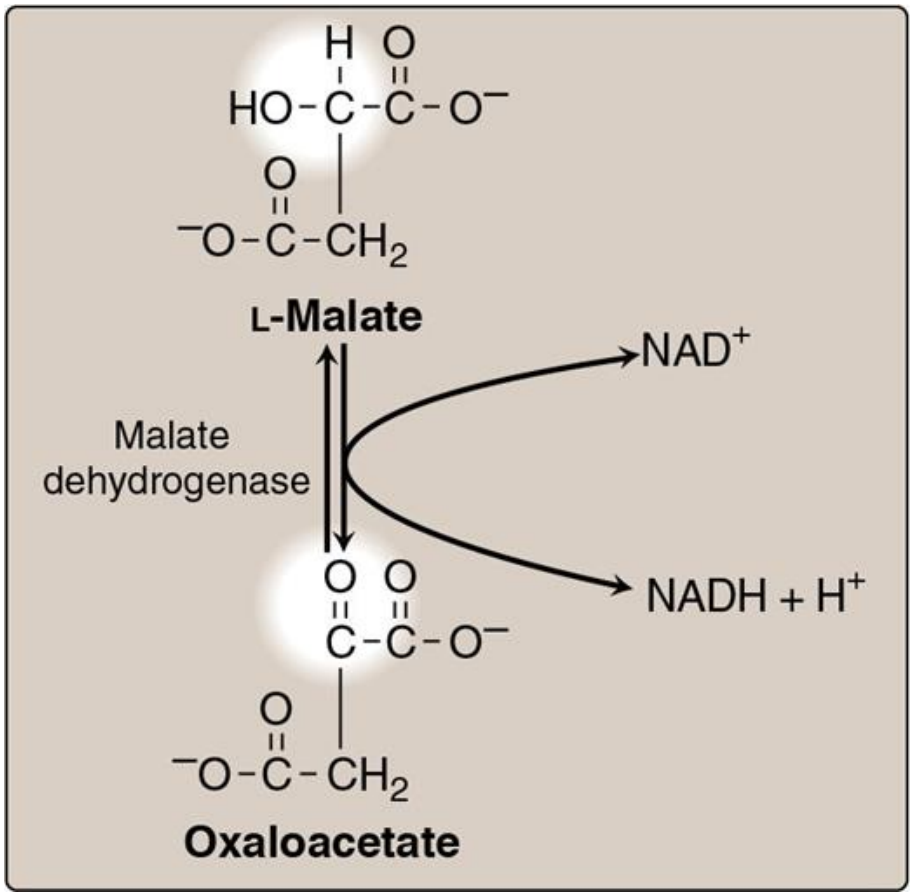
Fumarate hydration

Fumarate is hydrated to **malate** in a freely reversible reaction catalyzed by **fumarase** (fumarate hydratase, see Fig. 9.5). (Note: Fumarate is also produced by the urea cycle, in purine synthesis [see Fig. 22.7], and during catabolism of the amino acids phenylalanine and tyrosine.)

Malate oxidation

Malate is oxidized to OAA by malate dehydrogenase (Fig. 9.6). This reaction produces the third and final **NADH** of the cycle. The ΔG^0 of the reaction is positive, but the reaction is driven in the direction of OAA by the highly exergonic citrate synthase reaction. (Note: OAA is also produced by the transamination of the amino acid aspartic acid.)

FIGURE 9.6



Ene

Four pairs of electrons are transferred during one turn of the TCA cycle: three pairs reducing three NAD^+ to NADH and one pair reducing FAD to $FADH_2$. Oxidation of one NADH by the ETC leads to formation of three ATP, whereas oxidation of $FADH_2$ produces two ATP. The total yield of ATP from the oxidation of one acetyl CoA is shown in Figure 9.7. Figure 9.8 summarizes the reactions of the TCA cycle. (Note: The cycle does not involve the net consumption or production of intermediates. Two carbons entering as acetyl CoA are balanced by two CO_2 exiting.)

FIGURE 9.7

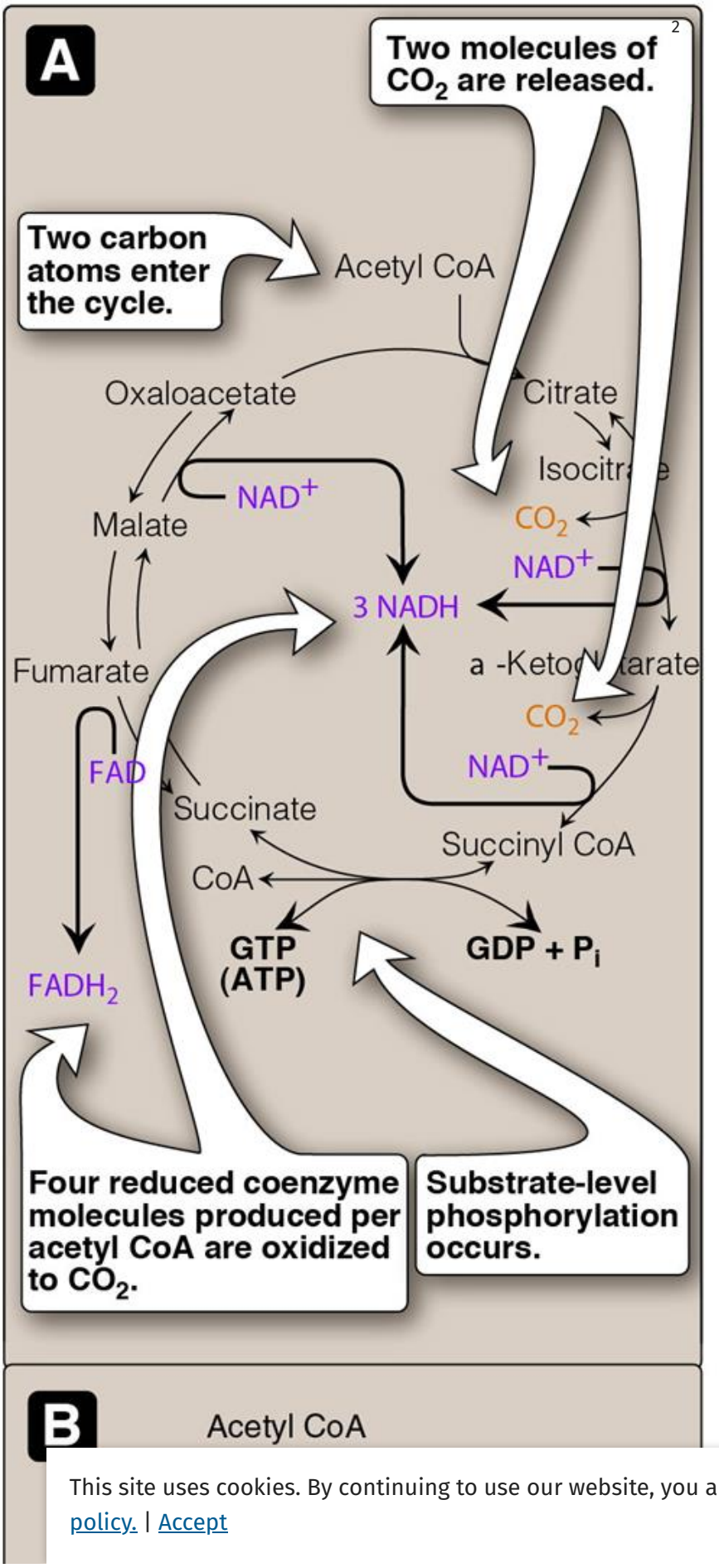
Energy-producing reaction	Number of ATP produced
$3\ NADH \xrightarrow{i} 3\ NAD^+$	9
$FADH_2 \longrightarrow FAD$	2
$GDP + P_i \longrightarrow GTP$	1
	12 ATP/acetyl CoA oxidized

…olecule of acetyl coenzyme A

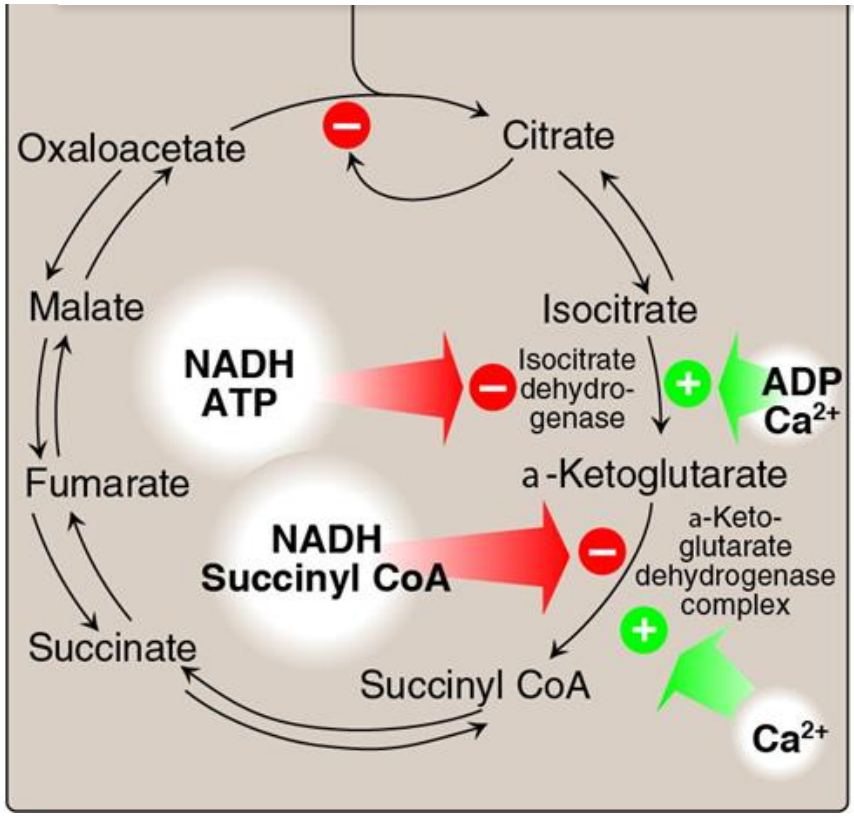
.

…DP and GTP = guanosine di- and

FIGURE 9.8



e tricarboxylic acid cycle. (Note: diphosphate kinase.) **B:** Inhibitors and



Cycle Regulation

Listen

In contrast to glycolysis, which is regulated primarily by PFK-1, the TCA cycle is controlled by the regulation of several enzymes (see Fig. 9.8). The most important of these regulated enzymes are those that catalyze reactions with highly negative ΔG^0: citrate synthase, isocitrate dehydrogenase, and the α-ketoglutarate dehydrogenase complex. Reducing equivalents needed for oxidative phosphorylation are generated by the PDHC and the TCA cycle, and both processes are upregulated in response to a decrease in the ATP/ADP ratio.

Chapter Summary

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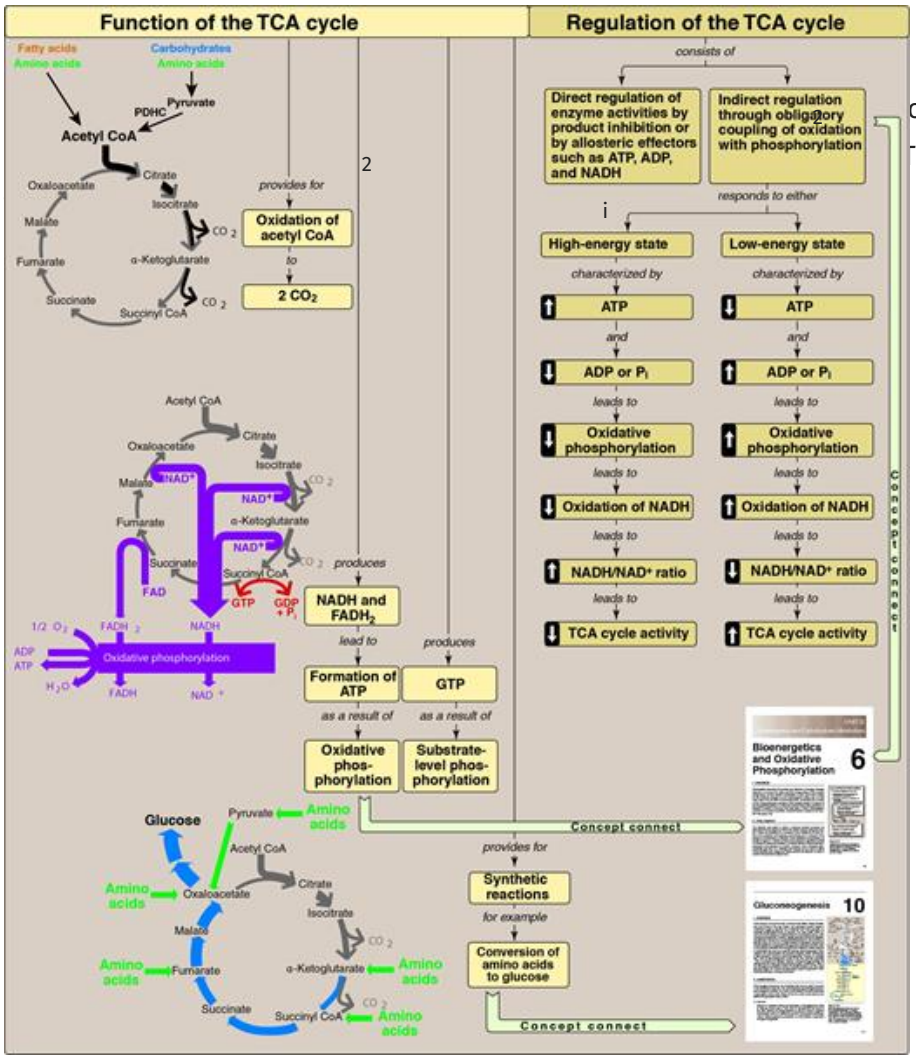
- In the **TCA cycle**, also called the **Krebs cycle, pyruvate** is oxidatively decarboxylated by the **PDHC**, producing **acetyl CoA** (Fig. 9.9).

FIGURE 9.9



- The multienzyme PDHC requires five coenzymes: **TPP**, **lipoic acid**, **flavin adenine dinucleotide** (**FAD**), **nicotinamide adenine dinucleotide** (**NAD^+**), and **CoA**.
- PDHC is regulated by covalent modification of **E1**, by **PDH kinase** and **PDH phosphatase**: Phosphorylation inhibits E1.
- PDH kinase is allosterically activated by ATP, acetyl CoA, and NADH and inhibited by pyruvate. The phosphatase is activated by calcium (Ca^{2+}).
- **Pyruvate decarboxylase deficiency** is the most common biochemical cause of **congenital lactic acidosis**. The brain is particularly affected in this **X-linked dominant** disorder.
- **Arsenic poisoning** causes inactivation of the PDHC by binding to lipoic acid. In the TCA cycle, **citrate** is synthesized from **OAA** and **acetyl CoA** by **citrate synthase**, which is inhibited by product.
- **Citr**
  dec is

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

- **α-Ketoglutarate** is oxidatively decarboxylated to **succinyl CoA** by the **α-ketoglutarate dehydrogenase complex**, producing CO_2 and NADH. The enzyme is very similar to the PDHC and uses the same coenzymes.
- The α-ketoglutarate dehydrogenase complex is activated by Ca^{2+} and inhibited by NADH and succinyl CoA but is not covalently regulated. Succinyl CoA is cleaved by **succinate thiokinase** producing **succinate** and **GTP**. This is an example of **substrate-level phosphorylation**.
- Succinate is oxidized to **fumarate** by **succinate dehydrogenase**, producing **$FADH_2$**. Fumarate is hydrated to **malate** by **fumarase** (**fumarate hydratase**), and malate is oxidized to OAA by **malate dehydrogenase**, producing **NADH**.
- **Three NADH** and **one $FADH_2$** are produced by one round of the TCA cycle.
- The generation of acetyl CoA by the oxidation of pyruvate via the PDHC also produces an NADH. Oxidation of the NADH and $FADH_2$ by the ETC yields 14 ATP. The terminal phosphate of the GTP produced by substrate-level phosphorylation in the TCA cycle can be transferred to ADP by nucleoside diphosphate kinase, yielding another ATP.
- Therefore, a total of 15 ATP are produced from the complete mitochondrial oxidation of pyruvate to CO_2.

Study Questions

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Choose the ONE best answer.

9.1. The conversion of pyruvate to acetyl coenzyme A and carbon dioxide:

A. involves the participation of lipoic acid.

B. is activated when pyruvate decarboxylase of the pyruvate dehydrogenase complex (PDHC) is phosphorylated by PDH kinase in the presence of ATP.

C. is reversible.

D. occurs in the cytosol.

E. requires the coenzyme biotin.

Correct answer = A. Lipoic acid is an intermediate acceptor of the acetyl group formed in the reaction. (Note: Lipoic acid linked to a lysine residue in E2 functions as a “swinging arm” that allows interaction with E1 and E3.) The PDHC catalyzes an irreversible reaction that is inhibited when the decarboxylase component (E1) is phosphorylated. The PDHC is located in the mitochondrial matrix. Biotin is utilized by carboxylases, not decarboxylases.

9.2. Which one of the following conditions decreases the oxidation of acetyl coenzyme A by the citric acid cycle?

A. A high availability of calcium

B. A high acetyl CoA/CoA ratio

C. A low ATP/ADP ratio

D. A low NAD^+/NADH ratio

Correct answer = D. A low NAD^+/NADH (oxidized to reduced nicotinamide adenine dinucleotide) ratio limits the rates of the NAD^+-requiring dehydrogenases. High availability of calcium and substrate (acetyl coenzyme A) and a low ATP/ADP (adenosine tri- to diphosphate) ratio stimulate the cycle.

9.3. The following is the sum of three steps in the citric acid cycle.

Choose the lettered answer that corresponds to the missing "A," "B," and "C" in the equation.

Reactant A	Reactant B	Product C
A. Succinyl CoA	GDP	Succinate
B. Succinate	NAD^+	Oxaloacetate
C. Fumarate	NAD^+	Oxaloacetate
D. Succinate	NAD^+	Malate
E. Fumarate	GTP	Malate

Correct answer = B. Succinate + NAD^+ + FAD + H_2O → oxaloacetate + NADH + $FADH_2$.

9.4. A 1-month-old male shows neurologic problems and lactic acidosis. An enzyme activity assay for pyruvate dehydrogenase complex (PDHC) performed on extracts of cultured skin fibroblasts showed 5% of normal activity with a low concentration of thiamine pyrophosphate (TPP) but 80% of normal activity when the assay contained a thousand-fold higher concentration of TPP. Which one of the following statements concerning this patient is correct?

A. Administration of thiamine is expected to reduce his serum lactate level and improve his clinical symptoms.

B. A high-carbohydrate diet would be expected to be beneficial for this patient.

C. Citrate production from aerobic glycolysis is expected to be increased.

D. PDH kinase, a regulatory enzyme of the PDHC, is expected to be active.

Correct answer = A. The patient appears to have a thiamine-responsive PDHC deficiency. The pyruvate decarboxylase (E1) component of the PDHC fails to bind thiamine pyrophosphate at low concentration but shows significant activity at a high concentration of the coenzyme. This mutation, which affects the K_m (Michaelis constant) of the enzyme for the coenzyme, is present in some, but not all, cases of PDHC deficiency. Because the PDHC is an integral part of carbohydrate metabolism, a diet low in carbohydrates would be expected to blunt the effects of the enzyme deficiency. Aerobic glycolysis generates pyruvate, the substrate of the PDHC. Decreased activity of the complex decreases production of acetyl coenzyme A, a substrate for citrate synthase. Because PDH kinase is allosterically inhibited by pyruvate, it is inactive.

9.5. Which coenzyme–cosubstrate is used by dehydrogenases in both glycolysis and the tricarboxylic acid cycle?

Oxidized nicotinamide adenine dinucleotide (NAD^+) is used by glyceraldehyde 3-phosphate dehydrogenase of glycolysis and by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase of the tricarboxylic acid cycle. (Note: E3 of the pyruvate dehydrogenase complex requires oxidized flavin adenine dinucleotide [FAD] and NAD^+.)