ЗМІСТ

Biological and Bioorganic Chemistry: in 2 books. Book 2. Biological Chemistry: textbook

Про книгу

This textbook contains a systematic presentation of the course of biological chemistry according to the educational program for students of higher medical (pharmaceutical) educational establishments. The core text of this book examines the structure of an enzyme, and the metabolic pathways of the major classes of biomolecules (proteins, amino acids, carbohydrates, lipids, nucleotides, porphyrins); structural features and properties of nucleic acids, DNA and RNA; molecular biology and genetics, biochemical foundations of the physiological functions of the human body and their neurohumoral regulation are highlighted. Considerable attention is paid to the molecular mechanisms underlying the functions of blood cells, liver, kidneys, muscles, connective tissue, immune and nervous systems. The biochemical basis of the pathogenesis of atherosclerosis, diabetes mellitus, obesity, diseases of the endocrine, immune, nervous systems and connective tissue are considered. In addition to informational material, each chapter of the textbook contains tests and tasks for self-control.

BIOLOGICAL AND BIOORGANIC Chemistry

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IN 2 BOOKS
2
BOOK

BIOLOGICAL CHEMISTRY

Second edition

APPROVED
by the Ministry of Education and Science of Ukraine as a textbook for students of higher medical educational establishments

PUBLISHED
pursuant to the Order of the Ministry of Health of Ukraine No. 502 as of 22 June 2010 as a national textbook for students of higher medical educational establishments

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CONTENT

Chapter 3
FUNDAMENTAL REGULARITIES OF METABOLISM. TRICARBOXYLIC ACID CYCLE

3.1. COMMON PATHWAYS OF PROTEIN, LIPID, AND CARBOHYDRATE METABOLISM

Metabolism is a set of life-sustaining chemical reactions taking place in the organism, i.e. a sequence of reactions that lead to the appearance of specified product.

Metabolism fulfills four specific functions:

— the supply of chemical energy resulting from the splitting of energy-yielding nutrients, synthesis of high-energy compounds (ATP, etc.) and their use for various types of work;

— the transformation of nutrient molecules into low molecular weight metabolites (building blocks), which are further used by the cell for a construction of macromolecules;

— the synthesis of proteins, lipids, polysaccharides, nucleic acids and other cellular components from these building blocks with the use of energy of ATP and NADPH;

— the synthesis and decomposition of low-molecular-weight biologically active compounds.

A metabolism involves pathways that are *anabolism* (from Greek *ana* — upward), which is intended for building molecules, and *catabolism* (from Greek *kata* — down) — breaking down of complex molecules. Compare the main features of these metabolic pathways (Table 3.1).

Table 3.1. **Pathways: catabolism and anabolism**

Catabolism	Anabolism
1. The breaking down of complex organic molecules into simpler end products. Important key reactions are oxidation of metabolites. Oxidized coenzymes are used, reduced ones are formed.	1. Synthesis of complex organic molecules from simple ones. Important key reactions — reduction. Reduced forms of coenzymes are used, oxidized are formed
2. A free energy is released (exergonic processes). Part of it is used for ATP formation	2. Energy is consumed (endergonic processes). The source of energy is ATP, that is as a result of catabolic processes
3. The same end products are formed from different starting substances.	3. The same starting substances form different end products
4. Intermediate products (metabolites) and end products of catabolism may serve as substrates (starting substances) for anabolism	4. The end products of anabolism serve as starting substances for catabolism

Thus, the catabolic and anabolic pathways are different, but at same time are closely interconnected through the system of ATP—ADP, reduced and oxidized forms of coenzymes ($NADP^+$, NAD^+, FAD^+), substrates and products. Catabolism and anabolism are conjugated complementary processes. The connection between catabolism and anabolism provides the optimal level of metabolism.

Metabolism has several consecutive stages.

1. Intake of nutrients — proteins, lipids, carbohydrates, vitamins, mineral elements, water into the body as part of food.
2. Transformation of nutrients — proteins, polysaccharides, fats in the digestive tract into simpler compounds: amino acids, monosaccharides, fatty acids, glycerol.
3. Transport (absorbtion) of digested products into the bloodstream or lymph, they passed through vessels wall and cell membrane to certain organs and tissues (liver, muscles, brain, kidneys, adipose tissue, etc.).
4. Intracellular metabolism of biomolecules in organs and tissues (intermediary metabolism, or intrinsic metabolism in the narrow sense).
5. Isolation (excretion) the waste products (carbon dioxide, ammonia, urea, water, products of conjugation of some organic molecules and products of their oxidation) through the kidneys, lungs, skin, gut.

The reactions of intracellular metabolism include the following biochemical transformations.

1. The breakdown of bioorganic molecules (glucose, fatty acids, amino acids, glycerol) to the end products of the intermediate metabolism (carbon dioxide, water, ammonia) with the release of chemical energy and its accumulation in the form of adenosine triphosphoric acid (adenosine triphosphate, ATP), other macroergic phosphates or proton potential that provide the energy needed to maintain body functions and carry out the activities of daily life.

Simple metabolites are subject to very specific cleavage reactions, which release a relatively small amount of energy: carbohydrates undergo anaerobic glycolysis, are involved in the reactions of the pentose phosphate pathway (PPP); fatty acids undergo β-oxidation; amino acids — deamination and transamination. During breakdown processes, products that are further involved in the tricarboxylic acid cycle (TCA cycle): acetyl-CoA, succinyl-CoA, α-ketoglutaric acid, oxalic acid, fumaric acid may be formed.

As a result, some of the cleavage reactions do not directly form compounds that participate in the TCA cycle: carbohydrates, some amino acids and glycerol are cleaved to pyruvic acid (PA), other amino acids and fatty acids with an odd number of carbons atoms form propionyl-CoA. However, pyruvic acid is then converted to acetyl-CoA, propionyl-CoA — to succinyl-CoA, and these compounds are already directly involved in the TCA cycle. These reactions are very important, and with their help a large number of metabolites, and partially carbohydrates, can enter the TCA cycle, where they are completely or partially cleaved. The conversion of pyruvic acid to acetyl-CoA, the TCA cycle and the electron transport chain in the mitochondria are referred to the *common pathway of catabolism* (Fig. 3.1).

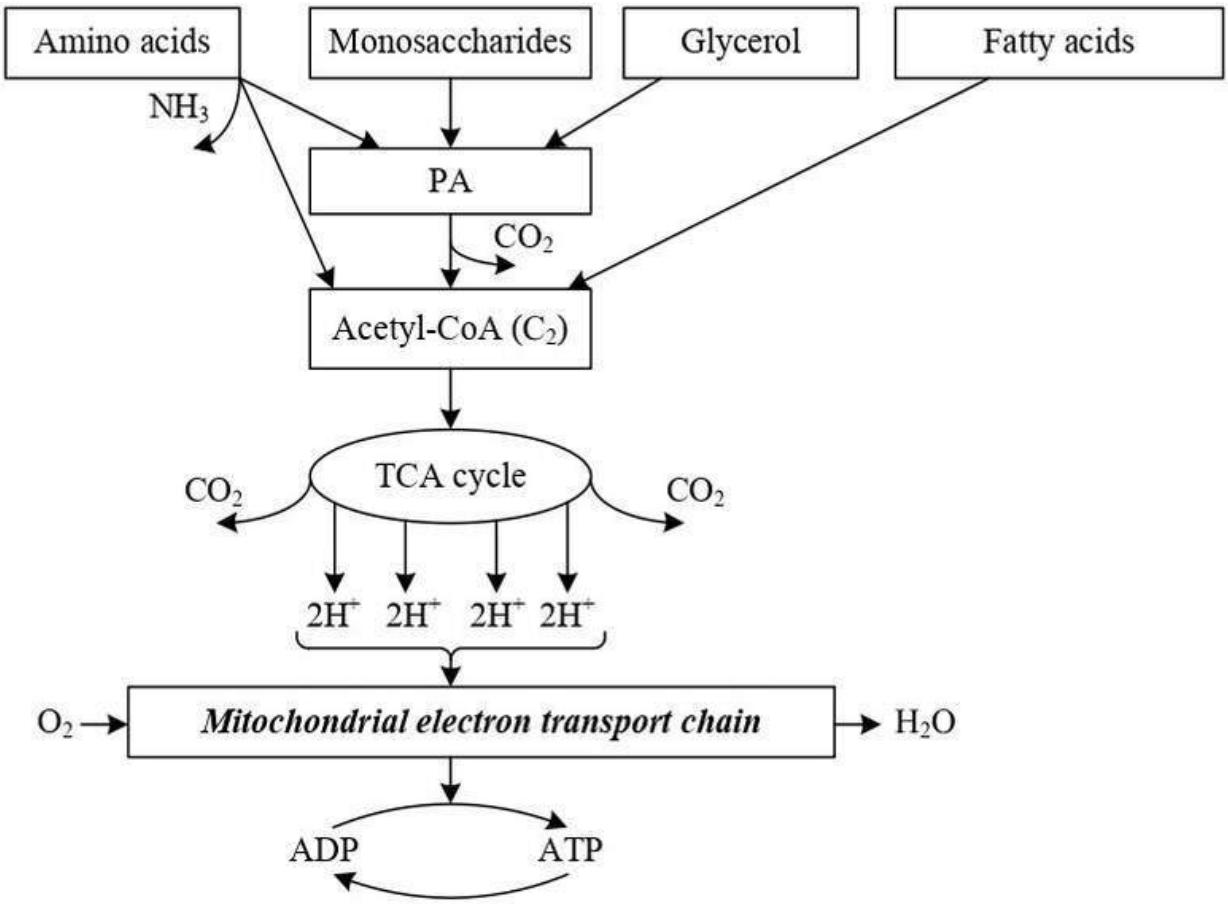


Fig. 3.1. **Scheme of common pathways of catabolism of biomolecules**

2. Synthesis of specific, genetically inherent biomolecules of certain organism (proteins, nucleic acids, polysaccharides, lipids, bioregulators, etc.), necessary for the formation of their own cellular and extracellular biostructures. These processes are called *anabolism* and require the energy in the form of ATP.

3. The using of energy (in the form of ATP or proton potential) provides such processes of cellular physiology as the muscle contraction, exo- and endocytosis, membrane potential generation, active transport of metabolites and inorganic ions.

The set of consecutive reactions of the conversion of a biomolecule to a specific product forms a *metabolic pathway*. To determine the metabolic pathways, the structures of substrate, the reactions of their transformation, the enzymes that catalyze these reactions and the regulatory mechanisms that ensure a normal metabolism, the rate of consecutive reactions at which the transformation of the substrate into the end product occurs, must be known. For example, substance *A* is converted to the end product *L* as the result of six consecutive enzymatic reactions:

$$A \xrightarrow{E_1} B \xrightarrow{E_2} C \xrightarrow{E_3} D \xrightarrow{E_4} G \xrightarrow{E_5} K \xrightarrow{E_6} L$$

Enzymes that catalyze consecutive stages form a multienzyme system — product of the first reaction serves as a substrate for the reaction, which is catalyzed by another enzyme, etc. The metabolic pathways are mostly linear, although they may be cyclic (Fig. 3.2).

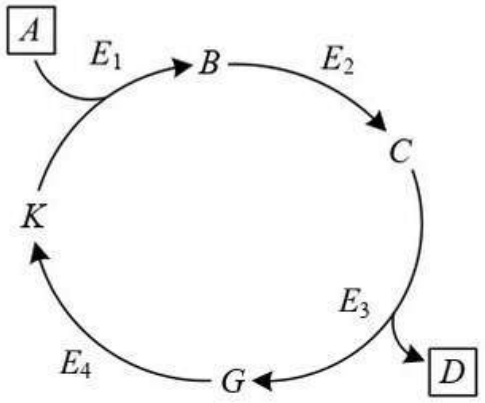


Fig. 3.2. **A scheme of cyclic metabolic partway of substance A transformation into a product D**

The transformation of proteins, lipids and carbohydrates is *central metabolic pathways*: the flow of metabolites in these pathways is quite large (hundreds and tens of grams). In the body, there are also specific metabolic pathways with a significantly smaller flow of metabolites (daily synthesis or breakdown is measured by milligrams). The central metabolic pathways, for example, include the synthesis of DNA, RNA, proteins, TAC cycle, the synthesis of fatty acids, etc. Specific metabolic pathways include the metabolism of glucuronic acid, sorbitol, carnosine, andzersin, etc.

All metabolic pathways are finally interrelated and in the event of a violation of any of them, all others undergo changes.

Energy and Metabolism. Metabolism is inextricably linked with conversion of energy, that is, metabolism would be impossible without accompanying exchange of energy. Each enzymatic reaction of the transformation of a substance is accompanied by the transformation of energy. At certain stages of catabolism, chemical energy is released and stored predominantly in the form of energy of phosphate bonds of ATP, and at some stages of anabolism it is consumed.

Energy relations cause a close interrelation between anabolic and catabolic pathways: each time the synthesis of complex molecules that consume energy must happen simultaneously with the processes that supply energy — breakdown of complex molecules or oxidation. Processes occurring with the release of energy are called *exergonic*, and with energy consumption — *endergonic*. The main exergonic reaction in the body is a synthesis of water during cellular respiration and basic endergonic reaction — synthesis of ATP from ADP and inorganic phosphate, which is associated with the release of energy during cellular respiration.

ATP plays a significant role in the bioenergetic processes. The ability of ATP to accumulate and supply energy, that is, to form an ATP—ADP system, occupying an intermediate position in the thermodynamic scale of phosphorylated compounds, determines the function of this system as the carrier of energy-rich phosphate groups from high-energy phosphorylated compounds, which are higher on the thermodynamic scale than ATP, to less energy-rich compounds that are activated by the adding phosphate. In the body, synthesis of many other macroergic compounds occurs in the presence of ATP. The formation of cre-

atine phosphate and nucleoside triphosphates (guanosine triphosphate (GTP), uridine triphosphate (UTP), cytidine triphosphate (CTP)), which, like ATP, can be a source of energy in biosynthetic processes, is of great importance in the energy metabolism.

It is interesting to give some calculations that characterize the amount of synthesized ATP in the human body. It turns out that a man with a body weight of 70 kg produces 75 kg of ATP per day, that is, more than its own weight. Certainly, it should be borne in mind that ATP molecules are always expended for the work, and new simply synthesized molecules ATP are formed in their place (75 kg of ATP, manufactured by industry, cost 150 thousand dollars).

Living systems require a constant flow of energy for their vital activity, the lack of energy in the cell is accompanied by a complete failure of functions. Life, growth, and cell integrity depend on food not only as a source of nutrients and various essential elements, but also as a source of energy.

3.2. OXIDATIVE DECARBOXYLATION OF PYRUVIC ACID

Pyruvate is one of the important oxidation substrates, which is formed as an intermediate product of catabolism of monosaccharides, amino acids, glycerol. Oxidation of pyruvate takes place in the mitochondrial matrix, where it comes from the cytoplasm. In addition to pyruvate, other substrates are oxidized in mitochondria. Some of them take part in the acceptance of the cytoplasmic hydrogen and transfer it to the mitochondrial respiratory chain. The value of pyruvate as a substrate of oxidation is not only that it is a source of hydrogen, but also acetyl-CoA, which can be referred to the main producers of hydrogen in mitochondria. Let us dwell on the enzymatic system of pyruvate oxidation.

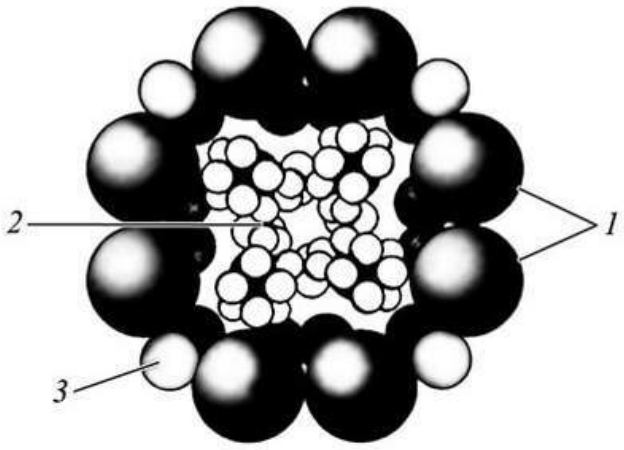


Fig. 3.3. **Structure of multienzyme pyruvate dehydrogenase complex**
(according to L.N. Voronina, V.F. Desenko, N.N. Madievskaya et al., 2000):
1 — pyruvate dehydrogenase; *2* — dihydrolipoyl acetyltransferase; *3* — dihydrolipoyl dehydrogenase

The oxidative decarboxylation of pyruvic acid is catalyzed by a *multienzyme pyruvate dehydrogenase complex*. This complex is found in the mitochondrial matrix, but dissolved, and is attached to the proteins of the inner mitochondrial membrane immersed in the matrix. Pyruvate dehydrogenase complex is an example of the structural organization of several different enzymes and has all the benefits of such an organization. The mass of the pyruvate dehydrogenase complex is $4 \cdot 10^6$ Da. It consists of three different enzymes: pyruvate dehydrogenase (E_1), dihydrolipoyl acetyltransferase (E_2) and dihydrolipoyl dehydrogenase (E_3) (Fig. 3.3).

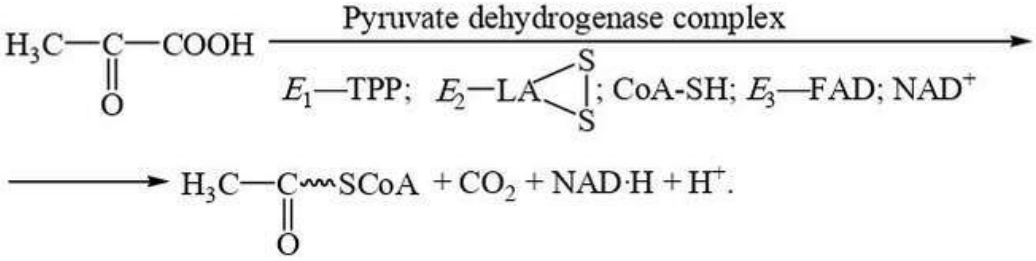
Pyruvate dehydrogenase consists of 24 molecules of the enzyme, each of which contains one residue of thiamine pyrophosphate (TPP), which is a coenzyme of pyruvate dehydrogenase. The total mass of this enzyme is approximately $2{,}16 \cdot 10^6$ Da.

Dihydrolipoyl acetyltransferase has a mass of about $0{,}76 \cdot 10^6$ Da, the quaternary structure of this enzyme consists of 24 subunits. Each subunit of dihydrolipoyl acetyltransferase contains one residue of lipoic acid (LA).

The pyruvate dehydrogenase complex consists of 12 molecules of *dihydrolipoyl dehydrogenase*, each of which contains one residue of FAD. The total mass of this enzyme complex is $0{,}66 \cdot 10^6$ Da.

Thus, all enzymes of pyruvate dehydrogenase complex are two-component and contain tightly bound coenzymes: thiamine pyrophosphate, lipoic acid and FAD. In addition, two external (unbound with complex) coenzymes — CoA-SH and NAD^+, which play the role of acceptors of the products of pyruvate oxidation, take part in the work of complex.

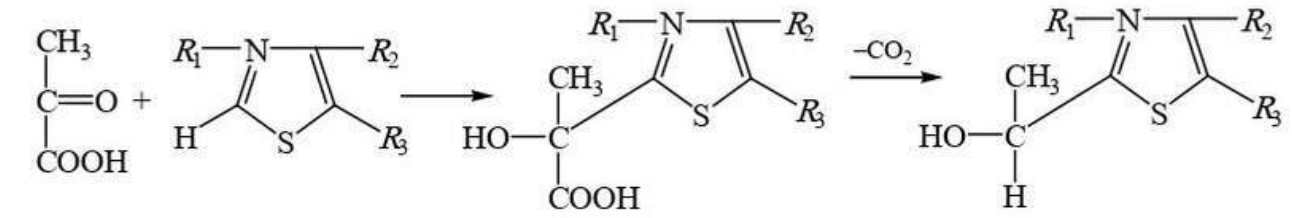
The conversion of pyruvate to acetyl-CoA is described by overall reaction:



In the course of this reaction, the oxidative decarboxylation of the pyruvate occurs, as a result of which the carboxyl group is removed in the form of CO_2, and the acetyl group is included in the acetyl-CoA — the main substrate of oxidation in the tricarboxylic acid cycle, and NAD is reduced.

Oxidative decarboxylation of pyruvate takes place in five stages:

1. Pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1), undergoing decarboxylation to from hydroxyethyl derivative:

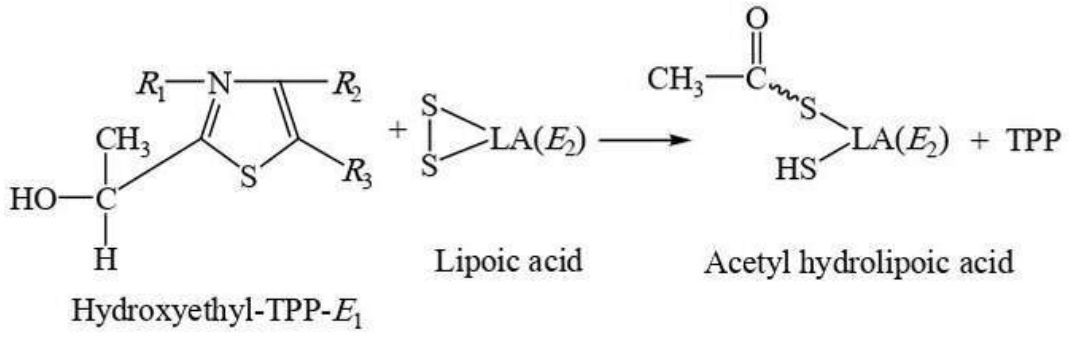


Pyruvate TPP-E_1 Hydroxyethyl-TPP-E_1

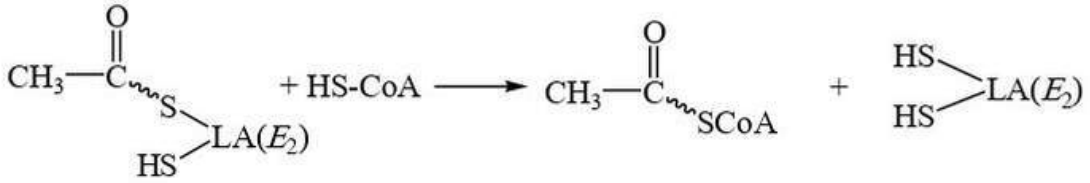
Oxidative decarboxylation of pyruvate is the only pathway of its catabolism; therefore, the deficiency of vitamins (primarily vitamin B_1) leads to a violation of the process, a decrease in the formation of ATP and manifests itself disorder of the central nervous system.

2. Oxidation of the hydroxyethyl group to the acetyl group and the simultaneous transfer of the acetyl group from the TPP to the oxidized form of lipoic acid that is part of the

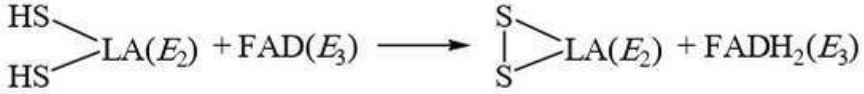
dihydrolipoyl acetyltransferase. The product of this reaction acetyl hydrolipoic acid has a macroergic thioester bond:



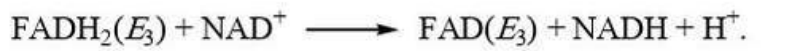
3. Transfer of acetyl group from acetyl hydrolipoic acid to HS-CoA yields molecule of acetyl-CoA. The second HS-group of lipoic acid is reduced due to HS-CoA to form dihydrolipoic acid. *Dihydrolipoyl acetyltransferase* catalyzes this reaction:



4. Oxidation of dihydrolipoic acid (reduced, sulfhydryl form of LA) to lipoic acid, its disulfide (oxidized) form. In the course of this reaction, two hydrogen atoms are transferred from dihydrolipoic acid to the FAD^+ , which is prosthetic group of dihydrolipoyl dehydrogenase:



5. Oxidation of $FADH_2$ to FAD^+. Hydrogen is transferred from $FADH_2$ to NAD^+ and NADH is formed. The same enzyme catalyzes the reaction:



Oxidation of one molecule of NADH gives three molecules of ATP.

Biological role of oxidative decarboxylation of pyruvate is as follows:

— the catabolism of pyruvate to one of the end products — CO_2 (removed from the body or used for synthesis);

— the formation of macroergic compound — acetyl-CoA (subject to further oxidation in the tricarboxylic acid cycle or used in the reaction of anabolism);

— the synthesis of reduced equivalent — NADH (oxidized in the mitochondrial electron transport chain).

Oxidative decarboxylation of pyruvate is regulated by changing the activity of pyruvate dehydrogenase in two ways. Firstly, an excess of the reaction products, such as acetyl-CoA

and NADH, inhibits the enzyme, and the glycolytic intermediate fructose-1,6-diphosphate, NAD^+, CoA are activators of pyruvate dehydrogenase. Allosteric effects are manifested very quickly.

Secondly, activity of pyruvate dehydrogenase is regulated by covalent modification through phosphorylation and dephosphorylation of the enzyme. In presence of ATP, pyruvate dehydrogenase is phosphorylated by protein kinase that resulting in loss of enzyme activity. However, phosphoprotein phosphatase causes the restoration enzyme activity by dephosphorylation of the enzyme. This mechanism of regulation is slower.

Pyruvate dehydrogenase deficiency leads to an increase in the concentration of lactate, pyruvate, alanine, which is accompanied by acidosis.

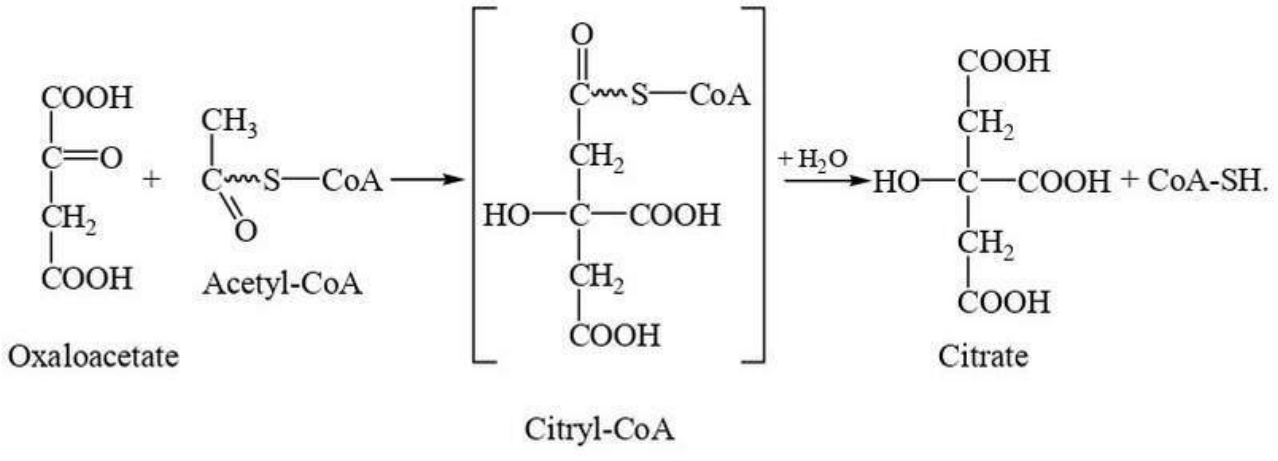
3.3. TRICARBOXYLIC ACID CYCLE OR KREBS CYCLE

The principal enzyme system, which acts as a generator of hydrogen for the mitochondrial electron transport chain, is the Krebs cycle. A German-English biochemist Hans Adolf Krebs based on his own experiments and data of researches of A. Szent-Gyorgyi suggested that the cells have an cyclic oxidative reaction system, which he called a citric acid cycle (CAC), because he believed that the first product of the cycle was a citric acid (citrate). It is also called the *tricarboxylic acid cycle (TAC cycle),* because at that time it was not known exactly whether the first substrate of the cycle was citric acid. Subsequently, it was shown that this cycle is the principal enzyme system of acetic acid residues (acetyl-CoA) oxidation and that its first reaction is the synthesis of citric acid. However, most often, this cycle is called the Krebs cycle, which for the first time has established a sequence of reactions in this process.

3.3.1. Individual reactions of the Krebs cycle

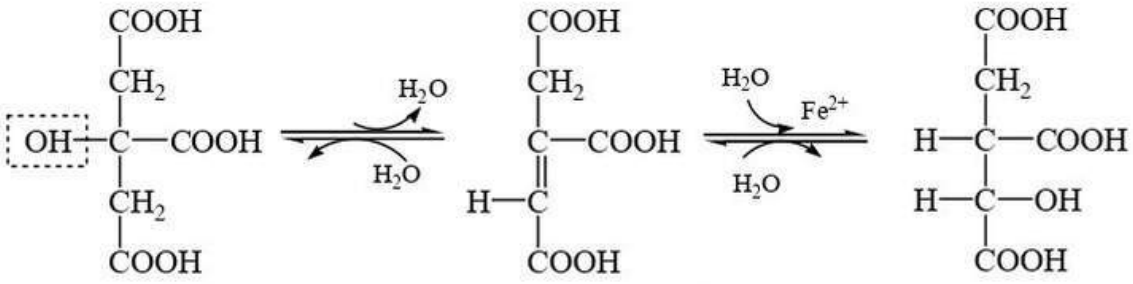
Acetyl-CoA, produced through the oxidation of pyruvate, fatty acids and amino acids, enters into the Krebs cycle:

1. The first stage of the cycle is the synthesis of citric acid, or citrate, catalyzed by *citrate synthase*:



The carbon atom of the methyl group of acetyl interacts with the carbon atom of oxaloacetate. An intermediate is considered to be a citryl-CoA, which is hydrolyzed to form free citrate. Hydrolysis of macroergic thioester bond shifts the equilibrium towards citrate formation and makes the reaction virtually irreversible under physiological conditions. Loss of energy during the hydrolysis of citryl-CoA provides entry of the acetyl fragment in the Krebs cycle with the formation of citrate.

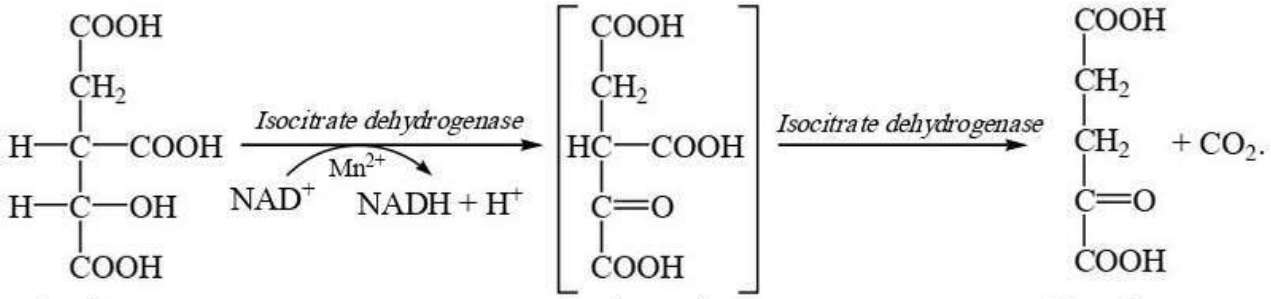
2. The second enzyme of the Krebs cycle — *cis-aconitate hydratase* catalyzes the reversible transformations of three tricarboxylic acids — *citrate, cis-aconitate* and *isocitrate*:



Citrate (91 %) *cis*-Aconitate (3 %) Isocitrate (6 %)

The equilibrium in the system is established when the ratio of the three substrates indicated in the chemical equation. Aconitate hydratase catalyzes the addition of H_2O to the trans-double bond of cis-aconitate. A feature of this enzyme is the need for the reaction of Fe^{2+} ions that form the metal-substrate complex. To shift the equilibrium of the aconitase reaction in one direction or another, it is required the consume of isocitrate or citrate

3. Enzymes that break down citrate are absent in the mitochondrial matrix, and the transformation of isocitrate is catalyzed the third enzyme of the Krebs cycle — *isocitrate dehydrogenase*. Like any dehydrogenase, this enzyme has a coenzyme — an acceptor of hydrogen that is split off from the substrate. True isocitrate dehydrogenase of the Krebs cycle is NAD-dependent enzyme, which is found only in the mitochondrial matrix and catalyzes the dehydrogenation of isocitrate according to the equation:



Isocitrate Oxalosuccinate α-Ketoglutarate

At the same time, decarboxylation of the intermediate (oxalosuccinate) occurs on the surface of the enzyme.