Chapter

Metabolomic Discovery of Microbiota Dysfunction as the Cause of Pathology

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Abstract

In the twenty-first century, metabolomics allowed evaluating the profile of metabolites of various classes of compounds in the human body. The most important achievement of the metabolic approach is to obtain evidence of the intersection of human biochemical pathways and its microbiota. The effect of certain microbial metabolites on the work of key enzymes involved in the biotransformation of amino acids and other substances becomes more important in patients at risk of developing neurological and mental disorders and also contributes to the development of life-threatening conditions up to multiple organ failure after operations, injuries, and serious diseases. The authors of this chapter call the microbiota an "invisible organ," emphasizing its functional significance, and not just taxonomy, as previously thought. This chapter will discuss the mutually beneficial integration of the metabolome/microbiome in the body of healthy people and will focus on the effects of microbiota dysfunction.

Keywords: homeostasis, microbiota, "invisible organ," bacterial metabolites

1. Introduction

Homeostasis is key for the normal performance of a human body. Many parameters are constantly maintained in fairly narrow vital ranges, such as temperature, acidity in the intracellular and intercellular spaces, the electrolyte concentrations, hormones, vitamins, etc. The traditional view is that the body itself is able to maintain the constancy of its internal environment due to a complex system of feedback (**Figure 1**). Each organ helps to maintain homeostasis, ensuring its specific function. It acts as a backward force that returns the system to equilibrium in the event of deviations from the normal state. Along with other organs, the microbiota plays an important role in maintaining homeostasis, despite being an "invisible organ."

By the way, in terms of weight, the microbiota should be attributed to the largest organ that can be compared only with the brain or liver: this can be easily ascertained using simple calculations based on known facts about the weight of human organs relative to the body weight of an adult (**Figure 2**). The human microbiota, which is a community of gut microorganisms, can be considered as an independent organ with many functions.

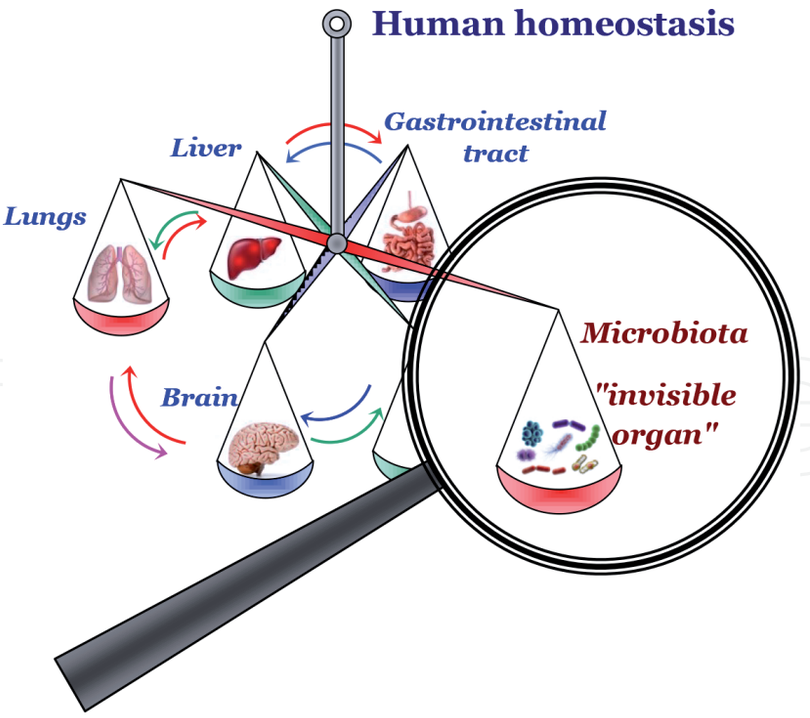
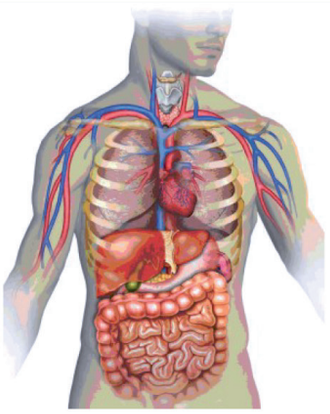


Figure 1.
Diagram of the interaction of organs that support the state of homeostasis.



Organ	Weight, g	Part of body mass, %
• *kidney*	*250*	*0,35*
• *heart*	*300*	*0,4*
• *lungs*	*1000*	*1,4*
• *brain*	*1500*	*2,1*
• *liver*	*1700*	*2,4*
• ***microbiota***	***1000 - 2000***	***1-3***

Figure 2.
Microbiota as a big but "invisible organ," % of body mass compared to other vital organs in an adult weighing 70 kg.

In the twenty-first century, a new insight on the processes occurring in the human body in health and disease on the basis of the new knowledge of the microbiota is formed. Detection and identification of the trillions of bacteria that form the microbiota of healthy and sick people are made possible by the use of modern technologies, for example, sequencing of the 16S rRNA gene.

Metabolite-based approaches (or metabolomics) to the study of the human microbiota are more significant progress in biology and medicine, searching for answers to the question "What are the chemical and pathophysiological results of the metabolic activity of the microbiota?" Today many research teams are searching for answers to this question [1].

The host organism is a habitat for the microbiota, so maintaining homeostasis is vital for the survival of hundreds of bacterial species. The microbiota seeks to restore homeostasis in the case of minor metabolic disorders that are not systemic in nature, and it has a huge amount of possibilities for this. If changes in the vital functions

of the body are serious, a new quality (pathology) is formed, the microbiota is also radically rebuilt: this is manifested not only in changes in the species composition of bacteria (taxonomy) but also in metabolic processes. Other non-normal products of microbial metabolism from the intestines enter the systemic circulation, and they can interfere with the endogenous metabolic pathways. When the microbiota works against the host, it is manifested by diseases, even death (sepsis).

The medical community has not yet formed an understanding of the role of the microbiota as a separate organ. A search query ("microbiota as an organ") or ("microbiome as an organ") in specialized databases, such as the Web of Science, Scopus, and Pubmed, gives a negative result. At the same time, a number of review articles are actually present which describe in detail the physiology and biochemistry of the close interaction of the intestinal microbiota with the host organism, in which there are many qualities and attributes of the organ.

This chapter formulates ideas about the microbiota as an organ, which has become possible due to the results of studies with metabolomic equipment of recent years. The material presented in this chapter relies primarily on articles published after 2010. Specialists working in both fundamental and clinical medicine are undoubtedly interested in the growing information about the role of microbiota in maintaining homeostasis, as well as the participation of microorganisms of the human body in the metabolic pathways, which are directly related to the development of various pathologies.

2. Microbiota in a healthy body

Food intake, its conversion, and excretion of waste products are material sources for the normal functioning of a human body. The aim of nutrition from a biochemical viewpoint is to maintain the body's critical parameters in narrowly defined value rates. The concept of a "living healthy organism" consists precisely in the ability to resist change and maintain the constancy of the composition and properties of its internal environment. The basis of digestion is a fairly universal mechanism, which includes splitting of the main components, such as carbohydrates (including polysaccharides), fats, biopolymers (proteins, macromolecules based on nucleotide sequences), etc., to individual low-molecular substances and then to the synthesis of low- and high-molecular weight compounds, which are the material basis for cells and organs as well as the energy source for biochemical reactions. Interest to low-molecular weight compounds has grown particularly in recent years. The Human Metabolome Database (HMDB) was created and is constantly updated by the international researcher group. Now it contains information on more than 100,000 individual low-molecular compounds (metabolites), constituting about 25,000 pathways of metabolism [2].

Food digestion is one of the main complex processes that form homeostasis. Transformation of the matter occurs throughout the gastrointestinal tract. Food undergoes ever-deeper processing as you move through it. Enzymes directly involved in this can potentially have endogenous and exogenous origin. The endogenous pathway is carried out with the participation of its own secrets produced by the body with the participation of organs that promote digestion and the excretion of waste products. The complex of biochemical reactions that coincide with the active participation of the microbiota, consisting of hundreds, sometimes reaching up to several thousand species, is presented as an alternative to it. In the literature there is no single point of view about the density of microorganism colonization of the human digestive system. According to [3], the relative content of microorganisms (cells/mL) in different parts of the gastrointestinal tract is duodenum, 10^1–10^3; jejunum and ileum, 10^4–10^7; cecum, 10^8; and large intestine, 10^{11}–10^{12}. A large number

of publications give the relative content of microorganisms in the range of 10^2–10^{13}, while the maximum values are recorded in the cecum and transverse colon.

The specificity of food digestion is due to the variety of enzymes capable of carrying out similar biochemical transformations, if not entirely, then at least of its many components, due to intestinal microbiota. The synthesis of specific proteins, including enzymes, is due to the presence of various nucleotide DNA sequences. The diversity of these sequences in a complex system consisting of hundreds, or even thousands, of individual species of microorganisms is significantly higher than that of human. The lifetime of a particular microorganism, depending on the immune response of the host organism, correlates with the function that promotes or interferes with its vital activity. The production of specific microorganism killer proteins is not observed in the case of symbiosis. Processes of synthesis of interleukins and phagocytosis are immediately activated in the alternative situation [4]. A big array of metagenomic studies of human intestinal microbiota collected in recent years in various information repositories, such as the National Center for Biotechnology Information (NCBI).

The role of microbiota is quite significant already at the stage of primary processing of nutrients. For example, in [5], the fact is given that only bacteroids of the *Bacteroides thetaiotaomicron* contain nucleotide sequences for the synthesis of 260 glycosidic hydrolases, while the entire human genome is capable of producing only 17 such enzymes, and 9 of them are not fully characterized. The author of the review [6] provides several specific metabolic pathways associated with intestinal microbiota. These include (i) cleavage of polysaccharides to monomers, followed by processing into short-chain fatty acids; (ii) depolymerization of proteins to amino acids, with further conversion of some of them (glycine, lysine, arginine, leucine, isoleucine, and valine) to nitrogen-containing heterocyclic compounds, for example, substituted indoles; (iii) neutralization and detoxification of arene-containing components from the external environment; and (iv) biotransformation of fats and bile acids and their inclusion in biochemical processes that promote energy cells, for example, in the Krebs cycle.

The species composition of the microbiota is specific for each person and depends on many factors, such as age, diet, use of antibiotics, etc. We can talk about two components of the microbiota—obligate or transient. A self-organizing ecosystem with the dominance of some species of microorganisms and the oppression of others arises in a normally functioning organism. The classification and systematization of information on the species and genetic diversity of the microbiota of the human body were carried out independently by two scientific communities in the United States and the European Union, which resulted in the appearance of two databases: Human Microbiome Project (HMP) [7] and Metagenomics of the Human Intestinal Tract (MetaHIT) [8].

Extensive information on the composition of the intestinal microbiota of a healthy person is contained in the literature. These studies indicate the dominance of several genera of strict anaerobes, and the main ones are *Bacteroides*, *Prevotella*, *Eubacterium*, *Ruminococcus*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium*. The data on the microbial community of the gastrointestinal tract are summarized in detail in the 2018 review [9] and presented in **Table 1**, which reflects the gradual change in the species composition of microorganisms as food progresses and digests.

A huge number of types of microorganisms perform the biochemical functions which we call the "conveyor" of the microbiota [10]. The diversity of species with different biochemical activity provides coordinated work of the microbiota. The final metabolite formation depends on many factors: the quality and quantity of substrate (food components); the function of the stomach, pancreas, liver, and gallbladder; bowel motility; etc. definitely influence the metabolism of microbiota. The normal biotransformation of any of the substrates in the intestinal lumen takes place sequentially [10]. Biochemistry of deep food transformation is in many respects similar to the metabolic characteristic of microorganisms. The main part of

Part of the gastrointestinal tract	The dominant species composition of the microbiota
Oral cavity	*Gemella*, *Granulicatella*, *Streptococcus, Prevotella*, *Veillonella*, *Porphyromonas*, *Neisseria*, *Rothia*, *Lactobacillus*, *Fusobacterium*
Throat, esophagus	*Streptococcus*, *Prevotella*, *Actinomyces*, *Gemella*, *Rothia*, *Granulicatella*, *Haemophilus*, *Veillonella*
Stomach	*Helicobacter pylori*, *Veillonella*, *Lactobacillus*
Small intestine	*Enterococcus*, *Escherichia coli*, *Klebsiella*, *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Bacteroides fragilis*, *Clostridium lituseburense*, *Gammaproteobacterium*
Cecum	*Lactobacillus*, *Enterococcus*, *Escherichia coli, Bacteroides*, *Clostridium leptum*, *Clostridium coccoides*
Rising gut	*Bacteroides*, *Lactobacillus*, *Bifidobacterium*
Colon	*Bacteroides*, *Clostridium*, *Desulfomonas*, *Desulfovibrio*

Table 1.
Differences in the composition of the microbiota throughout the gastrointestinal tract (adapted from [9]).

the individual amino acids that come from food after the cleavage of polypeptides is further spent on the synthesis of its own proteins, which are necessary for the functioning of the body. Residual amino acids can be transformed into other substances of a non-protein nature, performing a number of important functions not related to digestion or the building function.

This trend is most pronounced for aromatic amino acids such as phenylalanine, tyrosine, and tryptophan. The transformations of the phenylalanine-tyrosine pair occurring in the liver are contained in the human metabolome database (**Figure 3**). Phenylalanine and tyrosine are interchangeable in terms of metabolism. Phenylalanine is transformed into tyrosine under the action of a complex compound of Fe^{2+} ions with phenylalanine-4-hydroxylase with the participation of L-erythrotetrahydrobiopretin. Then both amino acids are transformed into 4-hydroxyphenylpyruvic acid, and then, by successive transformations, they are transformed into acetoacetic and fumaric acids—components of the Krebs cycle under the action of the same enzymes with the participation of the same substances [11]. There is no direct conversion of phenylpyruvic acid to 4-hydroxyphenylpyruvic acid in this metabolism scheme.

The pathway of tyrosine processing, namely, its biotransformation in tyramine further into three directions—dopamine, homovanillin, and dopachinone—is important for the normal functioning of human mental activity (**Figure 4**). All biochemical transformations that make up these metabolic pathways occur with the direct action of enzymes. However, enzymes for not all reactions are listed in the HMDB. The label "??" (**Figure 4**) refers to the absence of data on the enzyme. The pathway reactions can be divided into two types: "traditional" and "unusual."

The first type is rather trivial transformations, such as the conversion of an aldehyde to the corresponding carboxylic acid, for example, homovanillin to homovanillic acid. Such transformations are well known in classical organic chemistry. These reactions do not require enzymes; it is enough to have an oxidizing agent, such as molecular oxygen, hydrogen peroxide, reactive oxygen species, etc. The situation is different in the case of the formation of nitrogen-containing heterocycles formed from aromatic amino acids. The information about enzyme in HMDB is not available for the key dopachinone conversion reaction to leukodopachrome. A detailed study of the mechanism of this reaction, contained in [12], shows that nitric oxide (I) takes an active part in it. This fact is complicated only by understanding the base of interactions. Many reactions of

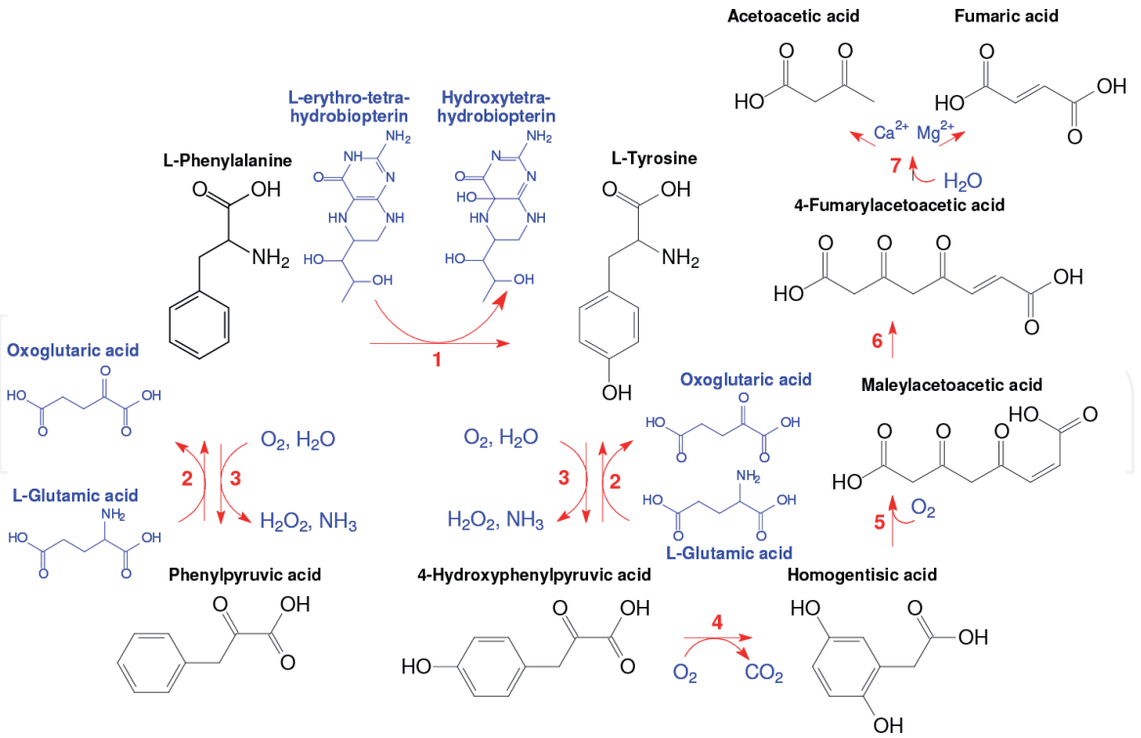


Figure 3.
Normal metabolism of phenylalanine and tyrosine in the liver. Enzymes (coenzymes): (1) phenylalanine-4-hydroxylase (Fe^{2+}); (2) aspartate aminotransferase, cytoplasmic tyrosine aminotransferase; (3) L-amino-acid oxidase (FAD); (4) 4-hydroxyphenylpyruvate dioxygenase (Fe^{3+}); (5) homogentisate 1,2-dioxygenase; (6) maleylacetoacetate isomerase; (7) fumarylacetoacetase (according to the HMDB).

tyrosine metabolism are supported by a complex of copper ions with tyrosinase, well known in the biochemistry of microorganisms and used in biotechnology (see, e.g., [13]).

A significant part of the reactions given in **Figure 4**, with a sufficient degree of confidence, occurs with the participation of enzymes of exogenous (microbiological) origin generated by the microbiota. The formation of metabolites not only with the benzene but also with the indole ring occurs as a result of tyrosine biotransformation, including the participation of microbial enzymes.

Indoles, including those synthesized using human microbiota enzymes, play an important role in metabolism. Such physiologically important substances as serotonin, tryptamine, and derivatives of quinic acid belong to them (**Figure 5**). Many of the compounds involved in the indole metabolism are able to pass through the blood–brain barrier. About 95% of tryptophan enters the brain as a conjugate with kynurenine compounds, whose final metabolic products are kinuric and quinolinic acids, 3-hydroxykynurenine [14].

Reactions associated with the presence of endogenous enzymes and enzymes of microbial origin are in a state of dynamic equilibrium with the normal functioning of biochemical processes in the body. Microbiota metabolism is able to quickly adjust in a direction that helps to maintain homeostasis with moderate deviations (abnormalities with dietary errors, travels with changing time zones, etc.). The dynamic metabolism of the "invisible organ" is provided by the potential of the metabolic pathways, such as catecholamine biosynthesis (**Figure 6**) with participation of numerous species of microorganisms.

Microbiota metabolism can also be seriously affected if the disorders are systemic under the influence of adverse external factors (e.g., massive antimicrobial therapy, severe poisoning, hypoxia, blood loss, etc.). These disorders can manifest themselves clinically by developing a critical state, which often puts the existence of the organism (its life) at risk.

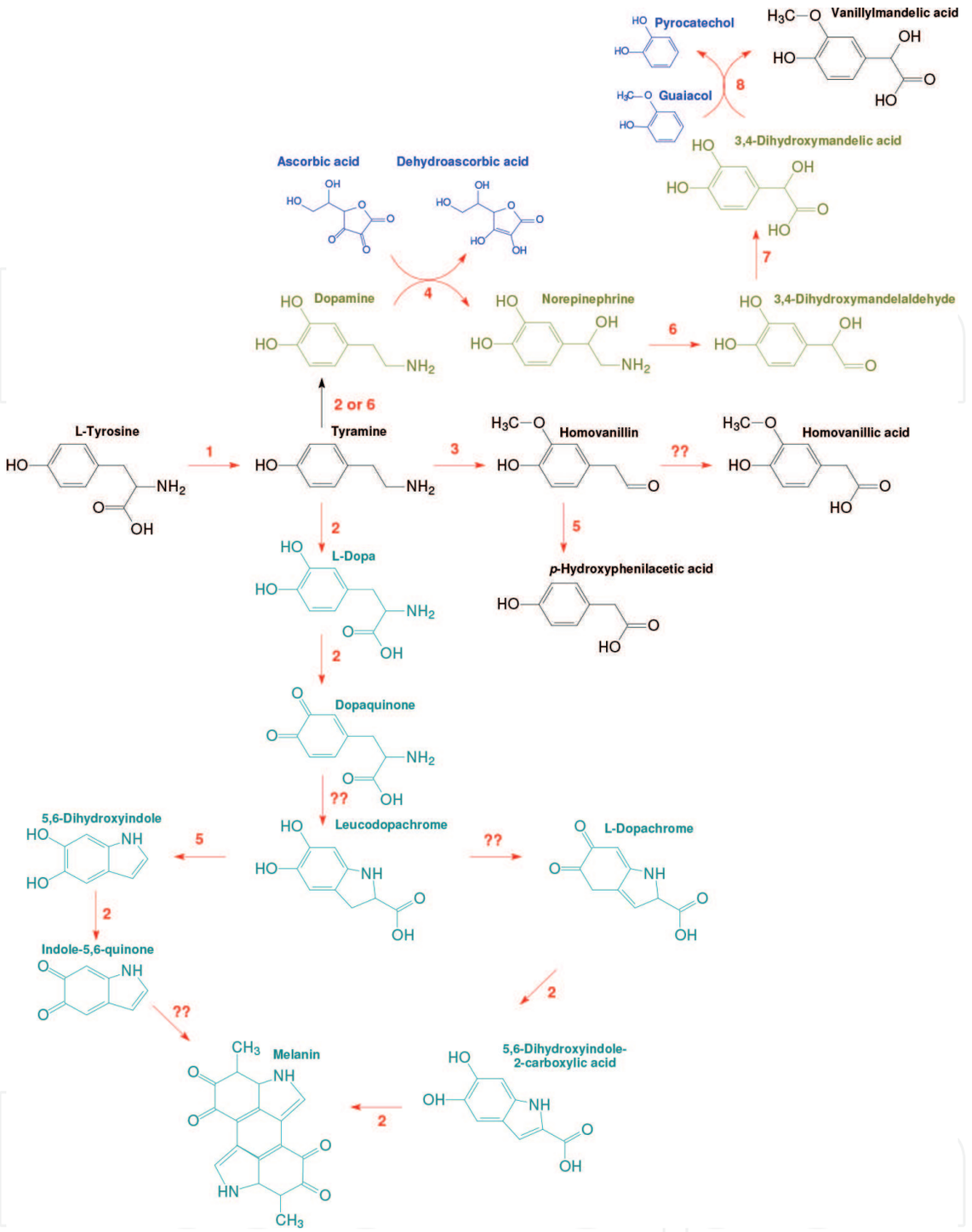


Figure 4.
Tyrosine metabolism. Enzymes (coenzymes): (1) aromatic-L-amino-acid decarboxylase, (Pyridoxal-5′-phosphate); (2) tyrosinase (Cu^{2+}); (3) amiloride-sensitive amine oxidase [copper-containing] (Cu^{2+}, Ca^{2+}, topaquinone); (4) dopamine beta-hydroxylase (Cu^{2+}, pyrroloquinoline, quinone); (5) aldehyde dehydrogenase (dimeric NADP-preferring); (6) amine oxidase [flavin-containing] A (FAD); (7) aldehyde dehydrogenase,(dimeric NADP-preferring); (8) catechol O-methyltransferase (Mg^{2+}) (according to the HMDB).

3. Microbiota dysfunction in pathology

3.1 Diseases of the digestive tract

Disturbances in the normal functioning of the gastrointestinal tract are largely due to changes in the digestion processes associated with the state of the microbiota. As noted above, the microbiota composition depends on the heredity and health

of the host, climate, nutrition, bad habits, etc. A system itself is able to return to a state of homeostasis in the case of mild disorders. The microbiota has mechanisms to adapt to the effects of antibacterial substances. Antibiotics are originally the products of bacteria which they use as competitive advantage in the conditions

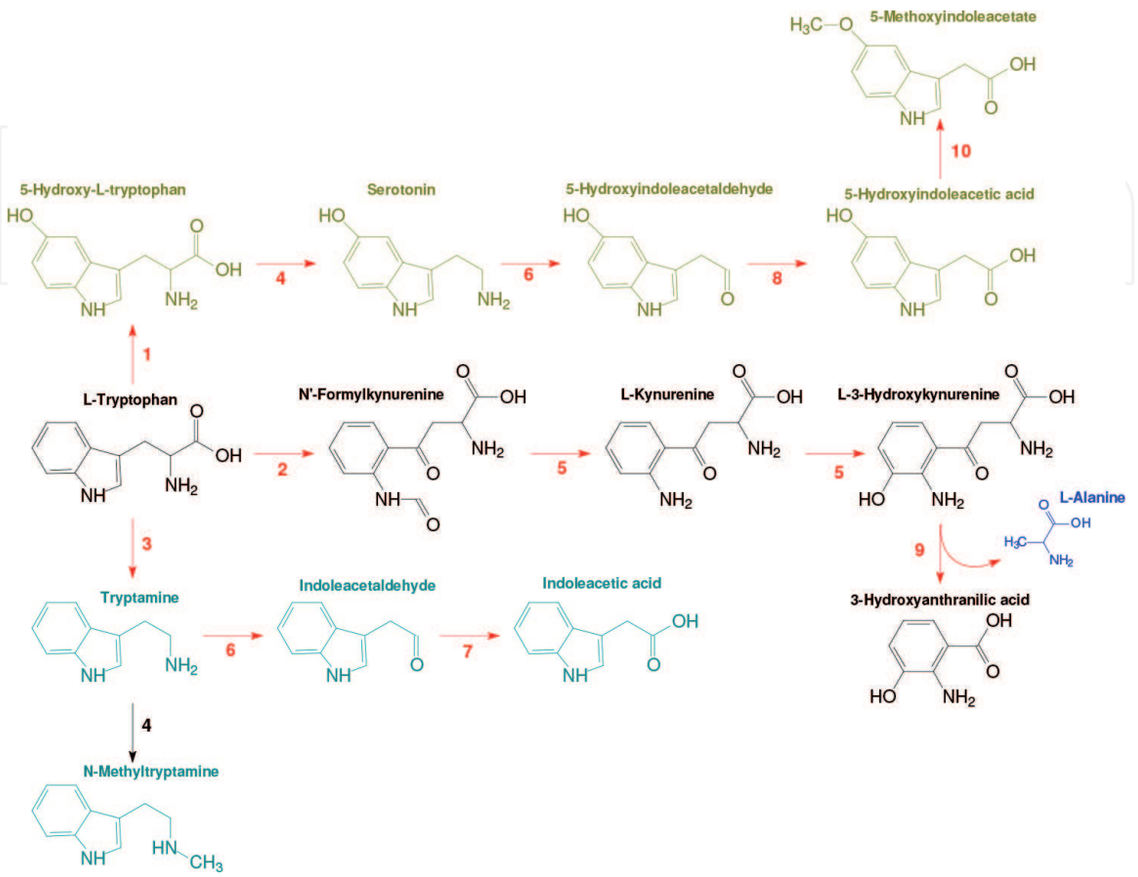


Figure 5.
Simplified scheme of normal tryptophan metabolism. Enzymes (coenzymes): (1) tryptophan 5-hydroxylase (Fe^{2+}); (2) tryptophan 2,3-dioxygenase (heme); (3) aromatic-L-amino-acid decarboxylase (pyridoxal-5'-phosphate); (4) indolethylamine N-methyltransferase; (5) kynurenine formamidase; (6) kynurenine 3-monooxygenase (FAD); (7) aldehyde dehydrogenase, mitochondrial (NAD); (8) aldehyde dehydrogenase, mitochondrial or aldehyde oxidase (FAD, molybdopterin, 2Fe-2S); (9) kynureninase (pyrophosphate); (10) acetylserotonin O-methyltransferase (S-Adenosyl methionine) (according to the HMDB).

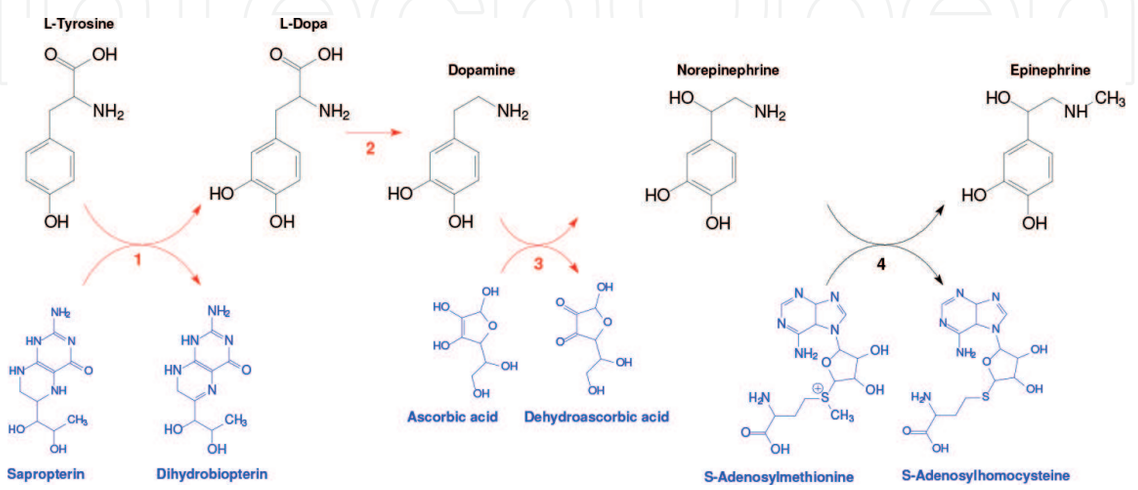


Figure 6.
Catecholamine biosynthesis. Enzymes (coenzymes): (1) tyrosine 3-monooxygenase (Fe^{2+}); (2) aromatic-L-amino-acid decarboxylase (pyridoxal-5'-phosphate); (3) dopamine beta-hydroxylase (Cu^{2+}, pyrroloquinoline, quinone); (4) phenylethanolamine N-methyltransferase) (according to the HMDB).

of nutrient substrate deficiency in their habitat. However, significant changes in species composition may be developed under the influence of broad-spectrum antibacterial drugs, since the massive use of antibiotics (xenobiotics) is violent and anti-biological and can disrupt biochemical processes.

The microbiota is involved in the transformation of xenobiotics and provides a range of reactions including acetylation, deacylation, decarboxylation, dehydroxylation, demethylation, etc. under the influence of low-quality products and synthetic drugs [15]. Modern possibilities of metabolic methods allow an extensive study of another important function of the microbiota—detoxification of the host organism, which maintains its normal state longer in the conditions of retention and self-repairing of the microbiota.

Disorders of the microbial products of short-chain fatty acids SCFA (acetic, propionic, butyric) are most thoroughly studied as a result of the suppression of the normal functioning of anaerobic bacteria. Normally, SCFA requires enterocytes as the main source of energy, respectively; their deficiency contributes to the violation of mucosal trophism, reduction of reparative processes, development of ulcers, and inflammation. Persistent indigestion disorders and chronic gastroenterological diseases are the clinical manifestations of serious changes in the species composition and dysfunction of the microbiota.

Different genera of anaerobic bacteria are called responsible for the production of SCFA. For example, large amount of carbohydrate dissimilation butyrate from dissimilation is associated with some *Clostridia* clusters, other SCFAs, and *Bifidobacterium* spp.

In his review, Nyangale et al. rightly noted that several members of the microbiota have been linked with diseases mainly affecting the gut, lake inflammatory bowel disease, such as ulcerative colitis, Crohn's disease, colorectal cancer, and irritable bowel syndrome, although mechanisms involved are still not yet fully understood [16]. The authors consider the possibilities of metabolite analysis to assess the metabolic activity of the microbiota, to measure volatile and nonvolatile metabolite in biological samples, and to give metabolic pathways the contribution of microbiota to which it is most pronounced. These pathways include also the transformation of glucose and amino acids into SCFA, amino acid, microbial degradation of tyrosine to *p*-hydroxyphenylacetic and *p*-hydroxyphenylbenzoic acids (including bypassing tyramine), and degradation of tryptophan to indolepropionate and indoleacetate (including bypassing tryptamine).

A metabolite composition, determined in the feces, may indicate the composition of microbiota and its changes associated with the use of antibiotics [17]. The use of chemometric approaches in relation to the primary mass spectral data of the samples under study allows one to reliably find the differences between patients with inflamed intestines and the control group. The authors consider that changes in the microbiota phenotype cause this kind of deviations. The ratio of the species composition of microbiota—obligate or transient—significantly affects the metabolite composition that enters the circulatory system from the bowel. Thus, the role of *Bacillus* and *Lactobacillus*, colonizing the epithelium of the gastrointestinal tract, is systematically examined in a review of Ilinskaya et al. [18]. The metabolite composition depends significantly on the activity of their enzymatic systems, even with a relatively low content of such microorganisms in the microbiota.

In such acquired endocrinological diseases as obesity, type 2 diabetes (not related to heredity) can be attributed to pathological conditions due to metabolic disorders involving the microbiota. Microbiota can influence the development of diabetes [19]. Changes in the microbiological composition—dysbacteriosis—caused, for example, by the use of antibiotics, may contribute to an increase in insulin dysfunction, a long-term consequence of which is the development of type 2 diabetes. Due diet may ensure opportune correction of the microbiota and prevent

further development of the disease. In a similar study for type 2 diabetes, cited in [20], the authors come to analogous conclusions. The authors agree that function is more important than taxonomy when discussing the role of microbiota in the development of metabolic disorders and diseases of the gastrointestinal tract [21].

In the future, methods of diagnosing gastrointestinal diseases and methods of treatment through the modulation of the microbiota based on information about intermediate metabolites and end products of microbial biodegradation of various compounds can be constructed and developed.

3.2 Microbial metabolites in oncology

Changes in the human body due to microbiota metabolism can affect cells and tissues and contribute to the development of benign and malignant tumors. The biochemistry and physiology of oncological processes is not completely clear, but certain metabolic shifts can be fixed instrumentally for some types of oncological diseases [22, 23]. The successful search for links between the patterns of normal functioning of the microbiota and the biochemistry of carcinogenesis is detailed in recent reviews [24, 25]. This indicates the prospects of such concept and allows us to call the microbiota "a key orchestrator of cancer therapy."

Most of the data on the correlation between a microbiota and cancer tumors is in the gastroenterology [26–31]. Such intestinal microorganisms as *Fusobacterium nucleatum*, *Streptococcus gallolyticus*, *Bacteroides fragilis*, *Escherichia coli*, and *Enterococcus faecalis* are most often mentioned as potential participants of the process. The inflammatory process in the epithelium or deeper tissues of the intestinal wall leads to increased local blood supply. At the same time, a favorable substrate is created for the massive multiplication of bacteria, the formation of microbial biofilms, which contributes to the activation of the enzymatic systems of bacteria, increasing concentrations of potentially dangerous mutagenic products of microbial metabolism. According to [30], the highest specificity of microorganisms contributing to the occurrence of colorectal cancer is noted in streptococci such as *Streptococcus bovis* and *Streptococcus gallolyticus.* Other authors indicate a violation of homeostasis in the intestine and emphasize the role of *Lactobacillus* deficiency in reducing the protective mechanisms [28].

The analysis of statistical data shows that there is an activation of the biosynthesis of fatty acids against the background of inhibition of the biosynthesis of amino acids and glycan in patients with colorectal cancer compared with the control group [26]. Statistically significant differences in the levels of metabolites of microbial origin, namely, an increase in the relative concentrations of phenylacetic, isobutyric, valeric, isovaleric acids, and hexose-phosphates with a simultaneous decrease in taurine, glutamine, β-alanine, isoleucine, galactose, xylose, glycerol, methanol, ornithine, guanidine, choline acid, and its derivatives, 4-aminohippuric acid, have been identified in a recent paper [32].

Certainty is not currently attainable regarding the use of volatile fatty acids as markers of oncology. Reducing the levels of SCFA (acetic, butyric), secondary bile acids, concomitant increase in amino acids (leucine, valine, proline, serine) valeric, isobutyric, isovaleric acid can be associated with the activity of enzymatic systems of *Ruminococcus* spp., *Fusobacterium*, *Porphyromonas*, *Clostridia*, *Lachnospiraceae*. Changes in the composition of the microbiota in patients with colorectal cancer, noted by the authors of the review [31], can be used as a diagnostic method. Also, the review authors [33] propose to use the following compound profile: short-chain fatty acids (mainly butyric acid), cholium-kilot on deoxycholic acid derivatives, bacterial toxin fragilis, and trimethylamine-N-oxide for the diagnosis of colorectal cancer. Other authors [34] also suggest a bacterial metabolite butyric acid as a marker for colorectal cancer.

An alternative concept is that volatile fatty acids, for example, butyric acid, may have a protective effect, which slows down the development of large intestine malignancies. Butyrate-producing bacteria contained in the microbiota of the gastrointestinal tract, such as *Faecalibacterium prausnitzii*, *Eubacterium rectale*, or *Roseburia*, promote an increase in the content of butyric acid [35].

A treatment of large amounts of information on substances of bacterial origin potentially capable of being included in human metabolism allows us to distinguish six groups of compounds, based on the profile of which early diagnosis of colorectal cancer can be built [29]. There are short-chain fatty acids, bile acids, indoles, cresols, phenolic (phenyl-containing fatty) acids, and polyamines. Analysis of literature data [27] shows that under the influence of microbiota, changes in the directions of chemical transformation of glucose, fats, and amino acids are possible.

The metabolic profile, largely formed by the microbiota, was used as a diagnostic method for cancer not directly related to the gastrointestinal tract. Statistically significant differences in the content of substances involved in the metabolism of glycerol lipids and retinol and ways of ethylbenzene degradation can be used to diagnose bladder cancer. Such metabolites are actively produced and/or absorbed with the participation of enzymes of *Herbaspirillum*, *Gemella*, *Bacteroides*, *Porphyrobacter*, *Faecalibacterium*, *Aeromonas*, and *Marmoricola* [36].

A change in the metabolic profile of amino acids such as valine, cysteine, tyrosine, and 6-hydroxynicotinic acid can be used as a method for diagnosing oral cancer [37]. Substances of microbial origin and components of the metabolism of *Helicobacter pylori* have a significant impact on the formation and growth of malignant neoplasms of the esophagus, large intestine, pancreas, and lung. A cross-sectional statistical analysis shows that the likelihood of oncological complications associated with *Helicobacter pylori* increases in smokers and patients diagnosed with chronic pancreatitis and diabetes [38].

The metabolites produced by the microbiota of the upper respiratory tract and lungs may influence the development of oncological processes in them. Three types of bacteria, *Granulicatella*, *Streptococcus*, and *Veillonella*, are mentioned most often in this connection. They probably have differences in the metabolism of polyamines, expressed in elevated levels of putrescine and similar products. According to other data, dysbiosis and an increase in *Streptococcus* and *Mycobacterium* are practically not associated with the development of lung cancer [39].

However, waste products of bacteria can contribute to the development of breast cancer [22, 40–42]. The waste products of bacteria of the gastrointestinal tract can contribute to the development of malignant tumors of any other location: lung cancer [38, 39], bladder [36], pancreas [38], including hormone-dependent forms of breast cancer [22, 38, 40–42], and prostate cancer [43].

Statistically significant correlations between the levels of secondary bile acids and the incidence of breast cancer were found in [22]. The authors believe that lithocholic acid, which is a product of the metabolism of microorganisms, is able to limit the proliferation of breast cancer cells both in vitro and in vivo by activating the TGR5 receptor. Changes in the metabolism of hormones, cysteine, and methionine and the biosynthesis of fatty acids associated with breast cancer were noted in a similar study [41], but there is no definite connection between them. The search for low-molecular markers of breast cancer, carried out in [42], allowed identification of 12 compounds (amino acids, organic acids, and nucleosides) that pretend to this role. These compounds are included in the metabolism of amino acid and nucleoside metabolism.

Microbiota metabolites are able to act as accelerants and inhibitors of oncological processes. Now a scientific search in this field of knowledge is in the stage of intensive development and accumulation of a critical amount of information. The use of metabolomic approaches in combination with modern methods of statistical processing of

large amounts of data undoubtedly contributes to the development of fundamental and applied medicine in the field of diagnosis and treatment of oncological diseases.

3.3 Neurological pathology and mental disorders

Some substances that form the amino acid metabolism can overcome the hemato-encephalic barrier and have a direct effect on the brain (**Figure 7**) [14, 44–46]. A search for such low-molecular compounds, quantitative determination, and their ratios can serve as the basis for the development of methods for early diagnosis, including cognitive and mental disorders [47]. It is important to note that metabolites can directly enter the region of the medulla, with blood through arteria vertebralis-arteria spinalis, bypassing the hemato-encephalic barrier, and that critical vital centers of respiration and circulation are located there.

It is unlikely that metabolites of microbial biotransformation of amino acids are the direct cause of mental or neurological diseases. At the same time, numerous experimental studies indicate the existence of a direct "intestine-microbiota-brain" link. Current evidence suggests that multiple mechanisms, including endocrine and neurocrine pathways, may be involved in gut microbiota-to-brain signaling and that the brain can in turn alter microbial composition and behavior via the autonomic nervous system [48].

The authors in literature sources traditionally attend to the aromatic amino acid tryptophan metabolism mainly due to its relationship with the synthesis of serotonin (5-HT) and melatonin [49]. Tryptophan biotransformation in humans can occur in different ways: either with the participation of endogenous enzymes that are synthesized by the intestinal cell wall or with the participation of bacterial enzymes. Accordingly, the ratios of end products of tryptophan metabolism will differ. This is easily seen by comparing the enzymes and metabolic products of tryptophan in **Figures 5** and **8**.

The traditional view is that the amino acid tryptophan is used primarily for protein synthesis or the formation of serotonin and melatonin. However, more than 90% of tryptophan was found to be metabolized into N-formyl-kynurenine followed by kynurenine (**Figure 8**) [50]. The presence of anthranilic and 3-hydroxy-anthranilic acids attracts particular attention as tryptophan metabolites. This pathway is not presented in mammalian metabolism. Such reactions of indole compounds are possible only with the participation of microbiota enzymatic systems. This also applies to picolinic and quinolinic acids, the formation of which

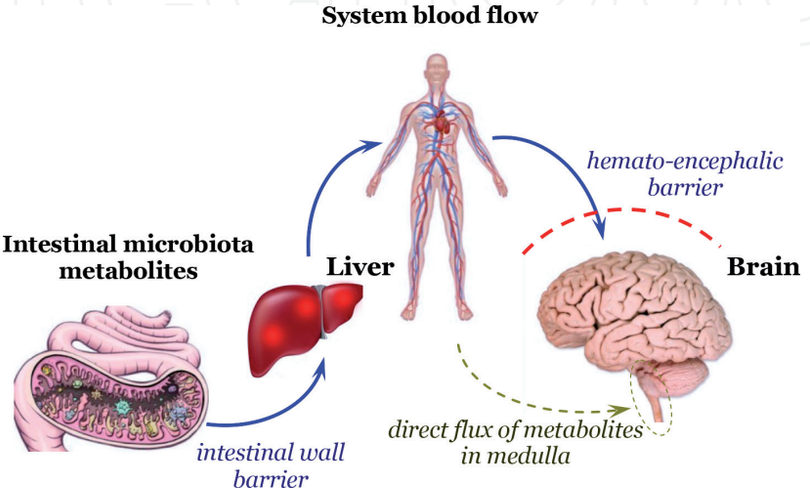


Figure 7.
Scheme of amino acid metabolite transport in the brain.

is associated with the opening of the indole ring, which can occur exclusively in the process of microbial biotransformation.

A decrease of tryptophan, xanthurenic, 3-hydroxyanthranilic, and quinolinic acids in the blood was recorded in the case of clinical occurrences of Alzheimer's disease. The same metabolites are given in [51] as potential markers of Alzheimer's disease. It can be assumed that one of the Alzheimer's disease triggers is a chronic deficiency of these substances.

Now there are two alternative hypotheses in the literature regarding products of tryptophan metabolism and their influence on the development of schizophrenia. One of them postulates that a chronic tryptophan deficiency results in failure of catabolism products, such as 3-hydroxykynurenine, quinolinic, picolinic, xanthurenic, kinureric, and anthranilic acids. Some authors maintain that such deficiency stipulates the psychosomatic symptoms of schizophrenia [52]. Other authors come to the opposite conclusion based on the analysis of statistical data [53]. They indicate a direct correlation of clinical manifestations of schizophrenia with an increased content of kynurenic acid in the cerebrospinal fluid relative to the control group. Such conflicting data emphasize once again the peculiarities of the metabolic approach. You should not limit yourself to searching and measuring one or two metabolites during clinical trials; it is important to evaluate the complex metabolic profile, to compare the indicators with positive and negative dynamics. In addition, other mechanisms that are not related to the metabolism of neurotransmitters may be the basis of mental and neurologic disorders.

Thus, attempts to search for low-molecular markers of autism [54] and depressive disorder [55] were unsuccessful. But the data indicating the potential role of the metabolism of aromatic amino acids were discovered in such a mental disorder as anorexia nervosa. Levels of tryptophan and phenylalanine were significantly reduced in patients compared with the healthy ones.

Changes in the distribution of the tryptophan metabolism products, such as kynurenine, 3-hydroxy kynurenine, kynurenic, and anthranilic acids, are observed

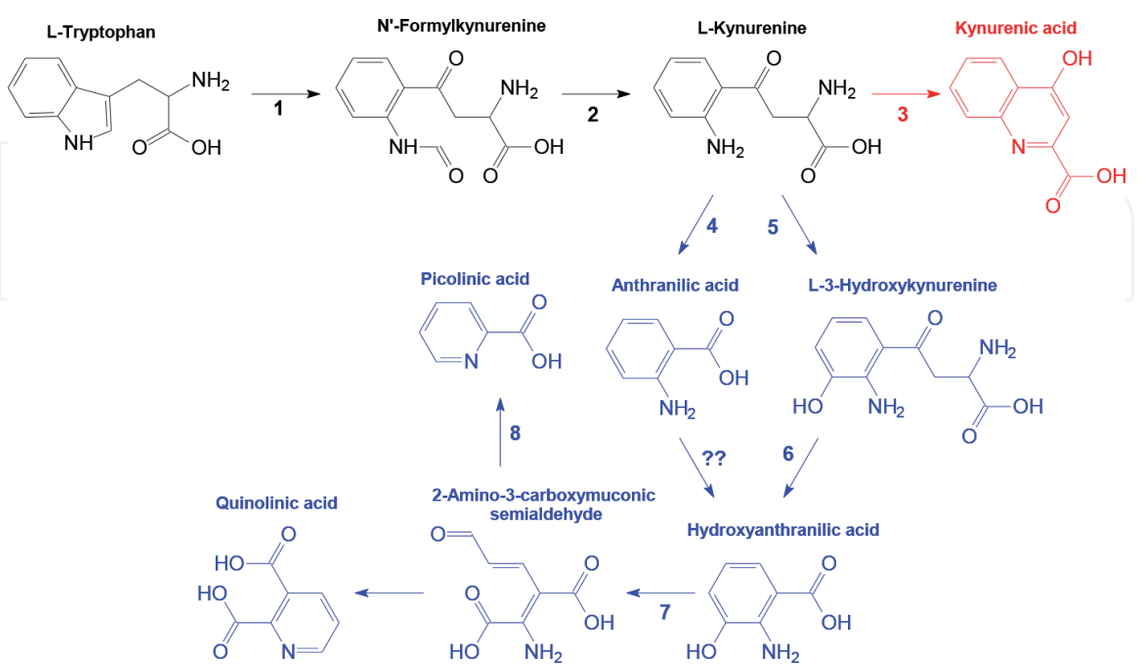


Figure 8.
Tryptophan metabolism. Enzymes: (1) indoleamine deoxygenase or tryptophan deoxygenase; (2) formidase; (3) kynurenine aminotransferase; (4) kynureninase; (5) kynurenine-3-monooxygenase; (6) kynureninase; (7) 3- hydroxyanthranilate 3,4-dioxygenase; (8) 2-amino-3- carboxymuconate-semialdehyde decarboxylase (according to [50]).

in patients with symptoms of Parkinson's disease [56]. Low levels of norepinephrine, dopamine, homovanillic acid, serotonin, and 5-hydroxyindoleacetic acid in the blood are fixed in these patients relative to the control group [57].

The failure of aromatic L-amino acid decarboxylase in combination with reduced levels of important metabolites such as serotonin, dopamine, and catecholamines leads to disruptions in the normal functioning of the whole organism, including brain activity. Crisis of oculomotor function along with muscular hypotonia and dystonia is observed in combination with other neurological syndromes in a similar state [58]. A decrease in the blood concentrations of homovanillic, 5-hydroxyindoleacetic acids, and 3-*o*-methyldopamine—substances included in the metabolism of tyrosine (**Figure 4**)—was observed in all patients. It can be noted with a high assurance that the deficiency of these metabolites is due to the lack of the transformation enzymes responsible for these reactions of the aromatic amino acids usually found in the microbiota.

3.4 Prospects for neurorehabilitation

Scientists have used metabolomics to gain new knowledge about the significance of the role that bacteria play in complex regulatory processes of higher nervous activity. Understanding the potential for managing this process cannot leave psychiatrists, neurologists, and neurorehabilitation specialists indifferent [59–61]. This fact is due to the relevance and high frequency of pathology of the nervous system. Prospects for the correction of microbiota metabolism for neurorehabilitation and the demand for this scientific search for new solutions in this area cannot be overestimated.

One of the areas discussed in the literature is the transformation of the species composition of the patient's microbiota to eliminate the deficiency of certain microorganisms. This idea has a scientific ground that many bacteria from the human microbiota in the in vitro study revealed the ability to produce hormones and neurotransmitters, that is, the presence of appropriate enzyme systems. These data are summarized in the reviews [44, 62] and in brief form are presented in **Table 2**.

Certain reports indicate that the treatment with large doses of *Lactobacillus casei* has a positive effect. Patients with chronic fatigue syndrome reported a decreased strain (n = 39). Patients who took a probiotic reported a significant decrease in symptoms of anxiety and had a substantial increase in the number of *Lactobacillus* and *Bifidobacteria* compared with the control group ($p = 0.01$). [63]. At the same time, treatment with live microorganisms (including fecal microbiota transplantation, FMT) is hardly predictable and can have negative consequences due to the variability of bacterial metabolism depending on the environment. For example, a randomized, double-blind, controlled study on the use of a drug based on lactobacilli in combination with prebiotic gives a negative result in patients with pancreatic necrosis: the mortality rate in the group receiving the biological product was significantly higher than in the control [64].

Neurorehabilitation of patients in modern clinics is considered as a component of acute cerebral therapy and starts from the earliest periods after injuries, strokes, and brain operations, even at the stage of the patient's stay in the intensive care unit. This is a multicomponent and long-term process aimed not only at saving lives but also at restoring motor activity, correcting neuro-endocrine, cognitive impairments, and emotional status. Different methods of monitoring the effectiveness of intensive care and the rehabilitation of the functional state of patients with various brain injuries are used [65].

The authors of this chapter believe that neurorehabilitation can be significantly enriched with a set of targeted measures aimed at correcting disorders in the development of which metabolic products associated with microbiota are actively involved. Our accumulated data on the magnitude of changes in the profile of

Hormone, neurotransmitter	Bacteria
Norepinephrine	*Bacillus subtilis*, *Bacillus mycoides*, *Proteus vulgaris*, *Serratia marcescens*
Dopamine	*B. subtilis*, *B. mycoides*, *Bacillus cereus*, *Staphylococcus aureus*, *P. vulgaris*, *S. marcescens*, *Escherichia coli*, *Morganella morganii*, *Klebsiella pneumonia*, *Hafnia alvei*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. bulgaricus
Dopamine precursor (DOPA)	*E. coli*, *B. cereus*, *L. helveticus*, *L. casei*, *L. delbrueckii* subsp. bulgaricus, *Toxoplasma gondii*
Serotonin	*S. aureus*, *Enterococcus faecalis*, *Rhodospirillum rubrum*, *B. subtilis*, *E. coli*, *M. morganii*, *K. pneumonia*, *H. alvei*, *Lactococcus lactis* subsp. cremoris, *L. lactis* subsp. lactis, *Lactococcus plantarum*, *L. helveticus*
Histamine	*M. morganii*, *P. vulgaris*, *Proteus mirabilis*, *Klebsiella* sp., *Enterobacter aerogenes*, *E. cloacae*, *Citrobacter freundii*, *Enterobacter amnigenus*, *Vibrio alginolyticus*, *Acinetobacter lowfli*, *Pseudomonas fluorescens*, *P. putida*, *Aeromonas* spp., *Clostridium* spp., *Photobacterium* spp., *Lactobacillus buchneri*, *Streptococcus thermophilus*
γ-Aminobutyric acid	*Bifidobacterium adolescentis*, *B. dentium*, *B. infantis*, *B. angulatum*, *Lactobacillus brevis*, *L. plantarum*, *L. paracasei*, *L. buchneri*, *L. helveticus*, *L. delbrueckii*, *L. reuteri*, *L. zymae*
Tyramine	*Lactobacillus* spp., *Lactococcus* spp., *Enterococcus* spp., *Carnobacterium*

Table 2.
Literary data on the ability of many bacteria: representatives of the human microbiota to participate in the production of hormones and neurotransmitters (adapted from [44, 62]).

microbiota metabolites and their connection with the course and outcome of the disease in patients with lesions of the central nervous system indicate the possibility of their use in choosing tactics for managing patients with this pathology. This complex may include several areas: (i) the first is the additional introduction into the body of substances that are associated with a shortage of other clinical manifestations of pathology. This can be achieved by nutritional correction or dietary supplements, including those obtained using industrial microbiology methods, as well as the administration of parenteral preparations containing the necessary metabolites of microbial origin. (ii) The second is the suppression of the metabolic activity of those types of bacteria in the composition of the microbiota, which in excess produce "unwanted" metabolites, through the selective use of antibacterial drugs with an appropriate mechanism of action. (iii) The third is the elimination of excess unwanted metabolites in the systemic circulation through the targeted use of extracorporeal blood purification procedures with filters/sorbents that remove specific substances.

Of course, the use of modern metabolic methods for an objective assessment of the dynamics of the profile of metabolites in parallel with the monitoring of the psychosomatic state, functions of the damaged brain, spasticity level, motor skills, etc. is necessary for the successful implementation of the above directions in a particular patient. But above all, reliable data on key microbial metabolites, the level of which must be monitored in patients in the process of neurorehabilitation to on must be obtained. For example, metabolites associated with the development of septic shock (p-HPhAA) [66, 67] and death (PhA, p-HPhLA) [10] were earlier established for patients with sepsis. At the same time, another metabolite—PhPA—was a characteristic for the metabolic profile of a healthy person. The study of metabolome is conducted using the GC–MS method for patients with affection of the central nervous system of various etiologies [68]. Currently, the purpose of this study is to detect microbial metabolites associated with changes in the neurological status of patients in the process of neurorehabilitation. Preliminary results indicate a number of significant features,

for example, positive neurological and psychosomatic dynamics is associated with the appearance and accumulation of the metabolite p-HBA in the intestine and the patient's blood, which is not observed in other groups of patients. The composition of the microbiota in patients with severe neurosomatic pathology using the method of metagenomic sequencing of the 16S pRNA is under study. Correlations with microbial blood metabolites are also being studied. Preliminary data demonstrate significant differences when comparing various patient groups [69]. The results of the multicenter study will serve as the basis for the development and objective evaluation of the effectiveness of the above technologies in the process of neurorehabilitation.

4. Conclusion

A new level of knowledge about the role of the microbiota in the human body was made possible by metabolomics. In the coming years, this will lead to new solutions in the diagnosis of many "difficult" diseases. Methods of active control of metabolic processes that will subordinate the dysfunction of the "invisible organ" to the benefit of the host will be found. It will lead to the increase in the effectiveness of treatment and successful rehabilitation of patients. In particular, in the field of neurorehabilitation, clinical studies are currently aimed at finding such methods for correcting the metabolism of microbiota that will achieve a balance of low-molecular metabolites as signaling molecules of microbiota to restore brain function.

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References

[1] Chernevskaya E, Beloborodova N. Gut microbiome in critical illness (review). General Reanimatology. 2018;**14**:96. DOI: 10.15360/1813-9779-2018-5-96-119

[2] Wishart DS, Feunang YD, Marcu A, Guo AC, Liang K, Vázquez-Fresno R, et al. HMDB 4.0: The human metabolome database for 2018. Nucleic Acids Research. 2018;**46**:D608. DOI: 10.1093/nar/gkx1089

[3] Hornung B, dos Santos VAPM, Smidt H, Schaap PJ. Studying microbial functionality within the gut ecosystem by systems biology. Genes and Nutrition. 2018;**13**:5. DOI: 10.1186/s12263-018-0594-6

[4] Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nature Reviews. Immunology. 2010;**10**:159. DOI: 10.1038/nri2710

[5] Cantarel BL, Lombard V, Henrissat B. Complex carbohydrate utilization by the healthy human microbiome. PLoS One. 2012;**7**:e28742. DOI: 10.1371/journal.pone.0028742

[6] Kim CH. Immune regulation by microbiome metabolites. Immunology. 2018;**154**:220. DOI: 10.1111/imm.12930

[7] HMP Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012;**486**:207. DOI: 10.1038/nature11234

[8] Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science. 2006;**312**:1355. DOI: 10.1126/science.1124234

[9] Yadav M, Verma MK, Chauhan NS. A review of metabolic potential of human gut microbiome in human nutrition. Archives of Microbiology. 2018;**200**:203. DOI: 10.1007/s00203-017-1459-x

[10] Beloborodova NV. Interaction of host-microbial metabolism in sepsis. In: Kumar V, editor. Sepsis. InTechOpen; 2017. pp. 3-19. DOI: 10.5772/68046

[11] Gertsman I, Gangoiti JA, Nyhan WL, Barshop BA. Perturbations of tyrosine metabolism promote the indolepyruvate pathway via tryptophan in host and microbiome. Molecular Genetics and Metabolism. Elsevier. 2015;**114**:431. DOI: 10.1016/j.ymgme.2015.01.005

[12] Land EJ, Ramsden CA, Riley PA. Pulse radiolysis studies of ortho-quinone chemistry relevant to melanogenesis. Journal of Photochemistry and Photobiology. B. 2001;**64**:123. DOI: 10.1016/S1011-1344(01)00220-2

[13] Faccio G, Kruus K, Saloheimo M, Thöny-Meyer L. Bacterial tyrosinases and their applications. Process Biochemistry. 2012;**47**:1749. DOI: 10.1016/j.procbio.2012.08.018

[14] van den Brink WJ, Palic S, Köhler I, de Lange ECM. Access to the CNS: Biomarker strategies for dopaminergic treatments. Pharmaceutical Research. 2018;**35**:64. DOI: 10.1007/s11095-017-2333-x

[15] Wilson ID, Nicholson JK. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Translational Research. 2017;**179**:204. DOI: 10.1016/j.trsl.2016.08.002

[16] Nyangale EP, Mottram DS, Gibson GR. Gut microbial activity, implications for health and disease: The potential role of metabolite analysis. Journal of Proteome Research. 2012;**11**:5573. DOI: 10.1021/pr300637d

[17] Bussche JV, Marzorati M, Laukens D, Vanhaecke L. Validated high resolution mass spectrometry-based approach for metabolomic fingerprinting of the human gut phenotype. Analytical Chemistry. 2015;**87**:10927. DOI: 10.1021/acs.analchem.5b02688

[18] Ilinskaya ON, Ulyanova VV, Yarullina DR, Gataullin IG. Secretome of intestinal bacilli: A natural guard against pathologies. Frontiers in Microbiology. 2017;**8**:1666. DOI: 10.3389/fmicb.2017.01666

[19] Han H, Li Y, Fang J, Liu G, Yin J, Li T, et al. Gut microbiota and type 1 diabetes. International Journal of Molecular Sciences. 2018;**19**:995. DOI: 10.3390/ijms19040995

[20] Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science. 2018;**359**:1151. DOI: 10.1126/science.aao5774

[21] Sitkin SI, Vakhitov TY, Demyanova EV. Microbiome, gut dysbiosis and inflammatory bowel disease: That moment when the function is more important than taxonomy. Almanac of Clinical Medicine. 2018;**46**:396. DOI: 10.18786/2072-0505-2018-46-5-396-425

[22] Mikó E, Vida A, Kovács T, Ujlaki G, Trencsényi G, Márton J, et al. Lithocholic acid, a bacterial metabolite reduces breast cancer cell proliferation and aggressiveness. BBA Bioenergitics. 2018;**1859**:958. DOI: 10.1016/j.bbabio.2018.04.002

[23] Mukherjee S, Joardar N, Sengupta S, Babu SPS. Gut microbes as future therapeutics in treating inflammatory and infectious diseases: Lessons from recent findings. The Journal of Nutritional Biochemistry. 2018;**61**:111. DOI: 10.1016/j.jnutbio.2018.07.010

[24] Alexander JL, Scott AJ, Pouncey AL, Marchesi J, Kinross J, Teare J. Colorectal carcinogenesis: An archetype of gut microbiota-host interaction. Ecancermedicalscience. 2018;**12**:865. DOI: 10.3332/ecancer.2018.865

[25] Roy S, Trinchieri G. Microbiota: A key orchestrator of cancer therapy. Nature Reviews. Cancer. 2017;**17**:271. DOI: 10.1038/nrc.2017.13

[26] Allali I, Boukhatem N, Bouguenouch L, Hardi H, Boudouaya HA, Cadenas MB, et al. Gut microbiome of Moroccan colorectal cancer patients. Medical Microbiology and Immunology. 2018;**207**:211. DOI: 10.1007/s00430-018-0542-5

[27] Zhou CB, Fang JY. The regulation of host cellular and gut microbial metabolism in the development and prevention of colorectal cancer. Critical Reviews in Microbiology. 2018;**44**:436. DOI: 10.1080/1040841X.2018.1425671

[28] Coleman OI, Haller D. Bacterial signaling at the intestinal epithelial interface in inflammation and cancer. Frontiers in Immunology. 2018;**8**:1927. DOI: 10.3389/fimmu.2017.01927

[29] Wang QQ, Li L, Xu R. A systems biology approach to predict and characterize human gut microbial metabolites in colorectal cancer. Scientific Reports. 2018;**8**:6225. DOI: 10.1038/s41598-018-24315-0

[30] Han S, Gao J, Zhou Q, Liu S, Wen C, Yang X. Role of intestinal flora in colorectal cancer from the metabolite perspective: A systematic review. Cancer Management and Research. 2018;**10**:199. DOI: 10.2147/CMAR.S153482

[31] Villéger R, Lopès A, Veziant J, Gagnière J, Barnich N, Billard E, et al. Microbial markers in colorectal cancer detection and/or prognosis. World Journal of Gastroenterology.

2018;**24**:2327. DOI: 10.3748/wjg.v24.i22.2327

[32] Gall GL, Guttula K, Kellingray L, Tett AJ, ten Hoopen R, Kemsley KE, et al. Metabolite quantification of fecal extracts from colorectal cancer patients and healthy controls. Oncotarget. 2018;**9**:33278

[33] Zou S, Fang L, Lee MH. Dysbiosis of gut microbiota in promoting the development of colorectal cancer. Gastroenterology Report. 2018;**6**:1. DOI: 10.1093/gastro/gox031

[34] Wu X, Wu Y, He L, Wu L, Wang X, Liu Z. Effects of the intestinal microbial metabolite butyrate on the development of colorectal cancer. Journal of Cancer. 2018;**9**:2510. DOI: 10.7150/jca.25324

[35] McNabney SM, Henagan TM. Short chain fatty acids in the colon and peripheral tissues: A focus on butyrate, colon cancer, obesity and insulin resistance. Nutrients. 2017;**9**:1348. DOI: 10.3390/nu9121348

[36] Wu P, Zhang G, Zhao J, Chen J, Yang C, Huang W, et al. Profiling the urinary microbiota in male patients with bladder cancer in China. Frontiers in Cellular and Infection Microbiology. 2018;**8**(167)

[37] Xie GX, Chen TL, Qiu YP, Shi P, Zheng XJ, Su MM, et al. Urine metabolite profiling offers potential early diagnosis of oral cancer. Metabolomics. 2012;**8**:220. DOI: 10.1007/s11306-011-0302-7

[38] Meng C, Bai C, Brown TD, Hood LE, Tian Q. Human gut microbiota and gastrointestinal cancer. Genomics, Proteomics and Bioinformatics. 2018;**16**:33. DOI: 10.1016/j.gpb.2017.06.002

[39] Mur LAJ, Huws SA, Cameron SJS, Lewis PD, Lewis KE. Lung cancer: A new frontier for microbiome research and clinical translation. Ecancermedicalscience. 2018;**12**:866. DOI: 10.3332/ecancer.2018.866

[40] Zhang A-h, Sun H, Qiu S, Wang XJ. Metabolomics in noninvasive breast cancer. Clinica Chimica Acta. 2013;**424**:3. DOI: 10.1016/j.cca.2013.05.003

[41] Fernández MF, Reina-Pérez I, Astorga JM, Rodríguez-Carrillo A, Plaza-Díaz J, Fontana L. Breast cancer and its relationship with the microbiota. International Journal of Environmental Research and Public Health. 2018;**15**:1747. DOI: 10.3390/ijerph15081747

[42] Chen Y, Zhang R, Song Y, He J, Sun J, Bai J, et al. RRLC-MS/MS-based metabonomics combined with in-depth analysis of metabolic correlation network: Finding potential biomarkers for breast cancer. The Analyst. 2009;**134**:2003. DOI: 10.1039/b907243h

[43] Porter CM, Shrestha E, Peiffer LB, Sfanos KS. The microbiome in prostate inflammation and prostate cancer. Prostate Cancer and Prostatic Diseases. 2018;**21**:345. DOI: 10.1038/s41391-018-0041-1

[44] Averina OV, Danilenko VN. Human intestinal microbiota: Role in development and functioning of the nervous system. Microbiology. 2017;**86**:1-18. DOI: 10.1134/S0026261717010040

[45] Umbrello G, Esposito S. Microbiota and neurologic diseases: Potential effects of probiotics. Journal of Translational Medicine. 2016;**14**:298. DOI: 10.1186/s12967-016-1058-7

[46] Willemsen MA, Verbeek MM, Kamsteeg E-J, de Rijk-van Andel JF, Aeby A, Blau N, et al. Tyrosine hydroxylase deficiency: A treatable disorder of brain catecholamine

biosynthesis. Brain. 2010;**133**:1810. DOI: 10.1093/brain/awq087

[47] Sadok I, Gamian A, Staniszewska MM. Chromatographic analysis of tryptophan metabolites. Journal of Separation Science. 2017;**40**:3020. DOI: 10.1002/jssc.201700184

[48] Mayer EA, Tillisch K, Gupta A. Gut/brain axis and the microbiota. The Journal of Clinical Investigation. 2015;**125**:926. DOI: 10.1172/JCI76304

[49] Kałużna-Czaplińska J, Gątarek P, Chirumbolo S, Chartrand MS, Bjørklund G. How important is tryptophan in human health? Critical Reviews in Food Science and Nutrition. 2019;**59**:72. DOI: 10.1080/10408398.2017

[50] Chatterjee P, Goozee K, Lim CK, James I, Shen K, Jacobs KR, et al. Alterations in serum kynurenine pathway metabolites in individuals with high neocortical amyloid-β load: A pilot study. Scientific Reports. 2018;**8**:8008. DOI: 10.1038/s41598-018-25968-7

[51] Lv C, Li Q, Liu X, He B, Sui Z, Xu H, et al. Determination of catecholamines and their metabolites in rat urine by ultra-performance liquid chromatography–tandem mass spectrometry for the study of identifying potential markers for Alzheimer's disease. Journal of Mass Spectrometry. 2015;**50**:354. DOI: 10.1002/jms.3536

[52] Kanchanatawan B, Sirivichayakul S, Thika S, Ruxrungtham K, Carvalho AF, Geffard M, et al. Physio-somatic symptoms in schizophrenia: Association with depression, anxiety, neurocognitive deficits and the tryptophan catabolite pathway. Metabolic Brain Disease. 2017;**32**:1003. DOI: 10.1007/s11011-017-9982-7

[53] Erhardt S, Schwieler L, Nilsson L, Linderholm K, Engberg G. The kynurenic acid hypothesis of schizophrenia. Physiology and Behavior. 2007;**92**:203. DOI: 10.1016/j.physbeh.2007.05.025

[54] Kałużna-Czaplińska J, Żurawicz E, Jóźwik J. Chromatographic techniques coupled with mass spectrometry for the determination of organic acids in the study of autism. Journal of Chromatography B. 2014;**964**:128. DOI: 10.1016/j.jchromb.2013.10.026

[55] Zheng P, Wang Y, Chen L, Yang D, Meng H, Zhou D, et al. Identification and validation of urinary metabolite biomarkers for major depressive disorder. Molecular and Cellular Proteomics. 2013;**12**:207. DOI: 10.1074/mcp.M112.021816

[56] Havelund JF, Andersen AD, Binzer M, Blaabjerg M, Heegaard NHH, Stenager E, et al. Changes in kynurenine pathway metabolism in Parkinson patients with L-DOPA-induced dyskinesia. Journal of Neurochemistry. 2017;**142**:756. DOI: 10.1111/jnc.14104

[57] Sitte HH, Pifl C, Rajput AH, Hörtnagl H, Tong J, Lloyd GK, et al. Dopamine and noradrenaline, but not serotonin, in the human claustrum are greatly reduced in patients with Parkinson's disease: Possible functional implications. The European Journal of Neuroscience. 2017;**45**:192. DOI: 10.1111/ejn.13435

[58] Manegold C, Hoffmann GF, Degen I, Ikonomidou H, Knust A, Laaß MW, et al. Aromatic L-amino acid decarboxylase deficiency: Clinical features, drug therapy and follow-up. Journal of Inherited Metabolic Disease. 2009;**32**:371. DOI: 10.1007/s10545-009-1076-1

[59] Dovrolis N, Kolios G, Spyrou GM, Maroulakou I. Computational profiling of the gut-brain axis: *Microflora dysbiosis* insights to neurological disorders. Briefings in Bioinformatics. 2017. bbx154. DOI: 10.1093/bib/bbx154

[60] Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology. 2015;**28**:203

[61] Singh V, Roth S, Llovera G, Sadler R, Garzetti D, Stecher B, et al. Microbiota dysbiosis controls the neuroinflammatory response after stroke. The Journal of Neuroscience. 2016;**36**:7428. DOI: 10.1523/jneurosci.1114-16.2016

[62] Lucas P, Landete J, Coton M, Coton E, Lonvaud-Funel A. The tyrosine decarboxylase operon of *Lactobacillus brevis* IOEB 9809: Characterization and conservation in tyramine-producing bacteria. FEMS Microbiology Letters. 2003;**229**:65. DOI: 10.1016/S0378-1097(03)00787-0

[63] Rao V, Bested AC, Beaulne TM, Katzman MA, Iorio C, Berardi JM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathogens. 2009;**1**:6. DOI: 10.1186/1757-4749-1-6

[64] Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, et al. Probiotic prophylaxis in predicted severe acute pancreatitis: A randomized, double-blind, placebo-controlled trial. Lancet. 2008;**371**:651. DOI: 10.1016/s0140-6736(08)60207-x

[65] Kiryachkov YY, Grechko AV, Kolesov DL, Loginov AA, Petrova MV, Rubanes M, et al. Monitoring of the effectiveness of intensive care and rehabilitation by evaluating the functional activity of the autonomic nervous system in patients with brain damage. Obshchaya Reanimatologiya. 2018;**14**(4):21. DOI: 10.15360/1813-9779-2018-4-21-34

[66] Beloborodova NV, Olenin AY, Pautova AK. Metabolomic findings in sepsis as a damage of host-microbial metabolism integration. Journal of Critical Care. 2018;**43**:246. DOI: 10.1016/j.jcrc.2017.09.014

[67] Beloborodova NV, Sarshor YN, Bedova AY, Chernevskaya EA, Pautova AK. Involvement of aromatic metabolites in the pathogenesis of septic shock. Shock. 2018;**50**:273. DOI: 10.1097/shk.0000000000001064

[68] Pautova AK, Bedova AY, Sarshor YN, Beloborodova NV. Determination of aromatic microbial metabolites in blood serum by gas chromatography–mass spectrometry. Journal of Analytical Chemistry. 2018;**73**:160. DOI: 10.1134/S1061934818020089

[69] Beloborodova NV, Chernevskaya EA, Pautova AK, Bedova AY, Sergeev AA. Altered serum profile of aromatic metabolites reflects the biodiversity reduction of gut microbiota in critically ill patients. Critical Care. 2018;**22**(Suppl 1):82. DOI: 10.1186/s13054-018-1973-5