INTERCELLULAR INTERACTIONS

Special Course of Lectures

Subtitle:

POPULATION STRUCTURES AND INTERCELLULAR COMMUNICATION IN MICROBIAL POPULATIONS

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INTRODUCTION

The present work is aimed at performing two main functions.

First, it is a brief guidebook on *population structures and intercellular communication in microbial populations.* This guidebook is mainly intended for students (bachelors and especially masters) majoring in immunology. Therefore, special attention will be paid to intercellular interactions implicated in the operation of the immune system. It is assumed that the students have already acquired sufficient knowledge concerning the mechanisms of immune responses. Even though much time will be spent on chiefly microbiological issues such as interactions among microbial cells, these issues will also be considered from the immunological viewpoint, taking into account, for instance, the response of the immune system to microbial antigens. The emphasis placed in these lectures on immunology does not imply that the lectures will be of no relevance to academic audience with a different background. It is hoped that microbiologists, neurophysiologists, ecologists, and even psychologists will also find this brief course of lectures sufficiently useful.

Second, the present book is to be considered a monograph that deals with the history and the present-day state-of-the-art of a subfield of microbiology referred to as the *population organization- and communication-centered paradigm (POCCP).* In this capacity, this work is focused on the main trends in research areas dealing with microbial social behavior, supracellular structures formed by microorganisms, and the communication mechanisms employed, with special emphasis on their ongoing interaction with multicellular forms of life including, importantly, the human organism.

This work is based on a number of recent relevant publications. I specifically recommend a book co-authored by me and late Prof. Shenderov, Boris, whose eminence and extremely important contribution to microbiology, immunology, and especially nutrition science should be emphasized here. The book details are as follows: *Oleskin, A. V., & Shenderov, B. A. (2020). Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers* © 2020. It is acknowledged that the present work includes some material from the Introduction and from

Sections 1.1-1.3, 2.1, 2.2, 2.4-2.6, 3.1-3.9 of the book cited; the material is reprinted, with permission from Nova Science Publishers, in an abridged and partly modified form.

Each new course of lectures usually begins with the definition of its subject. This course deals with **Population Structures and Intercellular Communication in Microbial Populations**. In short, the course is about how microbial populations develop, function, and form complex structures, e.g., biofilms. This course also includes communication, i.e., information exchange among microbial cells.

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ABBREVIATIONS

3-OHHL	N-(3-oxohexanoyl)-L-homoserine lactone
5-HIAA	5-hydroxyindoleacetic acid
ACTH	adrenocorticotropic hormone
ADHD	attention deficit hyperactivity disorder
AI	autoinducer (in a QS system)
AR	adrenoreceptor
BA	biogenic amine
BAS	biologically active substance
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
c-di-GMP	cyclic diguanylphosphate
CFU	colony-forming unit
CNS	central nervous system
DCreg	regulatory dendritic cell
DOPA	3,4-dihydroxyphenylalanine
DSF	diffusible signal factor used by QS systems
ENS	enteric nervous system
GABA	γ-aminobutyric acid
GALT	gut-associated lymphatic tissue
GBL	γ-butyrolactone, a QS signal
GDNF	glial cell line-derived neurotropic factor
GF	germ-free (animal)
GI tract	gastro-intestinal tract
HO	heme oxygenase
HPA	hypothalamus-pituitary-adrenal (axis)
IEC	intestinal epithelial cell
Ig	immunoglobulin
IL	interleukin
LPS	(microbial) lipopolysaccharide
MAMP	microbe-associated molecular pattern
MAO	monoamine oxidase
N-AHL	N-acylhomoserine lactone
NLR	nucleotide oligomerization domain-like receptor
NO	nitric oxide
NOS	NO synthase
PAMP	pathogen-associated molecular pattern

POCP	population organization and communication paradigm
PYY	peptide YY
QS	quorum sensing
SCFA	short-chain fatty acid
TGF	tumor growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T lymphocyte
VEGF	vascular endothelial growth factor

LECTURE 1. POPULATION ORGANIIZATION AND COMMUNICATION-CENTERED PARADIGM (POCCP) IN MICRONBIOLOGY. ITS SUBFIELDS AND HISTORY

1.1. Expounding the population organization and communication-centered paradigm. Starting from the early 1980's, much attention has been given by the global microbiological community to cell-cell interaction and signal exchange in the microbial world as well as to the structure and functioning of microbial colonies and biofilms. This area of research is referred to herein as the *population organization and communication-centered paradigm (POCCP)* in microbiology, and it includes the following main areas of research (Oleskin & Shenderov, 2020):

- **Heterogeneity of microbial populations and the polymorphism of microbial cells in them.**
- **Colony/biofilm architecture**
- **Social behaviors of microorganisms**
- **Chemical communication factors**
- **Relationships between microbial populations and macroorganisms in ecosystems**

The meaning of these areas of research will be explained hereinbelow.

The term *heterogeneity* means "consisting of dissimilar or diverse elements" according to the Merriam Webster Dictionary of American English (https://www.merriam-webster.com/dictionary/heterogeneity). If a microbial population, or any cell population, is heterogeneous, it contains different cell types; some of them may be larger in size than others; they may have different shapes, some being rounded in shape and others representing long and thin rods. Still more important, different cells in a heterogeneous population can specialize in different functions. Many microbial populations contain actively growing vegetative and dormant cells. Unlike vegetative cells, dormant cells do not grow. They do not consume any nutrients or energy. Their functions are different. Actively growing cells use all available medium components, such as carbon and energy sources. Dormant cells enable the survival of the population if no resources, no nutrients, and no nutrients are available. These two types and many other cell types were extensively investigated by Professor Smirnov (2004) at Ivanovo State Medical Institute.

The second important cornerstone of POCCP is the *architecture*, i.e. the structural organization, of microbial colonies and biofilms. Microbial cells form complex colonies and biofilms. In addition to structured groups of interacting cells, they also include complex organic substances. These biopolymers envelop microbial cells and provide the structural basis for the whole colony or biofilm. These polysaccharides, proteins, nucleic acids, and other biopolymers are located outside microbial cells. They are called the *matrix* of a colony or a biofilm.

The third component of the paradigm is *social behavior.* In human society, it is defined as the whole spectrum of interactions among human individuals and groups. We like or dislike one

another, we may talk, we may help each other, or sometimes we can quarrel and have conflicts with fellow human beings. Of relevance to this course is the fact that "social behavior does not only happen in higher organisms. In the microbial community, single-celled microbes have developed the capacity to work together for the common good through sophisticated cell-to-cell communication" (Zhao et al., 2017, p.516). Typical examples of microbial social behavior include collective hunting by *Myxococcus* spp., aggregation and subsequent programmed cell death as a stage of the development of the stalk in the fruiting body of *Dyctiostelium discoideum*, and biofilm formation, e.g., in *Pseudomonas fluorescens* and *Bacillus subtilis* (Tarnita, 2017); these and other examples will be revisited and considered in more detail in Lecture 2.

Chemical communication is widely spread in the microbial realm. Similar to humans and animals, microbial cells---and also immunocytes and other human cells---constantly engage in exchanging information. No cooperation, no social behavior is possible without communication. Importantly, many diffusible chemical signals are implicated in coordinating microbial growth, developmental processes, and the transition between the stages of the life-cycle of a microbial culture (culture ontogeny, Yerusalimsky, 1952). My question to the students might be whether they can give me examples of communication signals produced by immune cells. For instance, what do the diverse types of cytokines do? How do they transfer information from cell to cell?

Most microbial populations do not exist in isolation in nature. They constantly interact with other organisms. There is a wide variety of bacteria growing in or on plant organisms and sometimes causing agriculturally detrimental plant diseases. Likewise, there are many microorganisms that constantly interact with an animal or a human organism, and this is of special relevance to immunology. This has been an extremely dynamic and actively developing area of research, and the book recommended for students includes a chapter (Chapter 2) devoted to this subject.

1.2. Historical. Historians of science know that, before a new paradigm takes shape in a field of science, several decades are spent on disseminating new ideas that challenge pre-existing views. This trend was also characteristic of the development of POCCP. The main proponents of this novel paradigm include James Shapiro, Martin Dworkin, Eshel Ben-Jacob, Ian Sutherland, and other prominent researchers. However, their indisputably important contributions to the paradigm were antedated by the work of a whole school in Russian microbiology including Nikolai Yerusalimsky, Nikolai Krasil'nikov, Stanislav Smirnov, Galina El'-Registan, Vitaly Duda, Robert Pshenichny, Arsen Kapreliants, and others. Their studies were conducted in the 1950s-1980s; a recently defended dissertation on the history of microbiology describes their contributions (Kirovskaya, 2005).

These studies were foreshadowed by still earlier research that addressed the organization of life on the population and suprapopulation (ecosystem) levels as well as biological communication mechanisms in more general terms, with respect to a wide spectrum of forms of life. As early as at the turn of the 20th century, Vladimir Vernadsky considered the whole biosphere as one coherent system. Leontii Ramensky emphasized the continuity of the plant formations that cover the whole planet. Biological evolution was envisioned as a result of the formation of symbiotic systems by diverse biological species (Andey Famintsyn and Boris Kozo-Polyansky). The similarities between human society and the biosocial systems formed by various life forms were highlighted in the works by Peter Kropotkin as well as the representatives of the Russian "phytosociology" school of thought. Or more direct relevance to microbiology were the ideas put forward by Vasily Kedrovsky in 1910 who emphasized the similarity beween the structure of a microbial colony and that of a multicellular organism. These ideas foreshadowed Nikolai Yerusalimsky's views set forth in his dissertation (1952) as well as Shapiro's relatively recent hypothesis envisaging "bacteria as multicellular organisms" (1988).

A number of scientists around the globe were interested in collective microbial behaviors and what was later termed "microbial communication" at the beginning of the 20th century. William Penfold (1914) revealed that the culture liquid at the initial growth stage (the lag phase) of a bacterial culture contained substances promoting the culture's transition to the next stage (the exponential phase). Otto Rahn (1906) in Germany investigated substances that were produced by microbial populations and accelerated or decelerated their development. Drawing on these data, microbiologists were conducting research on the development of cultures of prokaryotic and eukaryotic microorganisms for almost a century.

In the 1930s, Rahn investigated the phenomenon called "mitogenetic radiation" that was identified as ultraviolet light. As early as in the 1920s and 1930s, Alexander Gurwitch and co-workers investigated ultraviolet radiation that is emitted by living cells and stimulates cell division. For example, the UV radiation produced by Nadsonia sp. yeast stimulated cell proliferation in Bacillus sp. cultures (Sewertzowa, 1929). Subsequently, these data provided the foundations for studies conducted in the 1990s by Yuri Nikolaev with the bacteria *Vibrio costicola, Pseudomonas fluorescens,* and others[1].

In the late 20th century and at the turn of the 21st century, extensive studies were carried out on the processes of communication, cooperation, and regulation in microbial populations and associations, including colonies, biofilms, flocs, etc. It was revealed that advanced social organization is characteristic of a large number of microorganisms, and their biosocial systems are, in important ways, similar to eukaryotic multicellular organisms (Shapiro, 1985, 1988, 1995; Shapiro & Trubatch, 1991; Shapiro & Dworkin, 1997; Vysotsky et al., 1991; Gray, 1997; Losick & Kaiser, 1997; Shenderov, 1998, 2008, 2013a, b, 2014, 2016. 2017; Shenderov et al., 2016, 2017; Oleskin, 1994, 2001, 2009, 2021; Oleskin & Shenderov, 2013, 2016, 2019, 2020; Oleskin et al., 2000, 2010, 2014a, b, 2016, 2017a, b, c; Greenberg, 2003; Waters & Bassler, 2005; Nikolaev, 1992, 2000; Nikolaev & Prosser, 2000; Nikolaev et al., 2006; Nikolaev & Plakunov, 2007). Currently, the idea that a bacterial culture is a homogeneous "soup" in which solitary cells independently develop, is being replaced by a new concept that focuses on coherent associations of communicating cells that are differentiated in functional terms within the whole supracellular "organism" composed of many microbial cells (Voloshin & Kapreliants, 2004). To re-emphasize, these data and concepts evoke the idea that bacteria actually are multicellular organisms (Shapiro, 1988; Shapiro, Trubatch, 1991; Shapiro, Dworkin, 1997).

Recently, microbiologists have been paying increasing attention to the diversity of microorganisms at the interspecies and also at the intraspecies level: the microbial population is envisioned as a system based on the *unity in diversity* principle. Emphasis is placed on the presence of several different types of microbial cells inside many populations; this enables some degree of functional differentiation and specialization among them. Over 50 years ago, this issue was in the focus of attention of Nikolai Yerusalimsky and other researchers of the same historical period. This enabled them, as early as in the 1950s, to set forth the main principles of the population organization and communication-centered paradigm (POCCP) in microbiology that are still valid and includes the following main points:

- *Phenotypic heterogeneity of the cells of a microbial population (culture)* exemplified by the formation of spores and other dormant forms as well as filterable cells and other L forms.
- *Integrity of a microbial population as a coherent system.* As pointed out by Yerusalimsky (1952), "under appropriate cultivation conditions, bacterial cells develop at different rates but in the same direction. Therefore, the totality of these cells, i.e., the bacterial culture as a whole, undergoes certain developmental changes referred to as the culture's *ontogeny*

[1] Reprinted from: Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers, pp. xi-xiv (abridged and partly modified)

<emphasis added, *O.A. & S.B* >… A manifestation of the ontogeny process is a gradual increase in the number of mature cells in the bacterial culture".

- *Microcolonial lifestyle of most microorganisms in nature.* Microorganisms in nature, e.g., in soil, form compact cell aggregates (microcolonies) that are separated by void areas. In multi-species microbial associations, the microcolonies of different species are spatially segregated, and there often are "no man's land" zones between them. Microcolonies also form within microbial biofilms.
- *Release of chemical factors that are produced by the cells of a microbial population, influence its development (enable the population's autoregulation), and, in many cases, enable the population to estimate its own density;* this phenomenon was denoted as "quorum sensing" long after Yerusalimsky's seminal work.
- *Constant interaction between a microbial population and environmental factors.* "In order to understand the driving forces of a microbial culture's ontogeny, account should be taken of the fact that it is the coherent system including both microbial cells and environmental factors that undergoes the development process" (Yerusalimsky, 1952)

Indesputably, Yerusalimsky's ideas were ahead of time; after a relatively short lag, they were confirmed in studies that were conducted by a large number of research teams around the globe. Indesputably, they were among the ideas that provided the foundations for the *population organization and communication-centered paradigm (POCCP)* in microbiology considered in this course of lectures.

The first item in the list of Yerusalimsky's ideas can be illustrated with studies on unusual microbial forms, including cells with disrupted division and defective cell walls, as well as cell wall-lacking forms (oval or spherical cells of the spheroplast or protoplast type called L forms, filamentous, giant, and miniscule cells). Such "monsters" are likely to contribute to the viability of bacterial populations and their adaptation under changeable environmental conditions. L forms can persist in an infected animal organism for a long time and cause a relapse of the infection once more favorable conditions are created (Vysotsky et al., 1991). Heterogeneity of microbial forms (heteromorphism) is also characteristic of cyanobacterial populations, especially when they establish a symbiotic relationship with plants (Fig. 1). In such symbiotic systems, they form a whole gamut of bizarre structural variants, including protoplasts and spheroplasts, giant and amophous cells, small microcells, minicells lacking the DNA, and cell wall-deficient elementary bodies (Baulina, 2012).

Fig. 1

Research on the colony architecture and the soclal behavior of microorganisms will be discussed in detail in Lecture 2.

As for communication, much research was conducted in this country. What chemicals do microorganism use for communicating messages? At the Institute of Microbiology in Moscow, the team headed by Prof. G.I. El'Registan specialized in studying autoregulatory substances., i.e. microbial metabolites that are released by a cell population, or its part, into the medium. Many autoregulators are not utilizable in constructive or energy metabolism but perform major communicative functions and, therefore, influence the physiological state and the reproductive potential of the cells involved (El'-Registan, 1988).

Autoregulators are exemplified (Fig. 2) by microbially produced factors d_1 (anabiosis factors) that represent alkylhyroxybenzenes (AHBs) and factors d_2 (autolysis factors) that belong to unsaturated fatty acids (El'-Registan, 1988; Plakunov & El'-Registan, 2004). AHBs induce the transition of bacterial cells to the dormancy state and increase their stress resistance. At sufficient concentrations, AHBs suppress the growth of microbial cultures and biofilms (Mart'yanov et al., 2015) and induce cell differentiation processes (El'-Registan et al., 2006). The mechanism of action of AHBs is based on their capacity to modify the structure and activity of cell biopolymers such as proteins and the DNA, to increase the microviscosity of biological membranes, and to influence ion transfer processes and the cell's water balance (Bukharin et al., 2005; El'-Registan et al., 2006). Importantly, AHBs change the activity of the cell effectors of

Fig. 2

the innate immunity system (Deryabin et al., 2013a, b) and the functional stability of antibodies (Deyabin et al., 2010).

Factors d_2 are unsaturated fatty acids. They uncouple membrane phosphorylation and, at high concentrations, damage cell membranes, resulting in cell death.

Interestingly, nonchemical distant communication also seems to make an important contribution to ifomation exchange among microbial cells. Electromagnetic and acoustic waves are likely to be involved in distant information transmission. As early as in the 1920s and 1930s, Alexander Gurwitch and co-workers investigated ultraviolet radiation that is emitted by living cells and stimulates cell division. For example, the UV radiation produced by *Nadsonia* sp. yeast stimulated cell proliferation in *Bacillus* sp. cultures (Sewertzowa, 1929). In the 1990s, Yuri Nikolaev revealed that a *Vibrio costicola* culture treated with a lethal dose of the antibiotic chloramphenicol produces a signal that stimulates the growth of another culture of the same species that was separated by a double quartz glass layer (Nikolaev, 1992, 2000). These data will be reiterated and discussed in more detail in the section on physical communication in Lecture 4.

Fig. 3

The historical evolution of the **population- and communication-centered paradigm (POCCP)** in microbiology resulted in developing its basic principles including (i) heterogeneity of microbial populations and the polymorphism of microbial cells in them; (ii) colony/biofilm architecture; (iii) social behaviors of microorganisms; (iv) chemical communication factors; and (v) relationships between microbial populations and macroorganisms in ecosystems.

LECTURE 2. MICROBIAL SOCIAL BEHAVIOR. BIOFILMS

2.1. Social behavior in microorgamisms. Much evidence has been presented that "microbes indulge in a variety of social behaviors involving complex systems of cooperation, communication, and synchronization" (West et al., 2007). Typical examples of microbial social behavior include collective hunting by *Myxococcus* spp. This bacterium is characterized by synchronously moving cells. Myxococcus cells engulf other bacterial cells and collectively digest them with exoenzymes.

Another illustrative example is provided by the aggregation of ameboid cells of the eukaryote *Dyctiostelium discoideum*; these cells conglomerate and produce a mushroom-like fruiting body (Fig. 4). The social interaction depends on a number of chemical signals including the widely spread multifuctional messenger cyclic AMP. The third important example of microbial sociality is biofilm formation e.g., in *Pseudomonas fluorescens* and *Bacillus subtilis* (Tarnita, 2017). A biofilm involves much social interaction, and it is metaphorically compared to a city of microbes, the biofilm-structuring extracellular biopolymer matrix being an analog of the buildings in a human city and biofilm formation, which will be considered in more detail in the final part of this lecture.

Fig. 4

In ethology[2] (and in the social sciences including social psychology), social behavior is often classified into the following two types (each types is subdivided into a number of behavior forms, see Fig. 5)[3]:

Fig. 5

[2] *Ethology* is defined as the field of biology dealing with animal behavior. Classical ethology emphasized research on innate species-specific behavior forms and mechanisms that were studied under natural conditions (during field studies).

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(a) *Agonistic behavior.* This type of behavior is associated with conflict among living organisms (Dewsbury, 1978). Importantly, "like any society… microbes face conflict" (Foster, 2010); agonistic behavior is further subdivided into aggression and avoidance.
(b) *Loyal* behavior including the totality of "friendly" interactions among living beings that consolidate their groups, families, colonies, or other *biosocial systems*; this type of behavior includes several subtypes exemplified by affiliation and cooperation.

2.1.1. Aggression. The classical definition given by Niko Tinbergen (1968) with regard to animals**)** is "approaching an opponent and inflicting damage on him or at least generating stimuli that cause him to submit." Imagine wolves in a flock fighting other wolves, or lions competing for dominance in a lion pride. Analogous behavior in the microbial realm includes, e.g., the production of antibiotics (including bacteriocins), toxins, or surfactants for destroying or inhibiting competitors. The cyanobacteria of the genus *Anacystis* suppress the growth of the green algae *Scenedesmus, Chlamydomonas, and Haematococcus* (Ostroumov, 1986). Undoubtedly, many antibiotics are not only "chemical weapons" because they also function as important developmental regulators in the antibiotic producer culture. Their involvement in microbial aggressive behavior, nonetheless, is consistent with the data that antibiotics are actually released in response to the presence of a competitor. Of special interest in this context is the fact that the fungus *Trichotecium roseus* produces 1.7 times more trichotecin (an anitibiotic) if its culture is supplemented with that of a competitor (*Penicillium chrysogenum,* Egorov & Landau, 1982).

These findings have direct relevance to the human gastro-intestinal (GI) tract that contains useful (symbiotic) and potentially pathogenic (opportunistic) microorganisms. Timur Vakhitov (2019, p.195) emphasizes that agonistic interaction between several bacterial species results in the competing species producing additional amounts of growth stimulators. A useful (probiotic) *E. coli* strain more efficiently develops in the presence of a pathogenic bacterium (*Streptococcus enteritidis*) than without it. Such aggressive behavior often results in destroying "outsiders", in an analogy to similar behavior in social insects. However, a competitor can be inactivated in a more "subtle" way. Some bacilli produce antibiotics that convert the cells of competing bacterial species into dormant spores (Bushell, 1989). As a result, the bacilli monopolize all available nutrient substrates.

Aggressive behavior in the microbial world does not only take the form of exchanging destructive/incapacitating chemical agents A series of micrographs in the work by Väth (1992) demonstrated the dynamics of a "battle" between an amoeba, the predator, and an infusorian, the prey. The fighting continued for 20 minutes and resulted in the death of both opponents.

There at least 4 different strains of the potential pathogen *Staphylococcus aureus.* Each strain produces a cyclic peptide. The peptide functions as a signal in the culture of the producer strain, but it also disrupts similar signaling systems in all other strains, like a wrong key that doesn't open a lock but instead spoils it, so that this lock doesn't work afterwards even with an otherwise fitting key.

All forms of aggression are considered as costly and risky behaviors, and evolution promotes the formation of aggression-mitigating mechanisms. Microorganisms do not lie on their back like wolves, exposing their vulnerable body parts to the aggressor as an appeasement signal. There are, however, microbial analogs of aggression-preventing strategy that are based on segregation, e.g., in the human GI tract where different representatives of the microbiota may inhabit different parts of the gut.

Generally, competition tends to select for individuals (cells) that utilize different resources than their competitors (Foster, 2010). If competition is mitigated in this way, this may promote cooperation among former competitors. For instance, the product synthesized by one of the strains/species is utilized as a substrate by another strain/species (or by the host organism).

2.1.2. Avoidance (isolation). In the animal kingdom (and in human society), avoidance behavior often manifests itself in marking the boundaries of one's own territory. For instance, song-birds sing in order to give others the message that the territory around their tree or nest is occupied by them, and rivals should not even try to enter their habitat. Isolation in the microbial world is based on strain- or clone-specific interaction among microbial cells. As an analog of behavior aimed at avoiding outgroup individuals in various animal species (including humans), isolation promotes the spatial structuring and segregation of microbial biosocial systems.

Avoidance behavior is displayed by various microbial species, including *Proteus mirabilis, E. coli, Vibrio alginolyticus, Bacillus subtilis, Pseudomonas putida, Rhodospirillum rubrum,* and *Rhodobacter sphaeroides*; it manifests itself, for instance, in the "colony separation" phenomenon: microbial colonies that share one petri plate typically do not merge even if they grow towards one another (Dienes, 1946; Shapiro, 1985; Budrene, 1985; Novikova, 1989; Oleskin, unpublished data). Moreover, the expansion of a single microbial colony on the agar surface may result in the formation of protrusions that separate from the original colony and never merge with it.

The wood-destroying fungus *Stereum hirsutum* forms spatially isolated mycelia that do not merge. Local aging proceeds in hyphae that grow towards a neighboring mycelium, and such hyphae contain pigments that are characterstic of an aging mycelium. Aging prevents the hyphae of the two mycelia from coming into contact (Rayner, 1988). Analogous local aging occurs during the tissue repulsion process in animals and the hypersensitive response of the plant immune system.

The following part of the lecture is concerned with microbial analogs of loyal behavior.

2.1.3. Affiliation is defined as a form of social behavior involving an individual animal's tending to approach and remain near conspecifics (*Dewsbury*, 1978), particularly those belonging to the same family or social group. Animals engage in greeting, play, and grooming behaviors. The cohesion of the cells of one clone and of one tissue in a muticellular organism is an obvious analog of animal affiliation. If cultivated kidney and liver cells are mixed experimentally, they tend to form separate aggregates, "like attracts like".

The colonies of many bacterial species contain cell aggregates (microcolonies). Interestingly, the addition of the brain neurochemicals dopamine, norepinephrine, serotonin, and histamine to an *E. coli* culture results in changing the ratio between solitary and aggregated cells (Anuchin et al., 2008).

The ameboid vegetative cells of the myxomycete (slime mold) *Dictyostelium discoideum* feed on bacteria. After all available bacteria have been consumed, the starving cells aggregate to form the motile pseudoplasmodium ("the slug") and, subsequently, the fruiting body with spores. Cell aggregation depends on chemical regulators such as cyclic adenosine monophosphate (cAMP) and chlorinated hexaphenones termed differentiation-inducing factors (DIF-1, DIF-2, and DIF-3). About 20% of the cells undergo programmed cell death (apoptosis), and the dead cells constitute the stalk of the mushroom-like fruiting body; this is considered an example of altruistic behavior in microorganisms.

Myxobacteria are prokaryotes that resemble eukaryotic myxomycetes in terms of social behaviors. When depleted of nutrients, the cells of *Myxobacterium xanthus* release factor A (a mixture of hydrophobic amino acids and short peptides) that induces cells to form compact groups. Subsequently, contact cell-cell communication comes into play. It involves non-diffusible factor C (attached to the cell producing it) that initiates fruiting body formation. Up to 90% of the cells involved undergo apoptosis, i.e., programmed cell death, during this process (Ulvestad, 2009).

2.1.4. Cooperation. In ethological terms, this kind of loyal behavior implies *interaction between two or more individuals or groups for the purpose of solving a problem or carrying out a task.* An

alternative, although in principle similar, approach to defining cooperation involves considering it from the viewpoint of a whole group (community). In these terms, *cooperators* are contrasted with *cheaters (free riders)*: cooperators contribute to the collective good within a distinct group at an individual cost, and cheaters exploit it (Hochberg et al., 2008, modified).

There are analogous phenomena at the cellular level (Crespi, 2001). Of relevance is the behavior of immune cells (macrophages and lymphocytes) inside an animal organism in response to a foreign invader. Macrophages bind the agent that has penetrated into the organism and present it to T lymphocytes. The activated T lymphocytes interact with B lymphocytes that produce antibodies neutralizing the agent.

Cooperation is widely spread among free-living prokaryotic and eukaryotic organisms. Like multicellular organisms, microorganisms cooperate to build a shelter, to forage, to reproduce, and to spread in the available area (Crespi, 2001; Ulvestad, 2009). Cooperation is characteristic of myxobacteria that coordinately move over the surface of the nutrient medium and pursue their prey, i.e., other bacteria they feed upon. Filamentous cyanobacteria form associations and display sophisticated behaviors aimed at securing the survival and integrity of the whole association (Sumina, 2006). If a cyanobacterial biofilm is damaged (ruptured), it tends to regenerate: filaments actively move towards the gap and close it.

Cooperation often implicates some degree of functional differentiation and specialization of the individuals (microbial cells) involved. “Nitrogen-fixing cells of *Rhizobium* and cyanobacteria filaments are specialized food providers analogous to the foraging classes of social insects” (Velicer, 2003, p. 330).

2.2. Biofilms. Biofilms[4] are *“matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces”* (Hall-Stoodley et al., 2004, p.95) that are mostly formed at interphase boundaries. Microbial biofilms are structurally heterogenous even if they contain cells of a single bacterial species because they include cells with different phenotypes. However, biofilms may include representatives of different species, genera, and even kingdoms or empires of life (Nikolaev & Plakunov, 2007). For instance, the film of a methanogenic association is composed of cells of eubacteria and archaea. Apart from prokaryotes, biofilms may be composed of fungal or protozoan cells (Vidyasagar, 2016).

Many biofilms are characterized by functional differentiation of the cell types they contain and coordinated behavior that enables the biofilm to develop as a single coherent entity with its life-cycle (ontogeny); like a multcelullar organism, a biofilm can reproduce and regenerate after injury (Sumina, 2006; Karatan & Watnick, 2009). In spite of their diversity, all microbial biofilms exhibit the following typical features (Nikolaev & Plakunov, 2007):

- *Spatial organization***,** i.e. the formation of two- and three-dimensional structures in a biofilm, exemplified by local cell aggregates (microcolonies), cavities (pores and channels), lipid membrane vesicles, the outer cover of the biofilm including the biofilm-coating lipid bilayer (Tetz et al., 2004), and the biofilm’s functional “organs” such as the O_2-transferring hemosomes of *Alcaligenes* (Duda et al., 1995, 1996, 1998) and fruiting bodies with maturating spores (in myxobacteria) or their analogs (in bacilli).
- *Metabolic organization* implying the existence of a directed metabolite flow in a biofilm.
- *Intercellular biopolymer matrix* that is responsible for maintaining the structural integrity of a biofilm, protecting microbial cells from deleterious environmental factors, masking the cells’ surface antigens to prevent their recognition by host immune cells, and creating a

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hydrophilic environment to promote the spreading of metabolites and signal molecules within the biofilm; these matrix features were considered in more detail above.

- *Adherence to a phase boundary* such as solid/liquid, solid/air, liquid/air, or liquid/liquid boundary.

Biofilms are comparable to human-made buildings: the matrix to the construction material(s), and the bacterial cells to the residents (Zhou & Cai, 2018).

The typical structure of a biofilm is formed stepwise (Fig. 6). Initially, a *transient attachment* of microbial cells (primary colonizers) occurs, which is due to their interaction with the substratum involving flagella, pili, fimbria, and the proteins of the outer membrane (in gram-negative bacteria). “Transport of *Ps. aeruginosa* bacteria to a surface before attachment is assumed to involve diffusive, convective, and active flagellum-driven transport” (Harmsen et al., 2010, p.253).

Fig. 6

This stage is followed by the *permanent attachment* of microbial cells to the surface. For example, motile bacterial cells first attach with one of their poles by means of flagella to a substratum; thereupon, one of their sides contacts the surface and is anchored there. At this stage, the microstructural features of the substrate surface play an important role. For instance, nano- and microscale surface roughness promotes bacterial adhesion, providing more area for cell attachment (Renner & Weibel, 2011).

Subsequently, microbial cells *spread* on the substratum colonized by them. This is accompanied by the formation of local cell aggregates, small microcolonies, and the intracellular matrix with characteristic cavities and the biofilm cover (Tetz et al., 1993, 2004; Pavlova et al., 2007; Zhou & Cai, 2018).

The development of a majority of biofilms includes the stage characterized by the *attachment of new microbial cells (secondary colonizers)* to the substratum-anchored cells, which results in the formation of multilayer biofilms. Cells attach to other cells and the substrate, and the attachment process largely depends on the matrix components with adhesive properties such as alginate, the linear anionic polysaccharide of *Ps. aeruginosa* (Skarlyachan et al., 2018).

Biofilm maturation is also often associated with the formation of wrinkles on its surface. They result from local cell death, the formation of empty spaces, and the shriveling of the matrix. Wrinkles increase the surface:volume ratio, promoting oxygen supply to aerobic biofilm cells, and facilitate the development of a decentralized network of liquid channels that accelerate liquid distribution within the biofilm (in an analogy to a circulatory system in a multicellular organism). In an aging biofilm of spore-forming bacteria, e.g., *B. subtilis,* wrinkles develop into protrusions that serve as sporulation sites (Mielich-Süss & Lopez, 2015).

Eventually, a single- or multiple-species biofilm with a developed structure is formed; this biofilm can display a lamellar structural pattern, contain mushroom- or pillar-shaped formations, and display a variety of other “architectural features” that are due to cell specialization, communication, and a complex spatio-temporal organization pattern that is “similar to those described for more sophisticated multicellular organisms” (Mielich-Süss & Lopez, 2015).

Like other cooperative acitivities, biofilm formation is vulnerable to cheating. For instance, *Ps. fluorescens* biofilm mats are based on a matrix that is made up of cellulose-like polymers (CLPs). Such biofilms colonize the air-liquid interface. Mutant cells fail to form CLPs but are able to invade and exploit biofilm-forming cooperators. However, upon reaching high frequency, these cheaters disrupt the biofilm structure, rendering it unable to float on the liquid medium surface; such a biofilm ultimately loses its viability (Velicer, 2003).

Chemical communication is actively involved in biofilm formation, spread, and dispersal.

Apart from chemical signals, physical factors such as electrical fields seems to be involved in communication among cells within a biofilm (Prindle et al., 2015). The potassium efflux from metabolically stressed, glutamate-deficient *B. subtilis* cells in the interior of a biofilm (that is caused by the operation of the YugO K^+ channel), results in depolarizing the membranes of other cells in the same biofilm and even of bacterial cells outside its boundaries (see:

Humphries et al., 2017). This decelerates the membrane potential-dependent influx of glutamate ions and, therefore, slows down metabolic processes in these cells. Hence, electrical communication results in reducing competition between biofilm cells for glutamate and other substrates and synchronizing the metabolic activities of the cells of a bacterial biofilm (Prindle et al., 2015).

The final stage of a biofilm's life-cycle involves its dispersal; microbial cells return to the planktonic mode of existence. This involves the detachment of the cells from the substratum and the separation of cell aggregates from the biofilm. Solitary cells can exit the film and start seeking new "accommodations" (Vidyasagar, 2016). Cells also detach from solid substrate surfaces (Davies, 2011). This is frequently accompanied by the synthesis of surfactants and enzymes, e.g., dispersin B and DNase, that degrade the matrix components (adhesins and extracellular DNA molecules, respectively) that are directly involved in the adherence of microbial cells to the substrate and to other cells. For instance, the oral cavity-inhabiting bacterium *Actinobacillus actinomycetemcomitans* produces an enzyme that degrades adhesin PNAG (Itoh et al., 2005; Romeo, 2006).

Biofilm dispersal also involves the suppression of *de novo* adhesin synthesis. Detailed studies conducted with the opportunistic pathogen *Ps. aeruginosa* that may inhabit various niches in the human organism have demonstrated that biofilm dispersal involves an endogenous prophage and the death of a part of the biofilm cells. This is associated with the transition of the remaining viable cells to the planktonic lifestyle. As a result, the surface-adherent microcolonies in biofilms undergo disintegration and become hollow shell-like structures. The whole phenomenon is termed "seeding dispersal" in the literature (Romeo, 2006).

Biofilm dispersal and the transition of microbial cells to the planktonic lifestyle results in a considerable increase in the cells' sensitivity to various agents, including antibiotics, detergents, disinfectants, bcteriophages, immne cells, and predatory bacteria. Therefore, biofilm-degrading enzymes such as proteases that cleave, e.g., the biofilms of *Staph. aureus* (Payne & Boles, 2016), or dispersin B that degrades PNAG in the biofilm matrix (Kaplan, 2014) are regarded as potentially efficient drugs for treating or preventing infections that are caused by biofilm-forming pathogens.

The biofilm formation process is influenced by environmental factors and regulatory agents formed by microbial cells. Cultivation conditions (pH, temperature, nutrient substrate concentration, pO_2, osmolarity, surface hydrophilicity/hydrophobicity degree, shear force, etc.) produce their effects on microbial biofilms. For instance, nutrient limitation results in enhanced biofilm formation by *Salmonella enterica* var. *Typhimurium.* This process involves the operation of stationary-phase σ factor RpoS (Gerstel & Romling, 2003). In contrast, the development of *Vibrio cholerae* biofilms is enhanced in a nutrient-rich medium, and RpoS represses the genes involved in biofilm formation (Yildiz et al., 2004). It was established that biofilm formation in pathogenic and non-pathogenic *E. coli* strains is dependent upon their cultivation conditions including medium composition. Most tested *E. coli* strains failed to form biofilms on the rich Luria-Bertani medium but formed them on a minimal medium and on diluted porcine intestinal mucus (Reisner et al., 2006).

Biofilms protect microorganisms under unfavorable conditions. "Bacterial biofilms can be likened to protective domiciles, such as nests or hives" (Velicer, 2003, p.330). For instance, marine bacteria in biofilms and structurally similar microbial mats "maintain the osmotic balance and resist the outside high-pressure environment" (Zhou & Cai, 2018) by activation matrix production.

The biofilms that are formed by microorganisms in the host macroorganism enhance the microorganisms' resistance to antibiotics (Foster, 2010); their lethal concentrations in biofilms are hundreds or even thousands of times higher than those killing plantonic cells of the same

species (Mathur et al., 2018) [5] . Biofilms also prevent immune cells from attacking microorganisms. This is largely due to the protective function of the matrix.

However, there are other important reasons why microorganisms form biofilms (Jefferson, 2004):

- *Sequestration to a nutrient-rich medium***,** the colonization of a favorable ecological niche. "A biofilm at an air-water interface has good access to oxygen and light…, and attachment to solid surfaces can yield similar advantages, particularly given that cells will often attach reversibly and swim off if they end up in a bad spot" (Foster, 2010; P.341). This strategy is also exemplified by biofilm formation in the GI tract; importantly, different species and strains, e. g. pathogenic and non-pathogenic *E. coli* strains, can compete for resources available in various areas of the GI tract (Bansal et al., 2007). Microbial cells in a biofilm engage in cooperation, and they can, therefore, more successfully adapt to environmental challenges (Aguilar et al., 2015).
- *Using the advantages of the social lifestyle,* including the functional specialization of microbial cells in metabolic terms; the biofilm lifestyle is unfavorable for non-cooperating cheaters that do not contribute to matrix synthesis and the production of other public goods (Aguilar et al., 2015). This is largely due to the spatially structured environment that is provided by a biofilm. This environment favors cooperation and communication. In a microbial biofilm, "the secretors <of enzymes, nutrients, regulatory substances, and other products used by the whole microbial biosocial system – O.A.> have the primary access to the substances produced, allowing the public good producers to easily outnumber the nonproducers" (Martin et al., 2016, p.2565). The protective extracellular matrix helps biofilms survive under extreme environmental conditions, e.g., in hot-water springs with very low or very high pH values and on the surface of glaciers.
- *Biofilms as the default mode of existence*, as the normal lifestyle of most microorganisms; over 90% of environmental microorganisms form biofilms (Zhou & Cai, 2018). The existence of microbial cells in suspensions (the planktonic lifestyle) represents, in these terms, a temporary adaptation aimed at searching for a new suitable habitat for biofilm formation or just an *in vitro* artifact.

Biofilm development and dispersal are subject to control by a complex of intra- and intercellular regulators. Microorganisms have special gene blocks involved in the planktonic cells-biofilm interconversion, including genes responsible for the adherence of microbial cells to substrata and to other cells, such as the *algC* gene required for the synthesis of alginate, a matrix component in *Ps. aeruginosa,* and *wcaB* gene involved in colanic acid synthesis in *E. coli.* Biofilm formation in *E. coli* implicates the expression of genes that are involved in the production of bacterial cell surface structures, such as the *csgA* gene required for the formation of curli fibers (reviewed, Jefferson, 2004).

Gene expression during biofilm formation is influenced by a variety of intracellular regulatory factors. Important functions are performed, particularly in gram-negative bacteria, by cyclic diguanylate monophosphate (c-di-GMP). Accordingly, the regulation of the activities of the c-di-GMP-synthesizing enzyme diguanylate cyclase (DGC) and of the c-di-GMP-degrading phosphodiesterase A (PDEA) are essential for the operation of the intracellular network of regulatory agents involved in biofilm formation/dispersal.

The intracellular c-di-GMP concentration decreases in response to environmental stimuli such as sudden changes in the nutrient concentration (in *Ps. aeruginosa,* this can be an increase in glutamate concentration) and oxygen depletion in the interior of the biofilm. The decrease in c-di-GMP concentration results, in a large number of bacterial species, in biofilm

[5] Nonetheless, a large number of bacterial biofilms are comparatively susceptible to the effects of baceriocins (antimicrobial peptides), including clinically impotant lantobiotics such as nisin and lantothionine (Mathur et al., 2018).

dispersal (Camilli & Bassler, 2006; Karatan & Watnick, 2009). The influence of environmental factors on the c-di-GMP pool is mediated by the chemotaxis protein BdlA that contains two PAS (Per-Arnt-Sint) domains involved in receiving a variety of extracellular signals including nitric oxide (Barraud et al., 2009a).

Biofilms are of paramount practical importance. A large number of biotechnological processes are carried out by means of microbial biofilms, as exemplified by the traditional French technology of producing vinegar with *Acetomonas* biofilms that are grown on woodchips. A thick multispecies biofilm containing bacterial and yeast cells is the producer of a useful beverage with medicinal properties, the "tea fungus" (kombucha, Yurkevich & Kutyshenko, 2002). Biofilms find application in bioremediation projects, including the removal of oil spills in the ocean and degradation of soil pollutants. Biofilms overgrow plant roots and cover the mucosa of the human/animal intestines; this "extracorporal organ" fulfills a number of important functions (see Lecture 5 below).

However, microbial biofilms can also do much harm. They cause the destruction of various materials and constructions (biofouling). For instance, biofilms growing on materials contained in cutlery and crockery produce biogenic amines (BAs). Although important neurotransmitters and histohormones (see Lectures 7-8 for details), such BAs as histamine, tyramine and putrescine are toxic and pose health risks if produced at high concentrations. It was established that 56 BA-producing strains belonging to *Enterococcus, Lactococcus,* and *Lactobacillus*, formed biofilms on polystyrene and adhered to stainless steel (Diaz et al., 2016).

A serious threat is posed by the biofilms of pathogenic microorganisms If bacteria succeed in forming biofilms inside our body, they may become invulnerable to antibiotics and cause chronic infection, e.g., in a surgical wound, in the lungs, or in the urinary tract. "Biofilm formation is an important aspect of many, if not most, bacterial diseases, including native valve endocarditis, osteomyelitis, dental caries, middle ear infections, ocular implant infections, and chronic lung infections in cystic fibrosis patients" (Jefferson, 2004, p.63). Biofilms overgrow catheters, contact lenses, and joint and intraocular implants. They cause gingivitis, bacterial vaginosis, and other urogenital infections (Jacubovics et al., 2013). As far as such harmful biofilms are concerned, "knowledge of the environmental cues, genetic elements, and molecular mechanisms that are involved in biofilm formation is necessary for a rational design of strategies to eliminate biofilms or to prevent biofilm formation" (Harmsen et al., 2010, p.253).

Fortunately, our modern-day knowledge enables us to overcome some of the pathogenic biofilms-caused problems (Saha et al., 2018). The following strategies of combating biofilms are practically used currently:

- Preventing biofilm attachment to surfaces by covering them with biofilm-repelling materials as exemplified by silver ion-containing substances
- Destroying the biopolymer-containing basis (matrix) of a biofilm, including the DNA that strengthens the matrix and can be degraded with DNases
- Introducing antimicrobials- or bacteriophages-containing lipid membrane vesicles (liposomes) into the biofilm matrix
- Using antibodies against pathogenic microorganisms or their toxins
- Using signal molecules such as nitric oxide that stimulate biofilm dispersal
- Applying surfactants including those of bacterial or fungal origin, as exemplified by *B. subtilis*-produced surfactin that degrades the biofilms of potential pathogens (*E. coli, Salmonella enterica, Proteus mirabilis,* etc.) growing, for instance, on vinyl catheters inserted into the bladder.

Microorganisms and other living cells engage in various kinds of **social behavior.** It is classified into (a) **agonistic** behavior associated with conflict among living organisms and including aggression and avoidance and (b) **loyal** behavior embracing "friendly" interactions

among living beings that are exemplified by affiliation and cooperation. Social interactions underlie the formation of advanced multicellular structures typified by **biofilms.**

LECTURE 3. CHEMICAL COMMUNICATION. QUORUM SENSING: MAIN PRINCIPLES

There is a large body of evidence that «… bacteria, like all other living organisms, process and use information about the environment during their life-sustaining activities. *Exchanging information and obtaining it from other living organisms is called communication*" (Nikolaev, 2000, p. 597, *emphasis added – O.A.*). Communication in microorganisms, as well as in any other kinds of biological systems, includes the three main stages (Zhao et al., 2017): (1) detecting a signal, e.g., via its binding with the cognate receptor; (2) recognizing the signal; for instance, a cyclic adenosine monophosphate molecule (cAMP) is interpreted by a myxomycete cell as the "start cell aggregation" instruction; (3) making a decision with regard to the response to the signal; in the aforementioned example with *D. discoideum,* it is cell competence (resulting from cell starvation) that determines the decision. In this respect, communicating cell groups are similar to neuronal networks or their artificial analogs such as perceptrons that contain specialized layers responsible for data perception, information processing, and decision-making, respectively[6].

Microorganisms including bacteria use contact, distant chemical, and, presumably, distant physical communication.

3.1. Contact communication. This type of communication is based on cell-cell contacts that represent cytoplasmic bridges (plasmodesms), outer membrane fusion sites (in gram-negative bacteria), or peptidoglycan fusion sites (in gram-positive bacteria; Tetz et al., 1990). Presumably, cytoplasmic bridges, or nanotubes, can function as wave conductors to transmit electromagnetic waves (belonging to various wavelength ranges) between bacterial cells (Vysotsky et al., 1991); electromagnetic communication is briefly discussed below.

The cells of the gram-negative bacterium *Myxococcus xanthus* aggregate and subsequently form fruiting bodies under conditions of nutrient deprivation. At the later stages of this process, the cells are densely packed, which enables spore formation. These developmental events are subject to regulation by non-diffusible factor C. Its precursor (p25) is the product of the *csgA* gene. In starving cells of *M. xanthus* the secreted protease PopC converts p25 to factor C (Stevens et al., 2012). "…The secretion of PopC is dependent on the stringent response protein RelA, which produces alarmone guanosine tetraphosphate (ppGpp)… The components providing the link between RelA/ppGpp and PopC were identified as PopD and FtsH: ppGpp directs activation of FtsH, an ATP-dependent protease that degrades PopD, which in turn inhibits PopC " (Stevens et al., 2012, p.2132). Factor C induces the expression of the genes that are involved in the maturation of fruiting bodies with spores and interact with transcription factors FruA and MrpC (reviewed, Zhao et al., 2017).

The strain *E. coli* EC93 inhibits the growth of the cultures of other strains of the same species in a mixed culture. The inhibition is based on direct cell–cell contact. Communication involves the CdiA/CdiB two-component system. CdiB is an outer membrane protein that is necessary for the secretion of protein CdiA that remains attached to the cell surface. Upon contacting the target cell, CdiA interacts with its receptor, BamA. The C terminal part of the CdiA molecule (CdiA-CT) is detached by a protease and transported into the target cell where it suppresses metabolic processes (Aoki et al., 2009; Otto, 2010; Zhao et al., 2017).

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Intercellular contacts involve a wide variety of surface structures, including microfibrils, cone-shaped protrusions, cell wall evaginates, and glycocalyx (reviewed, Oleskin et al., 2000).

Direct cell–cell contact is a prerequisite for communication via surface organelles such as pili and via the components of the exopolymer matrix that coats bacterial cells, their groups, and the whole colony/biofilm. Aggregation and spore formation in *M. xanthus* depend on type IV pili. Their homologues are formed by the pathogenic bacteria *Ps. aeruginosa* and *Neisseria gonorhoeae*, and they are responsible for socially coordinated cell movements in these species (Will et al., 1998). As for *M. xanthus,* its collective cell behaviors also involve polysaccharide-protein fibrils and the polysacharide O-antigen of the external layer of the outer membrane (Shapiro, 1995; Will et al., 1998). All these cell surface structures are synthesized with the help of S (social) genes that are necessary for collective coordinated cell translocation and the formation of multicellular structures. In contrast, the A (adventurous) genes of the myxobacteria are responsible for individual cell motility and enable cells to move away from their colony.

As already mentioned, some bacteria have been established to form membrane nanotubes for transferring macromolecules (proteins, DNA, and RNA) to adjacent cells. Such nanotubes form between the cells of the same species (*Bacillus subtilis)* and those belonging to different species, e.g., between *B. subtilis* and *E. coli* cells (Dubey & Ben-Yehuda, 2011; Zhao et al., 2017). In a similar fashion, networks of intercellular membrane nanotubes connect mammalian cells.

A large number of proteobacteria produce outer membrane vesicles (OMVs). Apart from other functions (virulence factor secretion and immunomodulation), OVMs, similar to nanotubes, are used for transferring chemical agents, including quorum-sensing signals (see below).

3.2. Distant chemical communication among spatially separated cells. Many diffusible chemical signals are implicated in coordinating microbial growth, developmental processes, and the transition between the stages of the life-cycle of a microbial culture (culture ontogeny, Yerusalimsky, 1952). Such signals are referred to as *autoregulatory substances,* or *autoregulators*. They are microbial metabolites that are released by a cell population, or its part, into the medium. Many autoregulators are not utilizable in constructive or energy metabolism but perform major communicative functions and, therefore, influence the physiological state and the reproductive potential of the cells involved (El'-Registan, 1988). This topic is only briefly mentioned here, and the students are invited to revisit Lecture 1 where the formula of one of the essential autoregulators of bacterial cultures is shown (see Fig. 2).

It was established that, during the initial stages of culture growth, the lag phase and the exponential phase, an *E. coli* culture releases substances (autostimulators) that, when added to another *E. coli* culture, stimulate its growth; during the later growth stages, the growth deceleration stage and the stationary phase, an *E. coli* culture releases autoinhibitors that suppress the growth of another culture (Vakhitov et al., 2003).

Autoregulatory substances that are produced by a microbial culture and influence the development of other cultures of the same strain also include glutamate that, together with lysine, methionine, and succinate, stimulates, and aspartate that, along with lactate and formate, inhibits the growth of the probiotic strain *E. coli* M-17. Aspartate, in contrast, stimulates the growth of another strain, *E. coli* BL (Vakhitov et al., 2000; Vakhitov & Sitkin, 2014).

3.3. Quorum sensing: basic principles. A large number of studies have been conducted on *quorum-sensing (QS)* systems that control, in a cell density-dependent fashion, many important processes in microbial cells and their groups (Fuqua et al., 1994; Gray, 1997; Waters & Bassler, 2005; Khmel', 2006; Tarighi & Taheri, 2011; Stevens et al., 2012; Bassler & Miller, 2013; Hagen, 2015; Kalia, 2015; Leoni & Rampioni, 2018), including bioluminescence, synthesis of antibiotics and enzyme complexes, cell-to-cell transfer of genetic information

(transformation and conjugation), cell aggregation, protein secretion, biofilm and gas vesicle formation, sporulation, virulence factor production, etc. "QS is an environmental sensing system that allows bacteria to monitor population density and to connect cell population density with gene expression" (Thornhill & McLean, 2018, p.3-4). Microbial populations estimate the density of their population from the concentration of the QS signal molecules (pheromones, autoinducers) that are released by each cell in the population. Once the QS signal concentrations reach specific thresholds, respective QS systems are either activated or repressed. Many QS systems function according to the positive feedback (autoinduction) principle (Duan & Surette, 2007).

"Bacteria use quorum sensing to communicate both within and between species. Both species-specific and species-nonspecific autoinducers exist" (Bassler & Miller, 2013, p.495). Some microbially produced substances, e.g., N-acylhomoserine lactones, only operate as QS signals. However, there are also multifunctional compounds, including factor AI-2 (see below) that, apart from operating as an interspecies QS signal, is used as the sink for metabolic waste products. Generally, QS signals form a part of a spectrum of evolutionarily conserved biologically active substances: a large number of them are multifunctional (Vakhitov, 2019).

In terms of the interaction between the microbiota and an animal host organism, it should be emphasized that QS "systems play global regulatory roles in bacterial virulence. They synchronize the expression of multiple virulence factors and they control and modulate bacterial antibiotic tolerance systems and host defense mechanisms" (Maura et al., 2018, p.227). Some of the main QS signals are shown in Fig. 7.

3.3.1. Quorum sensing systems in gram-negative bacteria. A majority of the QS signals of gram-negative bacteria are N-acylated homoserine lactons (N-AHLs), also called autoinducers-1 (AI-1s). Such QS systems are denoted as *luxI*-*luxR*-type QS systems; they are similar to the prototypical QS system of the marine luminescent bacterium *Aliivibrio fisheri*. N-AHLs bind to regulatory R proteins, and the resulting complex activates (or, alternatively, inhibits) the transcription of the genes that are responsible for diverse quorum-dependent processes. If the cell density is sufficiently high, bacteria engage in various collective behaviors. The prototypical system of *Aliivibrio fischeri* (Fuqua et al., 1994) enables this bacterium to emit light in concentrated cell populations. They inhabit the light organ of the bobtail squid *Euprymna scolopes*, in which the bacterial cell density may be as high as 10^{10}-10^{11} cells/mL.

Fig. 8

This QS system includes two main gene complexes (Fig. 8). One of them is the *luxICDABEG* operon. The *luxI* gene encodes the protein that is responsible for the synthesis of the QS signal, N-(3-oxohexanoyl)-L-homoserine lactone (3-OHHL). The other genes (*luxA, B, C, D, E,* and *G*) encode the components of the enzymes that are required for bioluminescence. The second gene complex includes the *lux R* gene. Its product, LuxR, binds to 3-OHHL. The LuxR-3-OHHL complex binds to the promoter site of the *luxICDABEG* operon and activates its transcription if the *V. fischeri* cell density and, accordingly, the signal concentration reach the threshold level. Most other QS systems in gram-negative bacteria function according to similar principles.

N-AHLs contain fatty-acid chains; their length is different, and they have different substituents. Some N-AHLs have aromatic radicals or branched amino acid side chains. For instance, the aromatic radical-containing N-AHL signals (aryl-HSLs) cinnamoyl-HSL and isovaleryl-HSL are produced by *Bradyrhizobium* species utilizing the BtaI and BjaI synthases, respectively (Stevens et al., 2012, p.2133). The binding of N-AHLs to respective R proteins results in conformational changes that enable the HTH domain of the R proteins to bind to the DNA at the *lux* sites of these QS-controlled genes. This allows the R protein-HSL complex to recruit the RNA polymerase and to activate transcription.

LuxR type proteins contain the acylhomoserine-binding domain at the N terminal and the DNA-binding domain at the C terminal (reviewed, Venturi et al., 2018).

Some bacteria of the genus *Erwinia* (*Erw. carotovora, Erw. chrysanthemii,* and others) cause the soft rot of potatoes, chrysanthemums, and other plants. They degrade plant cell walls using pectinases and cellulases. These enzymes are important virulence factors in *Erwinia,* and their formation is a quorum-dependent process (Fuqua et al., 1994; Revenchon et al., 1998). At a high population density, the synthesis of these enzymes is so rapid that plant cells are destroyed before their immune system responds to the pathogen. *Erwinia* contains the *expI-expR* system*,* an analog of the *luxI-luxR* system in *A. fischeri.* Protein ExpI, which is partly homologous to protein LuxI, is necessary for the synthesis of the diffusible communicative signal 3-OHHL (the same signal is used by *A. fischeri*). Since *Erwinia* and *A. fischeri* share the 3-OHHL signal*,* a plasmid containing all *lux* genes of *V. fischeri* except *luxI* brings about QS-dependent luminescence in *Erw. carotovora* (Revenchon et al., 1998)*.*

Apart from *expI-expR, Erw. carotovora* posseses the *carI-carR* gene system*.* The *carI-carR* system controls the synthesis of the antibiotic carbapenem in a quorum-dependent fashion. Activation of the antibiotic's synthesis at a high population density via the *carI-carR* system presumably helps *Erw. carotovora* eliminate bacterial competitors that attempt to use the products of plant cell degradation by *Erw. carotovora* exoenzymes (Fuqua et al., 1994; Salmond et al., 1995).

The bacterium *Agrobacterium tumefaciens* forms crown galls in a large number of plant species. The galls represent plant analogs of malignant tumors. The development of crown galls results from the transfer of oncogenic DNA fragments from the bacterium to the plant cell nucleus via specific Ti plasmids. Some of the genes of Ti plasmids induce the synthesis of opines that are utilized as nutrient substrates by *Ag. tumefaciens.* A homologue of *luxI-luxR,* the *traI-traR* gene system, stimulates the spreading of Ti plasmids within the bacterial population. Since the *traI-traR* is located on this plasmid, this mechanism conforms with the selfish DNA theory suggested by Richard Dawkins. The plasmid DNA aims to spread in a bacterial population. As soon as the population becomes quorate (sufficiently dense), plasmid-carrying cells are induced to conjugate with other bacterial cells (Greenberg et al. 1996). In addition, the conjugative transfer of Ti plasmids depends on opines. Therefore, efficient interaction between the microbiota and the macroorganism, a plant with an opine-producing tumor, are a prerequisite for carrying out this process. In particular, *traR* transcription is stimulated by factor OccR that is activated by octopine, one of the opines.

A large number of tested bacteria contain several QS systems. Their interactivity pattern is complex. In *Vibrio harveyi,* luminescence is subject to regulation by three QS systems. While internal signal transmission processes are carried out consecutively within a single QS system, several QS systems can interact both in a consecutive and a parallel fashion. QS systems may compete or inhibit each other's operation.

The pathogenic bacterium *Ps. aeruginosa* forms biofilms and releases virulence factors (involved in invading the human host and destroying human tissues) under the influence of several consecutive QS systems, including LasI-LasR and RhlI-RhlR (also called VsmI-VsmR)[7]. The functioning of the LasI-LasR system results in activating the RhlI-RhlR system (Waters & Bassler, 2005) via promoting the synthesis of protein RhlR that binds the N-AHL signal (Ganin et al., 2015).

In *Ps. aeruginosa,* the product of the *lasI* gene catalyzes the synthesis of the QS signal N-(3-oxododecanoyl)-L-homoserine lactone that forms a complex with transcription regulator LasR. LasR activates the expression of virulence factors-encoding genes such as *lasB* (elastase)*, lasA* (protease)*, toxA* (exotoxin A)*, aprA* (alkaline protease)*,* and *lasI* (the enzyme responsible for the synthesis of the QS signal). The *lasI-lasR* system also activates the *rhlI-rhlR*

[7] An additional QS system of *Ps. aeruginosa* depends on a quinolone signal, MvfR (PqsR), that is implicated in regulating the virulence of this pathogen and its interaction with the protective systems of the host organism (Ganin et al., 2015; Mauro et al., 2018). This system will be briefly discussed below.

system in which the product of the *rhlI* gene catalyzes the synthesis of the signal, N-butanoyl-L-homoserine lactone. This signal forms a complex with transcription regulator RhlR, and this complex activates *las B* and *aprA* expression. In addition, the *rhlI-rhlR* system activates the synthesis of the genes responsible for the synthesis of the surfactant rhamnolipid (that facilitates the migration of *Ps. aeruginosa* cells in a hydrophilic medium) and the pigment pyocyanin, as well as of the *rhlI* gene required for the biosynthesis of the QS signal N-butanoy-L-homoserine lactone (reviewed, Bassler & Miller, 2013; Fletscher et al., 2018). The same QS system is involved in biofilm formation in *Ps*. *aeruginosa.*

Another type of QS signals that is characteristic of a large number of gram-negative bacteria, including *Xanthomonas campestris, Xylella fastidiosa, Lysobacter enzymogenes, Stenotrophomonas maltophilia, Burkholderia cepacia,* and *Ps. aeruginosa,* comprises DSFs (diffusible signal factors). They represent unsaturated fatty acids such as cis-2-dodecenoic, cis-11-methyldodeca-2,5-dienoic, and cis-11-methyl-2-dodecenoic acid. Such QS systems regulate the expression of virulence and antibiotic resistance genes, cell motility and stimulate biofilm dispersal in, e.g., *X. campestris* (Zhao et al., 2017; Zhou & Cai, 2018). In *X. campestris,* the *cis*-11-methyl-dodecenoic acid signal is sensed by "the sensor kinase RpfC and the response regulator RpfG. RpfG has a… receiver domain attached to a HD-GYP domain that functions to degrade the second messenger cyclic di-GMP" (Stevens et al., 2012, p. 2135) that is involved in regulating motility and biofilm formation. Presumably, DSFs represent interspecies signals involved in infection; for instance, they are produced by the opportunistic bacteria *Burkholderia cepacia* and *Stenotrophomonas maltophila* in the lungs of individuals with cystic fibrosis (a hereditary disease characterized by excessive mucus formation in the lungs). The DSFs influence biofilm formation by *Ps. aeruginosa*, rendering the pathogen more resistant to antimicrobial agents (Stevens et al., 2012).

Apart from the LasI-LasR и RhlI-RhlR QS systems, *Ps. aeruginosa* contains a system that is dependent on 2-heptyl-3-hydroxy-4-quinolone (the *Pseudomonas* quinolone signal, PQS). This QS system is called the PQS system or the Mvf system; it consists of the PQS synthase (PqsI) and the regulatory protein PqsR (also referred to as MvfR). *P. aeruginosa* also produces PQS-like compounds such as 2-heptyl-4-hydroxyquinoline (HHQ), 2-nonyl-4-hydroxyquinoline (NHQ), and 2-heptyl-4-quinolone-N-oxide (HQNO). This QS system, along with the LasI-LasR and RhlI-RhlR system, is required for the production of virulence factors (pyocyanin, rhamnolipid, and lectin A). The same QS system is involved in releasing DNA molecules from the cells, which is associated with biofilm formation. Quinolone-type signals are also formed by other bacterial species, including the melioidosis pathogen *Burkholderia pseudomallei* (Fletscher et al., 2018).

3.3.2. Quorum sensing systems in gram-positive bacteria. Most QS systems of gram-positive bacteria are based on peptide signals that are either linear or contain a thiolactone ring. A peptide QS signal is produced by processing a longer precursor peptide and subsequently releasing it from the cell by means of an ATP-dependent ABC transporter (Bassler & Miller, 2013). Such QS systems are composed of two parts. A sensory histidine kinase binds the signal and phosphorylates the second part, the response regulator. A kinase cascade is initiated, which ultimately results in phosphorylating, and thereby activating, the protein that induces the transcription of the respective DNA operon.

For instance, one of the QS systems of *Staphylococcus aureus* (the Agr system, or SQS2) uses a peptide with a thiolactone ring (AIP, or AgrD). This QS system represses surface and attachment proteins, downregulates biofilm formation in *Staph. aureus* and upregulates the synthesis of toxins and exoenzymes, thereby facilitating infections caused by this dangerous pathogen (Shaw et al., 2007). The corresponding *agr* locus on the bacterial DNA is comprised of two suboperons with divergent promoters P2 and P3. P2 enables the transcription of the *agrBDCA* cluster. Among its protein products, AgrB is a membrane-associated protease that

cleaves and excretes a modified octapeptide form of AgrD (AIP). The peptide binds to sensor protein AgrC and regulates the synthesis of toxins, adhesion and colonization factors, proteases, and other agents involved in infection. AIP binding to AgrC results in its phosphorylation, which induces the phosphorylation of AgrA. It activates the P3 promoter of the *agr* operon. The RNA III molecules transcribed[8] encode the hemolysin protein with surfactant properties; they also stimulate the expression of extracellular proteases Aur and Spl ("staphopains") that degrade bioflims (Karatan & Watnick, 2009). "Purified staphopains were able to prevent biofilm formation" by *Staph. aureus* (Stevens et al., 2012, p.2138). Analogous AIP-dependent QS systems are characteristic of other *Firmicuta* including coagulase-negative staphylococci, enterococci, clostridia, and listeria (Murray & Williams, 2018).

In *B. subtilis,* spore formation efficiently proceeds at a high cell population density or after adding the culture liquid of a concentrated cell population. The process is subject to regulation by a QS system with an oligopeptide signal molecule that is encoded by the *pfrA* gene. Its expression results in formation of the inactive precursor with 41 amino acids. Upon excretion from the cell, the N-terminal amino acid sequence is detached from this peptide and may other signal proteins. The remaining peptide with 19 amino acids is further cleaved by an extracellular protease, resulting in the formation of an active signal pentapeptide (CSF, PEP5; Perego, 1997).

The CSF-mediated mechanism of spore formation activation in *B. subtilis* has been elucidated. CSF enters the cell via the oligopeptide permease. Once its concentration exceeds a certain threshold, CSF inhibits phosphatase RapA by forming an inactive complex with it. Without the phosphatase, the key sporulation factors, Spo0F и Spo0A, are in the active (phosphorylated) state.

The *rapA* phosphatase gene is co-transribed with the *pfrA* gene; they belong to the same operon. At low cell densities, the protein CSF formed by excreting and processing PfrA is present inside cells at low (sub-threshold) concentrations. Under these conditions, Spo0F and Spo0A are dephosphorylated by RapA, and spore formation does not start. Once the quorum level of cell density is achieved, the PfrA:CSF complex is formed, and the sporulation program is implemented (Mamson et al., 1998: Nakayama et al., 1998). Further research revealed two distinct levels of activation for phosphorylated Spo0A. A low activation level induces matrix production and a higher level results in sporulation (Fujita et al., 2005). It also renders cells insensitive to the Skf and Sdp toxins that are produced by them and kill sensitive cells. This is an analog of animal cannibalism "because dead cells serve as food to delay sporulation when nutrients are scarce" (Mielich-Süss & Lopez, 2015).

Another QS signal, ComX, activates the ComA QS system that turns on the transformation system (DNA transfer from cell to cell), rendering *B. subtilis* competent to transformation. The growth of the *B. subtilis* culture results in increasing the concentration of signal ComX produced by the cells. The signal is recognized by a two-component system that is composed of sensor kinase ComP and regulatory protein ComA. Upon binding the QS signal, ComA is phosphorylated. It activates the transcription of the *comS* gene. The product (protein ComS) protects another protein, ComK, against proteases-catalyzed degradation. Protein ComK activates the transcription of the genes that are responsible for DNA transfer between cells (transformation; Bassler & Miller, 2013).

Finally, activation of the third QS system results in phosphorylating DegU that promotes the secretion of exporoteases, enabling a subpopulation of cells to behave as "miners". They are

[8] The posited other QS system of *Staph. aureus*, referred to as SQS1, is based on the constitutive synthesis of ribosomal protein L2, or RNAIII-activating protein (RAP) that phosphorylates, at a sufficiently high concentration, the target protein (TRAP), which thereupon additionally stimulates the production of RNAIII molecules and, therefore, virulence. The role of SQS1 was called into question in a work in which no effect of an SQS1-inactivating mutation on virulence was detected (Shaw et al., 2007).

involved in producing “public goods”, i.e., degrading proteins into nutritive small peptides to be utilized by the whole population (Mielich-Süss & Lopez, 2015).

To sum up, the activation of master regulators Spo0A, DegU, and ComA leads to the development of several different cell subpopulations specializing in cannibalism and sporulation, DNA transformation, and biopolymer degradation, respectively.

Actinobacteria of the genus *Streptomyces* use QS systems that regulate antibiotic synthesis, aerial mycelium development, and spore formation. The signals that function in these systems are homoserine γ-butyrolactones (e.g., A factor in *S. griseus*), that bind to the transcription repressor. It loses its activity once bound to the QS signal. At least 15 homoserine γ-butyrolactones have been identified in *Streptomyces* and other prokaryotes (Biarnes-Carrera et al., 2018).

Communication is defined as exchanging information and obtaining it from other living organisms (Nikolaev, 2000). Microbial cells engage in **contact** and **distant communication** that can be based on chemical or physical (next lecture) signals. Of paramount importance is **quorum sensing (QS)** enabling bacteria to estimate the density of their population.

LECTURE 4. QUORUM SENSING: SPECIFIC SIGNALS. DISTANT PHYSICAL COMMUNICATION FACTORS.

This lecture places emphasis on some important communication signals that are widely spread in the microbial realm and are also involved in the dialogue between the animal/plant host and its symbiotic microbiota.

4.1. Furanone signals (AI-2). Both gram-positive and gram-negative bacteria use furanones as signals. While many homoserine lactones and peptides are species- (or strain-)specific, furanones are recognized as signals by a wide variety of bacterial species and, in all likelihood, are used for interspecies communication in microbial associations (Waters & Bassler, 2005; Khmel’, 2006; Shpakov, 2009; Zhao et al., 2017). There are at least four optical isomers of furanone AI-2 (2-methyl-2,3,4,5-tetrahydroxytetrahydrofuran), a regulator that seems to be very widely spread in the microbial world. However, *Salmonella enterica* serovar. *Typhimurium* produces a different furanone lacking the boron atom that forms a part of other furanones as organoboron compounds.

AI-2 regulates luminescence in *Vibrio harveyi*, virulence in *Vibrio cholerae* and other enteric pathogens, and spore formation in *Bacilus subtilis* (Waters & Bassler, 2005; Khmel’, 2006). Homologues of the *luxS* gene that encode AI-2 synthase were revealed in 537 tested bacterial genomes (Zhao et al., 2017).

A furanone QS signal that is implicated in virulence factor production and biofilm formation in *Ps. aeruginosa* is synthesized, in patients with lung cystic fibrosis, by normal respiratory tract microbiota, which, therefore, stimulates *Ps. aeruginosa*-dependent infection (Duan et al., 2003). This seems to account for the clinical data that antibiotics that fail to eliminate *P. aeruginosa,* nevertheless, ameliorate the symptoms of *Ps. aerugnosa*-caused infection. The antibiotics kill the normal microbiota, so that the pathogen is left without its microbial “friends”.

4.2. Neurotransmitter-like signals. *E. coli* (both its saprophytic and pathogenic strains), *Klebsiella pneumonia, Enterobacter cloacae, Shigella spp.,* and *Salmonella spp.* possess QS systems that are based on the AI-3 signal (Sircili et al., 2004; Walters & Sperandio, 2006). AI-3 is an aromatic compound. It binds to histidine kinases QseC and QseE involved in regulating the transcription of the genes which are responsible for the flagellar motility (*flhDC*) and the virulence (*LEE*) of the pathogenic strain *E. coli* O157:H7 (Clarke et al., 2006; Hughes et al., 2009; Shpakov, 2009) that produces “attaching and effacing lesions on the host’s intestinal

epithelial cells and eventually diarrhea" (Stevens et al., 2012, p.2138). The bacterial receptors bind, along with AI-3, neurochemicals such as catecholamines (Clarke et al., 2006) that produce stimulatory effects on *E.coli* motility and virulence.

To sum up, the quorum-dependent regulation of gene expression enables microorganisms to adjust their behavior, taking account of their population density and also diverse environmental factors. Moreover, QS systems provide for a coordinated expression of functional operons within the framework of a population or, with interspecies signals, of the whole microbial community, which, therefore, is comparable to a multicellular organism (Shapiro, 1988).

4.3. Eukaryotic QS signals. QS-like compounds are also produced by eukaryotic cells. Eukaryotes are likely to engage in "bluffing" bacterial cells into aimlessly carrying out costly quorum-dependent processes, even though the cell density is actually too low for the bacteria to be "quorate". This seems to be the reason why halogenated furanones formed by red algae of the genus *Delysea* are efficient antimicrobial agents (Givskov et al., 1998). The furanon of *D. pulchra* suppresses QS system-dependent swarming in *Serratia liquefaciens* and other bacterial species (Bassler & Miller, 2013).

Bacteria produce signals that are recognized by eukaryotes. The cells of a number of bacterial species, including *E. coli,* release an unidentified temperature- and pH-tolerant chemical factor. It induces the [GAR+] phenotype in the yeast *Saccharomyces cerevisiae,* enabling it to utilize various carbohydrates in the presence of glucose by overcoming catabolite repression. The bacteria of the genus *Sulfitobacter* stimulate cell division in diatomic algae by releasing the plant growth hormone auxin (indole-3-acetic acid: Zhao et al., 2017).

4.4. Host-microbiota interaction in terms of QS systems. Bacterial QS systems are involved in communication between the microbiota and the host macroorganism. For instance, there are LuxR-type proteins that bind signal molecules produced by the host, a plant (Gonzalez & Venturi, 2013) or an animal (catecholamines behave as homologues of the aforementioned signal AI-3). Bacterial QS systems may depend on signal molecules containing host-produced components. For instance, the bacterium *Rhodopseudomonas palustris* incorporates plant host-produced *p*-coumarate in its QS signal, *p*-coumaroyl-homoserine lactone (Cooley et al., 2008).

The host organism can specifically respond to bacterial QS signals. Some of them behave as immunomodulators (Ulvestad, 2009). 3-oxo-dodecanoyl-homoserine lactone, a major QS signal of *Ps. aeruginosa*, inhibits tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12) synthesis by immunocytes and stimulates the production of the proinflammatory γ-interferone as well as interleukin-8 (IL-8); this regulatory effect implicates transcription factor NF-κB and activator protein 2. Cytolysin, the signal that activates the *cyl* operon of *Enterococcus faecalis*, has been revealed to produce toxic effects on neutrophils, macrophages, epithelial cells, and erythrocytes (Kaper & Sperandio, 2005) The same signal affects intestinal epithelial cells, disrupting the function of tight junction proteins and, therefore, increasing the permeability of the intestinal epithelial barrier and facilitating bacterial translocation into the bloodstream. The gram-negative anaerobic rod *Fusobacterium nucleatum* that inhabits the human gut forms protein Fap2. It interacts with host immunocytes. Fap2 binds to the TIGIT receptor of NK (natural killer) cells, preventing them from efficiently eliminating tumor cells. Therefore, this bacterium promotes the development of the intestinal adenocarcinoma (Zhao et al., 2017).

4.5. Distant physical communication. Electromagnetic and acoustic waves are likely to be involved in distant information transmission. For several decades in the first half of the 20th century, as mentioned in the Introduction, this area of research was in the focus of attention of

Alexander Gurwich (1944) who presented his data on radiation that was produced by living cells and induced the division of other cells.

Recently presented data on communication via electrical fields are actually a variation on the "electromagnetic waves-mediated communication" theme, since oscillations in electrical fields are known to produce electromagnetic waves that can carry messages across long distances. Electrical field oscillations that are generated by transmembrane potassium pumps in *Bacillus subtilis* cells can spread within a biofilm formed by this bacterium and synchronize the metabolic activities of its cells (Prindle et al., 2015). Such electrical field oscillations can function as long-range signals and attract bacterial cells that are located outside the biofilm and may belong to the same (*B. subtilis*) or a different (*Ps. aeruginosa*) bacterial species; these cells may be induced to join the electrical signal-producing biofilm (Humphries et al., 2017).

In the 1990s, Yuri Nikolaev revealed that a *Vibrio costicola* culture treated with a lethal dose of the antibiotic chloramphenicol produces a signal that stimulates the growth of another culture of the same species that was separated by a double quartz glass layer; similar studies were subsequently conducted with other bacterial species (Nikolaev, 1992, 2000; Nikolaev et al., 2007, 2015). Lecture 1 contains a schematic representation of the equipment utilized by Nikolaev (see Fig. 3). Studies conducted by Nikolaev & Prosser (2000) demonstrated a synergistic effect of the physical and the chemical channels of intercellular communication. This was established in their experiments on the influence of a *Pseudomonas fluorescens* culture on the adhesive properties of another culture of the same species.

This lecture places quorum sensing systems in the ecological context considered in terms of interactions among different microbial species or between them and the host organism. Recent data on physical communication in the microbial world provide an incentive for further in-depth research.

This lecture is to be followed by a seminar during which the students are to be interviewed and tested.

LECTURE 5. SYMBIOTIC MICROBIOTA

Microbial communication facilities are involved in the dialogue between the microbiota and the host organism.

5.1. Functions of the microbiota. Symbiotic microorganisms inhabit various niches on and in an animal organism. Microorganisms grow on the skin (and their maximum concentrations are detected between the fingers, on the foot soles, in the inguinal folds and the armpits, and on the scalp), on the eye conjunctiva, and on the mucosa of the upper airways and the urogenital system[9]. Normally, the microbiota of each region of the human body performs a number of vital functions.

One of them is the *barrier function.* For instance, the symbiotic microbiota of the airways normally prevents their invasion by pathogenic microorganisms. The barrier function is also fulfilled by the vaginal microbiots. Representatives of the genus *Lactobacillus* consume the carbohydrates of the cells shed by the vaginal epithelium and form lactic acid that suppresses the growth of other microorganisms, including potential pathogens such as *Gardnerella*

[9] Reprinted from: Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers, pp. 75-78, including Fig. 8 (this material is abridged and partly modified)

vaginalis that can cause infections including bacterial vaginosis. Among lactobacilli, *Lact. crispatus* seems to be the most efficient protector from pathogens; the commercial preparation Lactin-V, a probiotic, has been developed from it for the purpose of treating vaginosis (Humphries, 2017). Naturally, the same functions are characteristic of the skin microbiota that is regarded as "the first layer of defense against infectious microorganisms and toxic agents" (Edmonds-Wilson et al., 2015, p.4); disruption of the barrier poses the threat of developing serious skin problems (eczema, psoriasis, etc.).

The symbiotic microbiota also contributes to the development (the *developmental function*) and the maintenance of the normal physiological state (the *homeostatic function*) of various organs and systems of the organism including the airways.

5.2. Distribution of the microbiota in the GI tract. While the presence of resident microorganisms in the small intestine is a debatable issue (Sharkey & Savidge, 2014), the microbial cell concentration in the large intestine may be as high as $10^{12}/cm^3$, and the total cell number is at least 10^{14} cells, which exceeds the human cell number in an adult human individual (Fig. 9). The total nucleotide number in the DNA of the human microbiota (the total *microbiome*) is ~150 times higher than that of the human DNA (Shenderov, 2014; Parashar & Udayabanu, 2016; Shenderov et al., 2017). The metagenome of the human GI microbiota, i.e., the total genome of the microbiome, contains over 1 million genes (Boddu & Divakar, 2018). Microbiome genes impact the nutritional and metabolic processes in the host organism; they influence the efficiency of drugs used to treat a diseased host organism (Rees et al., 2018). The microbiome is envisaged as our "second genome" that is comparable, in terms of its impact on human health, to the human organism's own genome (Shenderov, 2016; Shenderov et al., 2017; Herd et al., 2018).

The microbial *metabolome*, i.e. all low molecular weight (< 1500 Da) metabolites of microbial origin that are present in the GI tract, contains over 2.5 million different molecules, including about 1 million proteins and 300 thousand lipids (Shenderov et al., 2017). The total weight of the microbial biomass is 1,5—2 kG, i.e. it equals or exceeds the weight of such organs as the liver and the brain.

The total number of microbial species that are detectable in the GI tract may exceed ten thousand (probably, there are 15,000–36,000 species, Huang et al., 2019). However, only ~1500 species are cultivable. Among the predominantly occurring 160--300 bacterial species, only 18 species are invariably present in all tested individuals, 57% in 90%, and 75% in 50% of them. The most abundant microorganisms belong to the *Cytophaga-Flavobacterium-Bacteroides* (phylum *Bacteroidetes*) and *Clostridium-Lactobacillus-Enterococccus* (phylum *Firmicutes*) groups, each of them accounting for 30-40% of all detectable microorganisms in the colon. Less abundant but still sufficiently numerous microorganisms include *Actinobacterium* (especially *Bifidobacterium*), *Proteobacterium, Fusobacterium, Verrucobacterium,* and *Cyanobacterium.* In many human individuals, the colon harbors a significant number of methanogenic and methan-oxidizing archeans (Clarke et al., 2014; Shenderov, 2014; Shenderov et al., 2017; Logan et al., 2016; van de Wouw et al., 2017: Westfall et al., 2017; Rinninella et al., 2019).

GI microorganisms can exist as planktonic cells in the intestinal lumen or as biofilms in the mucus layer overlying the epithelium, mucus within intestinal crypts, and the surface of mucosal epithelial cells (Kaper & Sperandio, 2005). Apart from microbial cells, GI biofilms contain the matrix that includes microbial expolysaccharides and other biopolymers as well as host-produced components such as goblet cells-released mucin. Microbial biofilms line the most part of the intestinal mucosa, including that of the colon, the cecum, and the vermiform appendix.

The GI microbiota directly interacts with the barrier layer of the mucosal epithelium ("the firewall"); epithelial cells form tight junctions. This layer regulates the entry of ions and organic

molecules into the submucosal layer of the GI tract and subsequently into the bloodstream (Shenderov, 2014; Sharkey & Savidge, 2014).

5.3. Interindividual differences in microbiota composition. The composition of the symbiotic microbiota varies with different individuals, as far as the lowest taxonomic levels are concerned, including microbial genera, species, and especially strains. The microbiota is under the influence of the diet, the genotype, the epigenotype, and the state of the immune and the antioxidant system. Importantly, the influence of environmental factors tends to override that of hereditary factors (Shenderov, 2008; Shenderov et al., 2016; 2017; Herd et al., 2018; Rotschild, 2018)[10].

"An individual's gut microbiota composition is dynamic, changing in response to age, geographical location, diet, antibiotic use, and influx and efflux of external microbes... Based on their colonization ability, bacteria in the gut can be transient or permanent" (Yao et al., 2020).

Genetically unrelated individuals are characterized by a similar micobiota composition after living together for a long time (Liang et al., 2018). For instance, the microbiota of the husband is similar to that of the wife provided that they live together. The intestinal microbiotas of identical (monozygous) twins do not bear greater similarity than those of fraternal (dizigous) twins (Yatsunenko et al., 2012). In general, comparison of human individual microbiotas reveals that "dietary changes can account for up to 57% of gut microbiota changes, whereas genes account for no more than 12%" (Clark & March, 2016).

Apart from the shared environment and diet, the nongenetic similarity of the microbiota of cohabiting individuals may be due to direct microbiota transfer between them. This was demonstrated in studies with baboon troops (Herd et al., 2018). Interestingly, interindividual microbiota differences in bees reflect their status differences in the social hierarchy, because the status influences the bees' diet and stress level (Herd et al., 2018). In human society, interindividual microbiota differences tend to be more manifest in children than in adults (Yatsunenko et al., 2012).

Taking account of the impact of the diet and the regimen (which is under the influence of cultural factors) on the microbiota, we can subdivide the population of the planet into several major subpopulations that are characterized by different predominant microorganisms in the GI tract and live in the following regions (Shenderov, 2008):

- Tropical and subtropical areas
- Deserts
- Mountains
- Polar and circumpolar area
- West Europe and North America (including all those preferring a western-type diet)

The predominance of either carbohydrates or proteins and animal lipids in the diet results in the prevalence of either *Prevotella* or *Bacteroides* species in the intestinal microbiota (Wu et al., 2011; van de Wouw et al., 2017).

"Animal and human research indicates adolescence as a sensitive period when the gut-brain axis is fine-tuned, where dietary interventions to change the microbiome may have long-lasting consequences for mental health" (Kohen Cadosh et al., 2021).

. Sequencing colon content samples of several hundred humans within the framework of the MetaHIT Consortium (Metagenomics of the Human Intestinal Tract Consortium) project enabled classifying people into three *bacteriotypes*, depending on the predominance of *Prevotella, Bacteroides, or Ruminococcus* in the colon (Arumugam et al., 2011; Clarke et al., 2014).

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Importantly, the bacteriotype was not determined by the tested individuals' gender, age, nationality, or the height/weght ratio (Dinan et al, 2015).

However, the bacteriotype (alternatively termed the enterotype) of an individual is under the influence of the diet that is typical of his/her region and local cultural traditions. For instance, African traditional diets (based on millet, sorgo, and vegetables) result in the predominance of the *Prevotella* bacteriotype*,* while the modern European diet favors the *Bacteroides* bacteriotype (Rinninella et al., 2019). The bacteriotype-specific microbiota tends to restore itself after temporary alterations caused by antibiotics or unusual food.

The validity of the above classification is still open to question. The alternative suggestion is that there is a continuum of human individuals in terms of the ratio between the three types of bacteria, and that this ratio changes to some extent during an individual's lifespan, despite its relative stability.

Account should be taken of more traditional classifications of human types. One of them is based on classifying individuals into four temperaments, and the issue is how the three bacteriotypes can be combined with these four temperaments, namely, the sanguine, the phegmatic, the choleric, and the melancholic.

Of more relevance seems to be a more recent classification suggested by Helen Fischer (reviewed, Brown et al., 2013). She singled out four neurochemical types of people whose brain is dominated by four different neurochemical systems. These four systems in the brain depend on serotonin (sociable people), dopamine and norepinephrine (creative people), oxytocin plus estrogen (empathetic people), and testosterone (assertive, strong-willed people). Since not only human cells but also microbes produce most of the listed chemicals, an interesting idea to be explored in the future is to attempt to correlate these four neurochemical types with the three bacterial types, bearing in mind the neurochemical impact of bacteria-produced substances. One could also consider, within the microbial and neurochemical context, other psychological scales, such as the classical Kretschmer scale in which people are subdivided into schizothymic and cyclothymic subtypes.

5.4. Microbiota as the "microbial organ". Dysbiosis. The following aspects of the interaction between the microbiota and the host organism, including the nervous system, enable considering the microbiota as a special multifunctional organ (Lyte, 1993, 2010, 2011, 2013a, b; 2016; Lyte & Freestone, 2009; Oleskin et al., 2016):

1. The host nervous, immune, and other systems significantly influence the microbial organ.
2. In its turn, the microbial organ exerts an influence on the maintenance of the organism's adequate functional state and its neural, psychological, and metabolic homeostasis in health and disease.
3. The GI microbiota produces effects on other organs in the human body and responds to substances secreted by other organs; therefore, the symbiotic microbiota meets the essential criteria that enable us to consider the microbiota a special human organ (Lyte, 2010, 2011; Clarke et al., 2014; Oleskin et al., 2016).

"Accumulating evidence points to a major role of the gut microbiota in not only normal gut function but also in brain development and function… The recognition of such interactions between gut microorganisms and the brain has led to a new research field commonly referred to as the "microbiota-gut-brain" axis" (Sudo, 2019).

The human microbiota responds to changes in the human individual's physiological and even psychological state, including various pathological processes and stress. Stress activates the hypothalamus-pituitary-adrenal axis and the autonomous nervous system. This results in altering intestinal motility, increasing epithelial barrier permeability for microorganisms, and releasing neuropeptides and other biologically active substances into the intestinal lumen. All these effects influence the intestinal microbiota (Sharkey & Savidge, 2014).

The “micriobial organ” is sometimes also dubbed “the second liver”, because of its weight (1-2 kG) and multifunctional role. Microorganisms account for up to 60% of the dry feces weight (Siadat & Badi, 2019).

If this complex microbial organ ceases to perform its normal functions in the organism, a pathological condition referred to as *dysbiosis* may result. “Gut dysbiosis refers to alterations in the composition and function of the gut microbiota that have harmful effects on host health via qualitative and quantitative changes in the intestinal flora itself, changes in their metabolic activities, and/or changes in their local distribution” (Yao et al., 2020). “Many factors can be a cause of dysbiosis, including invasive intestinal pathogens, antibiotic treatment, physical damage to the mucosa, diet, or host genetic factors” (ibid.). Predominantly, “reduction in the relative proportion of obligate anaerobes and increases in facultative anaerobes including pathogens such as *E. coli, Salmonella, Shigella, Proteus,* and *Klebsiella* are common features of dysbiosis” (ibid.).

Dysbiosis may result in serious health problems including (a) local (intestinal) diseases such as irritated bowel syndrome (IBS), Crohn’s disease, ulcerous colitis, and colon cancer, (b) problems with more distant organs exemplified by the liver (liver dystrophy), joints (rheumatoid arthritis), the spine (spondylosis), (c) disruption of the operation of several organs (multiple organ failure), and (d) psychiatric problems (autism, Tourette’s syndrome, and attention deficit and hyperactivity disorder, ADHD). The influence of microbiota on our physical and mental health is mediated by chemical agents including neurotransmitters (see Lectures 6-8 below).

5.5. Impact of the microbiota on the nervous system. Within the framework of the multidirectional microbiota-host signalling system, microbial metabolites can modify the functioning of the nervous system via metabolic, epigenetic, and neuroendocirne mechanisms. We depend on myriads of essential neurochemical factors produced by microorganisms (Dinan et al., 2015). For instance, the serotonin-dependent (serotonergic) brain system that is responsible for many aspects of emotional behavior does not develop to the mature state without the microbiota (Clarke et al., 2013).

The intestinal microbiota directly interacts with the enteric nervous system (ENS), a semi-autonomous part of the nervous system. The ENS minimally contains about 0.5 million neurons, i.e., it includes more neurons than all peripheral ganglia taken together (Rao & Gershon, 2016). The ENS also contains auxiliary cells such as astroglia (enteroglia) that form a diffusion barrier between intestinal capillaries and the ENS ganglia. Glial cells perform a protective function vis-à-vis ENS neurons and provide them with nutrients (Sharkey & Savidge, 2014). The ENS is structurally similar to the central nervous system (CNS), and it uses virtually all types of neurochemicals that function in the CNS (Rao & Gershon, 2016). Unlike the other parts of the peripheral nervous system, ENS can operate without CNS control, even despite a chronic vegetative state of the brain in which most of its parts are inactive (Liang et al., 2018). ENS regulates the secretory activity of the gut, its motility, and the activity of the gut immune system; it helps maintain the mucosa in the functional state. An additional important function of the ENS is the regulation of permeability of the gut wall barrier for chemical factors and microbial cells.

The important role of the microbiota for the normal functioning of the ENS is highlighted by the fact that the ENS of GF mice is characterized by a decreased capacity to respond to external stimuli; this capacity is restored in a mouse if the intestine is colonized with a probiotic strain of *Lactobacillus reuteri* (Parashar & Udayabanu, 2016).

Apart from the ENS, the GI tract is innervated by the sympathetic and the parasympathetic nervous systems that directly communicate with the GI microbiota.

“Microbiota-gut-brain axis signaling can occur via several pathways, including via the immune system, recruitment of host neurochemical signaling, direct enteric nervous system routes and the vagus nerve, and the production of bacterial metabolites” (Long-Smith et al., 2020, p.17.1). The following factors are of paramount importance (Fig. 10):

Fig. 10

(1) *Microbiota-produced neuroactive compounds* that can cross the barrier between the gut wall and the bloodstream or the lymphatic system as well as the BBB and directly interact with the brain. Such microbial products are exemplified by *L*-3,4-dihydroxypehylalanine (DOPA) and γ-aminobutyric acid (GABA), and their brain effects will be discussed in the following lectures. Microorganisms also produce neurochemicals that do not cross the gut-blood barrier[11], e,g, serotonin, dopamine, and norepinephrine (Oleskin et al., 2010, 2016, 2017a, b; Oleskin & Shenderov, 2016; Shenderov et al., 2017; Lyte, 2016). Such neuroactive compounds exert a local effect, affecting the ENS that can systemically influence the whole organism;
(2) *GI tract-innervating nervus vagus* with afferent and efferent pathways (Dinan et al., 2015; Sampson & Mazmanian, 2015). This nerve forms a part of the organism's major regulatory systems that are implicated in the parasympathetic regulation of the functions of the heart, the bronchi and the GI tract. The density of sensory terminals of *n. vagus* is very high in all organs and tissues, and they supply the brain with spatially structured information concerning their activities (Ivashkin & Ivashkin, 2018). The microbiota affects *n. vagus* activity. Importantly, this nerve connects the ENS and the brain and sends, via afferent pathways, messages to the brain concerning the GI homeostatic state, including the feelings of fullness, satiety, and sickness. It is partly due to *n. vagus* that the microbiota influences behavior and mood. Opportunistic and potentially pathogenic bacteria such as *Citrobacter rodentium* and *Campylobacter jejunii* activate the transfer of stress-induced impulses along *n. vagus*. If the nerve is severed, this prevents many microbial effects, including *Lactobacillus rhamnosus* JB-1-induced activation of synthesis of the $GABA_B$ receptor to GABA in the cingulated gyrus of the mouse brain (Parashar & Udayabanu, 2016);
(3) *Immune system* that mediates some of the microbiota-produced effects on the CNS (see 5.6 for details).
(4) *Hypothalamus-pitiutary-adrenal system (HPA system)* that is direcrtly involved in the GI microbiota impact on the human organism and its CNS. Modification of the HPA system by harmful microbial compounds predisposes human individuals to depression, anxiety, the bipolar disorder, and emotional burnout and chronic fatigue syndromes. The HPA system is implicated in the effects of microbiota-disrupting factors (diet alteration, antibiotic, psychosocial stress, etc.) on an infant's nervous system. Subsequently, this results in psychological disorders in conformity with the Barker hypothesis (Barker & Osmond, 1986). According to this hypothesis, negative factors that affect the fetus or the neonate, including malnutrition, undernourishment, stress, and pathogen invasion, bring about long-term physiological alterations and increase the risk of developing serious health problems in adulthood (see Oleskin & Shenderov, 2020). Restoring the gut microbiota, especially by means of probiotics (see Lecture 6), decreases the risk of the development of mental problems. GF mice are distinguished by an abnormally intense response of the HPA system to stress, which is alleviated by administering the probiotic *Bifidobacterium infantis* to them. The HPA-dependent stress response is additionally intensified by colonizing the GI tract of GF mice with pathogenic *E. coli* strains (Liang et al., 2018).

There is evidence that, apart from the hypothalamus (dubbed the brain-visceral organs "interface"), other brain parts including the prefrontal areas of the brain cortex and the amygdala, are affected by microorganisms. The amygdala is directly involved in visceral pain perception, social behavior, and emotional responses. During the course of an individual lifecycle, childhood and old age are the critical periods in which the microbiota drastically changes and dysbiosis may develop. The same periods are characterized by the maximum risk of developing amygdala problems that manifest themselves in irritated bowel syndrome (IBS) and mental disorders (schizophrenia, autism, and others; Cowan et al., 2017).

[11] Such neuroactive substances may cross the gut-blood barrier and the BBB under stress that impairs these barriers.

In addition, there is convincing evidence for the involvement of gut microbiota in the pathogenesis of Alzheimer's disease and/or Parkinson's disease, which is partly due to modified activity of the microglia (brain immune cells) in the brain (Janssens et al., 2021).

5.6. Impact of the microbiota on the immune system. The microbiota's influence on the operation of the nervous system is also mediated by the immune system. The microbiota produces effects on both the innate and the adaptive immune system, including the cellular (T lymphocyte-dependent) and the humoral (B lymphocyte- and immunoglobulin-dependent) mechanisms of immune responses. "Studies of germ-free mice have shown several immunodeficiencies, including fewer splenic CD4+ T-cells, structural splenic disorganization, fewer intraepithelial lymphocytes, decreased conversion of follicular-associated epithelium to M-cells, decreased secretory IgA (SIgA), and decreased ability to induce oral tolerance. SIgA functions are the neutralization of bacterial toxins in the gut lumen" (Yao et al., 2020).

Involvement of symbiotic microbiota in the development and maturation of the innate and adaptive immune system (Hevia et al., 2015) includes the regulation of the number and activity of various rypes of T (especially CD^{4+}) and B lymphocytes. Under normal conditions, the microbiota stimulates the activity of anti-inflammatory T regulatory (T_{reg}) cells and suppresses that of proinflammatory Th1 and Th17 cells, limits the production of immunoglobulins IgE, and, therefore, decreases the risk of allergic processes (Rees et al., 2018). Evolution enabled the species-specific co-adaptation of the host organism with its immune system and the "microbial organ". For instance, the maturation of mouse immune cells is promoted by the mouse microbiota but not by the rat or human microbiota

Microbial cell components, especially lipopolysaccharides (LPSs), lipoproteins, flagellin, and CpG repeats-enriched DNA activate macrophages, neutrophils, dendritic cells, and other immune cells. They recognize these molecular motifs, including "stranger" (pathogen-associated molecular patterns, PAMPs) and "danger" (damage-associated molecular patterns, DAMPs) (Yesilyurt et al., 2021) using specific pattern-recognizing receptors (PRRs). They include Toll-like receptors (TLRs) nucleotide oligomerization domain-like receptors (NLRs), C type lectins, and cytosolic multiprotein oligomers of the innate immune system (inflammasomes) responsible for the activation of inflammatory responses (Ivashkin & Ivashkin, 2018; Liang et al., 2018). Similar receptors are present in gut mucosa cells (enterocytes). They recognize microorganisms and initiate an immune response to eliminate pathogens; normally, they are tolerant to the symbiotic microbiota.

Nervous cells, including those of the ENS, express receptors that recognize microbial patterns. This enables the nervous system to directly (and not only via the immune system) communicate messages about pathogenic microorganisms, causing a feeling of pain and activating the immune system (Lim et al., 2016). Bacterial LPS-activated TLR4 receptors were detected in the inferior ganglion of the vagal nerve of the rat. The same receptors and other types of TLRS (TLR-3 and TLR-7) are present in the jejunal plexus and the dorsal roots of the spinal cord, in mice and humans. The LPSs are recognized by the TLR4 receptors of the cells of endocrine organs, such as the thyroid, which stimulates the expression of the thyroglobulin gene (Mazzoli & Pessione, 2016). More information about the microbiota—immune system interaction will be provided in Lecture 10 below.

Fig. 11

Trilateral interactivity between the nervous system (especially the brain), the immune system, and the microbiota is essential for the physical and mental well-being of humans (Fig. 11). In particular, the brain sends messages to the immune system that impacts microorganisms. In turn, they influence both the immune and the nervous system. The operation of the whole triangle crucially depends on BASs including neurochemicals produced by all the three systems.

The neurochemical acetylcholine produced by the brain and the vagal nerve (and also immune cells themselves) exerts an influence on macrophages, suppressing TNF, IL-1, IL-6,

and IL-18 synthesis by them; acetylcholine also affects the microbiota, although there also is microbial acetylcholine (Wall et al., 2014). Adipose cells that form a part of the immune system contain receptors that bind neuropeptides produced, apart from immunocytes themselves, both by nervous cells and by microorganisms (Mazzoli & Pessione, 2016).

Apart form their direct impact on immunocytes, the immunological effects of neurochemicals, including those of microbial origin, may be due to their impact on the nervous system, especially the brain. The impact of neurochemicals on the CNS secondarily modifies their regulatory influence on the operation of the immune system.

Symbiotic microorganisms inhabit various niches on and in an animal organism, especially the **gastro-intestinal (GI)** tract. The microbial cell concentration in the large intestine may be as high as $10^{12}/cm^3$, and the total cell number is at least 10^{14} cells, which exceeds the human cell number in an adult human individual. They interact with the nervous and the immune system in health and disease. If this **microbial organ** ceases to perform its normal functions in the organism, a pathological condition referred to as **dysbiosis** may result.

LECTURE 6. PROBIOTICS AND PSYCHOBIOTICS. THE IMPACT OF NEUROTRANSMITTERS ON HOST-MICROBIOTA INTERACTION. THE ROLE OF CATECHOLAMINES.

6.1. Probiotics. In order to ameliorate the human microbiota, a wide variety of drugs, biologically active food additives, and functional nutrients are currently used. Much attention is currently given to preparations containing selected strains of lactobacilli, bifidobacteria, and other live microorganisms (*probiotics*), as well as to soluble food fibers and other organic substances that stimulate their growth (*prebiotics*). Prebiotics are exemplified by undigestible oligosaccharides degraded by beneficial gut microorganisms that produce SCFAs and other valuable organic acids (Shenderov, 2001; Boddu & Divakar, 2018).

According to the official definition given by FAO/WHO (2006), probiotics are live microorganisms that, "when administered in adequate amounts, confer a health benefit on the host". Commercially available probiotics are supplied in the form of drug preparations and biologically active food additives that contain microbial cultures.

The following health-promoting functions of probiotics have been documented in the literature (reviewed, Shenderov et al., 2017; Oleskin & Shenderov, 2020; Oleskin & Cao Boyang, 2022):

1. They help the human organism stabilize the GI microbiota and optimize its qualitative and quantitative composition. They also suppress harmful microorganism because they contain antimicrobial factors.
2. Low molecular-weight compounds contained in probiotics neutralize toxins and other metabolites that are harmful for the host organism.
3. Probiotics supply the host organism with nutrients, antioxidants, growth factors, enzymes, organic acids, polyphenols, vitamins, bile acids, gaseous substances, and other biologically active substances (BASs).
4. Probiotics exhibit anticarcinogenic activity, as exemplified by the strong anticancer effects of the *Lactobacillus acidophilus* 36YL strain on four tested cancer cell lines (AGS, HeLa, MCF-7, and HT-29), in which the strain induces cell death.
5. Probiotics produce anti-allergic, antidiabetic, and anti-inflammatory effects. The probiotic strain *Lactobacillus plantarum* 06CC2 relieved allergic symptoms in mice treated with the allergen ovalbumin.
6. Probiotics beneficially influence metabolism, and they can be used for treating obesity (metabolic syndrome).

7. Beneficial microbial agents can potentially be used to improve the symptoms of aging; this point was already made by Elia Metchnikoff (1904) in his famous work *Etudes sur la nature humaine: essai de philosophie optimiste*.
8. These agents promote the growth of blood vessels (angiogenesis) in the intestinal tissue by producing VEGF (vascular endothelial growth factor).
9. Some probiotics produce a pain-relieving effect, particularly with respect to abdominal pain. This effect may result in complete analgesia (a lack of pain sensitivity), which is attributable to the capacity of lactobacilli including *Lact. acidophilus* to induce the expression of μ-opioid and canabioid receptors in the intestinal epithelium (Cryan & Dinan, 2012).
10. Probiotics can relieve stress. This is characteristic of preparations that are based on bifidobacteria and lactobacilli contained in fermented dairy products. Tryptophan metabolism is optimized, which positively influences the production of the essential brain neurochemical serotonin from tryptophan (O'Mahony et al., 2015).
11. Probiotics regulate the activity of the intestinal part of the immune system, i.e. the gut-associated lymphoid tissue (GALT). They modulate immune responses, normalize the balance of pro- and anti-inflammatory cytokines, lower the antigen load of GALT, decrease gut wall permeability, increase immunoglobulin IgA secretion, induce the activity of anti-inflammatory Treg cells, and promote the production of anti-inflammatory interleukin-10 (Belkaid & Hand, 2014; Shenderov et al., 2017; Liang et al., 2018).
12. These agents systemically strengthen the whole immune system and the organism's natural barriers, including the gut–blood barrier and the BBB by increasing the expression of proteins involved in forming tight junctions between cells. In this fashion, they help prevent brain problems and, accordingly, cognitive and behavioral disorders (Liang et al., 2018).

Since probiotics provide the aforementioned health benefits thanks to their bioactive chemical components, studies are currently in progress on the use of *metabiotics* (the term suggested by Boris Shenderov, see: Shenderov et al., 2017; Oleskin & Shenderov, 2020). Metabiotics are "products containing non-living probiotic microorganisms… and/or their metabolites…" (Yesilyurt et al., 2021) and alternatively denoted as parabiotics and postbiotics in the literature. There is evidence that "these microbial compounds have more immunomodulatory activities than living microorganisms" (Yesilyurt et al., 2021).

6.2. Psychobiotics. Probiotics include a subgroup that is denoted as *psychobiotics*, i.e., live microorganisms that, when administered in adequate amounts, confer a health benefit on patients with psychiatric problems (Cryan & Dinan, 2012). There is a growing body of evidence that probiotics can significantly influence the brain and, therefore, affect behavior, mood, and cognition both in experimental and clinical settings. "Recent research on psychobiotics as active ingredients in host physiology shows influence on the nervous system, consequentially shaping psychological processes, behaviour and ultimately exerting health benefits in psychiatric conditions in preclinical animal research… and in humans" (Cohen-Kadish et al., 2021).

Administration of psychobiotic strains, e.g., of the species *Lactobacillus casei,* to patients with chronic fatigue syndrome (CFS) made them less anxious and stressed (reviewed, Oleskin & Shenderov, 2020). The GI microbiota of individuals with CFS became enriched in lactobacilli and bifidobacteria under the influence of the strain *Lact. casei* Shirota. In summary, "manipulation of the gut microbiome via psychobiotics may present a promising new avenue for treatment and prevention of anxiety", especially "in young people" (Cohen-Kadish et al., 2021).

Apart from relieving depression and anxiety, psychobiotics and dairy products containing them improve mood and cognitive capacities. For instance, the depression-relieving psyhobiotic strain *Lact. rhamnosus* JB-1 promoted information memorization and learning (Lyte, 2013b). The *Lact. acidophilus, Lact. fermentum,* and *B. animalis* subsp. lactis cocktail ameliorated the cognitive capacities and electroencephalographic data of subjects suffering from diabetes (Parashar & Udayabanu, 2016). In healthy volunteers, oral administration of the *Lact.*

helveticus B0052 and *B. longum* R0175 combination attenuated stress caused by psychological factors (Kerry et al., 2018).

One of the psychobiotics' mechanisms of action is based on mitigating systemic inflammation by suppressing the secretion of proinflammatory cytokines into the bloodstream. Proinflammatory cytokines increase BBB permeability and, therefore, the probability of the migration of potentially pathogenic agents into the brain. Psychobiotics inhibit proinflammatory cytokine production either directly or by increasing the anti-inflammatory cytokine content. Therefore, they decrease the probability of the translocation of pathogenic factiors into the CNS and improve the functioning of the BBB (Ivashkin & Ivashkin, 2018).

Alcoholism affects both the physical and mental health state, and it results in significant microbiota changes. Severe alcoholic hepatitis is associated "with a decrease in the abundance of *Bacteroidetes* and an enrichment of *Fusobacteria*, bacteria present mainly in the oral cavity" (Lynch et al., 2019, p.657). Useful bacterial strains such as *Akkermansia muciniphila* "are depleted by alcohol consumption in mice and humans, and supplementation of this bacterium in ethanol-induced experimental liver injury improves intestinal barrier function and relieves liver disease in mice" (ibid.).

Important data obtained with animal models demonstrate that "the earlier in the lifespan this intervention took place <i.e., the younger the subjects treated with psychobiotics – O.A.>, the more fully a normal stress response was restored. Thus, if these promising effects translate to humans, psychobiotics present candidate ingredients which could provide a measure of protection against stress-induced anxiety in adolescents which may carry over into adulthood" (Cohen Kadish et al., 2021). In other words, adolescent individuals (teenagers) are in the critical period when administering efficient health-promoting psychobiotics can prevent the development of mental problems in adulthood.

Of significant importance in terms of physical and mental health are the briefly mentioned *prebiotics*, "specific non-digestible food ingredients (including non-digestible oligosaccharides) which selectively feed intrinsic beneficial bacteria, consequentially stimulating their growth and activity" (Cohen Kadosh et al., 2021). For instance, "fructooligosaccharides (FOS) and galactooligosaccharides (GOS) have promising effects in animal and human trials". More specifically, "milk oligosaccharides administration can prevent stress-induced dysbiosis and anxiety-like behaviour in mice" (ibid.; based on Tarr et al., 2015). Diet optimization including sufficient supply of prebiotics such as fructans contributes to the proliferation of useful bacteria, e.g., Bifidobacterium, in the organism (Norris et al., 2013; Shenderov, 2014). Prebiotics also produce anti-inflammatory effects that are attributable to oligosaccharides' capacity to directly interact with the gut epithelium and to significantly decrease proinflammatory cytokine production (Ivashkin & Ivashkin, 2018, p. 15).

6.3. Neurochemicals. One of the most important mechanisms of action of useful bacteria such as psychobiotics on the brain involves chemical substances called *neurotransmitters* or, more broadly, *neurochemicals*[12]. They are low molecular weight substances that transmit messages between nervous cells (neurons) or from a neuron to a muscular of glandular cell and/or modulate the efficiency of impulse transmission. Neurochemicals are subdivided into the following groups (Fig. 12): (1) biogenic amines, including catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, octopamine, tyramine, and others; (2) amino acids (aspartic, glutamic, and γ-aminobutyric acid, glycine, and others); (3) peptides such

Fig. 12

[12] Reprinted from: Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers, pp. 155-160 (abridged and partly modified) © 2020 by Alexander Oleskin and Boris Shenderov, with permission from Nova Science Publishers, Inc.

as endorphins, enkephalins, dynorphins, substance P, etc.; (4) “gasotransmitters” including nitric oxide, carbon monoxide, and hydrogen sulfide, and (5) purines, e.g., adenosine and ATP.

Importantly, many neurochemicals are multifunctional agents: they combine the roles of neurotransmitters, hormones, and local tissue factors (histohormones). Some neurochemicals perform communicative and regulatory functions in diverse taxa of animals (Dubynin et al., 2010), plants (Roshchina, 1991, 2010, 2016), fungi (Buznikov, 1987, 2007), protozoans (Roshchina, 2010, 2016), and bacteria (reviewed, Lyte, 1993, 2010, 2014, 2016; Oleskin et al., 2010, 2016, 2017a, b; Oleskin & Shenderov, 2019, 2020), which allows us to use the more general term biomediators (Roshchina, 2010, 2016). Moreover, many neurotransmitters form a part of the ecosystem-level pool of signals that are concomitantly produced and recognized by a large number of ecosystem components (Oleskin & Postnov, 2022).

This lecture will focus on the group of neurotransmitters called biogenic amines (BAs). BAs regulate a large number of functions of the brain and the peripheral nervous system from homeostasis maintenance, i.e. from keeping up the state of equilibrium inside the organism to cerebral competence, i.e., to the normal operation of the brain.

Importantly, “biogenic amines including dopamine, norepinephrine, serotonin, and histamines are all generated by commensal gut microorganisms and are suggested to play roles as signaling molecules mediating the function of the “microbiota-gut-brain” axis” (Sudo, 2019).

6.4. Catecholamines. The first subgroup of BAs to be considered in this lecture are catecholamines (CAs). CAs (dopamine, norepinephrine, and epinephrine) are derived from the non-essential amino acid tyrosine (Fig. 13) whose hydroxylation yields L-3,4-dihydroxyphenylalanine (DOPA), the direct precursor of the catecholamine dopamine; its β-hydroxylation yields norepinephrine (noradrenaline). Its subsequent methylation produces epinephrine (adrenaline).

Fig. 13

In the mammalian organism, catecholamines are predominantly formed by the chromaffin cells of the adrenal medulla and by the axons of the sympathetic nervous system that effectuates the organism’s response to stress; they are also produced in the brain. Significant catecholamine concentrations are characteristic of the GI tract. For instance, about 50% of the dopamine contained in the human organism is located in the gut (Liang et al., 2018). Neurochemical functions are performed by dopamine and norepinephrine; a direct involvement of epinephrine in the operation of the nervous system is questionable (Boldyrev et al., 2010).

The biological activity of *dopamine* is largely due to its binding to specific D receptors. They are subdivided into five types (D1-5). All D receptors are coupled with G proteins. The receptors activate (the D1 and D5 receptors) or, conversely, inhibit (the D2-4 receptors) the adenylate cyclase enzyme, thereby increasing or decreasing the level of intracellular cyclic adenosinomonophosphate (cAMP). The recently discovered trace amine-associated receptor 1 (TAAR1) also influences intracellular adenylate cyclase activity.

As a CNS neurotransmitter, dopamine is produced by the neurons of several parts of the brain, including the substantia nigra, the tegmentum, and some hypothalamic nuclei (Dubynin et al., 2010). Release of dopamine by the ventral tegmentum results in its spreading along the axons toward the *nucleus accumbens* of the hypothalamus and the prefrontal cortex. The dopaminergic system of the brain induces active wakefulness, promotes hedonic, i.e., pleasure-seeking, behavior, and enhances the positive emotions that are caused by enjoying, e.g., tasty food or a videotape. Anticipating a reward results in increasing the dopamine concentration in the brain, and many addictive drugs stimulate dopamine release or block dopamine re-uptake by dopamine-producing neurons. The main functions of dopamine, as well as other neurochemicals, within the nervous system are summed up in Table 1.

Table 1

The dopamine precursor DOPA passes the gut-blood and the blood-brain barrier. Therefore, DOPA-producing microorganisms, including symbiotic bacteria such as *E. coli* K-12 (Fig. 14) probiotics, e.g., lactobacilli (Oleskin et al., 2014a, b), and potential pathogens such as

Fig. 14

Bacillus cereus (Oleskin et al., 2010), can cause euphoria, due to the conversion of microbial DOPA to dopamine in the brain. Such euphoria should be particularly impressive and bizarre when induced by pathogens and developing in spite of a severe bacterial infection and a worsening health state. The feeling of happiness will get stronger as more bacteria appear in the gut and produce more DOPA. This is a clinical paradox.

The second important catecholamine, *norepinephrine*, also known as noradrenaline, activates the brain and stimulates locomotive behavior. Norepinephrine increases cerebral blood supply and is involved in emotions associated with risk-taking and learning. Norepinephrine release into the bloodstream results in aggressive behavior and a significant increase in muscular strength. Norepinephrine promotes vigilant behavior, stimulates information memorization and retrieval, and is implicated in "fight or flight" behaviors.

The neurochemical and hormonal activities of norepinephrine are due to its binding to α- and β-adrenergic receptors. α-Receptors are subdivided into (i) the α1-subtype that increases the intracellular inositol-1,4,5-triphosphate and Ca2+ concentrations by activate phospholipase C and (ii)the α2-subtype that inhibits adenylate cyclase and, therefore, decreases the intracellular cAMP concentration. In contrast, β-type receptors (β_1, β_2, and β_3) represent G proteins; they activate adenylate cyclase upon binding norepinephrine.

In the brain, norepinephrine is predominantly produced by the neurons of the blue spot in the brain called *locus coeruleus*, the lateral reticular formation, the medulla oblongata, and the nuclei of the solitary tract.

Immunocytes respond to biogenic amines; they also synthesize and release them, including catecholamines. The immunological implications of catecholamines and other neurochemicals are shown in Table 2. Among catecholamine receptors, β_2-adrenoreceptors (β2-ARs) are predominantly expressed by immune cells. Stimulation of β_2-ARs chiefly results in an anti-inflammatory response of immunocytes including macrophages and monocytes. Antigen-presenting dendritic cell express both α- and β-adrenoreceptors. The binding of catecholamines to α-ARs mainly causes the stimulation of the immune response, whereas their interaction with β-ARs is more likely to inhibit the immune system and to mitigate inflammation. These data demonstrate the complexity of the functions of catecholamines in the immune system, as well as their importance from the immunological viewpoint.

Table 2

"Interestingly, catecholamines have emerged as potential inter-kingdom signaling molecules in the gut, in addition to their well-established roles as neurotransmitters in the central and peripheral nervous systems" (Sudo, 2019).

Therefore, the following is concerned with the impact of catecholamines on the microbiota. Table 3 summarizes the effects of catecholamines and other neurochemicals in microbial systems. This area of research, historically, was initiated when, in the 1930s, many people were reported to die of gangrene and other infectious complications after epinephrine (adrenaline) injections. Syringes were not so thoroughly sterilized after use and were often used several times. Therefore, bacterial spores contained in them started rapidly germinating if epinephrine was also contained in the syringe.

Table 3

There are several reasons for this stimulatory action. First, norepinephrine and other catecholamines exert an indirect influence on the microbiota by (Verbrugge et al., 2012)

- Suppressing immunoglobulin A synthesis and/or release
- Stimulating intestinal motility
- Promoting bile flow, which accelerates the growth of such bacteria as *Bacteroides*.

However, still more important, catecholamines produce a direct stimulatory effect on the growth of human organism-inhabiting microorganisms, including such pathogens as (reviewed, Oleskin & Shenderov, 2020):

- *Yersinia enterocolitica* causing intestinal inflammatory diseases,

- virulent *E. coli* strains that are responsible for intestinal infections and other serious problems including blood poisoning,
- *Shigella* causing dysentery,
- *Salmonella* responsible for food-caused infections,
- *Pseudomonas aeruginosa* involved in purulent inflammation in various organs,
- *Bordetella pertussis* and *B. bronchioseptica* responsible for whooping cough,
- *Aeromonas hydrophila*,
- *Staphylococcus epidermidis*.

Based on the data obtained in our lab (Anuchin et al., 2008), catecholamines stimulate biomass accumulation (estimated from optical density at 540 nm) and cell proliferation (determined from colony-forming unit number increase) in nonpathogenic *E. coli* K-12 (strain MC4100). It can be deduced from our data and facts presented in the literature that pathogenic bacteria are maximally stimulated by norepinephrine, whereas symbiotic bacteria generally prefer the other major catecholamine, dopamine.

Interesting findings were reported by us concerning the diametrically opposite effects of the two catecholamines on local cell aggregates developing into microcolonies, which is the initial stage of a biofilm's life cycle. From our data we concluded that dopamine inhibited cell aggregation, whereas norepinephrine stimulated it (Anuchin et al., 2008).

Fig. 15

Apart from prokaryotes, catecholamines produce significant effects on eukaryotic microorganisms such as yeast (Fig. 15). The maximum effect was detected with dopamine. The concentration dependence curve of its effect reached its maximum at 1 μM, thus resulting in approximately an 8fold stimulation of cell growth. Norepinephrine that, in contrast to dopamine, contained an OH group in the lateral chain caused almost no growth stimulation. The effect of apomorphine that specifically binds to D1 and D2 type receptors like dopamine was also tested. Apomorphine was characterized by a bell-shaped curve like dopamine; in an analogy to dopamine, the maximum growth stimulation with apomorphine was attained at 1 μM. The amplitude of the apomorphine effect was lower than that of dopamine.

The mechanism of action of catecholamines has been revealed. It was established that bacteria, like human nervous cells and immune cells, contain specific adrenoreceptors. In addition to catecholamines, dopamine and norepinephrine, these bacterial adrenoreceptors also bind a chemically related bacterial signal called AI-3. As briefly discussed in Lecture 4 above, the effects of AI-3 result from its binding to two-component quorum-sensing systems whose receptors are termed QseC and QseE. AI-3 binding causes the phosphorylation of the response regulators of these receptors. Upon phosphorylation, they function as kinases that phosphorylate the activators of transcription of the genes responsible for flagellar motility (the *flhDC* genes) and virulence (the *LEE* genes) in the virulent *E. coli* strain O157:H7 (Clarke and Sperandio, 2005).

To reiterate (see Lecture 3), QseC is capable of binding catecholamines in addition to AI-3 per se (Clarke et al., 2006), and QseE is presumably characterized by a similar capacity. These receptors, therefore, are regarded as bacterial analogs of the catecholamine-binding receptors of eukaryotic cells including neurons, even though QseC and QseE differ from the eukaryotic G proteins in structural terms that are known to be eukaryotic adrenoreceptors α and β.

Studies with *E. coli* O157:H7, *Salmonella enterica,* and *Yersinia enterocolitica* revealed that its interaction with norepinephrine, epinephrine, and AI-3, is specifically blocked by the α-adrenergic antagonists phentolamine, phenoxybenzamine, and prazosine, but not by the β-adrenergic antagonists propranolol and labetalol. Dopamine also loses its stimulatory effect if added in combination with chlorpromazine that selectively blocks D2 receptors; however, the dopamine effect is still observed if naclopride, a specific D1 receptor antagonist, or haloperidol, a non-selective antagonist of both dopamine receptors in eukaryotic systems, are added (Freestone et al., 2007).

The fact that the effects of dopamine and norepinephrine are suppressed by different antagonists apparently suggests that they are bound by different receptor sites of bacterial cells.

The question to raise is why human neurotransmitters are so important for bacteria?

First, they contribute to the cross-talk among various microorganisms in the GI tract, because AI-3 is an interspecies signal molecule; moreover, bacteria synthesize norepinephrine and dopamine and release them into the culture liquid (Shishov et al., 2009). In particular, pathogenic *E. coli* strains can receive growth-, virulence-, and biofilm formation-stimulating signals from the commensal microflora including nonpathogenic *E. coli* strains.

Second, they are involved in the chemical dialog between the microbiota and the host organism that specifically produces norepinephrine and epinephrine and releases them into the bloodstream (from which they penetrate into the intestinal lumen) in response to infection. Pathogenic microorganisms are more responsive to norepinephrine than to dopamine. In contrast, nonpathogenic *E. coli* prefers dopamine to norepinephrine. It also responds to other neurotransmitters that are characteristic of local inflammation rather than of serious systemic infection. Beneficial bacteria do not bring about serious infectious problems but they may thrive in a locally inflamed gut. This is due to the fact that local inflammation is associated with overproduction of some neurochemicals and hormones, including catecholamines and the proinflammatory factors serotonin and histamine.

Table 4

Table 5

Apart from responding to neurochemicals, microorganisms produce them (Table 4). High-performance liquid chromatography (HPLC) with amperometric detection was used to identify and quantitatively determine catecholamines (Table 5) in the cultures of a large number of prokaryotic and eukaryotic microorganisms (Tsavkelova et al., 2000). Norepinephrine was present at concentrations of 0.2-2 μM in the biomass of *Bacillus mycoides, B. subtilis, Proteus vulgaris,* and *Serratia marcescens*; dopamine at concentrations of 0.5-2 μM was found in the biomass of the majority of the tested prokaryotes. In the matrix-rich bacterium *B. subtilis* (the M variant), neurotransmitters are mainly contained in the matrix fraction. This fact supports the idea that these amines function as cell-cell communication signals, because the hydrophilic biopolymer components of the matrix promote the diffusion of low molecular weight signal molecules within the colony (biofilm).

Using the *E. coli* model, it was established (Shishov et al., 2009) that maximum (micromolar) catecholamine concentrations accumulate in biomass during the lag phase of culture growth. In light of these data, it should be suggested that neuroactive amines behave as triggers that activate growth processes and cell division during the initial phase of the ontogeny of the microbial culture. This is comparable with the effects of other known autoregulatory compounds. The biomass of all tested microorganisms, including *E. coli,* the yeast *S. cerevisiae*, the bacterium *B. cereus*, and lactobacilli also contained DOPA, the catecholamine precursor in animal cells.

Probiotics are live microorganisms that, "when administered in adequate amounts, confer a health benefit on the host" (FAO/WOS, 2006). One of their subgroups, **psychobiotics,** help patients with psychiatric problems. An important mechanism of action of useful bacteria on the brain involves chemical substances called **neurotransmitters** or, more broadly, **neurochemicals.** In this lecture, they are exemplified by **catecholamines** that. In addition to their neurochemical and hormone functions, they produce receptor-mediated effects on the microbiota.

LECTURE 7. THE ROLE OF SEROTONIN AND HISTAMINE

This lecture focuses on the functions of two major neurotransmitters, serotonin and histamine, with special emphasis on their role in host-microbiota interaction.

7.1. Serotonin. Serotonin, or, chemically, 5-hydroxytryptamine, is synthesized from the amino acid tryptophan via two different pathways. The pathway that is typical of animals, including humans, involves tryptophan hydroxylation as shown in Fig. 16:

Fig. 16

Tryptophan → 5- Hydroxytryptophan → Serotonin → 5-HIAA.

In plants and microorganisms, there is another pathway, involving conversion of tryptophan to tryptamine by removing the carboxyl grouping, which is followed by hydroxylation of tryptophan, resulting in serotonin formation.

Serotonin is mainly produced in the brain by 9 raphe nuclei, these are the structures of the brain located in its lower part called the brainstem. Serotonin spreads in various other parts of the brain. Serotonin limits the spreading of excitation waves in the brain caused by stimulus perception. As a result, stimulus processing is normally compartmentalized in specialized loci within the brain. In short, serotonin prevents the brain from being overexcited.

This stimulus compartmentalization is prevented by LSD, which disrupts the operation of serotonergic perceptive zones and, therefore, causes hallucinations. Serotonin at high concentrations has an additional effect: it “put the brain asleep”, and the serotonin-releasing raphe nuclei belong to the sleep-inducing zones of the brain.

Much serotonin is produced in the gut, and at high concentrations it causes diarrhea. Serotonin also accumulates in the blood where it is partly taken up by blood platelets. Too much serotonin in the blood activates brain receptors of the 5HT3 type that bring about nausea and vomiting. Blocking serotonin receptors in the brain with the drug LSD causes visual illusions and hallucinations.

In the animal kingdom, serotonin is involved in determining the social rank of individuals. In animal species ranging from lobsters to fireflies and vervet monkeys, the dominant male contains more serotonin in its blood (hemolymph) serum than a subordinate.

In human society, there is evidence that serotonin deficiency in the brain results in severe depression associated with anxiety, anger, and uncontrollable impulsive behavior (Masters, 1994). In addition, serotonin plays an important role in interactions between the neocortex, particularly its prefrontal area, and more primitive brain modules. Its lack limits the influence of the cortex over these modules, which may take over the control over human behavior under these conditions. Low serotonin levels in combination with a low baseline glucose concentration in the bloodstream (hypoglycemia) are correlated with recidivism for arson (setting houses on fire) and impulsive homicide (murder) (Virkkunen et al., 1994).

A lack of serotonin in the serotonin-dependent brain structures (the serotonergic system) is responsible for conditions such as seasonal affective disorder (SAD) and premenstrual syndrome (PMS). The symptoms of both disorders include depression, anxiety, and often impulsive behavior.

Serotonin and histamine (to be discussed below) represent efficient inflammation mediatiors. Nonetheless, their immunological effects can be both inflammation-stimulating and inflammation–inhibiting, depending on the micro-environment. Presumably, these compounds are implicated in inducing and potentiating the inflammatory response at its initial stages, but they may promote inflammation attenuation at the final stages of this process.

The effects of serotonin in microbial systems have not been as extensively studied as those of catecholamines. In contrast to them, serotonin does not stimulate the growth of such intestinal pathogens as the enterohemorrhagic strain (EHEC) of *E. coli* (M. Lyte, personal communication). It was established that serotonin stimulates cell proliferation and biomass accumulation in the non-pathogenic E. coli K-12 strain, although to a lesser extent than dopamine (Oleskin et al., 1998; Anuchin et al., 2008). In contrast to catecholamines, the

Fig. 17

serotonin effect is characterized by a bell-shaped, not linear, concentration dependence curve; the maximum stimulation of E. coli growth was attained with ~1 μM serotonin (Fig. 17). Serotonin also increases the growth of *Rhodospirillum rubrum,* a bacterium that lives in lakes and carries out photosynthesis. This bacterium is known to be closely related to the evolutionary ancestors of mitochondria, which once derived from free-living bacteria, in terms of the symbiogenesis theory on the origins of mitochondria. Serotonin is also a rather strong growth stimulator in yeast. Research with plants revealed a simulatory action of serotonin on the growth of plants such as radish shoots (Roshchina, 2010). Chemically, serotonin is closely related to the plant hormone auxin, or 3-indolacetic acid (IAA).

Biofilm formation is known to begin with cell conglomeration as the initial stage. This process is facilitated by serotonin (Fig. 18). This cell aggregation stimulation is observed both in *E. coli* and the soil myxobacterium *Polyangium* sp.

Fig. 18

The mechanism of action of serotonin is not well understood. In an analogy to catecholamines, one can assume the existence of serotonin-binding bacterial cell receptors, which could bind serotonin-like compounds, apart from serotonin per se.

One of the components of the serotonin molecule is a bicyclic aromatic moiety that also exists as an independent molecule called indole. Of particular interest, therefore, are recent data concerning the effects of *indole* in cell systems. Indole (the “backbone” of the molecule of serotonin, an indolamine) and its derivatives are widespread in the world of microorganisms (Domka et al., 2006). Indole often produces both stimulatory and inhibitory effects on biofilm formation, depending on the bacterial species involved (Lee et al., 2007a, b) although more research is needed to better understand its mode of action in the microbial world.

Useful symbiotic bacteria produce high concentration of indole in the gut, and they suppress the development of potentially pathogenic bacteria. In intestinal epithelial cells, indole was reported to induce the expression of the genes that are responsible for the barrier function, mucin formation, and the synthesis of anti-inflammatory cytokine IL-10, while concomitantly suppressing the synthesis of cytokine IL-8 (Bansal et al., 2010). Indole inhibits, in pathogens, biofilm formation and flagellar motility (reviewed, Oleskin & Shenderov, 2020). Thus, indole is involved in the protective action of beneficial bacteria that suppress pathogens. Indole probably owes its effect to its binding to quorum sensing receptors in bacteria. To reiterate, AI-1, or N-AHL, is a widespread signal in many gram-negative bacteria. Indole seems to mimic its effect and function as a cross-species signal in the gut microbial association. *E. coli* has an indole-binding receptor called SdiA. Indole, therefore, behaves as a functional analog of N-AHLs. Interspecies communication among bacteria within a biofilm can involve SdiA-type proteins and use N-AHLs and indole. Indole may convey the message “This niche is occupied” and allow new bacterial cells to adhere only to vacant sites of the substratum where the indole concentration is low.

7.2. Histamine. Histamine is a derivative of the amino acid histidine produced via enzymatic decarboxylation of the amino acid histidine (Fig. 19). Histamine combines two functions. It operates as a neurotransmitter in a small zone of the hypothalamus and also functions as a histohormone involved in local inflammation (that also results in releasing serotonin).

Fig. 19

“Interestingly, commensal microorganisms in the gut can produce histamine and related compounds under physiological conditions…, suggesting the potential role of luminal histamine in gut immunoregulation. In fact, a recent elegant study demonstrated that histamine can exert an anti-inflammatory effects on the host” (Sudo, 2019) by suppressing interleukin-18 production in the gut.

As part of an immune response to foreign pathogens, histamine is produced by basophils and by mast cells found in nearby connective tissues. Histamine increases the permeability of the capillaries to white blood cells and some proteins that engage pathogens in the infected tissues.

Histamine results from the decarboxylation of the amino acid histidine, a reaction catalyzed by the enzyme L-histidine decarboxylase present in a large number of bacteria (reviewed, Sudo, 2019; Oleskin & Shenderov, 2020). Bacteria also produce histamine in spoiled food, particularly fish, and this results in scombroid disease associated with sickness, headache, and sometimes red points on the skin (rash).

As for the brain, histamine is less widespread than other neurotransmitters. It is mainly produced in the hypothalamus, which is involved in many kinds of emotions. Histamine promotes awakening and maintains the active brain state. Accordingly, BBB-crossing antihistamine drugs cause somnolence (make people feel sleepy). Histamine facilitates locomotive activity (allows people to move fast), stimulates thirst, suppresses food-seeking behavior, and inhibits pain sensitivity.

In the microbial world, histamine efficiently stimulates cell proliferation and biomass accumulation in the nonpathogenic *E. coli* K-12 MC4100 strain. Like serotonin, histamine is characterized by a bell-shaped concentration dependence with a maximum effect at a concentration of 0.1 μM that stimulates *E. coli* growth twofold (Anuchin et al., 2008). Histamine promotes *E. coli* cell aggregation with microcolony formation (Anuchin et al., 2008), which, to re-emphasize, represents an early stage of a biofilm's life-cycle requiring a solid substratum. Micromolar histamine concentrations stimulate cell proliferation in the yeast *S. cerevisiae* (see Fig. 15, B, above), but, in contrast to the *E. coli* system, its stimulatory effect does not exceed that of serotonin and is significantly weaker than the effect of dopamine (Malikina et al., 2010).

7.3. Summarizing the data on the functions of catecholamines, serotonin, and histamine. The findings concerning the effects of biogenic amines on *E. coli* K-12 highlight the differences between the properties of the pathogenic and nonpathogenic (commensal) strains of *E. coli.* Norepinephrine was the most efficient growth-stimulating and cell adhesion- and biofilm formation-promoting agent with pathogenic *E. coli* strains such as EHEC that causes bloody diarrhea. Since norepinephrine is produced during stress caused by infection, the response of EHEC and other pathogenic intestinal microorganisms is to be regarded as an evolutionary adaptation. It enables the pathogens to use a product of the host's protective response to accelerate their own development. Clinically, this condition is characterized by a vicious circle, when the effect potentiates the cause.

In stark contrast, the symbiotrophic strain *E. coli* K-12 MC 4100 prefers a different neurochemical "landscape". Serotonin that is normally contained in the chromaffine granules of GI mucosa cells is not less efficient than norepinephrine. Dopamine, the minor component of the response to infection, stimulates proliferative activity and biomass accumulation to a greater extent than the major component, norepinephrine. Histamine, a characteristic factor of local inflammation, was the most efficient growth-stimulating agent among the tested neurotransmitters.

Presumably, the symbiotic strain, in contrast to the pathogenic strain(s), is adapted not to serious infection, but to mild local inflammation. It is characterized by the release of histamine and serotonin and, to a lesser extent, of catecholamines that extrude into the intestinal lumen from nerve terminals damaged by inflammation. The local inflammation of the intestinal mucosa may be due to microtraumas caused, e. g., by rough food.

This lecture focuses on the functions of two major neurotransmitters, serotonin and histamine, with special emphasis on their role in host-microbiota interaction. Symbiotic bacterial strains, in contrast to pathogens, seem to be adapted not to serious infection, but to mild local inflammation resulting in release of histamine, serotonin and, to a lesser extent, catecholamines.

This lecture should be followed by a seminar, with students invited to give talks on neurotransmitters and their roles in terms of host-microbiota interactions.

LECTURE 8. ROLE OF ACETYLCHOLINE, AGMATINE, NEUROACTIVE AMINO ACIDS, AND NEUROPEPTIDES

8.1. Acetylcholine. Another sufficiently important neurotransmitter, acetylcholine, is an ester of acetic acid and choline. It is widespread in living nature; it is synthesized by diverse microorganisms including bacilli and lactobacilli. As for unicellular eukaryotes, acetylcholine is produced by the protozoan *Acanthamoeba* sp. (Baig et al., 2018). The presence of acetylcholine receptors in unicellular eukaryotes and its regulatory influence on conjugation in infusorians and the growth and proliferation of *Acanthamoeba* sp. (that possesses a homologue of the neuronal muscarine receptor for acetylcholine) also suggest that acetylcholine is a highly evolutionarily conserved signal (Roschina, 2010, 2016; Baig & Ahmad, 2017).

In the brain, acetylcholine deals with motivation, attention, memory, and learning, as well as the plasticity and the general activity level of the brain. Memory problems that are characteristic of Alzheimer's disease are largely due to the disruption of the acetylcholinergic system of the brain. Acetylcholine stimulates the sensory perception of stimuli during the awakening process and regulates REM (rapid eye movement, people sleeping with quickly moving eyes) sleep that is associated with dreaming. The effects of acetylcholine are due to its binding to two types of receptors: (i) nicotine receptors that are responsible for tobacco addiction and (ii) muscarine receptors that bind muscarine contained in the fungus *Amanita muscari*.

Acetylcholine is released in neuromuscular junctions; it elicits skeletal (striated) muscle contraction. Disrupting this function by inhibiting the acetylcholine-degrading enzyme, choline esterase, has serious consequences ranging from convulsions to paralysis. Outside the CNS, important acetylcholine effects include deceleration of cardiac contraction, stimulation of GI peristalsis, induction of smooth muscle contraction, and regulation of the bronchial, perspiratory, lacrimal, and salivary glands (Boldyrev et al., 2010). Acetylcholine binds to the muscarine receptors of vascular endothelium, resulting in releasing nitric oxide, vasodilation, and blood pressure decrease (Kellogg et al., 2005)

In various tissues, macrophages and other immune cells express nicotine and muscarine acetylcholine receptors. By inhibiting the synthesis of pro-inflammatory factors, acetylcholine mitigates the immune response and inflammation. Of paramount importance is the interaction of acetylcholine with the nAchR$\alpha 7$ receptor that results in inhibiting the transfer of transcription factor NF-κB into the cell nucleus and, accordingly, in suppressing the production of pro-inflammatory cytokines TNF, IL-1β, IL-6, and HMGB1 (Ley et al., 2010). This mechanism appears to account for the anti-inflammatory effect of acetylcholine that is exerted via (i) afferent impulses to the CNS and (ii) efferent impulses in the branches of *nervus vagus*. Interestingly, acetylcholine does not suppress the secretion of anti-inflammatory cytokine IL-1 (Ley et al., 2010). Acetylcholine and the inhibitors of choline esterase manifest anti-inflammatory activity both in vivo and in vitro (Silva-Herdade & Saldanha, 2013). Apart from possessing acetylcholine receptors, lymphocytes and macrophages contain the complete cholinergic system. They can synthesize acetylcholine.

8.2. Agmatine. Curiously enough, some substances accumulating in a dead body have recently been revealed to work as neurotransmitters. An example is *agmatine,* which forms as a result of enzymatic decarboxylation of the amino acid arginine in a decomposing cadaver. Agmatine, (4-aminobutyl) guanidine, like other cadaveric decomposition-produced amines (ptomaines), such as cadaverine and putrescine, is formed via enzymatic decarboxylation of amino acids. Specifically, agmatine results from arginine decarboxylation, although it can also be produced by putrescine deamination (Doeun et al., 2017).

Agmatine is released into the medium by diverse microorganisms including some representatives of *Lactobacillus*. Putrescine and cadaverine are also produced by microorganisms, e.g., by many bacterial strains inhabiting pheasant carcasses (Buňková et al., 2016). Putrescine is present in many kinds of wine (Doeun et al., 2017). Agmatine exerts specific effects on microorganisms. For instance, it suppresses gut colonization by the parasitic protozoan *Cryptosporidium parvum* and prevents the infection caused by it (Lyte, 2016).

Following the discovery of endogenous agmatine synthesis in mammals in 1994, it was revealed that agmatine influences numerous molecular targets in the organism, such as ion channels, membrane transporters, and nitric oxide-synthesizing systems; it also affects polyamine metabolism, protein ADP-ribozylation, matrix metalloproteases, NADPH oxidase, etc. (Lyte, 2016). Therefore, it has been suggested that agmatine functions as a neurotransmitter (Piletz et al., 2013). Although no specific agmatine receptors have been detected up to now, agmatine has been revealed to bind to the receptors of other neurotransmitters. It binds to α_2-adrenergic and imidazoline receptors and blocks NMDA receptors, i.e. behaves as a meuromodulator and co-transmitter that affects the operation of other neurotransmitter systems.

Agmatine was established to lower blood pressure and decelerate the heart rhythm, decrease the glucose concentration in the blood, and stimulate the filtration function of the kidneys (Raasch et al., 2001; Satriano, 2004; Piletz et al., 2013). Agmatine inhibits the inducible NO synthase and exerts an anti-inflammatory effect. If administered after ischemic brain damage, admatine decreases the CD11b+ macrophage number in the spleen. It also reduces the Treg cell number. Administration of agmatine limits neural inflammation after experimental disruption of cerebral blood circulation in rats, decreases the necrosis area, and produces a vasoprotective effect (Uranchimeg et al., 2010). In RAW 264.7 macrophages, agmatine induces the activation of nuclear trascription factor Nrf2 and stimulates the production of antioxidant enzymes, which may contribute to its neuroprotective activity (Ahn et al., 2012; Chai et al., 2016).

8.3. Neuroactive amino acids. Neuroactive amino acids including glutamic and aspartic acid, glycine, taurine, and γ-aminobutyric acid (GABA) are present in the mammalian organism in the free and the bound form. They are formed via metabolic transformation of nutrients by intestinal and microbial enzymes. These amino acids are utilized by pro- and eukaryotic cells as nutrient substrates. For instance, glutamate is one of the main nutrient substrates for intestinal cells (enterocytes). Nevertheless, amino acids, often at low concentrations, serve as signal molecules that operate within the whole microbiota-nervous system-immune system triangle[13].

Although microorganisms utilize amino acids as nutrient substrates, they also recognize them as signals. The specific regulatory influence of neuroactive amino acids is exemplified by the data that glutamate (along with lysine, methionine, and succinate) stimulates and aspartate (along with lactate and formate) inhibits the growth of the probiotic strain *E. coli* M-17. Under the same conditions, aspartate, in contrast, produces a stimulatory effect on the strain E. coli BL (Vakhitov et al., 2000; Vakhitov & Sitkin, 2014).

The neurotransmitter *γ-aminobutyric acid (GABA)* increases the resistance of *Lactobacillus reuteri* to medium acidification (Lyte, 2014). GABA stimulates the expression of the pathogenic factors of *Candida albicans*, which manifests itself in the intensification of the synthesis of phospholipase B1-encoding mRNA, germ tube formation, and subsequent hypha development (Reyes-Garcia et al., 2012). GABA stimulates the virulence of *Ps. aeruginosa* by regulating the expression of six pathogenicity-related protein factors (Mazzoli & Pessione, 2016).

[13] Reprinted from: Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers, pp. 209-218 (abridged and partly modified)

The macro- and microstructure of *E. coli* colonies and, presumably, biofilms is formed under the influence of *aspartate*[14] (Budrene & Berg, 1991, 2002) as an attractant. Bacteria form concertedly moving clusters that generate complex patterns on the agar surface in the presence of aspartate (Mittal et al., 2003).

Pseudomonas fluorescens contains a periplasmic protein with a high affinity for GABA; the protein is related to one of the subunits of the ionotropic GABAA receptor of mammals. GABA-binding receptors were also detected in *Ps. aeruginosa* (PctC) and *Ps. putida* (McpG). A potassium-dependent glutamate channel (GluR0) was revealed in the cyanobacterium *Synechocystis* PCC6803 (Mazzoli & Pessione, 2016).

In human blood plasma and spinal fluid, GABA is present at concentrations of ~0.6 and ~0.3 μM, respectively (Abbott et al., 1982), which are close to those produced by lactobacilli. The strain of *Lact. delbrueckii* subsp. *bulgaricus* synthesized 0.3 μM GABA on a milk-containing medium (Oleskin et al., 2014a, b).

In the human organism, GABA is a prerequisite for normal pain sensitivity of the intestine and for the operation of the immune system. GABA mitigates inflammation processes and allergic responses by suppressing the activity of T lymphocytes (Auteri et al., 2015). An imbalance in the GI microbiota frequently results in decreased microbial production of GABA, which increases the risk of irritated bowel syndrome and other inflammatory intestinal diseases (Babin et al., 1994).

Neuroactive amino acids are involved in regulating the impulse transmission rate in the nervous system. They differ in their effects on the nervous system. Amino acids such as glutamate and aspartate activate specific structures in the nervous system. Other amino acids including GABA and glycine exert an inhibitory influence on the nervous system. Besides, amino acids produce multiple effects on the whole organism.

Of note are the roles of glutamate and GABA. Both amino acids along with glutamine form a part of a cycle that is necessary for the homeostatic operation of the CNS. Disruption of the GABA-glutamate-glutamine interconversion is associated with mental problems including anxiety, depression, bipolar disorder, and schizophrenia.

GABA and glutamate receptors are present in pre- and postsynaptic neurons and in glial cells such as astrocytes.

The major excitatory neurotransmitter glutamate is contained in the neocortex, olfactory bulbs, hippocampus, substantia nigra, cerebellum, and eye retina (Boldyrev et al., 2010). Glutamate is the predominant neurochemical in the nervous system of verterbrates (Meldrum, 2000); it is present in over 90% of all synapses in the human brain. Glutamate stimulates impulse transmission in the CNS and the energy metabolism of brain cells. It is involved in ammonia detoxification, helps improve behavioral symptoms in mentally retarded children, and mitigates stress. Glutamate is implicated in cognitive activities including learning and information memorization. Normally, almost no glutamate can cross the BBB ; it is synthesized in the brain from α-ketoglutarate in a transaminase-catalyzed process.

γ-Aminobutyric acid (GABA) performs the function of the main inhibitory neuromediator in the brain. There are three main classes of GABA receptors, denoted as $GABA_A$, $GABA_B$, and $GABA_C$. The five-subunit $GABA_A$ and $GABA_C$ form a part of ligand-gated ion channel complexes. Their activation results in increasing their permeability for chloride and bicarbonate, respectively. Metabotropic $GABA_B$ receptors, more widely spread in the peripheral nervous system, are G protein-coupled receptors that open or close ion channels.

GABA plays a major role in regulating the sleep-wake cycle, locomotor activity, vascular tone, and information memorization and recognition. GABA exhibits moderate antihypoxic and

[14] Since organic acids are predominantly present in the form of ions in biological systems, it is common to write glutamate and aspartate instead of glutamic and aspartic acid, respectively. This rule only does not apply to □-aminobutyric acid that is routinely abbreviated as GABA.

antiseizure activity, produces a sedative effect, promotes concentration, and can be used as as a non-addictive tranquilizer. GABA improves memory, promotes the restoration of locomotor activity and speech in patients with cerebral vascular disorders, ameliorates glucose utilization by brain cells, and facilitates the disposal of toxic metabolic products (Hevia et al., 2015).

Some GABA molecules cross the gut-blood barrier and the BBB (Boonstra et al., 2015). It seems likely, therefore, that many GABA effects are due to the combined impact of endogenous, microbial, and dietary GABA. Recently, it has been revealed that the GABA-producing bacteria *Bifidobacterium dentium* decrease the abdominal pain sensitivity of rats at the level of the dorsal roots of the spinal cord (Mazzoli & Pessione, 2016).

Glycine functions as a neurotransmitter in the brainstem and the spinal cord; it also inhibits neuronal activity by suppressing the release of glutamate, an excitatory amino acid, from neurons. Glycine promotes GABA formation and helps glutamate and aspartate perform their signal functions. Glycine also stimulates the functioning of the pituitary, improves metabolic processes in CNS cells, exerts an antistress effect, improves intellectual working capacity, produces a sedative effect, improves sleep, and enhances the organism's adaptive potential. The structure of the 5-subunit glycine receptor is generally similar to that of the $GABA_A$ receptor.

Aspartate, an excitatory amino acid, improves mood and prevents the state of fatigue. Aspartate promotes ammonia removal from the organism. Aspartate binds to the same receptors as glutamate but it is less widely spread in the CNS. The maximum aspartate content is characteristic of the midbrain (Boldyrev et al., 2010; Dubynin et al., 2010).

The expression of the genes of amino acid receptors is under the influence of the symbiotic microbiota of the GI tract. In mice raised aseptically (germ-free, or GF, mice), the expression level of the gene encoding subunit NR2B of the glutamate NMDA receptor in the hypothalamus and the amygdala of the brain is abnormally low (Rohrscheib & Brownlie, 2013).

Like other neurochemicals, amino acids are synthesized by immune cells including T cells, macrophages, and dendritic cells (Bhat et al., 2010; Fuks et al., 2012). $GABA_A$ and $GABA_B$ receptors are present on the surface of many immunocytes. Immunocytes possess α_1, α_2, β_1, β_3, δ, and plausibly other subunits of $GABA_A$ receptors (Jin et al. 2013). The immunotropic effects of GABA are complex; they vary depending on the GABA receptor types involved.

GABA's anti-inflammatory effect is due to suppressing T lymphocyte activity (Auteri et al., 2015). This is consistent with the fact that activation of GABA receptors on T cells and macrophages results in inhibition of the production of proinflammatory cytokines including interleukins IL-1β, IL-2, IL-6, IL-12, interferon-γ, and TNF-α (Bhandage et al., 2018). The work cited presents evidence that GABA influences (predominantly inhibits) the secretion of a wide spectrum of cytokines by immunocytes, e.g., CD4+ T cells.

Glycine can also exert immunotropic effects. Various cells of the immune system, including T lymphocytes and neutrophils, possess surface receptors for glycine. There is evidence that glycine exhibits anti-inflammatory activity in vivo and in vitro. In its presence, secretion of proinflammatory mediators such as IL-1 and TNFα is suppressed, while the synthesis of the anti-inflammatory mediator IL-10 is stimulated. In vivo, glycine mitigates the symptoms and prevents the consequences of experimental endotoxic shock. These effects are caused by very high glycine doses, corresponding to a 5% level of glycine in nutrients consumed per day (van den Eynden et al. 2009).

8.4. Neuropeptides. These essential neurochemicals represent short amino acid chains and predominantly function as neuromodulators: they increase/decrease the efficiency of signal transmission across synapses whose operation depends on other neurotransmitters. For instance, opioids (endorphins, enkephalins, and dynorphins) bind to specific neuron receptors and block impulse transmission along neuron axons, including those involved in pain perception. Opioids produced by the brain serve as positive reinforcement of altruistic acts; their

production encourages law-abiding people even in situations in which obeying the law causes negative consequences for the individual involved However, substance P (responsible for pain perceptiony) and some other peptides also directly perform the neurotransmitter function: they transmit impulses across synaptic clefts.

A large number of peptides combine the functions of hormones and neurochemicals. The aforementioned substance P is present in the hypothalamus, the amygdala, and the gray matter of the brain; apart from pain perception, it is implicated in anxiety development and stress responses. In addition to this neurotransmitter function, substance P operates as a hormone: it promotes blood vessel dilation, increases capillary permeability, stimulates mast cell degranulation, behaves as leukocyte attractant, causes smooth muscle contraction, and facilitates the release of prolactin, GI hormones, and inflammatory factors.

The diversity of neuropeptides is impressive, and many peptides produce microbial effects. This is exemplified by dynorphins, a subgroup of opiods. Preliminary studies demonstrated that the color of *Pseudomonas aeruginosa* colonies became intensely green (indicative of pyocyanine production) after exposure of *P. aeruginosa* to filtered intestinal contents of stressed mice which contain opioids. Importantly, pyocyanine production by this bacterium is correlated with virulence. Only κ-opioid receptor agonists dynorphin and its synthetic analog U50,488 cause a considerable stimulation of pyocyanin production. The effect increases with an increase in κ-agonist concentration and results in an approximately 4-fold stimulation of pyocyanin production at a U50,488 concentration of 1 μM (Zaborina et al., 2007).

While catecholamines behave as analogs of AI-3, dynorphin and its synthetic analog, in a similar fashion, perform the functions of the quorum-sensing autoinducer quinolone. The quinolone-dependent quorum-sensing system in *P. aeruginosa* is activated by the lasI-lasR system of this bacterium. Both quorum-sensing systems are involved in virulence factor synthesis and biofilm formation. It was demonstrated that the effects of dynorphin and U50,488 require the operation of these quorum-sensing systems. Mutations disrupting these systems prevent their effects.

Taken together, the data on various neurotransmitters (cf. the information in the lectures on biogenic amines) and their analogs suggest an important mechanism that links stress and the development of bacterial infection often accompanied by biofilm formation in the human/animal organism. In summary, vertebrate neurotransmitters whose synthesis and release are stimulated by stress factors can behave in an autoinducer-like fashion if they contact bacterial cells.

The data concerning microbially produced neuropeptides are meaningful but still rather fragmentary (Fetissov et al., 2008; Holzer & Farzi, 2014). It was established that *Staphylococcus aureus* synthesizes the autoregulator $[Met]_5$-enkephalin, a microbial opioid that functions as a neuromediator (Zagon & McLaughlin, 1992). Another opioid, β-endorphin, is synthesized by some unicellular eukaryotes, such as the infusorian *Tetrahymena pyriformis* and the amoeba *Amoeba proteus* (Lenard, 1992).

Importantly, the opioid [Met]5-enkephalin inhibits the growth of *Pseudomonas aeruginosa, Staph. aureus,* and *Serratia marcescens* (Zagon & McLaughlin, 1992). *Staph. aureus* possesses receptors to $[Met]_5$-enkephalin, and this opioid is present in its culture liquid at a concentration of up to 1.6 ng/mL. It was suggested that opioids had been performing their growth-modifying function millions of years before higher animals with their complex nervous system emerged (Zagon & McLaughlin, 1992). The macrophage- and polynuclear leucocyte-produced peptide LL-37 (catelicidin) stimulates the quinolone-dependent QS system that is involved in virulence factor synthesis in *Ps. aeruginosa* and concomitantly enhances the tolerance of *Ps. aeruginosa* to the antibiotics ciprofloxacin and gentamycin (Strempel et al., 2013).

To an extent, the boundary between hormones and neurochemicals is arbitrary and changeable. Microbial endocrinology is concerned with the operation and the functional roles of

both classes of compounds in microbial systems (Lyte, 1993, 2010, 2011). Many chemicals combine both functions. As a hormone, insulin increases the permeability of plasma membranes for glucose, stimulates the formation of glycogen from glucose in the liver, and suppresses the activities of glycogen- and lipid-degrading enzymes. As a neuromediator, insulin is involved in transmitting information concerning feelings of hunger and satiety into the brain. In this capacity, insulin functions in combination with other neuropeptides (ghrelin, leptin, and peptide YY). It was established that insulin is produced by *E. coli* and the fungus *Neurospora crassa*, which contains a gene that is homologous to the insulin gene of mammals. In *N. crassa*, insulin is implicated in the regulation of carbohydrate metabolism (Lenard, 1992).

Microorganisms are capable of producing corticotropin (*Tetrahymena pyriformis*), somatostatin (*B. subtilis* and *Plasmodium falciparum*), progesterone (*Trychophyton mentagrophytes*), and α-factor (*S. cerevisiae*), a homologue of the gonadotropin-liberating hormone of higher animals (Lenard, 1992) that, apart from its hormone function, regulates brain activity (Dubynin et al., 2010).

Acetylcholine, a major neurotransmitter, is synthesized by diverse microorganisms including bacilli and lactobacilli. **Agmatine** accumulating in a cadaver is also released into the medium by diverse microorganisms, and it seems to work as a neurochemical at low concentrations. **Neuroactive amino acids** serve as energy sources and signals in the microbial world. For instance, The macro- and microstructure of E. coli colonies and, presumably, biofilms is formed under the influence of aspartate. **Neuropeptides** function as neurochemicals, hormones, and microbial signals exemplified by dynorphins recognized as QS signals by *Ps. aeruginosa.* These chemical agents are in the focus of attention of **microbial endocrinology.**

LECTURE 9. ROLE OF SHORT-CHAIN FATTY ACIDS AND GASOTRANSMITTERS

9.1. Short-chain fatty acids (SCFAs). Short-chain fatty acids are saturated unbranched fatty acids with short carbon chains. Of paramount importance in biological terms are SCFAs with two to four carbon atoms in the chain, i.e., acetic, propionic, and butyric acid. Since they are mostly present in biological systems as anions, they will be referred to acetate, propionate, and butyrate, respectively. All the SCFAs represent volatile liquids under normal conditions, due to their low molecular weight.

SCFAs are among the major intermediate and final products of fermentation of complex dietary, bacterial, and endogenous biopolymers, including mucins, glycoproteins, and the proteins of shedded epithelial cells. Their concentrations may be as high as 70-140 mM in the upper and 20-70 mM in the lower part of the colon; acetate is the predominant SCFA (reviewed, Oleskin & Shenderov, 2020).

SCFAs provide the organism and, more specifically, the brain with energy, and, besides, they exert important regulatory effects. If their synthesis and metabolism are disrupted and their concentrations become abnormally low or abnormally high, this impedes the functioning of the nervous system and causes psychiatric problems.

In the gut, SCFAs regulate colonization resistance, the mechanism whereby the intestinal microbiota protects itself against incursion by new and often harmful microorganisms. The production of antimicrobial peptides, neurotransmitters, and hormones is subject to regulation by SCFAs. Literature data indicate that SCFAs are involved in maintaining the GI barrier and preventing bacterial translocation from the intestinal lumen to the bloodstream (Verbeke et al., 2015; El Aidy et al., 2016; Shenderov, 2008, 2013a, b, 2016; Oleskin & Shenderov, 2016; Yao et al., 2020). “For example, one important function of propionate is to limit pathogen expansion via facilitating the cytoplasmic acidification <i.e., lowering the pH inside the cells – A.O.> of *Salmonella* or *Shigella*, disrupting the intracellular pH homeostasis of the pathogens” (Yao et al.,

2020). In similar fashion, "*Bifidobacterium* reduces the intestinal pH during fermentation of lactose, thereby preventing the colonization by pathogenic *Escherichia coli*" (ibid.)

At low to medium concentrations, SCFAs are used as an important energy source by many representatives of the GI microbiota. However, at higher concentrations (> 100 μM), SCFA behave as antimicrobial agents, and they selectively eliminate pathogens while promoting the propagation of useful microorganisms. For instance, butyrate and propionate suppress the growth of *Salmonella* but stimulate the proliferation of lactobacilli. Propionate is widely used in Europe as a food additive (E280–E282) because it exhibits antifungal activity.

Depending on the concentrations, SCFAs can be both useful and harmful to the human organism. A currently widely spread problem is obesity. Many people are overweight, including a large number of children. They are characterized by markedly increased levels of SCFAs, especially propionate, which is correlated with a shift in the *Firmicutes:Bacteroidetes* ratio in favor of *Bacteroidetes*. Risperidone that is used for treating mental disorders in children and adolescents and causes weight gain (as a side effect), increases SCFA production by their GI microbiota These data seem to be not quite consistent with the fact that SCFA administration to mice brings about a weight loss and normalizes the composition of the microbiota (van de Wouw et al., 2017).

Human individuals with a loss of appetite (anorexia) and a decreased weight are characterized by lowered levels of acetate and propionate; their levels remain low even if their weight becomes normal (van de Wouw et al., 2017).

The activity of the sympathetic nervous system is subject to regulation by SCFAs (e.g., by propionate) via their interaction with G-protein-coupled receptors (GPRs) such as the GPR41 and GPR43 receptors of the ganglia of the enteric nervous system.

Approximately 60% of GI tract diseases are accompanied by neuropsychological disorders. They may result from changes in the energy level of nervous cells that depend on the availability of SCFAs. SCFAs, including those of microbial origin, can cross the gut-blood barrier and the BBB and, therefore, directly influence brain biochemistry. SCFAs can affect calcium influx into cells, intracellular pH maintenance, lipid metabolism, the gap junction-dependent cell barrier function, gene expression, and immune system activity (MacFabe, 2012).

SCFAs promote the maturation and active operation of microglial cells that represent CNS immunocytes. SCFAs also strengthen the BBB; they prevent microbial LPSs from disrupting the BBB by activating the immune system of the organism.

There are three main aspects of the molecular mechanisms of action of SCFAs:

- they regulate the expression of the gene coding for tryptophan hydroxylase, the key enzyme of the serotonin biosynthesis pathway
- they decrease the activity of chromosome histone deacetylases (HDACs), thereby facilitating the access of repair enzymes to the DNA. This promotes the improvement of the health state of patients with excessive activity of these enzymes that is characteristic of Parkinson's disease, depression, and schizophrenia.
- they induce the synthesis of a large number of neurochemicals.

Injection of butyric acid mitigates depression and anxiety. This seems to be due to an increased synthesis of the brain-derived neurotropic factor (BDNF) in the cells of the hippocampus and the frontal cortex (Schroeder et al., 2007). The brain serotonin concentration increases under the influence of acetate (Ivashkin & Ivashkin, 2018). Butyrate activates the synthesis of the glial cell line-derived neurotrophic factor (GDNF; Westfall et al., 2017).

However, high SCFA concentrations act as neurotoxins. Injecting propionate into the brain ventricles causes autistic symptoms in rats that become less social and stop recognizing their group mates. Propionate also brings about seizures, affects locomotive behavior, and causes metabolic acidosis (acidification of an organism's internal medium) and GI symptoms.

Currently, there are increasing numbers of people affected by autism and related psychiatric disorders. Children with autistic spectrum disorders (ASDs) are characterized by elevated concentrations of SCFAs, especially of propionate, in the intestines. The parents of autistic children reported that their behavioral symptoms and GI problems increased after consuming processed food items that were rich in carbohydrates (used by bacteria to synthesize SCFAs including propionate) or contained propionate as a preservative (MacFabe, 2012). Intraventricular administration of propionic acid to rodents results in autism-like behavioral disorders (Shultz et al., 2009; MacFabe, 2012).

The behavioral problems of autistic people may result from propionate's capacity to modulate the expression of many autism-related genes, predominantly those associated with mitochondrial processes. As studies with models such as mice and also human subjects have revealed, probiotic bacteria help overcome the symptoms of autism, such as social withdrawal and repetitive behaviors (e.g., playing with the same toys for several days).

Butyrate administered to volunteers by enema decreased visceral pain sensitivity. It also relieved the sense of discomfort in the colon area by elevating the sensitivity threshold of visceral mechanoreceptors and increasing the production of peptide YY that suppresses phasic contractions of the circular smooth muscles of the colon (Canani et al., 2011; Erofeev et al., 2012).

Many CNS diseases, including Parkinson's disease, are associated with siginificant microbiota changes; importantly, they are often characterized by a decreased SCFA level in the intestinal content, which affects the enteric nervous system and downregulates GI motility. By stimulating BDNF synthesis (see above), butyrate prevents the destruction of dopaminergic brain neurons. A decrease in microbial butyrate production, therefore, promotes neurodegenerative processes in the brain that are characteristic of Parkinson's disease (Westfall et al., 2017).

SCFAs, including those of microbial origin, possess the capacity to modulate host immune responses. This is achieved via (a) activation of chemoattractant membrane receptors including free fatty acid receptors (GPR41 and GPR43), the niacine/butyrate receptor (GPR109a), and the olfactory receptor Olfr-78 that are located on immune and intestinal epithelial cells and (b) inhibition of histone deacetylases (Shenderov, 2013; Correâ-Oliveira et al., 2016; Yao et al., 2020). Microbial SCFAs promote the functional differentiation of B lymphocytes that produce IgA in the blood plasma (Rees et al., 2018).

Research on animal models revealed that modifying the microbiota of pregnant females with a diet enriched in fibers results in forming an increased amount of SCFAs and preventing the development of allergic diseases. Allergic responses can be suppressed by directly introducing SCFAs, e.g., acetate into the maternal organism during the pregnancy period. Upon entering the fetal bloodstream. SCFAs stimulate T_{reg} cell formation. Airway eosinophil cell numbers and serum IgE concentrations are decreased and Th2-dependent immune responses with IL-5 and IL-13 production are inhibited. It is of relevance that females with low SCFA levels give birth to children with an increased risk of developing allergic problems, such as recurrent bronchial obstruction during the first life year (Logan et al., 2016).

The anti-inflammatory effect of microbial SCFAs seems to at least partially account for the health-promoting influence of the SCFAs-enriched Mediterranean diet that decreases the risk of allergic processes, depression, and cardio-vascular diseases (Logan et al., 2016).

SCFAs can inhibit neutrophil chemotaxis and suppress immunocyte migration from the bloodstream to the inflammation area. SCFAs are also implicated in regulating the production of cytokines (TNF-α, IL-2, IL-6, and IL-10), eicosanoids, and chemokines' (MCP-1 and CINC-2). Acetate and butyrate affect inflammatory neutrophil and macrophage responses by inducing, in epithelial and killer cells, the production of cytokines that regulate leukocyte chemotaxis and suppress the formation of adhesion molecules (reviewed, Oleskin & Shenderov, 2020; Yao et al., 2020).

9.2. Gasotransmitters. The final part of my lecture deals with gaseous substances that function as neurochemicals. They include such simple molecules as nitric oxide, carbon oxide, and hydrogen sulfide, which apparently are among the most ancient gas molecules that can perform neurochemical functions.

Presumably, some other gases (hydrogen, methane, ammonia, carbon dioxide, etc.) also exhibit neurochemical activities. Both host tissue-dependent and microbial synthesis of gases with proven neurotransmitter functions is carried out by specific enzymes. For example, synthesis of NO from arginine is catalyzed by NO synthases (NOSs) and that of CO by heme oxygenases (HOs) that cause heme degradation. H_2S is predominantly synthesized from L cysteine, and this reaction is catalyzed by at least three different enzymes (Althaus & Clauss, 2013; Oleskin & Shenderov, 2016).

As for the GI tract, it contains about 20 ml of various gaseous products. The volume of intestinal gases that is produced per day varies between 400 and 1200 ml. These gaseous substances enter the GI tract with air and food; in addition, they are formed by various eukaryotic and prokaryotic cells via enzymatic or nonenzymatic processes. Hydrogen and methane only result from microbial fermentation. Unlike other neurochemicals, gases working as neurotransmitters possess several distinctive properties:

- they produce their effects on the cells that synthesize them (autocrine effect), adjacent cells (paracrine effect), and even remote tissues/organs (endocrine effect).
- they do not bind to specific receptors on cell membranes and do not accumulate in synaptic vesicles; upon their synthesis, they are usually released from the synthesizing cells
- they easily penetrate into the cells of the nervous, vascular, and immune systems.
- at the molecular level, they interact with intracellular enzymes and ion channels.

9.2.1. Nitric oxide (NO). This is a small short-lived signal molecule that can modify diverse proteins by binding to thiol groups and other amino acid sites (Farrugia & Szurszewski, 2014). In the human organism, NO is formed via both enzymatic and non-enzymatic reactions.

NO can protect bacteria from antibiotics. NO-dependent antibiotic resistance is due to chemical modification of toxic components and to mitigation of antibiotic-induced oxidative stress (Gusarov et al., 2009, 2013; Tinajero-Trejo et al., 2013). In the human organism, NO plays the following main roles:

- Dilation of blood vessels.
- Activation of the brain zones responsible for grooming behavior in animals and petting in humans; the reason why we feel happy when touching each other; the release of NO associated with physical contact brings about a state of euphoria.
- Cell differentiation, apoptosis (programmed cell death), and cell proliferation.
- Cytotoxic effect in terms of the immune response to foreign or tumor cells. The two latter effects require very high concentrations of NO.

To reiterate, NO is synthesized by NO synthase. This enzyme is subject to regulation by Ca^{2+}/calmodulin in neurons; NO directly activates the membrane-bound guanylate cyclase. In contrast to other neurochemicals, the NO gas

- Does not accumulate in vesicles
- Does not bind to specific membrane receptors
- Does not use any specific degradation mechanism; instead, it rapidly converts into NO_2

A serious infection results in NO production by the human/animal organism. NO produces a toxic effect on the whole organism, causing a life-threatening septic shock. The organism may kill itself before it is destroyed by the pathogen. According to V.P. Skulachev (1999), this prevents the pathogen from spreading in the population at the expense of the lives of some individuals in it. This is the essence of Skulachev's "altruistic death" hypothesis. Like a single

cell undergoing apoptosis, a whole human/animal individual altruistically kills itself with NO, the organism prevents the spreading of the virulent bacterium in the population.

When applied at nanomolar concentrations, NO predominantly performs regulatory functions, whereas its higher (micro- and millimolar) concentrations are toxic to both mammalian cells and microbial symbionts. Blood immune cells (macrophages) release high NO concentrations that exert a cytotoxic effect on tumor cells and other kinds of foreign cells. By interacting with protein FeS groups, NO binds to cytochrome hemes. Interaction of NO with molecular oxygen and superoxide radical yields toxic compounds, such as NO_2, N_2O_3, and especially $ONOO^-$ (peroxynitrite) that inactivate the thiol groups of organic molecules and react with the tyrosyl residues of proteins and the nitrogenous bases of the DNA (Tinajero-Trejo et al., 2013; Robinson et al., 2014; Oleskin & Shenderov, 2016). Apart from immune cells, NO is synthesized by hepatocytes, vascular endothelium cells, and others. NO production enables the cells to destroy pathogenic protozoans, helminths (James, 1995), and bacteria (Chen et al., 2015).

Microorganisms respond to high (micromolar) NO concentrations using the stress response mechanism. This may account for the fact that high NO concentrations stimulate biofilm formation in *P. aeruginosa*. However, low (pico- to nanomolar) NO concentrations are normally generated by various microbial species, and perform regulatory functions in microbial systems.

NO formation is one of the stages of the denitrification process :

$NO^{3-} \rightarrow NO \rightarrow N_2O$

Similar to eukaryotes, prokaryotes use NO (at low concentrations) as a regulatory agent. For instance, they downregulate biofilm formation and cause biofilm destruction. Low NO concentrations enhance the capacity of antimicrobial compounds, e. g., the antibiotic tobramycin, hydrogen peroxide, and the detergent sodium dodecylsulfate, to remove microbial biofilms from water distribution and treatment systems.

The biofilm of the NO synthase-deficient ΔnirS mutant of *P. aeruginosa* did not disperse after 6 days of cultivation, in contrast to the wild-type strain. The biofilm of the ΔnorCB mutant lacking the NO reductase dispersed to a greater extent than the wild-type biofilm, so that numerous hollow voids were formed and cell death was enhanced. NO as a signal molecule is likely to be implicated in the operation of quorum-sensing systems. Quorum sensing signals (acylated homoserine lactones, oligopeptides, furanones, and quinolones, see Lecture 3 and 4 above) activate processes that depend on high microbial population density. NO is similar to QS signals: its size is small, it accumulates extracellularly, and rapidly penetrates into the cell. Unlike other, more specific, signals, NO is capable of interacting with diverse targets (Schreiber, 2006).

Microbially produced NO exerts multifarious effects on eukaryotic organisms. In the flatworm *Caenorhabditis elegans,* NO synthesized by *B. subtilis* and *E. coli* behaves as a transcription activator. Its effect on *C. elegans* enterocytes increases the flatworm's heat resistance and prolongs its life expectancy (Gusarov & Nudler, 2005). A similar mechanism may operate in higher animals, enabling the intestinal microbiota to slow down the host organism's aging process. The microbiota includes gram-positive bacteria of the genera *Lactobacillus, Streptococcus,* and *Lactococcus* that posess NO synthases (Yarullina et al., 2011; Oleskin & Shenderov, 2016). Both microbiota- and host-produced NO, can perform cyto-, vaso, and neuroprotective functions (Medinets et al., 2015).

Lact. plantarum probiotic strains are efficient NO producers. The probiotic strains-synthesized NO is rapidly degraded by *E. coli* and *Staph. aureus* both *in vitro* and in the intestines of test animals (Midtvedt, 2006).

In mammals, NO is involved in regulating impulse transfer across synaptic clefts, regional blood flow, intestine peristalsis, and water and electrolyte transport. NO influences the operation of the immune and cardiovascular systems and regulates energy metabolism (Ivashkin &

Drapkina, 2001; Schreiber, 2006; Larsen et al., 2011; Lundberg & Weitzberg, 2013; Gusarov et al., 2014; Hezel & Weitzberg, 2015; Oleskin & Shenderov, 2016).

At low concentrations, NO behaves as a neurochemical both in the brain and in the peripheral nervous system. It is implicated in learning and cognition activities. Mice with a defective nNOS are characterized by elevated locomotive activity, virility that is retained for a long time, high fertility, and long-term depression (LTD). Male mice lacking neuronal isoform (NOS-1-/- or nNOS-/-)-encoding genes are more aggressive than wild-type males (Nelson et al., 1995). nNOS-containing mice are more resistant to experimental stroke caused by ligaturing the middle cerebral artery.

NO also affects the functions of ionotrophic glutamate receptors (iGluRs) and acid-sensitive ion channels (ASICs) that are present in various areas of the central nervous system and in other mammalian tissues. Dysfunctional ion channels pose the threat of neurological disorders. NO can modify iGluRs and ASICs either directly, by S-nitrosylation of cysteine, or indirectly, via cGMP protein kinase G (PKG)-dependent phosphorylation (Wang et al., 2012).

To sum up, the following conclusions can be drawn from the above data:

• The regulatory effects of NO on biofilm dispersal in symbiotic and/or parasitic microorganisms seem to suggest that it is involved in the chemical communication between them and the host organism that contains various types of cells producing NO.

• The fact that NO is produced by a wide variety of microbial species enables it to function as an interspecies signal molecule within the microbial association inhabiting the GI tract and other niches in the human/animal organism.

9.2.2. Carbon monoxide (CO). CO has long been considered as the most widespread air pollutant and a “silent killer” because of its high affinity for reduced iron in hemoglobin that transports oxygen to the tissues of the animal/human organism.

Endogenous CO was discovered in the human organism in 1950. Various plants and animals, including humans, have been revealed to synthesize CO as an intermediate product formed during heme degradation by heme oxygenases termed the inducible (HO-1) and the constitutive (HO-2) heme oxygenase, respectively.

Fig. 20

In spite of these detrimental effects, CO is also formed by bacteria, including pathogens, plant and animal symbionts, and soil and marine species that contain heme oxygenases (Fig. 20). Some bacteria contain the specific *coo* operon that codes for CO dehydrogenase. Paradoxically, like many other poisons, CO becomes useful when diluted to very low concentrations. Unlike NO with a very short lifetime, CO is a sufficiently stable molecule that easily enters cells because it readily crosses cell membranes. Its biological effects include anti-apoptic, antiproliferative, anti-inflammatory, and cytoprotective activities. The molecular basis of these effects is that CO regulates ion channels/transporters in various subtypes of epithelial cells.

Recently, convincing evidence has been presented that CO possesses all typical properties of a “gasotransmitter” with a broad biological action spectrum (Berne et al., 2012; Tinajero-Trejo et al., 2013). The protective influence of CO on the central nervous system was investigated in model systems. CO inhalation (up to 250 ppm) protects test animals against I/R brain injury and ischemic stroke (Wang et al., 2012; Zeynalov & Dore, 2009). The same CO concentrations prevent neurological damage (neuronal apoptosis) in a pig model of deep hypothermic circulatory arrest.

CO is a physiological signal molecule regulating the functions of membrane channel proteins and transporters (Peers et al., 2015). The antimicrobial and anti-inflammatory effects of CO and CO-releasing molecules (CO-RMs), e.g. metal carbonyl CO-RM-3, RU(CO)3Cl, and glycinate, implicate the opening of K+/Na+ channels in eukaryotic and bacterial cells. This decreases the proton motive force and disrupts ion transport. The mechanisms of protection of the nervous and cardiovascular systems in the presence of CO-RMs have not been completely

elucidated yet. Mitochondria represent the main target of CO. This does not rule out an additional effect of CO-RMs, the stimulation of ROS production in mitochondria. It was established that the CO released at low CO-RM concentrations can produce a cardioprotective effect, due to its antioxidant properties. Recently, increasing attention has been paid to the use of heme oxygenases, CO inhalation, and CO-RMs for treating various infection and inflammation processes as well as cardiovascular and, potentially, neurological problems (Smith et al., 2011; Berne et al., 2012; Wegiel et al., 2013; Almeida et al., 2015; Peers et al., 2015).

9.2.3. Hydrogen sulfide (H_2S). This is a highly water-soluble gas that readily penetrates into cells. At a concentration of 1 ppm, it can be recognized because of its rotten egg odor; 4 ppm H_2S causes a headache; at still higher concentrations (500 ppm and above), H_2S can produce a lethal effect (Sitdikova & Zefirov, 2010; Gadalla & Snyder, 2010). Intoxication is due to H_2S binding to the iron of cytochrome c oxidase, which inactivates the enzyme and abolishes oxidative phosphorylation in mitochondria (Sitdikova & Zefirov, 2010). Despite its toxic effect, H_2S has recently been established to play a vital role in bacteria, plants, and invertebrate and vertebrate animals, including mammals.

H_2S synthesis is catalyzed by three enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE or CTH), and 3-mercaptopyruvate sulfur transferase (3-MST) (Farrugia & Szurszewski, 2014). H_2S-synthesizing enzymes are expressed, to a different extent, in the cardiovascular, nervous, immune, urinary, respiratory, and GI system (Polhemus & Lefer, 2014; Oleskin & Shenderov, 2016).

The microbiota of the large intestine is implicated in the generation of H_2S in the human organism. A red meat-enriched diet stimulates H_2S synthesis by the indigenous microbiota by supplying the large intestine with a significant amount of sulfated proteins. Some other dietary ingredients also provide substances from which H_2S can be produced, including those present in garlic, onions, and other food stuffs.

Based on the literature data referenced above, sources of microbial H_2S include, e.g., *E. coli* strains that possess two enzymes (L-cysteine transaminase and 3-mercaptopyruvate sulfur transferase) which catalyze its formation. Some representatives of intestinal bacteria (*Prevotella, Bacteroides, Helicobacter, Peptococcus,* and *Akkermansia*) produce glycosyl sulfatases or similar enzymes that promote production of sulfates from sulfomucins. Sulfate-reducing bacteria compete with methanogenic microorganisms for H_2 molecules both in vitro and in vivo. If the oxygen content is low (under microaerophilic conditions), H_2S at millimolar concentrations can serve as an electron donor and an energy source. H_2S behaves either as a potential toxin or as a signal molecule, depending on its concentration. At high (millimolar) concentrations, as already mentioned, H_2S is a highly toxic compound that causes a whole spectrum of pathological processes, including those brought about by inhibiting mitochondrial functions; it also produces genotoxic effects by damaging the DNA.

In contrast, when applied at low (micromolar) concentrations, H_2S serves as an inorganic electron donor for mitochondria. In addition, H_2S regulates a number of physiological processes:

- the inflammatory response,
- apoptosis,
- cell proliferation,
- neuronal impulse transfer,
- smooth muscle tone.

The varied regulatory effects of H_2S are due to its capacity for modifying proteins via reducing disulfide (S=S) bonds or attaching a sulfur atom to a thiol group (-SH). As a result, -SH is converted into a hydropersulfide residue (- SSH). The physiological effects of H_2S are due to the influence of this molecule on various molecular targets in diverse tissues, including heme-containing proteins, ion channels, and signal proteins. channels as well as calcium and chloride channels. H_2S enhances the activity of transporter systems by facilitating the release of

antioxidants that are required for protecting the systems against toxic substances-caused damage (reviewed, Oleskin & Shenderov, 2016, 2020).

In the nervous system, H_2S is an active neuromodulator and neuroprotector in various brain cells Cystathionine-β-synthase present in the cells of various brain areas is responsible for generation of H_2S. It activates transmembrane ATP-associated channels (in neurons both inside and outside the brain) via modulating glutamate-dependent N-methyl-D-aspartate receptors (Sitdikova & Zefirov, 2010). H_2S regulates the activity of serotonergic neurons and induces the release of corticotrophin-releasing hormone.

In astrocytes (a type of glial cells in the nervous system), H_2S influences the intracellular level of calcium that plays a major role in intercellular communication. The intracellular calcium level rapidly increases upon the addition of H_2S; subsequently, it slowly decreases. These effects of H_2S and various H_2S donors were revealed in astrocyte cultures and in the glia of hippocampal sections (Ishigami et al., 2009). H_2S was established to exert an influence on the operation of the peripheral nervous system, which in volves modulating pain perception and transferring pain signals to the relevant brain areas (Sitdikova & Zefirov, 2010). The effects of H_2S are removed by NMDA antagonists.

The clinically important aspects of H_2S include the following points (reviewed, Oleskin & Shenderov, 2016, 2020):

1. Human subjects with seizures (like epilepsy), psychiatric disorders, or abnormal electroencephalograms mostly lacked the enzymes (CBS) that are involved in H_2S synthesis.
2. Patients with Down syndrome, in contrast, are characterized by abnormally high concentrations of these enzymes in the brain tissue.
3. The H_2S content in the brain tissue was decreased by over 50% in Alzheimer patients
4. H_2S prevented nervous cell damage and apoptosis in a model system in which Parkinson disease was caused by administering the toxin rotenone to test animals.
5. H_2S functions as a signal molecule in the visual system of mammals. H_2S synthesis-catalyzing enzymes (CBS and CSE) were detected in various kinds of eye cells, and H_2S was found to regulate sympathetic and glutamatergic neurotransmission during the signal transduction processes in this system.

Even though the use of gaseous H_2S for therapeutic purposes is hardly feasible, chemical compounds that release H_2S in the human organism either rapidly (NaHS) or slowly (GYY 4137) can apply. This gives grounds for the suggestion that H_2S should be used for medical purposes (Pouokam & Diener, 2012). H_2S treatment is considered an efficient therapeutic technique for a number of diseases (e.g., lung cystic fibrosis and kidney problems in patients with hereditary hypertension) that are characterized by enhanced Na+ influx into cells (Hine et al., 2015). In all likelihood, the employment of chemical donors or microbial producers of H_2S for medical purposes will hold much promise as a potential pharmacological approach to the treatment of neurodegenerative diseases.

Short-chain fatty acids (SCFAs) interact with the host's nervous and immune system via (a) activation of chemoattractant membrane receptors including free fatty acid receptors (GPR41 and GPR43), the niacine/butyrate receptor (GPR109a), and the olfactory receptor Olfr-78 and (b) inhibition of histone deacetylases. **Gas molecules (gasotransmitters)** including those of nitric oxide (NO), carbon oxide (CO), and hydrogen sulfide (H_2S) perform neurochemical functions and are produced by microbiota. Importantly, **NO** functions include dilation of blood vessels, activation of the brain zones responsible for grooming behavior in animals, cell differentiation, apoptosis (programmed cell death), and cytotoxic effect in terms of the immune response to foreign or tumor cells.

LECTURE 10. INTERACTION BETWEEN THE MICROBIOTA AND THE IMMUNE SYSTEM INCLUDING CHEMICAL SIGNAL EXCHANGE

The final part of my course concentrates on the interaction between the microbiota and the immune system. The main principles are as follows:

1. The immune system-microbiota alliance allows the induction of protective responses to pathogens and the maintenance of regulatory pathways involved in tolerance to innocuous agents
2. The immune system is composed of a complex network of innate and adaptive components endowed with an extraordinary capacity to adapt and respond to highly diverse challenges
3. Collectively this cellular network acts as an important regulator of host homeostasis allowing to sustain and restore tissue function in the context of intestinal and environmental factors (Belkaid & Hand, 2014; Hevia et al., 2015; Oleskin et al., 2017a; Yao et al., 2020).

10.1. Historical. The field discussed in this lecture may be termed *microbial immunology.* Its development actually dates back to 1892 when Albert Doderlein revealed that bacteria such as lactobacilli stabilize the vaginal ecosystem preventing the intrusion of potentially pathogenic microorganisms. It was more recently established that mother milk contains some immunoglobulins A and stimulates beneficial bacteria exemplified by *Bifidobacterium*. The interest in this developing field of research was also generated by problems caused by antibiotics and an unhealthy diet in Western countries. Sometimes even measures aimed at eliminating parasitic worms such as nematodes proved to be detrimental to the microbiota. A disrupted microbiota results in immune problems including both low immune activity fraught with infections and malignant tumors as well as abnormally high immune responsiveness bringing about autoimmune disorders.

Promising research was conducted with animals raised under aseptic conditions. These germ-free (GF) animals showed manifest signs of disrupted immune system development. GF mice had small Peyer's patches (which are the immune organs located in the intestinal wall), and a decreased number of CD4+ T cells and IgA-producing plasma cells.

10.2. Immunological implications of microbiota-host interaction. This lecture reiterates some of the key points that were emphasized in the preceding lectures. Therefore, there will be relatively few new references. Symbiotic beneficial microorganisms are often referred to as *commensals*, although there are some terminological issues involved. They make the gut wall more resistant to opportunistic pathogens. They strengthen the intestinal barrier (prompt epithelial cell maturation and angiogenesis, i.e. blood vessel formation).

There is a nice old English saying: *Good fences make good neighbors.* You might be on friendly terms with your neighbors but there should be a sufficient distance from them, in order to prevent possible conflicts. A similar strategy is widely used by our gut.

The central strategy utilized by the host is to minimize contact between microorganisms and the epithelial cell surface, thereby limiting tissue inflammation and microbial translocation. In other words, microorganisms should not be allowed to penetrate the epithelium, the mucosa, and enter the submucosal tissue, which might result in their spreading in the organism and getting as far as the bloodstream. The distance between the resident microbiota and the gut wall proper is secured by the so-called firewall in the gut. It incorporates epithelial cells, the mucus, IgA, antimicrobial peptides (AMPs), and immune cells, including intestinal dendritic cells. It seems likely that low-intensity chronic inflammation of the gut as a normal phenomenon. This low-intensity chronic inflammation enables the immune system to constantly produce antibodies and T cells specific to commensals. The immune system is maintained in a sufficiently active state; it is ready to respond to an infectious agent. Human serum normally contains antibodies

and T cells specific to commensals suggesting that a certain degree of commensal recognition is a common occurrence and in most cases is not associated with pathological responses (Belkaid & Hand, 2014; Hevia et al., 2015; Rees et al., 2018).

Such constitutive sensing of commensals plays an important homeostatic role, while acute responses to the microbiota are associated with pathogenesis. Even useful bacteria are not allowed to penetrate the mucosa, and they are destroyed by macrophages upon crossing the epithelial barrier. Of paramount importance are Th17 cells that regulate the functions of epithelial cells and their homeostatic interaction with the microbiota, control AMP production, and promote epithelial regeneration and mucus formation. Studies with GF animals having underdeveloped immunocytes and other recent data indicate that tolerance—the active suppression of inflammatory responses to food and other orally ingested antigens—cannot be induced in the absence of gut microbiota-derived signals. Foxp3+ regulatory T (T_{reg}) cells maintain peripheral and mucosal homeostasis and help develop tolerance to commensal and environmental antigens (a combined effect of thymally and GI-induced T_{reg}s). These T_{reg}s are induced by antigen-presenting cells (CD103+CD11β+ DC.) that produce cytokine TGF-β and retinoic acid. A part of induced Tregs in the colonic tissue is specific for antigens derived from the commensal microbiota. Induction of Treg cells is one of the mechanisms of action of probiotics. Some of the regulatory effects of probiotics in the context of inflammatory diseases and atopic eczema in neonates and infants are associated with the induction or expansion of T_{reg}s (Yano et al., 2015; Ayres, 2016; Liang et al., 2018).

The useful probiotic bacterium *Bacteroides fragilis* protects mice from colitis caused by *Helicobacter hepaticus*. The probiotic produces polysaccharide A (PSA) that induces and expands IL-10-producing Treg cells, engages the TLR2 expressed by T cells, and limits Th17 responses. The link between the microbiota and the induction of regulatory cells can enable the identification of the next generation of probiotics with superior capacity to induce T_{reg} cells (Chiu et al., 2014).

Bacterial neuroactive products including SCFAs control various aspects of the immune response. Butyrate regulates the size and function of the regulatory T cell network by promoting the induction and fitness of regulatory T cells in the colonic environment. The probable mechanism is that butyrate regulates gene expression epigenetically by inhibiting histone deacetylases (HDAs). Importantly, recognition of the commensal-derived metabolites SCFAs by innate immune cells is critical for the regulation of inflammation in response not only to intestinal damage, but also to arthritis (joint inflammation) and allergy. Importantly, in the gut, TLR activation by commensals promotes tissue repair and host survival. Commensals can also tune the function of inflammatory monocytes, a population of cells involved in the control of pathogens (reviewed, Oleskin & Shenderov, 2016, 2020; Yao et al., 2020). Importantly, intestinal epithelial cells (IECs) “are an integral component of the innate immune system and affect the intestinal microenvironment through the identification and uptake of SCFAs” (Yao et al., 2020).

These results highlight a major role for the microbiota in shaping the repertoire, number, and activation of tissue-resident T cells of various types and in the maintenance of host-microbe mutualism at barrier sites (see Belkaid & Hand, 2014; Mazzoli & Pensione, 2016; Liang et al., 2018; Oleskin et al., 2017a; Oleskin & Shenderov, 2019, 2020; Yao et al., 2020).

Interestingly, the immunotropic activity of the microbiota can be enhanced by optimizing the diet. The diet exerts a strong influence on the microbiota. In the absence of complex natural carbohydrates, the microbiota produces little SCFAs (e.g., butyrate) and predominantly carries out proteolytic fermentation that may result in forming potentially toxic proinflammatory compounds, including various amines and ammonia. These compounds are implicated in gut dysbiosis and pose the risk of the development of nonspecific ulcerous colitis and colorectal cancer. SCFAs (see also 9.1 above) produced by bacteria from food fibers, in contrast, possess anti-inflammatory and anticarcinogenic properties (Carlucci et al., 2016, Chen & Vitetta, 2018).

Importantly, the gut wall contains the gut-associated lymphoid tissue (GALT). “GALT comprises Peyer’s patches (PPs), interdigitating lymphocytes, plasma cells and lymphocytes present in the lamina propria, and mesenteric lymph nodes. The role of GALT is to manage the immune response via up-take of gut luminal antigens through M-cells, and to initiate antigen-specific immune responses in the host” (Yao et al., 2020).

10.3. Colonization resistance. Colonization resistance, or protection of the host from exogenous pathogens by commensal bacteria, is based upon several essential resistance strategies. Commensals compete with potential pathogens, and their SCFAs downregulate the expression of virulence genes (type 3 secretion system in *Salmonella enterica* and *S. typhimurium*).

“The maintenance of mucosal immunologic homeostasis is an enormous task demanding discrimination between billions of beneficial microbes and rare, pathogenic invaders. Gut homeostasis is characterized by the dominance of obligate anaerobic members of *Firmicutes* and *Bifidobacteriaceae*, whereas an expansion of facultative anaerobic *Enterobacteriaceae* is a common marker of gut dysbiosis” (Yao et al., 2020).

Commensals make the environment unsuitable for pathogens both in the GI tract and in other important niches (e.g., lactobacilli in the vagina lower the pH; likewise, intestinal microbiota is known to produce SCFAs that acidify the cell interior of pathogenic bacteria, disrupting the operation of their membranes, see 9.1 above). The nocuous pathogen “*Shigella flexneri* requires oxygen for the competent secretion of virulence factors, but commensal facultative anaerobes, including members of the Enterobacteriaceae family, consume the residual oxygen, leading to incomplete expression of *Shigella* virulence factors in the gut lumen” (Yao et al., 2020).

Microbial SCFAs such as butyrate stimulate peroxisome proliferator-activated receptor gamma (PPAR-γ), resulting in mitochondrial β-oxidation of SCFAs and increased oxidative phosphorylation in gut epithelial cells. This brings about a decrease in local oxygen concentration in the gut. “The obligate anaerobic SCFA-producing bacteria grow vigorously in such an environment, while the facultative anaerobic enteric pathogens’ growth is suppressed… Conversely, inhibition of the PPAR-γ signaling pathway induces metabolic reprogramming, gut dysbiosis, and SCFA exhaustion” (Yao et al., 2020). Commensals produce antimicrobial peptides: host-friendly *E. coli* releases bacteriocin that kills the pathogenic *E. coli* strain EHEC.

Finally, commensals prepare innate cells for responding to pathogens: gut microbes control the production of prointerleukins, e.g., pro-IL-1β by intestinal macrophages (converted to mature IL-1β in response to infection) Commensals and pathogens contain the same markers that act on Toll-like and Nod-like receptors (see Belkaid & Hand, 2014; Mazzoli & Pensione, 2016; Liang et al., 2018; Oleskin et al., 2017a; Oleskin & Shenderov, 2019, 2020; Yao et al., 2020).

My question to the students: why is the existence of the same markers so important in terms of immune system readiness to respond to potentially pathogenic microorganisms?

10.4. Miroorganisms’ role in terms of immune responses. The following is direct evidence for the involvement of the microbiota in building up adaptive immune responses. Relevant data include the following (Belkaid & Hand, 2014; El Aidy et al., 2016; Oleskin & Shenderov, 2020; Yao et al., 2020):

- impaired host immune responses to pathogens in mice treated with antibiotics or raised under germ-free conditions.
- experimental small intestine infection with the protozoan parasite *Encephalitozoon cuniculi* in which protective Th1 and Th17 responses are compromised in the absence of commensals.

Any vaccination is only successful if the microbiota is normal and promotes the development of the immune system; it fails to take effect with dysfunctional microbiota

(resulting, e.g., from malnutrition). Therefore, vaccination of starved children in Africa does not protects them against infections.

Not only can changes in gut microbiota composition and density affect local immune responses. These changes can also alter immunity and inflammation in organs distal from the intestine. The products of GI microbes in the bloodstream produce important systemic effects, including the following:

- function as TLR or NOD ligands
- improve the killing of *Streptococcus pneumonia* and *Staph. aureus* by bone-marrow derived neutrophils in a NOD1-dependent manner
- contribute to steady-state hematopoiesis (blood cell development)
- promote monocyte liberation from the bone marrow
- promote the inflammasome-mediated induction of IL-1β and IL-18 secretion
- control the levels of TNF-α and ROS in tumor (e.g., myeloid) cells

Antibiotics that destroy commensals compromise immune responses. All these data reveal a major role for the microbiota in shaping the repertoire, number, and activation of immune cells. This role includes, importantly, the impact of the microbiota on tissue-resident Treg cells and on the maintenance of host-microbe mutualism at barrier sites.

Fig. 21

The picture shown on this page (Fig. 21) is concerned with an interesting clinical paradox in which a negative effect of anticancer treatment, nonetheless, has positive consequences for the patient. It is for the students to explain the situation (a creative task).

In light of all the above, some general principles can be formulated:

- Commensals control various aspects of immunity including those associated with anti-tumoral responses
- Exposure to microbial ligands influences systemic immunity both in the steady state and in the context of inflammation
- Commensal bacteria-derived signals influence the gene expression profiles of immune cells via epigenetic modification of genes involved in innate responses thus enabling baseline expression of host defense factors and rapid responses upon encounter with a pathogen
- Commensal bacteria establish a threshold of activation and regulation required for immune fitness.

To use an English idiom, the microbiota *keeps the powder dry* in the immune system.

10.5. Local immunity and the microbiota. Importantly, different part of our body, and not only the intestine, have their own microbial helpers (Belkaid & Hand, 2014; Oleskin et al. 2017a; Oleskin & Shenderov, 2020). Local immunity is influenced by commensals residing in the lung, skin, and other barrier sites. Each barrier tissue is a complex and, in some cases, unstable composite of microbes and host structural, hormonal, nervous, and immunological networks, with each of these systems controlled by microbiota. This is exemplified by skin commensals (esp. *Streptococcus epidermidis*). It modulates the dermal T cell function, resulting in the production of IL-1α. It, in turn, exerts a significant influence on the production of inflammatory cytokines (IFN-γ and IL-17A). In similar fashion, the oral microbiota brings about an increase in local inflammatory activity. This results in an increase in IL1β content.

Attention should be paid, nevertheless, to destructive role of even the normally useful microbiota under specific circumstances. This is exemplified by normally beneficial bacteria that help a helminth, the parasitic nematode *Trichuris,* develop from its egg by attaching to one of the nematode egg's poles and promoting the hatching of the nematodes (Vejzagic et al., 2015). This is what the English idiom *A fly in the ointment* is about.

Nonetheless, the role of our normal indigenous microbiota is predominantly beneficial (it includes probiotics), and this seems to be the most important message you should get from this lecture as well as from the whole course.

The **immune system-microbiota alliance** allows the induction of protective responses to pathogens and the maintenance of regulatory pathways involved in tolerance to innocuous agents. In particular, the microbiota shapes the repertoire, number, and activation of tissue-resident T cells and promotes the functioning of host-microbe **barrier sites**. Commensals compete with potential pathogens, and their SCFAs downregulate the expression of virulence genes. Vaccination is only successful if the microbiota is normal and promotes the development of the immune system, *keeping the powder dry* in it.

Note: It is suggested that this lecture should be followed by the final seminar that may include evaluating the students' performance and marking them.

ACKNOWLEDGMENTS

Thank you for attending this hopefully useful course of lectures. I wish all of you a successful and efficient scientific career.

I wish to thank, most sincerely, my wife Julia for her continuous support during the course of the work on this guidebook. I gratefully acknowledge the insightful questions and comments made by my students that have helped me produce the guidebook.

GLOSSARY

AGONISTIC BEHAVIOR. Comprises all conflict-related forms of social behavior.
AGGRESSION. In ethology, the term means approaching an opponent and inflicting damage on him/her or at least generating stimuli that cause him/her to submit (Tinbergen, 1968).
AUTOREGULATORS (AUTOREGULATORY SUBSTANCES). Microbial metabolites that are released by a cell population, or its part, into the medium. Many autoregulators are not utilizable in terms of constructive or energy metabolism but perform major communicative functions and, therefore, influence the physiological state and the reproductive potential of the cells involved (El'-Registan, 1988).
AFFILIATION. Social behavior involving an individual animal's tending to approach and remain near conspecifics (Dewsbury, 1978), particularly those belonging to the same family or social group
BACTERIOTYPES (ENTEROTYPES). Putative classification of human individuals into three bacteriotypes (entrotypes), depending on the dominance of the genera *Prevotella, Bacteroides* or *Ruminococcus* in the gut microbiota (Arumugam et al., 2011; Clarke et al., 2014).
BIOGENIC AMINES. A group of nitrogen-containing organic compounds performing neurochemical and/or hormonal functions and serving as signals in cell systems. They include catecholamines (dopamine, norepinephrine, and epinephrine), serotonin, histamine, octopamine, tyramine, etc.
BIOFILMS. "Matrix-enclosed microbial accretions that adhere to biological or non-biological surfaces" (Hall-Stoodley et al., 2004, p.95).
COMMUNICATION. Exchanging information and obtaining it from other living organisms (Nikolaev, 2000).
CONTACT COMMUNICATION. Communication based on direct contact between livng organisms, e.g., microbial cells.
COOPERATION. Interaction between two or more individuals for the purpose of solving a problem or carrying out a task. Alternatively, cooperation is defined from the viewpoint of a whole group (biosocial system): cooperators contributing to the collective good are contrasted with cheaters (free riders) exploiting it (Hochberg et al., 2008, modified).

DISTANT CHEMICAL COMMUNICATION. Distant information transmission among living organsms based on signal molecules.
DISTANT PHYSICAL COMMUNICATION. Distant information transmission among living organisms involving electromagnetic and/or acoustic waves or other physical communication channels.
DYSBIOSIS. Microbiota disruption (in the GI tract) manifesting itself in a decrease in the number of useful microorganisms and impoverishment of taxonomic diversity of the microbiota, which is frequently accompanied by an increase in the number of potential pathogens.
ENTERIC NERVOUS SYSTEM (ENS). The semi-autonomous part of the nervous system located in the intestinal wall.
ETHOLOGY. A field of biology dealing with animal behavior. Many ethological concepts are applicable to the behavior of free-living (microbial) cells as well as cells within the tissues and organs of multicellular organisms.
GASOTRANSMITTERS. Gaseous substances, such as nitric oxide, carbon oxide, hydrogen sulfide, and, probably, other gases that perform nerochemical functions.
GASTRO-INTESINAL (GI) MICROBIOTA. A complex organized consortium of communicating microorganisms (“the microbial organ”) that supplies the host organism with indispensible organic substances from vitamins to hormones and neurochemicals and also performs many other vitally important functions.
GERM-FREE (GF) ANIMALS. Animals raised under aseptic (germ-free) conditions.
HETEROMORPHISM. Formation, in a microbial population, of abnormal cell types, including cells with disrupted division and defective cell walls as well as cell wall-lacking forms (oval or spherical cells of the spheroplast or protoplast type), filamentous, giant, and miniscule cells such as L forms.
INTESTINAL IMMUNE SYSTEM. Composed of immune cells in the gut-associated lymphatic tissue (GALT).
ISOLATION (AVOIDANCE). Conflict-mitigating behavior that does not directly involve aggression and implies avoiding a potential opponent.
LOYAL BEHAVIOR. All kinds of friendly interactions among individuals; loyal behavior helps consolidate a biosocial system.
MATRIX. Biopolymer substances that bind together and envelop the cells of a microbial colony or biofilm.
METABIOTICS. Biologically active substances that are produced by symbiotic (probiotic) microorganisms and exert a positive influence on various physiological processes and activities (Shenderov et al., 2017, p. 27).
MICROBIAL ENDOCRINOLOGY. The area of research dealing with the role of hormones and neurochemicals in communication among microorganisms and in the host–microbiota dialogue (Lyte, 2010, 2011, 2013a, b; 2016).
MICROBIAL METABOLOME. Low molecular weight (< 1500 Da) metabolites of microbial origin.
MICROBIOME. Total genome of all microorganisms, e.g., of the GI microbiota of the human organism.
MICROBIOTA-GUT-BRAIN AXIS. Incorporates the whole gut microbiota, the enteric, parasympathetic, sympathetic nervous system, and the CNS; of paramount importance is the interaction of these systems with the endocrine and immune system.
NEUROCHEMICALS. Substances that transmit messages between nervous cells (neurons) or from a neuron to a muscular or glandular cell (that carries out the neuron’s command) and/or modulate the efficiency of impulse transmission. In this work, we do not pay special attention to the differences between *neurotransmitters* that directly transmit impulses across the synaptic cleft between nervous cells and *neuromodulators* that modulate neurotransmitter effects; the more general term neurochemicals is mostly preferred throughout this work.

NEUROPEPTIDES. Peptide neucrochemicals that often perform neuromodulatory functions by altering the efficiency of impulse transmission across synapses that use other agents as neurotrans-mitters.
NUTRITIONAL PSYCHIATRY. A recently developed subfield of psychiatry that is based on using the diet, including food-associated microorganisms and their products, for the purpose of preventing and treating mental diseases (Sarris et al., 2015).
POPULATION ORGANIZATION AND COMMUNICATION-CENTERED PARADIGM (POCCP) in modern microbiology. A subfield of microbiology that focuses on cell-cell interactions and signal exchange in the microbial world as well as on the structure and functioning of microbial colonies and biofilms.
PREBIOTICS. “Specific non-digestible food ingredients (including non-digestible oligosaccharides) which selectively feed intrinsic beneficial bacteria, consequentially stimulating their growth and activity” (Cohen Kadosh et al., 2021).
PROBIOTICS. Live microorganisms that, “when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2006).
PSYCHOBIOTICS. Live microorganisms that, when administered in adequate amounts, confer a health benefit on patients with psychiatric problems (Cryan & Dinan, 2012).
QUORUM-SENSING (QS) SYSTEMS. Signaling systems that control, in a cell density-dependent fashion, many important microbial processes including bioluminescence, synthesis of antibiotics and enzyme complexes, intercellular transport of genetic information (transformation and conjugation), cell aggregation, protein secretion, biofilm and gas vesicle formation, sporulation, virulence factor production, etc.
SHORT-CHAIN FATTY ACIDS (SCFAs). Saturated unbranched fatty acids with short carbon chains. Of paramount importance in biological terms are SCFAs with two to four carbon atoms in the chain, i.e., acetic, propionic, and butyric acid, or, according to their anion names, acetate, propionate, and butyrate.
SOCAL BEHAVIOR. Any behavior that affects another individual’s (cell’s) evolutionary fitness (Ulvestad, 2009).
SOCIOMICROBIOLOGY. The subfield of microbiology that is concerned with communication and collective behavior in microorganisms (Sekowska et al., 2009).

REFERENCES

Note: the literature specifically recommended for students is **bold-typed**.

Abbott, R. J., Pye, I. F., & Nahorski, S. R. (1982). CSF and plasma GABA levels in Parkinson's disease. Journal of Neurology, Neurosurgery, & Psychiatry, 45, 253-256.

Aguilar, C., Eichwald, C., & Eberl, L. (2015). Multicellularity in bacteria: from division of labor to biofilm formation. In: I. Ruiz-Trillo & A. M. Nedelcu (Eds.), Evolutionary Transitions in Multicellular Life (pp.79-85). Dodrecht: Springer-Verlag + Business Media.

Ahn, S. K., Hong, S., Park, Y. M., Choi, J. Y., Lee, W. T., Park, K. A., & Lee, J. E. (2012). Protective effects of agmatine on lipopolysaccharide-injured microglia and inducible nitric oxide synthase activity. Life Sciences, 91(25),1345-1350.

Almeida, A. S., Figueiredo-Pereira, C., & Vieira, H. L. A. (2015). Carbon monoxide and mitochondria-modulation of cell metabolism, redox response and cell death. Frontiers in Physiology, 6, 33.

Althaus, M. & Clauss, W. G. (2013). Gasotransmitters: novel regulators of ion channels and transporters. Frontiers in Physiology, 4, 27.

Anuchin, A. M, Chuvelev, D. I., Kirovskaya, T. A, & Oleskin, A. V. (2008). Effect of neuromediator monoamines on the growth characteristics of *Escherichia coli K-12.* Microbiology (Russia), 77(6), 758-765.

Aoki, S. K., Webb, J. S., Braaten, B. A., & Low, D. A. (2009). Contact-dependent growth inhibition causes reversible metabolic downregulation in *Escherichia coli.* Journal of Bacteriology, 191(6), 1777-1786.

Arreola, R., Becerril-Villanueva, E., Cruz-Fuentes, C., Velasco-Velazquez, M. A., Garces-Alvarez, M. E., Hurtado-Alvarado, G., Quintero-Fabian, S., & Pavon, L. (2015). Immunomodulatory effects mediated by serotonin. Journal of Immunological Reserarch. 2015. 354957.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., Fernandes, G. R., Tap, J., Bruls, T., Batto, J.-M., Bertalan, M., Borruel, N., Casellas, F., Fernandez, L., Gautier, L., Hansen, T., Hattori, M., Hayashi, T., Kleerebezem, M., Kurokawa, K., Leclerc, M., Levenez, F., Manichanh, C., Bjørn, N. H., Nielsen, T., Pons, N., Poulain, J., Qin, J., Sicheritz-Ponten, T., Tims, S., Torrents, D., Ugarte, E., Zoetendal, E. G., Wang, J., Guarner, F., Pedersen, O., de Vos, W. M., Brunak, S., Doré, J., MetaHIT Consortium (additional members), Weissenbach, J., Dusko, E. S., & Bork, P. (2011). Enterotypes of the human gut microbiome. Nature, 473, 174-180.

Auteri, M., Zizzo, M. G., & Serio, R. (2015). GABA and GABA receptors in the gastrointestinal tract: from motility to inflammation. Pharmacological Research, 93, 11-21.

Ayres, J. S. (2016). Cooperative microbial tolerance behaviours in host-microbiota mutualism. Cell, 165, 1323-1331.

Babin, V. N., Domaradsky, I. V., Dubinin, A. V., & Kondrakova, O. A. (1994). Biochemical and molecular aspects of the symbiosis of the human being and his microflora. Rossyskiy Khimicheskiy Zhurnal (Russian Chemical Journal), 38, 66-78.

Baig, A. M. & Ahmad, H. R. (2017). Evidence of a M1-muscarinic GPCR homolog in unicellular eukaryotes: featuring Acanthamoeba spp. bioinformatics 3D-modelling and experimentations. Journal of Reception & Signal Transduction Research, 37(3), 267-275.

Baig, A. M., Rana, Z., Tariq, S., Lalani, S., & Ahmad, H. R. (2018). Traced on the timeline: discovery of acetylcholine and the components of the human cholinergic system in a primitive unicellular eukaryote Acanthamoeba spp. ACS Chemical Neuroscience, 9(3), 494-504.

Bansal, T., Englert D., Lee, J, Hegde, M., Wood, T. K., & Jayaraman, A. (2007). Differential effects of epinephrine, norepinephrine, and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. Infection & Immunity, 75, 4597-4607.

Bansal, T., Alaniz, R. C., Wood, T. K., & Jaraman, A. (2010). The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proceedings of the National Academy of Sciences of the USA, 107(1), 228-233.

Barker, D. J. & Osmond, C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet, 1, 1077-1081.

Barraud, N., Hassett, D. J., Hwang, S.-H., Rice, S. A., Kjelleberg, S., & Webb, J. S. (2006). Involvement of nitric oxide in biofilm dispersal of *Pseudomonas aeruginosa.* Journal of Bacteriology, 188, 7344-7353.

Barraud, N., Schleheck, D., Klebensberger, J., Webb, J. S., Hassett, D. J. Rice, S. A., & Kjelleberg S. (2009a). Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. Journal of Bacteriology, 191, 7333-7342.

Barraud, N., Storey, M. V., Moore, Z. P., Webb, J. S., Rice, S. A., & Kjelleberg, S. (2009b). Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. Molecular Microbiology, 2. 370-378.

Barrett, E., Ross, R. P., O'Toole, P. W., Fitzgerald G. F., & Stanton C. (2012). γ-Aminobutyric acid production by culturable bacteria from the human intestine Journal of Applied Microbiology, 113(2), 411-417.

Bassler, B. L. & Miller, M. B. (2013). Quorum sensing. In: E. Rosenberg et al. (Eds.), The Prokaryotes – Prokaryotic Communities and Ecophysiology (pp.495-509), Berlin & Heidelberg: Springer-Verlag.

Baulina, O. I. (2012). Ultrastructural Plasticity of Cyanobacteria. Heidelberg: Springer-Verlag.

Belkaid, Y. & Hand, T. (2014). Role of the microbiota in immunity and inflammation. Cell, 157(1), 121-141.

Berne, J. P., Lauzier, B., Rochette, L., & Vergely, C. (2012). Carbon monoxide protects against ischemia-reperfusion injury in vitro via antioxidant properties. Cell Physiology & Biochemistry, 29, 475−484.

Bhandage, A. K., Jin, Z., Korol, S. V., Shen, Q., Pei, Y., Deng, Q., Espes, D., Carlsson, P.-O., Kamali-Moghaddam, M., & Birnir, B. (2018). GABA regulates release of inflammatory cytokines from peripheral blood mononuclear cells and CD4+ T cells and is immunosuppressive in type 1 diabetes. EBioMedicine, 30, 283-294.

Bhat, R., Axtell, R., Mitra, A., Miranda, M., Lock, C., Tsien, R. W., & Steinman, L. (2010). Inhibitory role for GABA in autoimmune inflammation. Proceedings of the National Academy of Sciences of the US A, 107, 2580-2585.

Biarnes-Carrera, M., Lee, C. K., Nihira, T., Breitling, R.& Takano, E. (2018). Detection and quantification of butyrolactones from *Streptomyces*. In: L. Leoni & G. Rampioni (ed.), Quorum Sensing. Quorum Sensing: Methods and Protocols (pp.117-128). Rome: Humana Press.

Boddu, R. S. & Divakar, K. (2018). Metagenomic insights into environmental microbiome and their application in food/pharmaceutical industry. In: J. K. Patra, G. Das, & H.-S. Shin (Eds.), Microbial Biotechnology. Vol. 2 Application in Food and Pharmacology (pp.23-38), Singapore: Springer Nature Singapore Pte. Ltd.

Boldyrev, A. A., Yeschenko, N. D., Ilyukha, V. A., & Kyaivyaryainen, E. I. (2010). Neurochemistry. Moscow: Drofa (in Russian).

Boonstra, E., de Kleijn, R., Colzato, L. S., Alkemade, A., Forstmann, B.. U., & Nieuwenhuis, S. (2015). Neurotransmitters as food supplements: the effects of GABA on brain and behavior. Frontiers in Psychology, 6, 1520.

Brown, L. L., Acevedo, B., & Fisher, H. E. (2013). Neural correlates of four broad temperament dimensions: testing predictions for a novel construct of personality. Plos One, 8(11), e78734.

Budrene, E. O. (1985). Formation of spatially ordered structures in the colonies of motile bacteria on the agar. USSR Academy of Sciences Doklady, 383(2), 470-473.

Budrene, E. O. & Berg, H. C. (1991). Complex patterns formed by motile cells of Escherichia coli. Nature, 349, 630-633.

Budrene, E. O. & Berg, H. C. (2002). Dynamics of formation of symmetrical patterns by chemotactic bacteria. Nature, 376, 49-53.

Bukharin, O. V., Gintsburg, A. L., Romanova, Yu. M., & El'-Registan, G. I. (2005). Mechanisms of Bacterial Suvival. Moscow: Meditsina (In Russian).

Buňkova, L., Gál, R., Lorencová, E., Jančová, P., Doležalová, M., Kmeť, V., & Buňka, F. (2016). Microflora of farm and hunted pheasants in relation to biogenic amine production. European Journal of Wildlife Research, 62, 341-352.

Bushell, M. E. (1989). Biowars in the bioreactor. New Scientist, 124, 42-45.

Buznikov, G. A. (1987). Neurotransmtters in Embryogenesis. Moscow: Nauka (in Russian).

Buznikov, G. A. (2007). Preneural transmitters as embryogenesis regulators: the present-day state-of-the-art. Ontogeny (Russia), 38(4), 262-270.

Camilli A. & Bassler, B. L. (2006). Bacterial small-molecule signaling pathways. Science, 311, 1113-1116.

Canani, R. B., Di Costanzo, M., Leone, L., Pedata, M., Meli, R., & Calignano, A. (2011). Potential beneficial effects of butyrate in intestinal and extra-intestinal diseases. World Journal of Gastroenterology, 17(12), 1519-1528.

Carbonero, F., Benefiel, A. C, Alizadeh-Ghamsari, A. H, & Gaskins, H. R. (2012). Microbial pathways in colonic sulfur metabolism and links with health and disease. Frontiers in Physiology, 3, 448.

Carlucci, C., Petrof, E. O., & Allen-Vercoe, E. (2016). Fecal microbiota-based therapeutics for recurrent *Clostridium difficile* infection, ulcerative colitis and obesity. EBioMedicine, 13, 37-45.

Chai, J., Luo, L., Hou, F., Fan, X., Yu, J., Ma, W., et al. (2016). Agmatine reduces lipopolysaccharide-mediated oxidant response via activating PI3K/Akt pathway and up-regulating Nrf2 and HO-1 expression in macrophages. PLoS One, 11(9), e0163634.

Chen, J. & Vitetta, L. (2018). Inflammation-modulating effect of butyrate in the prevention of colon cancer by dietary fiber. Clinical Colorectal Cancer, 17(3), e541-e544.

Chen, T., Nai-Kei Wong, N.-K., Jiang, X., Luo, X., Zhang, L., Yang, D., Ren, C., & Hu, C. (2015). Nitric oxide as an antimicrobial molecule against *Vibrio harveyi* infection in the hepatopancreas of Pacific white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology, 42, 114-120.

Chiu, C.-C., Ching, Y.-H., Wang, Y.-C, Liu, J.-Y., Li Y.-P., Huang Y.-T., & Chuang H.-L. (2014). Monocolonization of germ-free mice with *Bacteroides fragilis* protects against dextran sulfate sodium-induced acute colitis. BioMed Research International, 2014, 675786.

Clark, A. & March, N. (2016). Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. Journal of the International Society of Sports Nutrition, 13, 43.

Clarke, M. B., Hughes, D. T., Zhu, C., Boedeker, E. C., & Sperandio, V. (2006). The QseC sensor kinase: A bacterial adrenergic receptor. Proceedings of the National Academy of Sciences of the USA, 103, 10420-10425.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiota-gut-brain axis during early life regulates the hippocampal serotonin system in a sex-dependent manner. Molecular Psychiatry, 18, 666-673.

Clarke, G., Stilling, R. M., Kennedy, P. J., Stanton, C., Cryan, J. T., & Dinan, T. G. (2014). Gut microbiota: the neglected endocrine organ. Molecular Endocrinology, 28,1221−1238.

Cohen Kadosh, K., Basso, M., Knytl, P., Johnsyone, N., Lau, J. Y. F., & Gibson, G. R. (2021). Psychobiotic interventions for anxiety in young people: a systematic review and meta-analysis, with youth consultation. Translational Psychiatry, 11, 352.

Cooley, M., Chhabra, S. R. & Williams, P. (2008). N-Acylhomoserine lactone-mediated quorum sensing: a twist in the tail and a blow for host immunity. Chemistry & Biology, 15,1141-1147.

Correâ-Oliveira, R. O, Fachi, J. L., Vieira, A., Sato, F. T., & Vinolo, M. A. R. (2016). Regulation of immune cell function by short-chain fatty acids. Clinical & Translational Immunology, 5(4), e73.

Cosentino, M. & Marino, F. (2012). Nerve-driven Immunity. Vienna: Springer.

Cosentino, M., Marino, F., & Kustrimovic N. (2013). Endogenous catecholamines in immune cells: discovery, functions and clinical potential as therapeutic targets. Brain Immune Trends. Available from: http://brainimmune.com/endogenous-catecholamines-in-immune-cells-discovery-functions-and-clinical-potential-as-pharmacotherapeutic-targets-3.

Cowan C. S. M., Hoban A. E., Ventura-Silva A. P., Dinan T. G., Clarke G., & Cryan J. F. (2018). Gutsy moves: the amygdala as a critical node in microbiota to brain signaling. BioEssays, 40, 1700172.

Crespi, B. J. (2001). The evolution of social behavior in microorganisms. Trends in Ecology & Evolution, 16(4), 178-183.

Cryan, J. F. & Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behavior. Nature Reviews Neuroscience, 13, 701-712.

Davies, D. G. (2011). Biofilm dispersion. In: H. Fleming et al. (Eds.), Biofilm Highlights: Springer Series on Biofims 5 (pp.1-28), Berlin & Heidelberg: Springer-Verlag.

Deryabin, D. G., Romanenko, N. A., & El'-Registan, G. I. (2010). Influence of alkylhydroxybenzenes on the functional and operational stability of antibodies. Microbiology (Russia), 79 (3),1-7.

Deryabin, D. G, Romanenko, N. A., & El'-Registan, G. I. (2013a). Changes in antibody specifity in the presence of alkylhydroxybenxenes, chemical analogs of bacterial autoregulatory molecules. Immunology (Russia), No. 1, 27-30.

Deryabin, D. G, Sviridova, T. G., El'-Registan, I., & Chereshnev, V. A. (2013b). Impact of bacterial autoregulatory molecules (homoserine lactones and alkylhydroxybenzenes) on the oxidative metabolism of the cell effectors of native immunity. Microbiology (Russia), 82(2), 147-156.

Devalia, J. L., Grady, D., Harmanyeri, Y., Tabaqchali, S., & Davies, R. J. (1989). Histamine synthesis by respiratory tract micro-organisms: possible role in pathogenicity. Journal of Clinical Pathology, 42, 516-522.

Dewsbury, D. A. (1978). Comparative Animal Behavior. New York: McGraw-Hill.

Diaz, M., Ladero, V., del Rio, B., Redruello, B., Fernández, M., Martin, M. C., & Alvarez, M. A. (2016). Biofilm-forming capacity in biogenic amine-producing bacteria isolated from dairy products. Frontiers in Microbiology, 7, 591.

Dienes, L. (1946). Reproductive processes in *Proteus* cultures. Proceedings of the Society for Experimental Biology and Medicine, 63, 265-270.

Dinan, T. G., Stilling, R. M., Stanton, C., & Cryan, J. F. (2015). Collective unconscious: how gut microbes shape human behavior. Journal of Psychiatric Research, 63, 1-9.

Doeun, D., Davaatseren, M., & Chung, M.-S. (2017). Biogenic amines in food. Food Science & Biotechnology, 26(6), 1463-1474.

Domka, J., Lee, J., & Wood, T. K. (2006). YliH (BssR) and YceP (BssS) regulate *Escherichia coli* K-12 biofilm formation by influencing cell signaling. Applied & Environmental Microbiology, 72, 2449-2459.

Dubey, G. P. & Ben-Yehuda, S. (2011). Intercellular nanotubes mediate bacterial communication. Cell, 144, 590-600.

Dubynin, V. A., Kamenskii, A. A., Sapin, M. R., & Sivoglazov, V. N. (2010). Regulatory Systems of the Human Organism. Moscow: Drofa (in Russian).

Duda, V. I., Vypov, M. G., Sorokin, V. V., Mityushina, L. L., & Lebedinsky, A. V. (1995). Formation of extracelular hemosome-containing structures by bacteria. Microbiology (Russia), 64(1), 69-73.

Duda, V. I., Dmitriev, V. V., Suzina, N. E., Shorokhova, A. P., & Mishina, G. V. (1996). Ultrastructural organization of gas vesicles and surface films in the colonies of the gram-negative bacterium Alcaligenes sp., strain d2. Microbiology (Russia), 65(2), 222-227.

Duda, V. I., Il'chenko, A. P., Dmitriev, V. V., Shorokhova, A. P., & Suzina, N. E. (1998). Isolation and characterization of a hemopflavoprotein from the gram-negative bacterium *Alcaligenes* sp., strain d2. Mikrobiology (Russia), 67, 12-18.

Duncan, S. H., Holtrop, G., Lobley, G. E., Calder, A. G., Stewart, C. S., & Flint, H. J. (2004). Contribution of acetate to butyrate formation by human faecal bacteria. British Journal of Nutrition, 91(6), 915-923.

Edmonds-Wilson, S. I., Nurinova, N. I., Zapka, C. A., Fierer, N., & Wiloson, M. (2015). Review of human hand microbiome research. Journal of Dermatology, 80, 3-12.

Egorov, N. S. & Landau, N. S. (1982). Biosynthesis of biologically active compounds by mixed cultures of microorganisms. Applied Biochemistry & Microbiology (Russia), 18, 835-849.

El Aidy, S., Stilling, R. M., Dinan, T. G., & Cryan, J.F. (2016). Microbiome to brain: unraveling the multidirectional axes of communication. In: Lyte, M. (Ed.), Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health. Advances in Experimental Medicine and Biology 874 (pp.301-336), Cham, Heidelberg, New York, & London: Springer.

El Karim, I. A., Linden, G. J., Orr, D. F., & Lundy, F. T. (2008). Antimicrobial activity of neuropeptides against a range of microorganisms from skin, oral, respiratory and gastrointestinal tract sites. Journal of Neuroimmunology, 200, 11-16.

El'-Registan, G. I. (1988). Role of Membranotropic Autoregulatory Factors in the Growth and Development of Microorganisms. D. Sci. (Biol.) Dissertation. Moscow: Institute of Microbiology of the USSR Academy of Sciences (in Russian).

El'-Registan, G. I., Mulyukin, A. L., Nikolaev, Yu. A., Suzina, N. E., Gal'chenko, V. F., & Duda, V. I. (2006). Adaptogen functions of microbial extracellular autoregulators. Microbiology (Russia), 75(4), 446-456.

Erofeev, N. P., Radchenko, V. G., & Seliverstov, P. V. (2012). Clinical Physiology of the Colon. Mechanism of Action of Short-Chain Fatty Acids in Health and Disease. Saint-Petersburg: Forte-Print (In Russian).

FAO/WHO (2006). Probiotics in Food. Health and Nutritional Properties and Guidelnes for Evaluation. Rome: World Health Organization. Available at: https://www.who.int/news-room/fact-sheets/detil/depression.

Farrugia, G. & Szurszewski, J. H. (2014). Carbon monoxide, hydrogen sulfide, and nitric oxide as signaling molecules in the gastrointestinal tract. Gastroenterology, 147, 303-313.

Fetissov, S. O., Sinno, M. H., Coëffier, M., Bole-Feysot, C., Ducrotté, P., Hökfelt, T., & Déchelotte P. (2008). Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulaton by gut microflora. Nutrition, 24, 348-359.

Filippova, S. N., Surguchan, N. A., Kasaikin, O. T., Kragovov, D. A., & Galchenko V. F. (2010). Growth induction and stabilization of population composition in *Saccharopolyspora erythraea* by catecholamine compounds. Microbiology (Russia), 79(2), 191-196.

Fletscher, M. P., Diggle, S P., Camara, M., & Williams, P. (2018). Detection of 2-alkyl-4-quinolones using biosensors. In: L. Leoni & G. Rampioni (ed.), Quorum Sensing. Quorum Sensing: Methods and Protocols (pp.25-34), Rome: Humana Press.

Foster, K. R. (2010). Social behaviour in microorganisms. In: T. Székely, A. J. Moore, & J. Komdeur (Eds.), Social Behaviour: Genes, Ecology and Evolution (pp.331-356). Cambridge: Cambridge University Press.

Fraikin, G. Ya, Strakhovskaya, M. G., & Ivanova, E. V. (1985). Effect of serotonin on the yield of UV-induced thymine dimers. Biophysics (USSR), 30(4), 564-567.

Freestone, P. P. E. & Lyte, M. (2008). Microbial endocrinology: Experimental design issues in the study of interkingdom signaling in infectious disease. Advances in Applied Microbiology, 64, 75-108.

Freestone, P. P. E., Haigh, R. D., Williams, P. H., & Lyte, M. (1999). Stimulation of bacterial growth by heat-stable, norepinephrine-induced autoinducers. FEMS Microbiology Letters, 172, 53-60.

Freestone, P. P. E., Haigh, R. D., & Lyte, M. (2007). Blockade of catecholamine-induced growth by adrenergic and dopaminergic receptor antagonists in Escherichia coli O157:H7, Salmonella enterica and Yersinia enterocolitica. BMC Microbiology, 7, 8.

Fujita, M., Gonzalez-Pastor, J. E., & Losick, R. (2005). High- and low-threshold genes in the Spo0A regulon of *Bacillus subtilis*. Journal of Bacteriology, 187, 1357-1368.

Fuks, J. M., Arrighi, R. B., Weidner, J. M., Kumar Mendu, S., Jin, Z., Wallin, R. P., Rethi, B., Birnir, B., & Barragan A. (2012). GABAergic signaling is linked to a hypermigratory phenotype in dendritic cells infected by Toxoplasma gondii. PLoS Pathogenesis, 8, e1003051.

Fuqua, W. C., Winans, S. C., & Greenberg, E. P. (1994). Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. Journal of Bacteriology, 176(2), 269-275.

Gadalla, M. M. & Snyder, S. H. (2010). Hydrogen sulfide as a gasotransmitter. Journal of Neurochemistry, 113, 14-26.

Ganin, H., Yardeni, E. H., & Kolodkin-Gal, I. (2015). Biofilms: maintenance development, and disassembly of bacterial communities are determined by QS cascades. In: V. C. Kalia (Ed.), Quorum Sensing vs. Quorum Quenching: A Battle with no End in Sight (pp.23-37), New Delhi, Heidelberg, & New York: Springer-Verlag.

Ganor, Y. & Levite, M. (2014). The neurotransmitter glutamate and human T cells: glutamate receptors and glutamate-induced direct and potent effects on normal human T cells, cancerous human leukemia and lymphoma T cells, and autoimmune human T cells. Journal of Neural Transmission, 121(8), 983-1006.

Gao, C., Major, A., Rendon, D., Lugo, M., Jackson, V., Shi, Z., Mori-Akiyama, Y., & Versalovic, J. (2015). Histamine H2 receptor-mediated suppression of intestinal inflammation by probiotic *Lactobacillus reuteri.* Molecular Biology, 6(6), e01358-15.

Gardini, F., Rossi, F., Rizotti, L., Torriani, S., Grazia, L., Chiavari, C., Coloretti, F., & Tabanelli, G. (2012). Role of *Streptococcus thermophilus* PRI60 in histamine accumulation in cheese. International Dairy Journal, 27, 71-76.

Gerstel, U. & Romling, U. (2003). The *csgD* promoter, a control unit for biofilm formation in *Salmonella typhimurium*. Research in Microbiology, 154, 659-666.

Givskov, M., Östling, J., Eberl, L., Lindum, P. W., Christensen, A. B., Christiansen, G., Molin, S., & Kjelleberg, S. (1998). Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. Journal of Bacteriology, 18(3), 742-745.

Gonzalez, J. F.,& Venturi, V. (2013). A novel widespread interkingdom signaling circuit. Trends in Plant Science, 18,167-174.

Gray, K. M. (1997). Intercellular communication and group behavior in bacteria. Trends in Microbiology, 5(5), 184-188.

Greenberg, E. P. (2003). Tiny teamwork. Bacterial communication. Nature, 424, 134.

Greenberg, E. P, Winans, S., & Fuqua, C. (1996). Quorum sensing by bacteria. Annual Review of Microbiology, 50, 727-751.

Gurvich, A. G. (1944). The Theory of Biological Field. Moscow: Soviet Science (in Russia).

Gusarov, I. & Nudler, E. (2005). NO-mediated cytoprotection: instant adaptation to oxidative stress in bacteria. Proceedings of the National Academy of Sciences of the USA, 102, 13855-13860.

Gusarov, I., Shatalin, K., Starodubtseva, M., & Nudler, E. (2009). Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. Science, 325, 1380-1384.

Gusarov, I., Gautier, L., Smolentseva, O., Shamovsky, I., Eremina, S., Mironov, A., et al. (2013). Bacterial nitric oxide extends the lifespan of *C. elegans*. Cell, 152, 818-830.

Hagen, S. J. (Ed.). (2015). The Physical Basis of Bacterial Quorum Communication. New York, Heidelberg, Dordrecht & London: Springer.

Halász, A., Baráth, Á., Simon-Sarkadi, L., & Holzapfel, W. (1994). Biogenic amines and their production by microorganisms in food. Trends in Food Science and Technology, 5, 42-49.

Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. Nature Review Microbiology, 2, 95-108.

Hamilton, W.D. (1964). The genetical evolution of social behavior. Journal of Theoretical Biology, 7, 1-52.

Hansen, C. J., Burnell, K. K., & Brogden, K. A. (2006). Antimicrobial activity of Substance P and Neuropeptide Y against laboratory strains of bacteria and oral microorganisms. Journal of Neuroimmunology, 177, 215-218.

Harmsen, M, Yang, L., Pamp, S. J., & Tolker-Nielsen T. (2010). A update on *Pseudomonas aeruginosa* biofilm formation, tolerance and dispersal. FEMS Immunology & Medical Microbiology, 59, 253-268.

Helinck, S., Perello, M.-C., Deetae, P., de Revel, G., & Spinnler, H.-E. (2013). *Debaryomyces hansenii, Proteus vulgaris, Psychrobacter sp.* and *Mycobacterium foliorum* are able to produce biogenic amines. Dairy Science & Technology, 93, 191-200.

Herd, P., Palloni, A., Rey, F., & Dowd J. B. (2018). Social and population science approaches to understand the human microbiome. Nature Human Behavior, 2, 808-815.

Hevia, A., Delgado, S., Sanchez, B., Margolles, A. (2015). Molecular players involved in the interaction between beneficial bacteria and the immune system. Frontiers in Microbiology, 6, 1285.

Hezel, M. P. & Weitzberg, E. (2015). The oral microbiome and nitric oxide homoeostasis. Oral Diseases, 21, 7-16.

Hine, C., Harputlugil, E., Zhang, Y., Ruckenstuhl, C., Lee, B. C, Brace, L, & et al. (2015). Endogenous hydrogen sulfide production is essential for dietary restriction benefits. Cell, 160,132−144.

Hochberg, M. E., Rankin, D. J., & Taborsky, M. (2008). The coevolution of cooperation and dispersal in social groups and its implications for the emergence of multicellularity. BMC Evolutionary Biology, 8, 238.

Holzer, P. & Farzi, A. (2014). Neuropeptides and the microbiota-gut-brain axis. In: M. Lyte & J. F. Cryan (Eds.), Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease. Advances in Experimental Medicine and Biology. 817 (pp.195-219). New York: Springer.

Hsu, S. C., Johansson, K. R., & Donahne, M. J. (1986). The bacterial flora of the intestine of *Ascaris suum* and 5-hydroxytryptamine production. Journal of Parasitology, 72, 545-549.

Huang, X., Fan, X., Ying, J., & Chen, S. (2019). Emerging trends and research foci in gastrointestinal microbiomes. Journal of Translational Medicine, 17, 67.

Hughes, D. T., Clarke, M. B., Yamamoto, K., Rasko, D. A., & Sperandio, V. (2009). The QseC adrenergic signaling cascade in enterohemorrhagic *E. coli* (EHEC). PLoS Pathogenesis, 5, e1000553.

Humphries, C. (2017). Detecting diversity. Nature, 550, S12-S14.

Humphries, J., Xiong, L., Liu, J., Prindle, A., Yuan, F., Arjes, H. A., Tsimring, L., & Süel, G. M. (2017). Species-independent attraction to biofilms through electrical signaling. Cell, 168(1-2), 200-209.

Ishigami, M., Hiraki, K., Umemura, K., Ogasawara, Y., Ishii, K., & Kimura, H. (2009). A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxidants & Redox Signaling, 11, 205−214.

Itoh, Y., Wang X., Hinnebusch B. J., Preston I., J. F., & Romeo, T. (2005). Depolymerization of beta-1,6-N-acetyl-D-glucosamine disrupts the integrity of diverse bacterial biofilms. Journal of Bacteriology, 187, 382-387.

Ivashkin, V. T. & Drapkina, O. M. (2001). Clinical Importance of Nitric Oxide and Heat Shock Proteins. Moscow: GEOTAR-MEDIA (in Russian).

Ivashkin, V. T. & Ivashkin, K. V. (2018). The gut microbiome as a factor regulating the activity of the enteric and central nervous system. Russian Journal of Gastroenterology, Hepatology, & Colorecto-proctology, 27(5), 11-19.

Jacubovics, N. S., Shields, R. C., & Rajarajan, N. (2013). Life after death: the critical role of extracellular DNA in microbial biofilms. Letters of Applied Microbiology, 57(6), 467-475.

James, S. L. (1995). Role of nitric oxide in parasitic infections. Microbiology Reviews, 59, 533-547.

Janssens, Y., Debunne, N., De Spiegeleer, A., Wynendaele, E., Planas, M., Feliu, L., Quarta, A., Claes, C., Van Dam, D., De Deyn, P. P., Ponsaerts, P., Blurton-Jones, M., & De Spiegeleer, B. (2021). PapRIV, a BV-2 477 microglial cell activating quorum sensing peptide. Science Reports, 11, 10723.

Jefferson, K. K. (2004). What drives bacteria to produce a biofilm? FEMS Microbioogy Letters, 236, 163-173.

Jin, Z., Mendu, S. K., & Birnir, B. (2013). GABA is an effective immunomodulatory molecule.Amino Acids, 45(1), 87-94.

Johnson, K. V.-A. & Foster, K. R. (2018). Why does the microbiome affect behavior? Nature Reviews Microbiology, 16, 647-655.

Kalia, V. C (Ed.). (2015). Quorum Sensing vs. Quorum Quenching: A Battle with No End in Sight. New York: Springer-Verlag.

Kaper, J. B. & Sperandio, V. (2005). Bacterial cell-to-cell signaling in the gastrointestinal tract. Infection and Immmunity, 73, 3197-3209.

Kaplan, J. B. (2014). Biofilm matrix-degrading enzymes. In: G. Donelli (Ed.), Mcrobial Biofilms: Methods and Protocols. Methods in Molecular Biology. Vol. 1147. (pp. 203-213). New York: Springer-Verlag + Business Media.

Karatan, E. & Watnick, P. (2009). Signals, regulatory networks, and materials that build and break bacterial biofilms. Microbiology & Molecular Biology Reviews, 73, 310-347.

Kellogg, D. L., Zhao, J. L., Coey, U., & Green, J. V. (2005). Acetylcholine-induced vasodilation is mediated by nitric oxide and prostaglandins in human skin. Journal of Applied Physiology, 98(2), 629-632.

Kerry, R. G., Pradhan, P., Samal, D., Gouda, S., Das, G, Shin, H.-S., & Patra J. K. (2018). Probiotics: the ultimate nutritional supplement. In: J. K. Patra, G. Das, & H.-S. Shin (Eds.), Microbial Biotechnology. V. 2 Application in Food and Pharmacology (pp.141-152), Singapore: Springer Nature Singapore Pte. Ltd.

Khmel', I. A. (2006). Quorum-sensing regulation of gene expression: implications for fundamental and applied scence; involvement in bacterial communication. Microbiology (Russia), 75(4), 457-464.

King, G. M. & Weber, C. F. (2007). Distribution, diversity and ecology of aerobic CO-oxidizing bacteria. Nature Reviews Microbiology, 5, 107-118.

Kinney, K. S., Austin, C. E., Morton, D. S., & Sonnenfeld G. (1999). Catecholamine enhancement of Aeromonas hydrophila growth. Microbial Pathogenesis, 25, 85-91.

Kirovskaya, T. A. (2005). Research on Microbial Population Organization and Intercellular Communication in the Soviet Union (Russia) in the Second Half of the 20th Century. Cand. Sci. (Biol.) Dissertation. Moscow: Institute of History of Science and Technology. Available at the MSU Library.

Ko, C. Y., Lin, H. T. V., & Tsai, G. J. (2013). Gamma-aminobutyric acid production in black soybean milk by *Lactobacillus brevis* FPA 3709 and the antidepressant effect of the fermented product on a forced swimming rat model. Process Biochemistry, 48, 559-568.

Kowalska, K., Carr, D. B., & Lipkowski, A. W. (2002). Direct antimicrobial properties of substance P. Life Sciences, 71, 747-750.

Larsen, F. J., Schiffer, T. A., Borniquel, S., Sahlin, K.,Ekblom, B., Lundberg, J. O., & et al. (2011). Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metabolism, 13, 149-159.

Lee, J., Jayaraman, A., & Wood, T. K. (2007a). Indole is an inter-species biofilm signal mediated by SdiA. BMC Microbiology, 7, 42.

Lee, J., Bansal, T., Jayaraman, A., Bentley, W. E., & Wood T. K. (2007b). Enterohemorrhagic *Escherichia coli* biofilms are inhibited by 7-hydroxyindole and stimulated by isatin. Applied & Environmental Microbiology, 73, 4100-4109.

Lee, B.-J., Kim, J.-S., Kang, Y. M., Lim, J.-H., Kim, Y.-M., Lee, M.-S., Jeong, M.-H, Ahn, C.-B., & Je, J.-Y. (2010). Antioxidant activity and γ-aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. Food Chemistry, 122, 271-276.

Lenard, J. (1992). Mammalian hormones in microbial cells. Trends in Biochemical Science, 17, 147-150.

Leoni, L. & Rampioni, G. (2018). Preface. In: L. Leoni & G. Rampioni (Eds.), Quorum Sensing: Methods and Protocols (pp. vii-viii). Rome: Humana Press.

Levite, M. (2016). Dopamine and T cells: dopamine receptors and potent effects on T cells, dopamine production in T cells, and abnormalities in the dopaminergic system in T cells in autoimmune, neurological and psychiatric diseases. Acta Physiologica, 216(1), 42-89.

Ley, S., Weigert, A., & Brüne, B. (2010). Neuromediators in inflammation–a macrophage/nerve connection. Immunology, 215, 674-684.

Liang, S., Wu, X., & Jin, F. (2018). Gut-brain physiology: rethinking psychology from the microbiota-gut-brain axis. Frontiers in Integrative Neuroscience, 12(Art.33), 1-24.

Liao, W.-C., Wang, C.-Y., Shyu, Y.-T., Yu, R.-C., & Ho, K.-C. (2013). Influence of preprocessing methods and fermentation of adzuki beans on γ-aminobutyric acid (GABA) accumulation by lactic acid bacteria. Journal of Functional Foods, 5, 1108-1115.

Lin, C.-S., Tsai, H.-C., Lin, C.-M., Huang, C.-Y., Kung, H.-F., & Tsai Y.-H. (2014). Histamine content and histamine-forming bacteria in mahi-mahi (*Coryphaea hippurus*) fillets and dried products. Food Control, 42, 165-171.

Logan, A. C., Jacka, F. N., & Prescott, S. L. (2016). Immune-microbiota interactions: dysbiosis as a global health issue. Current Allergy & Asthma Reports, 16(2), 13.

Long-Smith, C., O'Riordan, R., Clarke, G., Stanton, C., Dinan, T. G., & Cryan, J. F. (2020). Microbiota-gut-brain axis: new therapeutic opportunities. Annual Review of Pharmacology & Toxicology, 60, 17.1-17.6.

Losick, R. & Kaiser, D. (1997). Why and how bacteria communicate. Scientific American, February, 68-73.

Lundberg, J. O. & Weitzberg, E. (2013). Biology of nitrogen oxides in the gastrointestinal tract. Gut, 62, 619-629.

Lynch, S. V., Ng, S. C., Shanahan, F., & Tilg, H. (2019). Translating the gut microbiome: ready for the clinic? Nature, 16, 656-661.

Lyte, M. (1993). The role of microbial endocrinology in infectious disease. Journal of Endocrinoogy, 137, 343-345.

Lyte, M. (2010). The microbial organ in the gut as a driver of homeostasis and disease. Medical Hypotheses, 74, 634-638.

Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. Bioessays, 33, 574-581.

Lyte, M. (2013a). Microbial endocrinology and nutrition: A perspective on new mechanisms by which diet can influence gut-to brain-communication. Pharma Nutrition, 1, 35-39.

Lyte, M. (2013b). Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. Plos Pathogen, 9(11), p.e. 1003726.

Lyte, M. (2014). Microbial endocrinology and the microbiota–gut–brain axis. In: M. Lyte & J. F. Cryan (Eds.), Microbial Endocrinology: The Microbiota–Gut–Brain Axis in Health and Disease. Advances in Experimental Medicine and Biology. 817 (pp.3-24), New York: Springer.

Lyte, M. (2016). Microbial endocrinology: an ongoing personal journey. In: M. Lyte (Ed.), Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health (pp. 1-24). New York: Springer.

Lyte, M., & Ernst, S. (1993). Alpha and beta adrenergetic receptor involvement in catecholamine-induced growth of gram-negative bacteria. Biochemical & Biophysical Research Communications, 190(2), 447-452.

Lyte, M. & Freestone, P. (2009). Microbial endocrinology comes of age. Microbe, 4(4), 169-176.

MacFabe, D. F. (2012). Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. Microbial Ecology in Health and Disease, 23, 1926

Malikina, K. D., Shishov, V. A., Chuvelev, D. I., Kudrin, V. S., & Oleskin, A. V. (2010). The regulatory role of monoamine neuromediators in *Saccharomyces cerevisiae* cells. Applied Biochemistry & Microbiology (Russia), 46, 1-5.

Mamson, M. D., Armitage, J. D., Hoch, J. A., & Macnab R. M. (1998). Bacterial locomotion and signal transduction. Journal of Bacteriology, 180(5), 1009-1022.

Mano, Y., Hyodo, M.,Kaneko, T., Otani, S., & Yoshimura,S. (1996). *Bacillus carbonifillus* cells respond to growth-promoting physical signals from cells of homologous and heterologous bacteria. Journal of General & Applied Microbiology, 42, 315-323.

Martin, M., Hölscher, T., Dragoš, A., Cooper, V. S., & Kovács, Á. T. (2016). Laboratory evolution of microbial interaction in bacterial biofilms. Journal of Bacteriology, 198, 2564-2571.

Mart'yanov, S. V., Zhurina, M. V., El'-Registan, G. I., & Plakunov, V. K. (2015). The activating effect of azitromycin on biofilm formation and the prevention of this phenomenon. Microbiology (Russia), 84(1), 27-36.

Masters, R. D. (1994). Why study serotonin, social behavior and the law? In: R. D. Masters & M. T. McGuire (Eds.), The Neurotransmitter Revolution. Serotonin, Social Behavior and the Law (pp.3-16). Carbondale; Edwardsville: Southern Illinois University Press.

Mathur, H., Feld, D., Rea, M. C., Cotter, P. D., Hill, C., & Ross, R. P. (2018). Fighting biofilms with lantobiotics and other groups of bacteriocins. Biofilms & Microbiomes, 4, 9.

Matsuhashi, M., Shindo, A., Ohshina, H., Tobi, M., Endo, S., Watanabe, H., & Pankrushina, A. N. (1996a). Cellular signals regulating antibiotic sensitivities of bacteria. Microbial Drug Research, 2(1), 91-93.

Matsuhashi, M., Pankrushina, A. N., Endoh, K., Watanabe, H., Ohshima, H., Tobi, M., Endo, S., Mano, Y., Hyodo, M.,Kaneko, T., Otani, S., & Yoshimura,S. (1996b). *Bacillus carbonifillus* cells respond to growth-promoting physical signals from cells of homologous and heterologous bacteria. Journal of General & Applied Microbiology, 42, 315-323.

Maura, D., Bandyopadhaya, A., & Rahme, L. G. (2018). Animal models for *Pseudomonas aeruginosa* quorum sensing studies. In: L. Leoni & G. Rampini (Eds.), Quorum Sensing: Methods and Protocols (pp.227-241), Rome: Humana Press. Environmental Microbiology, 17(3), 555-565.

Mayr, A., Hinterberger, G., Dierich, M. P, & Lass-Flörl, C. (2005). Interaction of serotonin with *Candida albicans* selectively attenuates fungal virulence in vitro. International Journal of Antimicrobial Agents, 26, 335-337.

Mazzoli, R. & Pessione, E. (2016). The neuro-endocrinological role of microbial glutamate and GABA signaling. Frontiers in Microbiology, 7, 1934.

Medinets, S., Skiba, U., Rennenberg, H., & Butterbach-Bahl, K. (2015). A review of soil NO transformation: Associated processes and possible physiological significance on organisms. Soil Biology & Biochemistry, 80, 92-117.

Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: review of physiology and pathology. Journal of Nutrition, 130(4S Suppl), 1007S-1015S.

Metchnikoff, É. (1904). Etudes sur la nature humaine: essai de philosophie optimiste. Paris: Masson & Cis.

Mielich-Süss, B. & Lopez, D. (2015). Molecular mechanisms involved in *Bacillus subtilis* biofilm formation. Environmental Microbiology, 17(3), 555-565.

Midtvedt, T. (2006). Defense mechanisms of the innate system: NO as gastrointestinal eco-regulator. In: T. Midtvedt, J. Bienenstock,P. Heidt,V. Rusch, & D. van der Waaij (Eds.), Defense Mechanisms of the Innate System: Influence of Microbes (pp.113-116).Germany: Old Herborn University Seminar.

Mittal, N., Budrene, E. O., Brenner, M. P. & Van Oudenaarden, A. (2003). Motility of *Escherichia coli* cells in clusters formed by chemotactic aggregation. Proceedings of the National Academy of Sciences of the USA., 100, 13259-13263.

Murray, E. J. & Williams, P. (2018). Detection of Agr-type autoinducing peptides produced by *Staphylococcus aureus*. In: L. Leoni & G. Rampioni (Eds.), Quorum Sensing. Quorum Sensing: Methods and Protocols (pp. 673-88), Rome: Humana Press.

Nakayama, J., Takanami, Y., Horii, T., Sakuda, S., & Suzuki, A. (1998). Molecular mechanism of peptide-specific pheromone signaling in *Enterococcus faecalis*: functions of pheromone receptor TraA and pheromone-binding protein TraC encoded by plasmid pPD1. Journal of Bacteriology, 180(3), 449-456.

Neish, A. S. (2009). Microbes in gastrointestinal health and disease. Gastroenterology, 136(1), 65-80.
Nelson, R. J., Demas, G. E., Huang, P. L., Fishman, M. C., Dawson, V. L., Dawson, T, M, & et al. (1995). Behavioral abnormalities in male mice lacking neuronal nitric oxide synthase. Nature, 378, 383-386.
Nikolaev, Yu. A. (1992). Distant informational interactions between bacterial cells. Microbiology (Russia), 61(6), 1066-1071.
Nikolaev, Yu. A. (2000). Distant informational interactions in bacteria. Microbiology (Russia), 69(5), 597-605.
Nikolaev, Yu. A. & Prosser, J. (2000). Extracellular factors influencing the adhesion of *Pseudomonas fluorescens* to glass. Microbiology (Russia), 69(2), 231-236.
Nikolaev, Yu. A. & Plakunov, V. K. (2007). The biofilm: a "microbial city" of an analog of the multicellular organism? Microbiology (Russia), 76(2), 148-163.
Nikolaev, Yu. A., Mulyukin, A. L., Stepanenko, I. Yu., & El'-Registan, G. I. (2006). Autoregulation of the stress response of microorganisms. Microbiology (Russia), 75(4), 489-496.
Nikolaev, Yu. A., El-Registan, G. I., & Desu, S. B. (2007). Distant interaction during germination of *Bacillus subtilis* spores.. In: L. V. Beloussov L.V., V. L. Voeikov V.L., & V. S. Martynyuk (Eds.), Biophotonics and Coherent Systems in Biology (pp. 159-166), New York: Springer.
Nikolaev, Yu. A., Surgucheva, N. A., & Filippova, S. N. Effect of distant interactions on the growth and development of streptomycetes (2015). Microbiology (Russia), 84(6), 673–679.
Norris, V., Molina, F., & Gewirtz, A. T. (2013). Hypothesis: bacteria control host appetites. Journal of Bacteriology, 195, 411-416.
Novikova, I. Yu. (1989). Possible mechanisms of sterile zone formation. Microbiology (Russia), 58, 127-157.
O'Mahony, S. M, Clarke, G., Borre, Y. E., Dinan, T. G., Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behavior & Brain Research, 277, 32-48.
Olas, B. (2015). Hydrogen sulfide in signaling pathways. Clinica Chimica Acta, 439, 212-218.
Oleskin, A. V. (1994). Social behaviour of microbial populations. Journal of Basic Microbiology, 34(6), 425-439.
Oleskin, A. V. (1996). Making a new case for voluntary cooperation-based fission-fusion structures in human society. Social Science Information, 35(4), 619-627.
Oleskin, A. V. (2001). Ecologically important properties of microbial populations. Soros Educational Journal, 7(8), 7-12.
Oleskin, A. V. (2009). Biosociality of unicellular organisms as exemplified by prokaryotes. Journal of General Biology (Russia), 70(3), 35-60.
Oleskin, A. V. (2014). Network Structures in Biological systems and in Human Society. New York: Nova Science Publishers.
Oleskin, A. V., & Shenderov, B. A. (2016). Neuromodulatory effects and targets of the SCFAs and gasotransmitters produced by the human symbiotic microbiota. Microbial Ecology in Health & Disease, doi: 10.3402/mehd.v27.30971.
Oleskin, A. V., & Shenderov, B. A. (2019). Probiotics and psychobiotics: the role of microbial neurochemicals. Probiotics & Antimicrobial Proteins, 11(4),1071-1085.
Oleskin, A. V., & Shenderov, B. A. (2020). Microbial Communication and Microbiota-Host Interactivity: Neurophysiological, Biotechnological, and Biopolitical Implications. New York: Nova Science Publishers.
Oleskin, A. V. & Cao, B. (2022). Microalgae in terms of biomedical technology: probiotics, prebiotics, and metabiotics. Applied Biochemistry and Microbiology, 58(6), 635–648.
Oleskin, A. V. & Postnov, A. L. (2022). Neurotransmitters as communicative agents in aquatic ecosystems. Moscow University Biological Sciences Bulletin, 77(1), 6–12.
Oleskin, A. V., Kirovskaya, T. A., Botvinko, I. V., & Lysak, L. V. (1998). Effect of serotonin (5-hydroxytrypamine) on microbial growth and differentiation. Microbiology (Russia), 67(3), 306-311.
Oleskin, A. V., Botvinko, I. V., & Tsavkelova, E. A. (2000). Colonial organization and intercellular communication in microorganisms. Microbiology, 69(3), 309-327.
Oleskin, A. V., Shishov, V. A., & Malikina, K. D. (2010). Symbiotic Biofilms and Brain Neurochemistry. New York: Nova Science Publishers.
Oleskin, A. V., Zhilenkova, O. G., Shenderov, B. A., Amerhanova, A. M., Kudrin, V. S., & Klodt, P. M. (2014a). Starter cultures of lactobacilli as producers of neuromediators such as biogenic amines and amino acids. Dairy Industry (Russia), No. 9, 42-43.

Oleskin, A. V., Zhilenkova, O. G., Shenderov, B. A., Amerhanova, A. M., Kudrin, V. S., & Klodt, P. M. (2014b). Lactic-acid bacteria supplement fermented dairy products with human behavior-modifying neuroactive compounds. Journal of Pharmacy and Nutrition Sciences, 4P, 199-206.

Oleskin, A. V., El'-Registan, G. I.,& Shenderov, B. A. (2016). Role of neuromediators in the functioning of the human microbiota: "business talks" among microorganisms and the microbiota-host dialogue. Microbiology (Russia), 85(1), 1-22.

Oleskin, A. V., Shenderov, B. A., & Rogovsky, V. S. (2017a). Role of neurochemicals in the interaction between the microbiota and the immune and the nervous system of the host organism. Probiotics & Antimicrobial Proteins, 9(3), 215-234.

Oleskin, A. V., Zhilenkova, O. G., & Vodolazov, I. R. (2017b). Biotechnology and microbial neurochemistry: Biotechnological implications of the role of neuromediators in microbial systems. Advances in Biotechnology and Microbiology, 2(4), 001-004.

Oleskin, A. V., Sorokina, E. V., Zarubina, A. P., & Parkhomenko, I. M. (2017c). Testing neurotransmitters for toxicity with a luminescent biosensor: implications for microbial endocrinology. Journal of Pharmacy & Nutrition Sciences, 7, 88-94.

Orlova, E, Paschenkov, M, Davydovskaya, M, Klimova, S, Khozova, A, Mugutdinova, B, & Boiko, A (2012). Role of dopamine in regulating the interaction of the nervous and immune system in multiple sclerosis. Zhurnal Nevrologii i Psikhiatrii (Journal of Neurology & Psychiatry), 112(2), 34-40.

Ostroumov, S. A. (1986). Introduction to Biochemical Ecology. Moscow: Moscow State University Publishers.

Otto, K. (2010). Signalling processes implicated in *Escherichia coli* biofilm formation. In: W. C. Bayley (Ed.) Biofilms: Formation, Development and Properties (pp.195-225). New York: Nova Science Publishers.

Özogul, F. (2004). Production of biogenic amines by *Morganella morganii, Klebsiella pneumonia* and *Hafnia alvii* using a rapid HPLC method. European Food Research & Technology, 219, 465-469.

Özogul, F. & Özogul, Y. (2005). Formation of biogenic amines by Gram-negative rods isolated from fresh, spoiled, VP-packed and MAP-packed herring (*Clupea harengus*). European Food Research & Technology, 221, 575-581.

Özogul, F. & Özogul, Y. (2007). The ability of biogenic amines and ammonia production by single bacterial cultures. European Food Research & Technology, 225, 385-394.

Özogul, F., Kuley, E., Özogul, Y., & Özogul, I. (2012). The function of lactic acid bacteria on biogenic amines production by food-borne pathogens in arginine decarboxylase broth. Food Science & Technology Research,18, 795-804.

Parashar, A. & Udayabanu, M. (2016). Gut microbiota regulates key modulators of social behavior. European Neuropsychopharmacology, 26, 78-91.

Paulose, J. K. & Cassone V. M. (2016). The melatonin-sensitive circadian clock of the enteric bacterium *Enterobacter aerogenes*. Gut Microbes, 7, 424-427.

Pavlova, I. B., Levchenko, K. M., & Bannikova, D. A. (2007). Atlas of the Morphology of the Populations of Pathogenic Bacteria. Moscow: Kolos (in Russian).

Payne, D. E. & Boles, B. R. (2016). Emerging interactions between matrix components during biofilm development. Current Genetics, 62, 137-141.

Peers, C., Boyle, J, P., Scragg, J. L., Dallas, M.L., Al-Owais, M. M., Hettiarachchi, N. T, & et al. (2015). Diverse mechanisms underlying the regulation of ion channels by carbon monoxide. British Journal of Pharmacology, 172,1546−1556.

Penfold, W. J. (1914). On the nature of the bacterial lag. Journal of Hygiene, 14, 215-241.

Perego, M. (1997). A peptide export-import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. Proceedings of the National Academy of Sciences of the USA, 94(16), 8612-8617.

Piletz, J. E., Aricioglu, F., Cheng, J. T., Fairbanks, C. A., Gilad, V. H., Haenisch, B., Halaris, A., Hong, S., Lee, J. E., Li, J., Liu, P., Molderings, G. J., Rodrigues, A. L., Satriano, J., Seong, G. J., Wilcox, G., Wu, N., & Gilad, G. M. (2013). Agmatine: clinical applications after 100 years in translation. Drug Discovery Today, 18(17-18), 880-893.

Plakunov, V. K. & El'-Registan, G. I. (2004). Research on microbial physiology and biochemistry in the Institute of Microbiology of the Russian Academy of Sciences. Microbiology (Russia), 73 (5), 659-667.

Polhemus, D. J. & Lefer, D. J. (2014). Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. Circulation Research, 114, 730-737.

Pouokam, E & Diener, M. (2012). Modulation of ion transport across the rat distal colon by cysteine. Frontiers in Physiology, 3, 43.

Prindle, A., Liu, J., Assali, M., Ly, S., Garcia-Ojavo, J., & Süel, G. M. (2015). Ion channels enable electrical communication within bacterial communities. Nature, 527(7576), 59-63.

Prud'homme, G. J., Glinka, Y., & Wang, Q. (2015). Immunological GABAergic interactions and therapeutic applications in autoimmune diseases. Autoimmunity Reviews, 14(11), 1048-1056.

Raasch, W., Schäfer, U., Chun, J., & Dominiak, P. (2001). Biological significance of agmatine, an endogenous ligand at imidazoline binding sites. British Journal of Pharmacology, 133(6), 755-780.

Rahman, M. A., Azuma, Y., Fukunaga, H., Murakam, T., Sugi, K., Fukushi, H., Miura, K., Suzuki, H., & Shizai, M. (2005). Serotonin and melatonin, neurohormones for homeostasis, as novel inhibitors of infection by the intracellular parasite chlamydia. Journal of Antimicrobials & Chemotherapy, 56, 861-868.

Rahn, O. (1906). Über den Einfluß der Stoffwechselprodukte auf das Wachstum der Bakterien [On the influence of metabolic products on the growth of bacteria]. Zentralblatt für Bakteriologie und Parasitenkunde,16,.417-429.

Ramírez-Mata, A., Fernández-Domínguez, I. J., Nuñez-Reza, K. J., Xiqui-Vázquez, M. L., & Baca, B. E. (2014). Redes de señalización en la producción de biopelículas en bacterias: quorum sensing, di-GMPc y óxido nitric [Signaling pathways in biofilm production in bacteria: quorum sensing, di-GMP, and nitric oxide]. Revista Argentina de Microbiologia, 46, 242-255.

Rao, M. & Gershon, M. D. (2016). The bowel and beyond: the enteric nervous system in neurological disorders. Nature Reviews. Gastroenerology & Hepatology, 13, 517-528.

Raqib, R., Sarker, P., Bergman, P., Ara, G., Lindh, M., Sack, D. A., Islam, K. M. N., Gudmundsson, G. H., Andersson, J., & Agerberth, B. (2006). Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic. Proceedings of the National Academy of Sciences of the USA, 103(24), 9178-9183.

Rayner, A. D. M. (1988). Life in a collective: lessons from the fungi. New Scientist, 120, 49-53.

Rees, T., Bosch, T., & Douglas, A. E. (2018). How the microbiota challenges our concept of self. Plos Biology, 16(2), e2005358.

Reisner, A., Krogfelt, K. A., Klein, B. M., Zechner, E. L., & Molin, S. (2006). In vitro biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. Journal of Bacteriology, 188(10), 3572-3581.

Revenchon, S., Bouillant, M. L., Salmond, G., & Nasser, W. (1998). Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemii*. Molecular Microbiology, 29, 1407-1418.

Reyes-García, M.G., Hernández-Hernández, F., & García-Tamayo, F. (2012). Gamma-aminobutyric acid (GABA) increases in vitro germ-tube formation and phospholipase B1 mRNA expression in Candida albicans. Mycoscience, 53, 36-39.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G. A. D., Gasbarrini, A., & Mele, M. C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and disease. Microorganisms, 7, 14.

Robinson, J. L., Adolfsen, K. J., & Brynildsen, M. P. (2014). Deciphering nitric oxide stress in bacteria with quantitative modeling. Current Opinions in Microbiology, 19, 16-24.

Rohrscheib, C. E. & Brownlie, J. C. (2013). Microorganisms that manipulate complex animal behaviours by affecting the host's nervous system. Springer Science Reviews, 1, 133-140.

Romeo, T. (2006). When the party is over: a signal for dispersal of *Pseudomonas aeruginosa* biofilms. Journal of Bacteriology, 188, 7325-7327.

Roshchina, V. V. (1991). Biomediators in Plants. Puschino: NTS (in Russian).

Roshchina, V. V. (2010). Evolutionary considerations of neurotransmitters in microbial, plant, and animal cells. In: M. Lyte, P. & P. E. Freestone (Eds.), Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health (pp.17-52), New York: Springer.

Roshchina, V. V. (2016). New trends in perspectives in the evolution of neurotransmitters in microbial, plant, and animal cells. In: M. Lyte (Ed.), Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health. Advances in Experimental Biology and Medicine 874 (pp.25-72), New York: Springer International Publishing AG.

Rotschild, D. (2018). Environment dominates over host genetics in shaping human gut microbiota. Nature, 555, 210-215.

Roig-Sagués, A. X., Molina, A. P., & Hernandez-Herrero, M. M. (2002). Histamine and tyramine-forming microorganisms in Spanish traditional cheeses. European Food Research & Technology, 215, 96-100.

Saha, P., Nath, D., Choudhury, M. D., & Talukdar, A. D. (2018). Probiotic biosurfactants: a potential therapeutic exercise in biomedical sciences In: J. K. Patra, G. Das, & H.-S. Shin (Eds.), Microbial Biotechnology. Vol. 2, Application in Food and Pharmacology (pp. 499-514), Singapore: Springer Nature Singapore Pte. Ltd.

Salmond, G. P. C., Bycroft, B. W., Stewart, C. S. A. B., & Williams, P. (1995). The bacterial "enigma": cracking the code of cell-cell communication. Molecular Microbiology, 16(4), 615-624.

Sampson, T. R. & Mazmanian, S. K. (2015). Control of brain development, function, and behavior by the microbiome. Cell Host & Microbe, 17(5), 565-576.

Sarris, J., Logan, A. C., Akbaraly, T. N., Amminger, G. P., Balanza-Martinez, V., Freeman, M. P, Hibbeln, J., Matsuoka, Y., Mischoulon, D., Mizoue, T., Nanri, A., Nishi, D., Ramsey, D., Rucklidge, J. J., Sanchez-Villegas, A., Scholey, A., Su, K. P., Jacka, F. N., & International Society for Nutritional Psychiatry Research. (2015). Nutritional medicine as mainstream in psychiatry. Lancet Psychiatry, 2,271-274.

Satriano, J. (2004). Arginine pathways and the inflammatory response: interregulation of nitric oxide and polyamines: review article. Amino Acids, 26(4), 321-329.

Schroeder, F. A., Lin, C. L., Crusio, W. E., & Akbarian, S. (2007). Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. Biological Psychiatry, 62(1), 55-64.

Sekowska, A., Masson, J. B., Celani, A., Danchin, A., & Vergassola, M. (2009). Repulsion and metabolic switches in the collective behavior of bacterial colonies. Biophysical Journal, 97, 688-698.

Sewertzowa, L. B. (1929). Über den Einfluß der mitogenetischen Strahlen auf die Vermehrung der Bacterien [On the influence of mitogenetic rays on bacterial reproduction]. Biologisches Zentralblatt, 49, 212-225.

Shajib, M. S. & Khan, W. I. (2015). The role of serotonin and its receptors in activation of immune responses and inflammation. Acta Physiologica, 213(3), 561-574.

Shapiro, J. A. (1985). Scanning electron microscope studies of *Pseudomonas putida* colonies. Journal of Bacteriology, 164(3), 1171-1181.

Shapiro, J. A. (1988). Bacteria as multicellular organisms. Scientific American, June, 82-89.

Shapiro, J. A. (1995). The significance of bacterial colony patterns. BioEssays 17(7), 597-607.

Shapiro, J.A. & Trubatch, D. (1991). Sequential events in bacterial colony morphogenesis. Physica D, 4(1-2), 214-223.

Shapiro, J. A. & Dworkin, M. (eds). (1997). Bacteria as Multicellular Organisms. Oxford: Oxford University Press.

Sharkey, K. A. & Savidge T. C. (2014). Role of enteric neurotransmitters in host defense and protection of the gastrointestinal tract. Anatomic Neuroscience: Basic and Clinical, 182, 70-82.

Shaw, L. N., Johnson, I.-M., Singh, V. K., Tarkowki, A., & Stewart, G. C. (2007). Inactivation of trap has no effect on the Agr quorum-sensing system or virulence of *Staphylococcus aureus*. Infection and Immunity, 75(9), 4519-4527.

Shenderov, B. A. (1998). Medical Microbial Ecology and Functional Nutrition. Vol.2. Social and Ecological Consequences of Imbalanced Microbial Ecology of Animals and Humans. Moscow: Grant (in Russian).

Shenderov, B. A. (2001). Medical Microbial Ecology and Functional Nutrition. Vol.3. Probiotics and Functional Nutrition. Moscow: Grant (in Russian).

Shenderov, B. A. (2008). Functional Nutrition and its Role in Preventing Metabolic Syndrome. Moscow: DeLi Print (in Russian).

Shenderov, B. A. (2011). Probiotic (symbiotic) bacterial languages. Anaerobe, 17, 490-495.

Shenderov, B. A. (2013a). Targets and effects of short-chain fatty acids. Sovremennaya Meditsinskaya Nauka (Russia), No.1-2, 21-50.

Shenderov, B. A. (2013b). Metabiotics: novel idea or natural development of probiotic conception. Microbial Ecology in Health & Disease, doi:10.3402/mehd.v24.20399.

Shenderov, B. A. (2014). Microbial ecology and its role in maintaining health. Metamorphozy (Russia), No. 5, 72-80.

Shenderov, B. A. (2016). The microbiota as an epigenetic control mechanism. Chapter 11. In: Nibali. L, & Henderson, B. (Eds.). The Human Microbiota and Chronic Disease: Dysbiosis as a Cause of Human Pathology. 1st ed, J. Wiley & Sons, pp.179-197.

Shenderov, B. A. (2017). Metabiotics: an overview of progress, opportunities and challenges. Journal of Microbial Biochemistry & Technology.http://doi.org/10.4172/1948-5948-C1-029

Shenderov, B.A., Golubev, V. l., Danilov, A. B., & Prischepa, A. V. (2016). Human gut microbiota and neurodegenerative diseases. Neurology (Russia), No. 1, 7-13.

Shenderov, B. A., Sinitsa, A. V., & Zakharchenko, M. M. (2017). Metabiotics Yesterday, Today, and Tomorrow. Saint-Petersburg: Kraft (in Russian).

Shenderov, B. A., Yudin, S. M., Zagaynova , A. V., Shevyreva, M. P. (2018). The role of commensal gut bacteria in the aetiopathogenesis of inflammatory bowel disease: *Akkermansia muciniphila*. Experimental and Clinical Gastroenterology (Russia), 159 (11): 4-13.

Shireen, T., Basu, A., Sarkar, M., & Mukhopadhyay, K. (2015). Lipid composition is an important determinant of antimicrobial activity of alpha-melanocyte stimulating hormone, Biophysical Chemistry, 196, 33-39.

Shishov, V. A., Kirovskaya, T. A., Kudrin, V. S., & Oleskin, A. V. (2009). Amine neuromediators, their precursors, and oxidation products in the culture of Escherichia coli K-12. Applied Biochemistry & Mikrobiology (Russia), 45, 494-497.

Shpakov, A. O. (2009). Nonpeptide bacterial signal molecules. Microbiology (Russia), 78(2), 163-175.

Shultz, S. R., MacFabe, D. F., Martin, S. Jackson, J.,Taylor, R., Boon, F., Ossenkopp, K. P, & Cain, D. P. (2009). Intercerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. Behavioural and Brain Research, 200, 33-41.

Siadat, S. D. & Badi, S. A. (2019). The symbiosis or dysbiosis of gut microbiota: friend or foe? Acta Scientific Microbiology, 2(3), 54-55.

Silva-Herdade, A. S. & Saldanha, C. (2013). Effects of acetylcholine on an animal mode of inflammation. Clinical Chemorheology & Microcirculation, 53(1-2), 209-216.

Sircili, M. P., Walters, M., Trabulsi, L. R., & Sperandio, V. (2004). Modulation of enteropathogenic *Escherichia coli* virulence by quorum sensing. Infection & Immunity, 72, 2329-2337.

Sitdikova, G. F., & Zefirov, A. L. (2010). The hydrogen sulfide: from the sewers of Paris to the signal molecule. Nature (Russia), No. 9, 29−37.

Skarlyachan, S., Sridhar, V. S., Packirisami, S., Kumargowda, S. T., & Challapilli, S. B. (2018). Recent perspectives on the molecular basis of biofilm formation by *Pseudomonas aeruginosa* and approaches for treatment and biofilm dispersal. Folia Microbiologica, 63(4), 413-432.

Skulachev, V. P. (1999). Phenoptosis: the organism's programmed death. Biochemistry (Russia), 64(12), 1679-1688.

Smirnov, S. G. (2004). Bacterial Ethology. Ivanovo: Ivanovo State Medical Academy (in Russian).

Smith, E. A. & Macfarlane, G. T. (1996). Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. Journal of Applied Bacteriology, 81,288-302.

Smith, H., Mann, B. E., Motterlini, R., Poole, R. K. (2011). The carbon monoxide-releasing molecule, CORM-3 (Ru(CO)3Cl(glycinate)), targets respiration and oxidases in Campylobacter jejuni, generating hydrogen peroxide. IUBMB Life, 63, 63−71.

Stevens, A. M., Schuster, M., & Rumbaux, K. P. (2012). Working together for the common good: cell-cell communication of bacteria. Journal of Bacteriology, 194, 2131-2141.

Strakhovskaya, M. G, Ivanova, E. V, & Fraikin, G. Ya. (1993). Stimulatory influence of serotonin on the growth of the yeast *Candida guillermondii* and the bacterium *Streptococcus faecalis*. Microbiology (Russia), 62, 46-49.

Strempel, N., Neidig, A., Nusser, M., Geffers, R., Vieillard, J., Lesouhaitier, O., Brenner-Weiss, G., & Overhage J. (2013). Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in *Pseudomonas aeruginosa*. Plos One, 8(12), e82240.

Sudo, N. (2019). Biogenic amines: signals between commensal microbiota and gut physiology. Frontiers in Endocrinology, 10, 504.

Sumina, E. L. (2006). Behavior of filamentous cyanobacteria in lab culture. Microbiology (Russia), 75(4), 532-537.

Tarighi, S. & Taheri, P. (2011). Different aspects of bacterial communication signals. World Journal of Microbiology and Biotechnology, 27,1267-1280.

Tarnita, C. E. (2017). The ecology and evolution of social behavior in microorganisms. Journal of Experimental Biology, 220, 18-24.

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′Sialyllactose and 6′Sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. Brain, Behavior, and Immunity, 50, 2015, 166-177.

Tetz, V. V., Rybalchenko, O. V., & Savkova, G. A. (1990). Ultrastructural features of microbial colony organization. Journal of Basic Microbiology, 30, 597-607.

Tetz, V. V., Rybalchenko, O. V. & Savkova, G. A. (1993). Ultrastructure of the surface film. Journal of General Microbiology, 139, 855-858.

Tetz, V. V., Korobov, V. P., Artemenko, N. K., Lemkina, L. M, Panjkova, N. V., & Tetz, G. V. (2004). Extracellular phospholipids of isolated bacterial communities. Biofilms, 1, 149-155.

Thornhill, S. G. & McLean, R. J. C. (2018). Use of whole-cell biassays for screening quorum signaling, quorum interference, and biofilm dispersion In: L. Leoni & G. Rampioni (Eds.), Quorum Sensing: Methods and Protocols (pp.3-24), Rome: Humana Press.

Tinajero-Trejo, M., Jesse, H. E., & Poole, R. K. (2013). Gasotransmitters, poisons, and antimicrobials: it's a gas, gas, gas! F1000 Prime Report, 5, 28.

Tinbergen, N. (1968). On war and peace in animals and man. Science, 160, 1411-1418.

Tsavkelova, E. A., Botvinko, I. V., Kudrin, V. S., & Oleskin, A. V. (2000). Detecting neuromediator amines in microorganisms with high performance liquid chromatography. Dokl. Ross. Akad. Nauk (Russian Academy of Sciences Reports), 372, 840-842.

Ulvestad, E. (2009). Cooperation and conflict in host-microbe relations. Acta Pathologica, Microbiologica et Immunologica Scandinavica, 117, 311-322.

Uranchimeg, D., Kim, J. H., Kim, J. Y., Lee, W. T., Park, K. A., Batbaatar, G., Tundevrentsen, S., Amgalanbaatar, D., & Lee, J. E. (2010). Recovered changes in the spleen by agmatine treatment after transient cerebral ischemia. Anatomy & Cell Biology, 43(1), 44-53.

Vakhitov, T. Ya. (2019). Principles of Metabolic Regulation and Communication in Bacteria. Moscow: URSS (In Russian).

Vakhitov, T. Ya., Yashina, O. Yu., Petrov, L. N., & Korolyuk, A. M. (2000). Investigation of the effect of the growth autostimulator preparation from Escherichia coli M-17 (the Actoflor preparation) on the growth of pure and mixed bacterial cultures. Zhurnal Mikrobiologii (Journal of Microbiology), No.3, 20-24.

Vakhitov, T. Ya., Protasov, E. A., Visnold, N. V., Tolparov, Yu. N., & Petrov, L. N. (2003). Isolation and identification of the growth autostimulators of Escherichia coli. Zhurnal Mikrobiologii (Journal of Microbiology), No. 2, 7-12.

Vakhitov, T. Ya & Sitkin, S. I. (2014). The superorganism concept in biology and medicine. Experimental & Clinical Gastroenterology (Russia),107(7), 72-85.

van de Wouw, M., Schellekens, H., Dinan, T. G., & Cryan J. F. (2017). Microbiota-gut-brain axis: modulator of host metabolism and appetite. Journal of Nutrition, 147. 727-745..

van den Eynden, J., Ali, S. S., Horwood, N., Carmans, S., Brone, B., Hellings, N., Steels, P., Harvey, R. J., & Rigo, J. M. (2009). Glycine and glycine receptor signalling in non-neuronal cells. Frontiers in Molecular Neuroscience, 2, 9.

Väth, R. (1992). Amöbe gegen *Paramecium*: zwei Verlierer. Eine Beobachtung in einer Amöbenkultur [An amoeba fights a *Paramecium*; both lose out. An observation in an amoeba culture]. Mikrokosmos, 81, 272-275.

Vega, N. M., Allison, K. R., Khalil, A. S., & Collins, J. J. (2012). Signaling-mediated bacterial persister formation. Nature Chemical Biology, 8, 431-433.

Vejzagić, N., Adelfio, R., Keiser, J., Kringel, H., Thamsborg, S., & Kapel, C. (2015). Bacteria-induced egg hatching differs for Trichuris muris and Trichuris suis. Parasites & vectors. 8. 371.

Velicer, G. J. (2003). Social strife in the microbial world. Trends in Microbiology, 11(7), 330-337.

Venturi, V., Subramoni, S., Sabag-Daigle, A., & Ahmer, B. M. M. (2018). Methods to study solo/orphan quorum sensing receptors. In: L. Leoni & G. Rampini (Eds.), Quorum Sensing: Methods and Protocols (pp.145-160), Rome: Humana Press.

Verbeke, K. A., Boobis, A. R., Chiodini, A., Edwards, C. A., Franck, A, Kleerebezem, M., Nauta, A., Raes, J., van Tol, E. A. F., & Tuohy, K. M. (2015). Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutritional Research Reveiws, 28, 42-66.

Verbrugge, E., Boyen, F., Gaastra, W., Bekhuis, L., Leyman, B., van Paris, A., Haesebrouck, F., & Pasmans, F. (2012). The complex interplay between stress and bacterial indections in animals. Veterinary Microbiology, 155, 115-127.

Vidyasagar A. (2016). What are biofilms? Live Science. Online Publication: http://livescience.com/57295-biofilm.html.

Virkkkunen, M., DeJong, J., Bartko, J., Goodwin, F. K., & Linnoila, M. (1994). Relationship of psychobiological variables to recidivism in violent offenders and impulsive fire setters: a follow-up study. In: R. D. Masters & M. T. McGuire (Eds.), The Neurotransmitter Revolution. Serotonin, Social Behavior, and the Law (pp.86-95) Carbondale & Edwardsville: Southern Illinois University Press.

Vodolazov, I. R., Dbar, S. D., Oleskin, A. V., & Stoyanova, L. G. (2018). Exogenous and endogenous neuroactive biogenic amines. Applied Biochemistry & Microbiology (Russia), 54(6), 603-610.

Voloshin, S. A. & Kapreliants, A. S. (2004). Intercellular interactions in bacterial populations. Biochemisty (Russia), 69(1), 1555-1564.

Vysotsky, V. V., Zaslavskaya, P. L., Mashkova, A. V., & Baulina, O. I. (1991). Polymorphism as a developmental pattern in populations of prokaryotic organisms. Biological Sciences, No.12, 5-17.

Wall, R., Cryan, J. F., Ross, R. P., Fitzgerald, G. F., Dinan, T. G., & Stanton, C. (2014). Bacterial neuroactive compounds produced by probiotics. Advances in Experimental Medicine & Biology, 817, 221-239.

Walters, M. & Sperandio, V. (2006). Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. Infection & Immunity, 74, 5445-5455.

Wang, R. (2012). Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiology Reviews, 92, 791-896.

Waters, C.M. & Bassler, B.L. (2005). Quorum sensing: cell-to-cell communication in bacteria. Annual Reviews in Cell Development Biology, 21, 319-346.

Wegiel, B., Hanto, D. W., & Otterbein, L. E. (2013). The social network of carbon monoxide in medicine. Trends in Molecular Medicine, 19,3−11.

West, C. A., Diggle, S. P., Buckling, A., & Griff, S. A. (2007). The social lives of microbes. Annual Review of Ecology, Evolution, and Systematics, 38, 53-71.

Westfall, S., Lomis, N., Kahouli, I., Dia, S. Y., Singh, S. P., & Prakash, S. (2017). Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. Cellullar & Molecular Life Sciences, 74, 3769-3787.

Will, D., Wu, S. S., & Kaiser, D. (1998). Contact stimulation of Tgl1 and type IV piliin *Myxococcus xanthus*. Journal of Bacteriology, 180(3), 759-761.

Wilson, E. O. (1975). Sociobiology: The New Synthesis. Cambridge (Mass.): Harvard University Press.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y. Y., Keilbaugh, S. A., Bewtra, M., Knights, D., Walters, W. A., Knight, R., Sinha, R., Gilroy, E.,Gupta, K., Baldassano, R., Nessel, L., Li, H., Bushman, F. D., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science, 334, 105-108.

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., Nagler, C. R., Ismagilov, R. F., Mazmanian, S. K., & Hsiao, E. Y. (2015). Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell, 161, 264-276.

Yao, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., McSkimming, D.I. (2020). Gut microbiota and immune system interaction // Microorganisms, 8, 1587.

Yarullina, D. R., Smolentseva, O. A., & Ilinskaya, O. N. (2011). Modulation of nitric oxide (NO) biosynthesis in lactobacilli. Moscow University Biological Science Bulletin, 66(2), 79-80.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldrassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozapone, C. A., Lauber, C., Clemente, J. C., Knights, D., Knight, R., & Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. Nature, 486, 222-227.

Yerusalimsky, N. D. (1952). The Physiology of the Development of Pure Bacterial Cultures. D. Sci. (Biol.) Dissertation. Moscow: Institute of Microbiology. USSR Academy of Sciences (in Russian).

Yesilyurt, N., Yılmaz, B., Agagündüz, D., & Capasso, R. (2021). Involvement of probiotics and postbiotics in the immune system modulation. Biologics, 1, 89–110.

Yildiz, F. H., Liu, X. S., Heydorn, A., & Schoolnik G. K. (2004). Molecular analysis of rugosity in a *Vibrio cholerae* O1 El Tor phase variant. Molecular Microbiology, 53, 497-515.

Yunes, R. A., Poluektova, R. U, Dyachkova, M. S., Klimina, K. M., Kovtun, A. S., Averina O.V., Orlova, V. S., & Danilenko, V. N. (2016). GABA production and structure of *gadB/gadC* genes in *Lactobacillus* and *Bifidobacterium* strains from human microbiota. Anaerobe, 42, 1-8.

Yurkevich, D. I. & Kutyshenko, V. P. (2002). The medusomycete (the tea fungus): history of research, composition, and physiological and metabolic peculiarities. Biophysics (Moscow), 47, 1116-1129.

Zaborina, O., Lepine, F., Xiao, G., Valuckaite, V., Chen, Y., Li, T., Ciancio, M., Zaborin, A., Petroff, E., Turner, J. R., Rahme, L. G., Chang, E., & Alverdy, J. C. (2007). Dynorphin activates quorum sensing quinolone signaling in *Pseudomonas aeruginosa*. PloS Pathogens, 3, e 35.

Zagon, I. S. & McLaughlin, P. J. (1992). An opioid growth factor regulates the replication of microorganisms. Life Sciences, 50, 1179-1187.

Zampeli, E. & Tiligada, E. (2009). The role of histamine H4 receptor in immune and inflammatory disorders. British Journal of Pharmacology, 157, 24-33.

Zeynalov, E. & Dore, S. (2009). Low doses of carbon monoxide protect against experimental focal brain ischemia. Neurotoxicity Research, 15, 133-137.

Zhao, X., Liu, X., Xu, X., & Fu, Y. V. (2017). Microbe social skill: the cell-to-cell communication between microorganisms. Science Bulletin, 62, 516-524.

Zhou, J. & Cai Z.-H. (2018). Microbial social interactions in biofilms. In: P. V. Bramchachari (Ed.), Implications of Quorum Sensing Systems in Biofilm Formation and Virulence (pp. 29-46). Singapore: Springer Nature Singapore Ltd.

Zumft, W. G. (1993). The biological role of nitric oxide in bacteria. Archives of Microbiology, 160, 253-264.

Note: the highlighted publications can be used by students wishing to prepare presentations.

TABLES

Table 1. Functions of neurochemicals in the nervous system (based on the following publications: Boldyrev et al., 2010; Dubynin et al., 2010; Sitdikova & Zefirov, 2010; Duan et al., 2015; Oleskin & Shenderov, 2016; Oleskin et al., 2017a, b).

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Neurochemicals	**Neurophysiological and psychological effects**	
Dopamine	Activation of the sympathetic nervous system; involvement in cognition, information memorization, and emotions	Maintenance of the wakeful state and stimulation of hedonic behavior; involvement in effectuating voluntary movements
Norepinephrine		Stimulation of locomotor activity aggressiveness and mitigation of anxiety
Serotonin	Regulation of the emotional state, memorization and learning processes, and dominant behavior. Appetite suppression. “Putting the brain asleep” at high concentrations.	
Histamine	Involvement in regulating appetite, pain sensitivity, the cognitive activity of the brain, and the sleep-wake rhythm	
Acetylcholine	Regulation of brain processes related to motivation, attention, memory, learning, plasticity, and the general activity level of the brain	
Agmatine	Hypothetic neurochemical function, consistent with the data on synthesis of agmatine in the brain, its accumulation in synaptic vesicles, and release upon membrane depolarization	
Glutamate	Main excitatory neurochemical in the CNS that exerts a stress-relieving effect and is involved in learning and information memorization	
GABA	Main inhibitory neurochemical in the CNS. Involvement in regulating the sleep-wake cycle, locomotor activity, conditioned reflex formation, and information memorization and recognition	
Glycine	Inhibitory neurochemical with a stress-relieving and relaxing effect	
Aspartate	Auxiliary excitatory neurochemical. Mood improvement, mitigation of the state of fatigue	
SCFAs	Mitigation of depression and anxiety, pain relief. Antidepressant effect (especially butyrate). Propionate at high concentrations causes locomotive behavior disruption and accelerates autism progression. Appetite suppression (acetate).	
Nitic oxide	Involvement in pain perception; mood improvement during grooming behavior.	
Hydrogen sulfide	Involvement in neuronal activity regulation, cognitive activities, and memory. Neuroprotective effect.	
Opioids (endorphins, enkephalins, and dynorphins)	Inhibition of impulse transmission, pain-relieving effect. Mood improvement, which may result in euphoria. Soporific effect at high concentrations	
Substance P	Involvement in pain perception, anxiety stimulation	

Neuropeptide Y	Pain relief, stress and anxiety mitigation, food intake stimulation
Cholecystokinin	Involvement in foraging behavior and pain perception. A fragment of the cholecystokinin molecule causes anxiety and fear.

Table 2. Functions of neurochemicals in the immune system

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Neurochemicals	**Effects in the immune system**
Dopamine	Complex and partly contradictory effects involving multiple receptors. Overwhelmingly, catecholamines exhibit anti-inflammatory and immunosuppressive activity. Norepinephrine can promote the development of Th2-asociated diseases, such as allergic processes (Orlova et al., 2012; Cosentino & Marino, 2012; Cosentino et al., 2013; Levite, 2016).
Norepinephrine	
Serotonin	Both compounds are implicated in effectuating and potentiating immune responses at the initial inflammation stages. However, at the final inflammation stages, they may be involved in mitigating inflammation. Their immunotropic effects can be both stimulatory and inhibitory, depending on the microenvironment. Serotonin activates phagocytosis at low IFN-□ levels and inhibits this process at high IFN-γ levels□ As for Th1-dependent pathological processes, e.g., rheumatoid arthritis, serotonin can attenuate inflammation. Histamine's capacity to stimulate T lymphocyte differentiation can be used for treating autoimmune diseases such as multiple sclerosis. Histamine decreases the risk of an immunocyte attack on the myeline sheath of neurons (Zampeli & Tiligada, 2009; Ley et al., 2010; Arreola et al., 2015; Gao et al., 2015; O'Mahoni et al., 2015; Shajib & Khan, 2015).
Histamine	
Acetylcholine	Immunosuppressive and anti-inflammatory activity. Of paramount importance is the interaction of acetylcholine with the nAchRα_7 receptor that results in suppressing proinflammatory cytokine production; stimulation of the efferent activity of the vagal nerve inhibits the systemic inflammatory response (Ley et al., 2010)
Agmatine	Inhibition of the inducible NO synthase, an anti-inflammatory and neuroprotective effect (Satriano, 2004; Uranchimeg et al., 2010; Ahn et al., 2012; Chai et al., 2016)
GABA	Predominantly, an anti-inflammatory effect that is due to suppressing T lymphocyte activity and downregulating proinflammatory cytokine production. Protection from experimental autoimmune encephalomyelitis, type 1 diabetes, contact dermatitis, and other autoimmune problems (Auteri et al., 2015; Prud'homme et al., 2015; Bhandage et al., 2018).
Glycine	Predominantly, an anti-inflammatory effect; inhibition of the secretion of proinflammatory cytokines and stimulation of the synthesis of anti-inflammatory mediators (van den Eynden et al., 2009)
Glutamate and aspartate	Complex immunotropic effects; predominantly immunosuppressive activity at high concentrations (characteristic of glutamate; Ganor & Levite, 2014).
SCFAs	Inhibition of neutrophil adherence and chemotaxis and suppression of immunocyte migration from the bloodstream to the inflammation area Acetate and butyrate suppress T cell proliferation and activation, decrease the antibody content in the bloodstream, and induce apoptosis in immunocytes. Butyrate and propionate increase the production and stimulate the activity of extrathymic (intestinal) T_{reg} cells (Shenderov, 2013a, b; Verbeke et al., 2015; Correâ-Oliveira et al., 2016)
Nitric oxide	Complex and partly contradictory effects on all parts of the immune system. A cytotoxic effect at high concentrations (used by T killers)

Table 3. Effects of neurochemicals in microbial systems

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Neurochemicals	Effects	Subjects and sources
Biogenic amines and their precursors, derivatives, and metabolites		
Catecholamines (dopamine, norepinephrine, epinephrine)	Stimulation of growth and, in pathogens,of virulence, flagellar motility, and adherence to host cells	*Escherichia coli* (commensal and pathogenic strains), *Shigella* and *Salmonella* species, *Pseudomonas aeruginosa* (Lyte & Ernst, 1993; Freestone et al., 1999, 2007; Anuchin et al., 2008); *Bordetella pertussis*, *B. broncioseptica,* (Freestone & Lyte, 2008); *Aeromonas hydrophila* (Kinney et al., 1999); *Helicobacter pylori, Haemophilus influenza, Klebsiella pneumonia* (reviewed, Shpakov, 2009); *Listeria monocytogenes* (Verbrugge et al., 2012), *Saccharomyces cerevisiae* (Malikina et al., 2010) *Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of growth and medium acidification	
Additional effects of individual catecholamines: Dopamine		
	Inhibition of cell aggregation Promotion of spore survival and germination	*E. coli* K-12 (Anuchin et al., 2008) *Saccharopolyspora erythraea* (Filippova et al., 2010)
	Stimulation of growth and antibacterial activity	*Lactococcus lactis subsp.lactis* strain 194, F-116, K-205, 729 (Vodolazov et al., 2018)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
	Stimulation of cell aggregation	*E. coli* K-12 (Oleskin et al., 1998a; Anuchin et al., 2008), *Polyangium* sp. (Oleskin et al., 1998a)

Neurochemicals	Effects	Subjects and sources
Norepinephrine	Growth inhibition	*Mycoplasma hyopneumoniae* (Oneal et al., 2008)
	Balance shift in the human GI tract	Increase in the *Clostridium:Bacteroides* ratio (Bailey et al., 2011)
	Stimulation of growth and antibacterial activity	*Lactococcus lactis subsp.lactis* strain 194(Vodolazov et al., 2018)
	Inhibition of luminescence	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
Serotonin	Growth stimulation	Commensal (Oleskin et al.,1998a; Anuchin et al., 2008) and, to a lesser extent, pathogenic (M.Lyte, personal communication) strains of *E. coli, Enterococcus faecalis*(Strakhovskaya et al.,1993); *Rhodospirillum rubrum* (Oleskin et al., 1998a); *Polyangium* sp. (Oleskin et al., 1998a); *Candida guillermondii* (Strakhovskaya et al., 1993); *Saccharomyces cerevisiae* (Malikina et al., 2010; Oleskin et al.,2010)
	Stimulation of growth and antibacterial activity	*Lactococcus lactis subsp.lactis* strain 194(Vodolazov et al., 2018)
	Stimulation of growth and medium acidification	*Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
	Stimulation of cell aggregation	*E.coli* K-12(Oleskin et al.,1998a;Anuchin et al., 2008), *Polyangium* sp.(Oleskin et al.,1998a).
	Photo- and radioprotective effects	*S. cerevisiae* (Fraikin et al., 1985)
	Growth inhibition	Chlamydia (Rahman et al., 2005)
	Virulence attenuation	*Candida albicans* (Mayr et al., 2005)
Melatonin	Swarming stimulation	*Enterobacter aerogenes* (Paulose & Cassone, 2016)
Indole	Growth stimulation	*Salmonella enterica* var. *enteritidis* (Vakhitov & Sitkin, 2014)
	Stimulation of biofilm formation	*Pseudomonas aeruginosa, Ps. fluorescens* (Lee et al., 2007b)
	Inhibition of biofilm formation	*E. coli* (Lee et al., 2007a)
Histamine	Growth stimulation	*E.coli* K-12(Anuchin et al., 2008).
	Stimulation of cell aggregation	*E. coli* K-12 (Anuchin et al., 2008)
	Stimulation of growth and medium acidification	*Lactobacillus acidophilus* NK-1 (Vodolazov, Zhilenkova, and Oleskin, unpublished)
	Stimulation of luminescence (at low concentrations)	*E. coli* TGI with the *lux* operon (Oleskin et al., 2017c)
Acetylcholine	Regulation of conjugation	Infusorians (reviewed, Roschina, 2010),

Neurochemicals	Effects	Subjects and sources
	and growth	*Acanthamoeba* sp. (Baig & Ahmad, 2017)
Agmatine	Inhibition of colon colonization	*Cryptosporidium parvum* (Lyte, 2016)
Short-chain fatty acids and their derivatives		
SCFAs in general	Antimicrobial activity	Gram-negative bacteria (Neish, 2009; Shenderov 2013a)
Acetate	Growth stimulation	*Roseburia* spp., *Faecalibacterium prausnitzi* (Duncan et al., 2004)
Propionate	Antifungal activity	Various groups of fungi (van de Wouw et al., 2017)
Phenylbutyrate	Induction of endogenous antimicrobial peptides	Various groups of bacteria (Raqib et al., 2006)
Neuroactive amino acids		
Aspartate	Regulation of colony macro- and microstructure	*E.coli* (Budrene &Berg, 1991, 2002; Mittal et al., 2003)
	Growth stimulation	*E. coli* BL — Vakhitov et al., 2000; Vakhitov & Sitkin, 2014; Vakhitov, 2019
	Growth inhibiion	*E. coli* M-17 — Vakhitov et al., 2000; Vakhitov & Sitkin, 2014; Vakhitov, 2019
Glutamate	Growth stimulation	*E. coli* M-17 — Vakhitov et al., 2000; Vakhitov & Sitkin, 2014; Vakhitov, 2019
GABA	Increase in resistance to acidification	*Lact. reuteri* (Lyte, 2014)
	Virulence stimulation	*Ps aeruginosa* (Mazzoli & Pessione, 2016)
	Virulence and germination stimulation	*C. albicans* (Reyes-Garcia et al., 2012)
Neuropeptides		
Dynorphin	Stimulation of virulence,pyocyanine production, and antagonistic activity	*Ps. aeruginosa* (Zaborina et al., 2007)
$[Met]^5$-Enkephalin	Growth inhibition	*Ps. aeruginosa, Staph.aureus*, *Serratia marcescens* (Zagon & McLaughlin,1992)
α–MSH	Growth inhibition	*Saph. aureus* (Shireen et al., 2015)
LL-37 (catelicidin)	Stimulation of virulence and antibiotic resistance	*Ps. aeruginosa* (Strempel et al., 2013)
Insulin	Regulation of carbon metabolism	*Neurospora crassa* (Lenard, 1992)
Substance P	Antimicrobial activity	Many gram-positive and gram-negative bacteria and fungi: the data are discordant (Kowalska et al., 2002; Hansen et al., 2006; El Karim et al., 2008)
Neuropeptide Y	Antimicrobial activity	Many gram-positive and gram-negative bacteria and fungi: the data are discordant (Kowalska et al., 2002; Hansen et al., 2006; El Karim et al., 2008)

Neurochemicals	Effects	Subjects and sources
Gasotransmitter		
Nitric oxide: Low (nanomolar) concentrations	Inhibition of biofilm formation and acceleration of biofilm dispersal	*Ps. aeruginosa* (Barraud et al., 2006), *S. marcescens*, *Vibrio cholerae, E. coli* (pathogenic strain BW20767), *Staphylococcus epidermidis*, *Bacillus licheniformis*, *C. albicans* (Barraud et al., 2009a, b)
High (micro- and millimolar) concentrations	Stimulation of biofilm formation, cytotoxic and stressor effects	*Ps. aeruginosa* (Barraud et al., 2006); *Azospirillum brasilense*, *Neisseria gonorrhoeae* (reviewed: Medinets et al., 2015); *Mycobacterium tuberculosum* (Robinson et al., 2014)

Table 4. Production of neurochemicals by microorganisms.

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Neurochemicals	Subjects	Sources
Biogenic amines and their precursors		
Dopamine	*Bacillus cereus, B. mycoides, B. subtilis, Staphylococcus aureus, E. coli, Ps. aeruginosa, Serratia marcescens, Proteus vulgaris, Saccharomyces cerevisiae*	Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010; ; Oleskin et al., 2010
	Morganella morganii, Klebsiella pneumonia, Hafnia alvei	Özogul, 2004
	Lactobacillus helveticus NK-1, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
	Lactococcus lactis subsp. *lactis,* strains K-205 and F-116	Vodolazov et al., 2018
Norepinephrine	*B. mycoides, B. cereus, B.subtilis, P. vulgaris, S. marcescens, E. coli, S. cerevisiae, Pennicilum chrysogenum*	Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010; Oleskin et al., 2010
	Lact. helveticus 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *Bulgaricus*	Oleskin et al., 2014 a, b
DOPA	*E. coli* K-12, *S. cerevisiae, B. cereus*	Shishov et al., 2009; Malikina et al., 2010; Oleskin et al., 2010
	Lact. helveticus 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
	Lactococcus lactis subsp. *lactis,* strains K-205 and F-116	Vodolazov et al., 2018
	Toxoplasma gondii	Rohrscheib & Brownlie, 2013
Serotonin	*Staph. aureus*	Hsu et al., 1986
	Enterococcus faecalis	Strakhovskaya et al., 1993
	Rhodospirillum rubrum, *B. subtilis, Staph. aureus, E. coli* K-12, *S. cerevisiae*	Oleskin et al., 1998a; Tsavkelova et al., 2000; Shishov et al., 2009; Malikina et al., 2010

	Morganella morganii, Klebsiella pneumonia, Hafnia alvei	Özogul, 2004
	Lactococcus lactis subspecies *cremoris* MG 1363, *L. lactis* subspecies *lactis* IL 1403, *Lact. plantarum* NCFB2392.	Özogul et al., 2012
	Lact. helveticus 100ash	Oleskin et al., 2014a, b
Histamine	*Morganella mogranii, Proteus vulgaris, Pr. mirabilis, Klebsiella* spp., *Enterobacter aerogenes, Enterococcus. faecalis, Citrobacter freundii, Raoultella orhithinolytica, Pantooea agglomerans, Allivibrio fischeri, Vibrio alginolyticus, V. harveyi, Acinetobacter lowfli, Pseudomonas fluorescens, Ps. putida, Ps. aruginosa, Aeromonas* spp., *Clostridium* spp., *Photobacterium* spp., *Branhamella catarrhalis,Haemophilus parainfluenza, Streptococcus thermophilus, Bacillus licheniformis, B. coagulans, Lactobacillus buchneri, Lact. reuteri, Lact. casei, Lactococus lactis*; the yeast *Debaryomyces hansenii* and *Yarrowia lypolytica*	Devalia et al., 1989; Halász et al., 1994; Shenderov, 1998; Roig-Sagués et al., 2002; Özogul & Özogul, 2005, 2007; Roshchina, 2010; Gardini et al., 2012; Helinck et al., 2013; Lin et al., 2014; Doeun et al., 2017; van de Wouw et al., 2017
Tyramine	*Lactobacillus brevis, Lact. plantarum, Lact. delbrueckii, Lact. casei, Lactococcus lactis, Leuconostoc mesenteroides,* the yeast *D. hansenii* and *Y.lypolytica*	Roig-Sagués et al., 2002; Doeun et al., 2017
Indole	*E. coli, Bacteroides ovatis,*	Smith & Macfarlane, 1996; Lee et. al.,

	Clostridium bifermentes, Ps. aeruginosa, Ps. fluorescens	2007; Vega et al., 2012
Acetylcholine	*Bacillus* spp., *Lactobacillus* spp.	Wall et al., 2014; Johnson & Foster, 2018
Neuroactive amino acids		
Agmatine	*Lactobacillus* spp.	Reviewed, Oleskin et al., 2017a
Glutamate	*E. coli, Corynebacterium glutamicum*, *Brevibacterium lactofermentum, B. flavum, Lactobacillus helveticus* 100ash, *Lact. helveticus* NK-1, *Lact. casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Vakhitov & Sitkin, 2014; Oleskin et al., 2014a b; Mazzoli & Pessione, 2016
Aspartate	*E. coli*	Vakhitov & Sitkin, 2014
GABA	*Lactobacillus brevis, Lact. rhamnosus, Lactococcus lactis, Lact. helveticus* 100ash, *L. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. b*ulgaricus, Bifidobacterium adolescentis,* and other lactobacilli and bifidobacteria,	Lee et al., 2010; Barrett et al., 2012; Ko et al., 2013 ; Liao et al., 2013; Oleskin et al., 2014a, b; Mazzoli & Pessione, 2016; Yunes et al, 2016
Glycine	*Lact. helveticus* 100ash, *Lact. helveticus* NK-1, *Lact.casei* K3III24, *Lact. delbrueckii* subsp. *bulgaricus*	Oleskin et al., 2014a, b
Taurine		
Short-chain fatty acids		
SCFAs in general	Various representatives of the GI micrbiota	Reviewed, Oleskin & Shenderov, 2016; Oleskin et al., 2017a
Propionate	*Propionibacterium* spp.	MacFabe, 2012
Neuropeptides		
β-Endorphin	*Tetrahymena pyriformis, Amoeba proteus*	Lenard, 1992
[Met]^5-Enkephalin	*Staph. Aureus*	Zagon & McLaughlin, 1992
Insulin	*E. coli,Neurospora crassa*	Lenard, 1992
Corticotropin	*Tetrahymena pyriformis*	
Somatostatin	*B. subtilis, Plasmodium falciparum*	

α-Factor, a homologue of gonadotropin-liberating factor	*S. cerevisiae*	
Gasotransmitters		
Nitric oxide	Many microorganisms including *Firmicutes*, *Actinobacteria*, *Proteobacterium,* and archaeans (e.g., *Euryarchaeota*)	Zumft, 1993; Barraud et al., 2006; Ramírez-Mata et al., 2014; Medinets et al., 2015
Carbon (mono)oxide	Many hemoxidase-containing microorganisms	King & Weber, 2007; Tinajero-Trejo et al., 2013;
Hydrogen sulfide	*E. coli* and many other GI bacteria	Carbonero et al., 2012; Olas, 2015

Table 5. Concentrations of biogenic amines and their metabolites in microbial cells.
The cells were ultrasonically disintegrated, BA contents were measured by HPLC with an amperometric detector (data from the authors' work: Tsavkelova et al., 2000). All concentrations are expressed in micromoles/kg of biomass. Designations: NE, norepinephrine; DA, dopamine; 5-HT, serotonin; DHPAA, dihydrophenylacetic acid; 5-HIAA, 5-hydroxyindolylacetic acid.
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Subject	NE	DA	DHPAA	5-HT	5-HIAA
Bacillus cereus	-	2.13	0.69	0.85	0.95
B. mycoides	0.32	0.25	0.81	-	0.33
*B.subtilis:*Total fraction	0.25	0.36	-	-	0.42
Cells	-	-	-	-	-
Matrix	0.26	0.34	-	-	0.52
Staph. aureus	-	1.35	1.54	2.2	-
E. coli	-	1.61	2.64	-	0.81
Proteus vulgaris	0.6	0.73	0.46	-	0.4
Ps. aeruginosa, var. R	-	-	1.62	-	2.7
Ps.aeruginosa, var. S	-	-	3.74	-	2.1
Serratia marcescens	1.87	0.6	1.47	-	0.51
Zoogloea ramigera	-	-	14.2	-	0.34
Saccharomyces cerevisiae	0.21	-	-	-	0.26
Penicillum chrysogenum	21.1	-	88.9	-	10.8

FIGURE CAPTIONS

Fig. 1. Various cell forms in the population of the cyanobacterium *Anabaena variabilis* CALU 458 located in the callus tissue of tobacco (reproduced, with permission, from the work: Baulina, 2010. Fig. 46). Bar, 5 μ.

Fig. 2. The formula of factors d_1 (alkylhydroxybenzenes, AHBs) according to: El'-Registan, 1988. R, carbon side chain.

Fig. 3 Equipment used to detect distant interaction between bacterial cells. 1, inner flask; 2, outer flask; 3, cotton bung; 4, foil. The culture in the outer flask was supplemented with a stress factor (chloramphenicol, an antibiotic); the culture in the inner flask received the signal from the outer flask, which resulted in accelerating its growth. According to: Nikolaev, 1992, with the author's permission.

Fig. 4. Under the influence of the signal molecule cAMP, solitary amoebas of *Dictyostelium discoideum* form a multicellular slug-like body (the pseudoplasmodium) that converts into a mushroom-like fruiting body with a stip and a cap (Samuilov et al., 2000; modified). The conversion of the multicellular body into the "mushroom" is regulated, apart from cAMP, by another signal molecule called DHMG, or 1-(3,5-dichloro-2,6-hydroxy-4-methoxyphenyl)-1-hexanone). PCD is programmed cell death. PCD is a prerequisite for the formation of the "mushroom's" stip that consists of dead cells

Fig. 5. Main forms of social behavior (according to: Oleskin, 2012).

Fig. 6. Stages of biofilm formation by *Staphylococcus epidermidis* 33 in the human oral cavity (a scheme). The Figure demonstrates the consecutive stages of the transition from a planktonic lifestyle (1) via the attachment of primary colonizers (2) and extracellular matrix synthesis (2, 3) to the formation of three-dimensional pillar- and mushroom-like structures (4). Balls, *Staph. epidermidis* cells; Pale halos around them, matrix elements; Spirals, extracellular DNA and RNA; Dots, proteins and peptides including enzymes and communicative signals. The picture is a gift from Dr. Vladimir P. Korobov.

Fig. 7. Some types of QS signals: a, N-acylhomoserine lactones (AI-1 signals); b, peptide signals used by gram-positive bacteria; c, γ-butyrolactone of *Streptomyces*; d, AI-2 signals; e, DSFs; f, quinolones.

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Fig. 8. The QS system of *Aliivibrio fischeri*. The C, D, A, B, F, and G genes that encode luciferase components are cotranscribed with the I gene; its protein product catalyzes the synthesis of the signal (3-OHHL). All these genes are efficiently transcribed provided that the R gene product binds to the signal and their complex attaches to the promoter (filled rectangle; according to: Oleskin, 2001).

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Fig. 9. Abundance and composition of the microbiota in various GI tract parts (according to: Oleskin & Shenderov, 2020).

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Fig. 10. The main pathways used by microbial neuroactive substances in terms of microbiota-gut-brain interaction: (i) via the vagus nerve; (ii) via the immune system that produces BBB-

crossing neuroactive cytokines and other compounds, and (iii) by crossing the gut-blood barrier and the BBB. Abbreviations: DOPA, L-3,4-dihydroxyphenylalanine (the precursor of catecholamines); 5-HTP, 5-hydroxytryptophan (the precursor of serotonin); GABA, γ-aminobutyric acid. Note: In addition to the vagus nerve mentioned in the Figure, the effects of microbial substances on the brain may be mediated by other neuronal pathways within the peripheral nervous system and its part located in the intestines (the enteric nervous system). According to: Oleskin & Shenderov, 2020, p.146, modified.

Fig. 11. The microbiota-nervous system-immune system triangle.

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Fig. 12. The formulas of some important neurochemicals.

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Fig. 13. Catecholamine biosynthesis pathway.

Fig. 14. *E.coli* cultures produce DOPA whose concentration increases with cultivation time on M-9 medium. Horizontal axis: 1, lag phase; 2, early exponential phase; 3, late exponential phase; 4, stationary growth phase; 5, supernatant of the medium with the inoculum. Vertical axis: DOPA concentration (micromoles/L) in the cultivation liquid. According to Vladimir Shishov's Cand Sci. (Ph. D.) dissertation (2010).

Fig. 15. Effects of biogenic amines on *S. cerevisiae* proliferation on Sabouraud agar (15 h culture). A, effects of norepinephrine (1), apomorphine (2), and dopamine (3); B, effects of serotonin (1) and histamine (2). Vertical axis, cell number per field of view.

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Fig. 16. Serotonin synthesis pathway in animals.

Fig. 17. Stimulation of biomass accumulation by serotonin in *E. coli* K-12 (1) and *Rhodospirillum rubrum* (2). According to: Oleskin et al., 1998a. Designation: -lgM is -lg [Serotonin concentration in moles per liter].

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Fig. 18. Effect of serotonin on microcolony formation in *E. coli* K-12 on LB agar: a, control; b, with 1 μM serotonin. According to: Oleskin, 2001. Magnification, 1500.

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Fig. 19. Histamine biosynthesis.

Fig. 20. Enzymatic production of carbon monoxide.

Fig. 21. The negative influence of anticancer chemotherapy or radiation therapy on the intestinal microbiota, paradoxically, might help fend off the tumor. The reason is that anti-commensal IGs formed by Th17 cells also prove lethal to the tumor cells. This conforms to the English saying *Every cloud has a silver lining.*

FIGURES

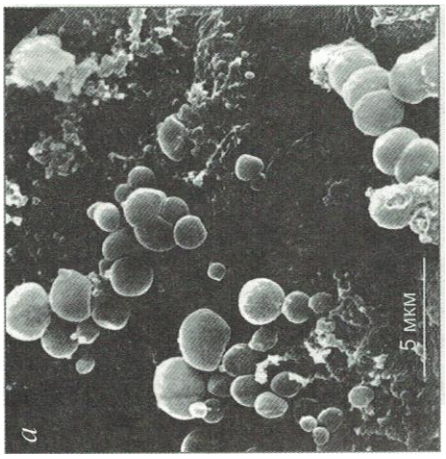


Fig. 1

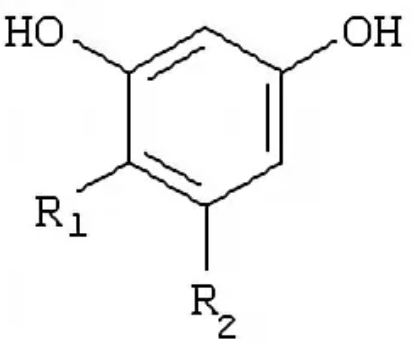


Fig. 2

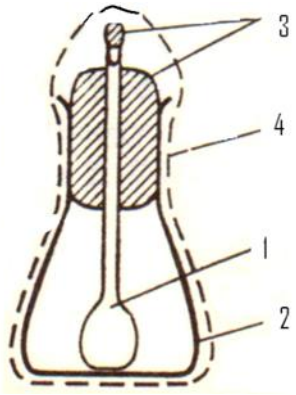


Fig. 3

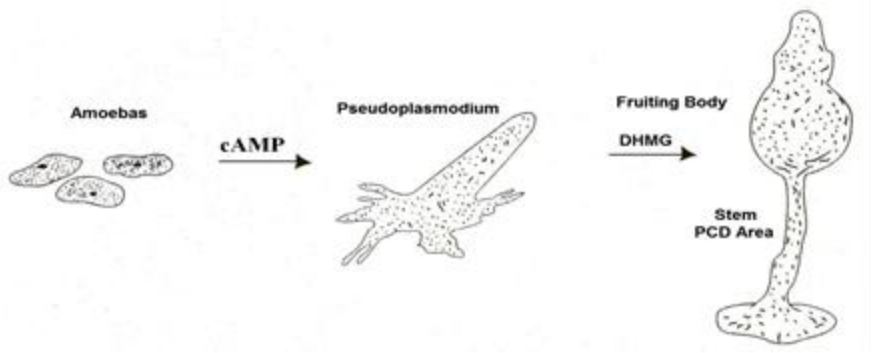


Fig. 4

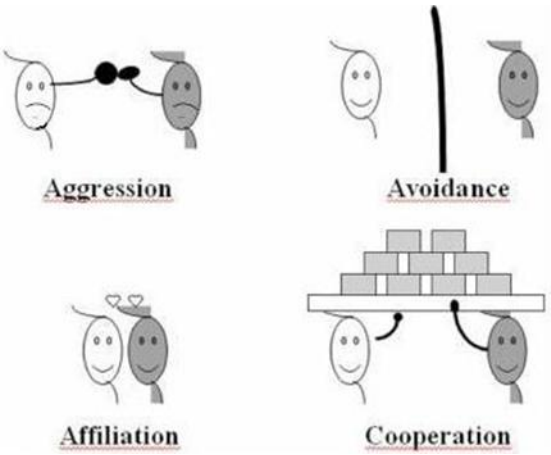


Fig. 5

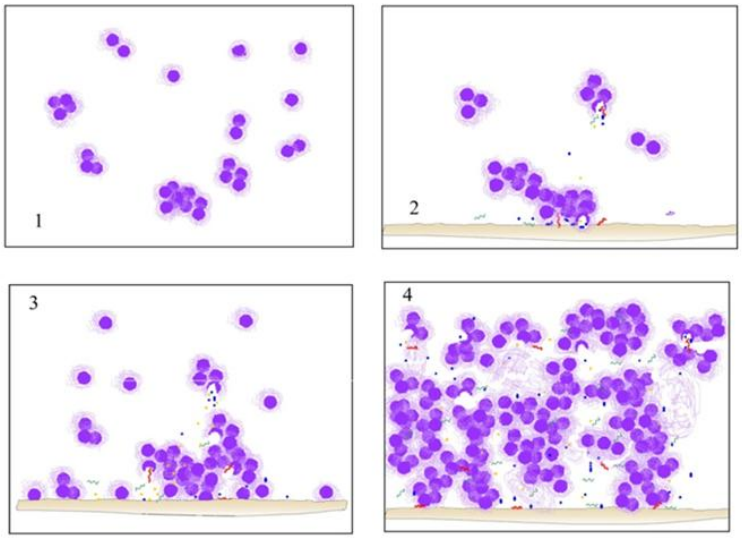


Fig. 6

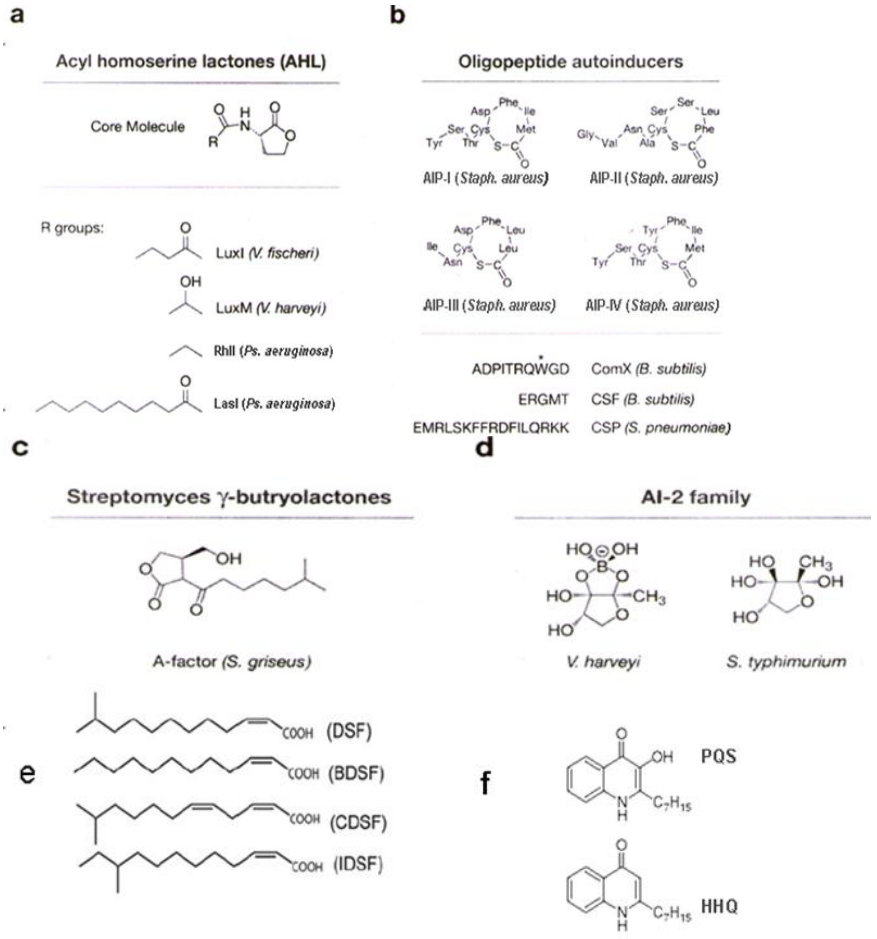

a
Acyl homoserine lactones (AHL)
Core Molecule
R groups:
LuxI (V. fischeri)
LuxM (V. harveyi)
RhlI (Ps. aeruginosa)
LasI (Ps. aeruginosa)
b
Oligopeptide autoinducers
AIP-I (Staph. aureus)
AIP-II (Staph. aureus)
AIP-III (Staph. aureus)
AIP-IV (Staph. aureus)
ADPITRQWGD ComX (B. subtilis)
ERGMT CSF (B. subtilis)
EMRLSKFFRDFILQRKK CSP (S. pneumoniae)
c
Streptomyces γ-butyrolactones
A-factor (S. griseus)
d
AI-2 family
V. harveyi
S. typhimurium
e
(DSF)
(BDSF)
(CDSF)
(IDSF)
f
PQS
HHQ

Fig. 7

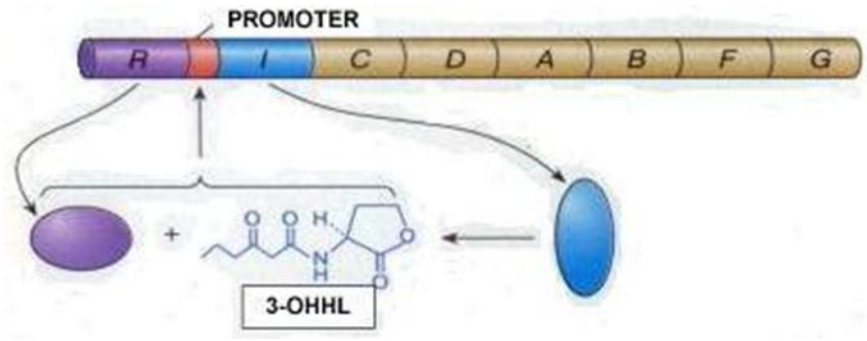

PROMOTER
R
I
C
D
A
B
F
G
3-OHHL

Fig. 8

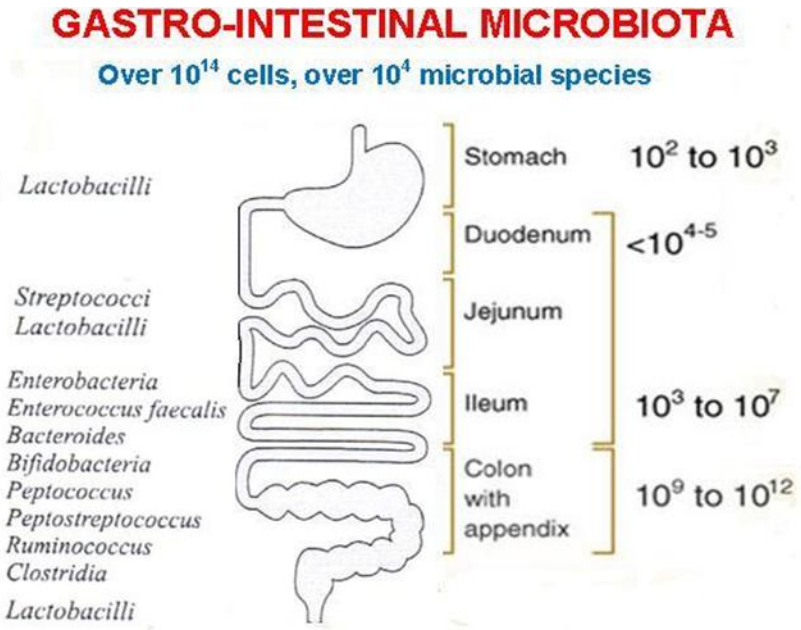

GASTRO-INTESTINAL MICROBIOTA
Over 10^{14} cells, over 10^{4} microbial species
Lactobacilli
Streptococci
Lactobacilli
Enterobacteria
Enterococcus faecalis
Bacteroides
Bifidobacteria
Peptococcus
Peptostreptococcus
Ruminococcus
Clostridia
Lactobacilli
Stomach
10^{2} to 10^{3}
Duodenum
Jejunum
$<10^{4-5}$
Ileum
10^{3} to 10^{7}
Colon with appendix
10^{9} to 10^{12}

Fig. 9

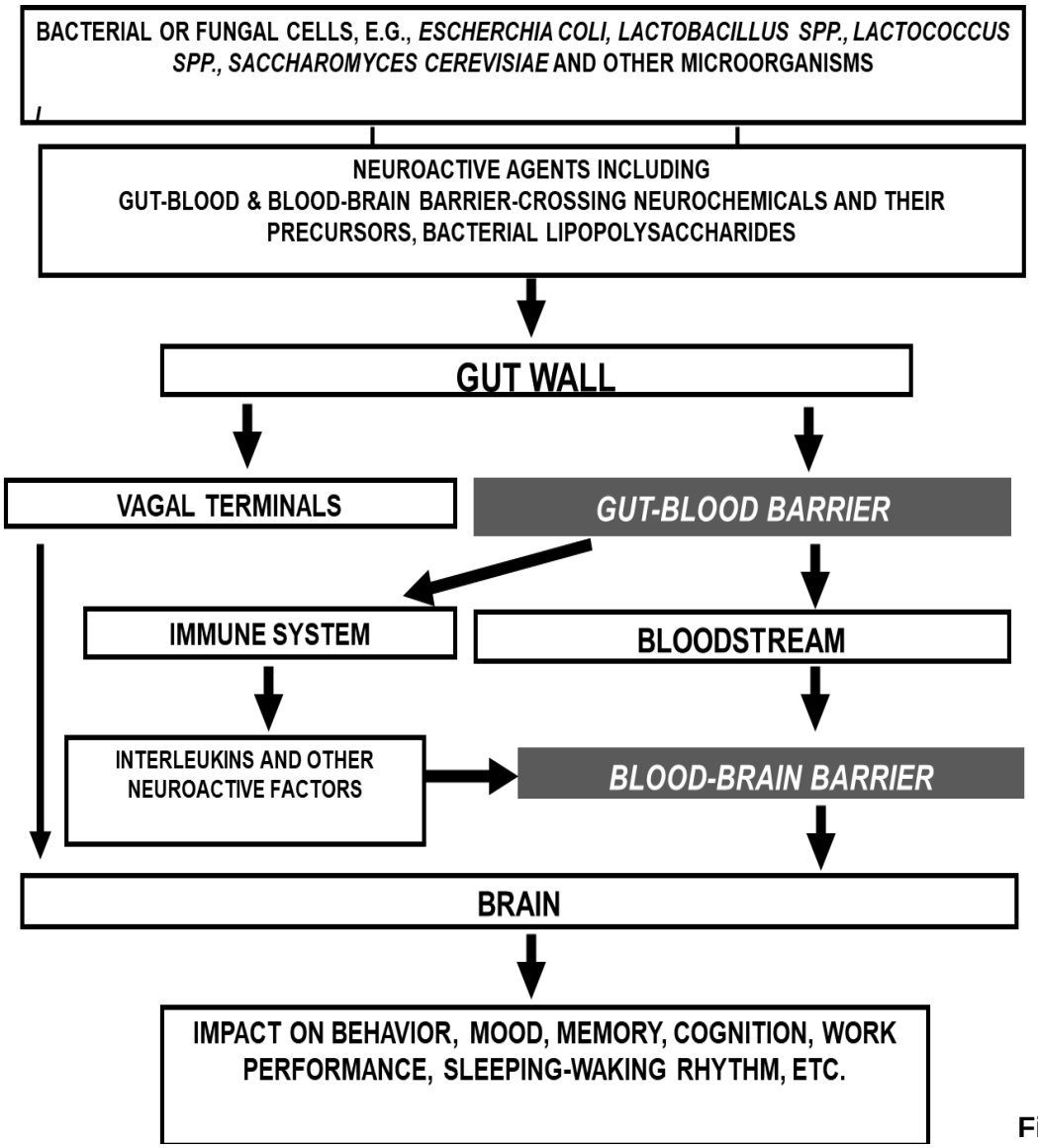

BACTERIAL OR FUNGAL CELLS, E.G., *ESCHERCHIA COLI, LACTOBACILLUS SPP., LACTOCOCCUS SPP., SACCHAROMYCES CEREVISIAE* AND OTHER MICROORGANISMS
NEUROACTIVE AGENTS INCLUDING GUT-BLOOD & BLOOD-BRAIN BARRIER-CROSSING NEUROCHEMICALS AND THEIR PRECURSORS, BACTERIAL LIPOPOLYSACCHARIDES
GUT WALL
VAGAL TERMINALS
GUT-BLOOD BARRIER
IMMUNE SYSTEM
BLOODSTREAM
INTERLEUKINS AND OTHER NEUROACTIVE FACTORS
BLOOD-BRAIN BARRIER
BRAIN
IMPACT ON BEHAVIOR, MOOD, MEMORY, COGNITION, WORK PERFORMANCE, SLEEPING-WAKING RHYTHM, ETC.

Fig. 10

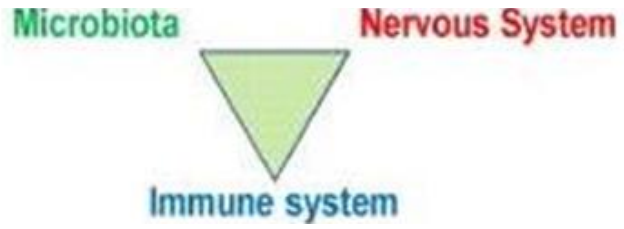

Microbiota
Nervous System
Immune system

Fig. 11

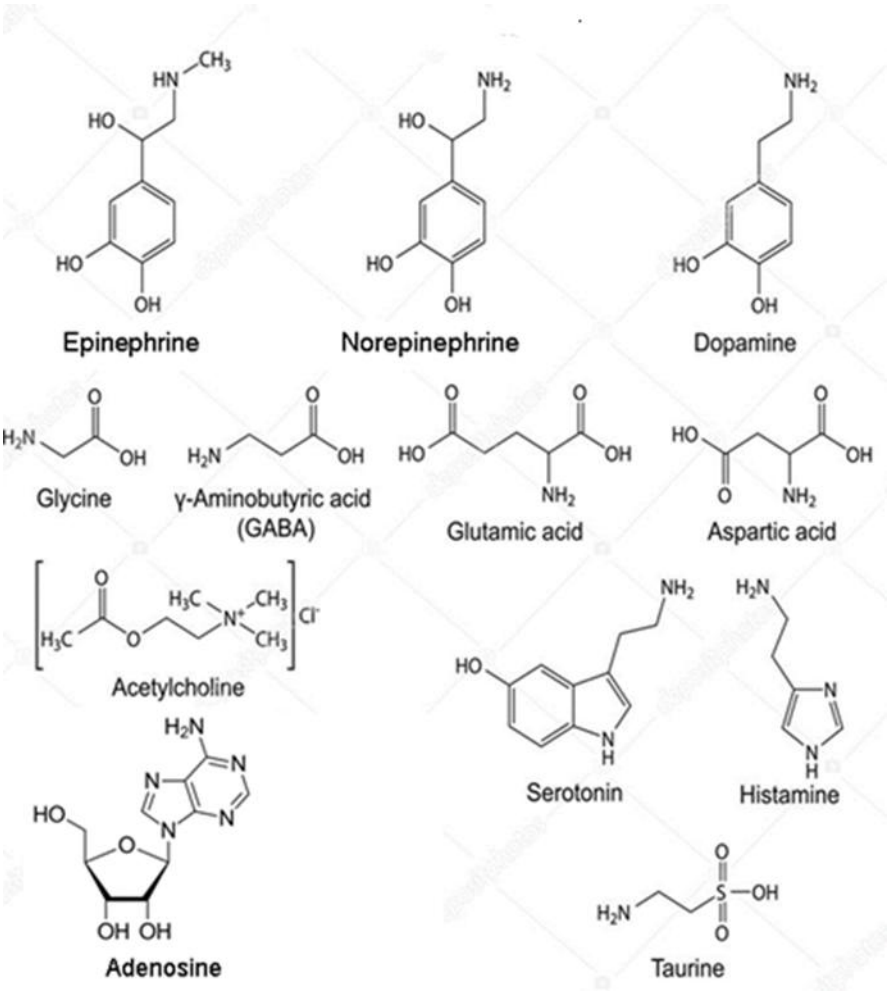

Epinephrine
Norepinephrine
Dopamine
Glycine
γ-Aminobutyric acid (GABA)
Glutamic acid
Aspartic acid
Acetylcholine
Serotonin
Histamine
Adenosine
Taurine

Fig. 12

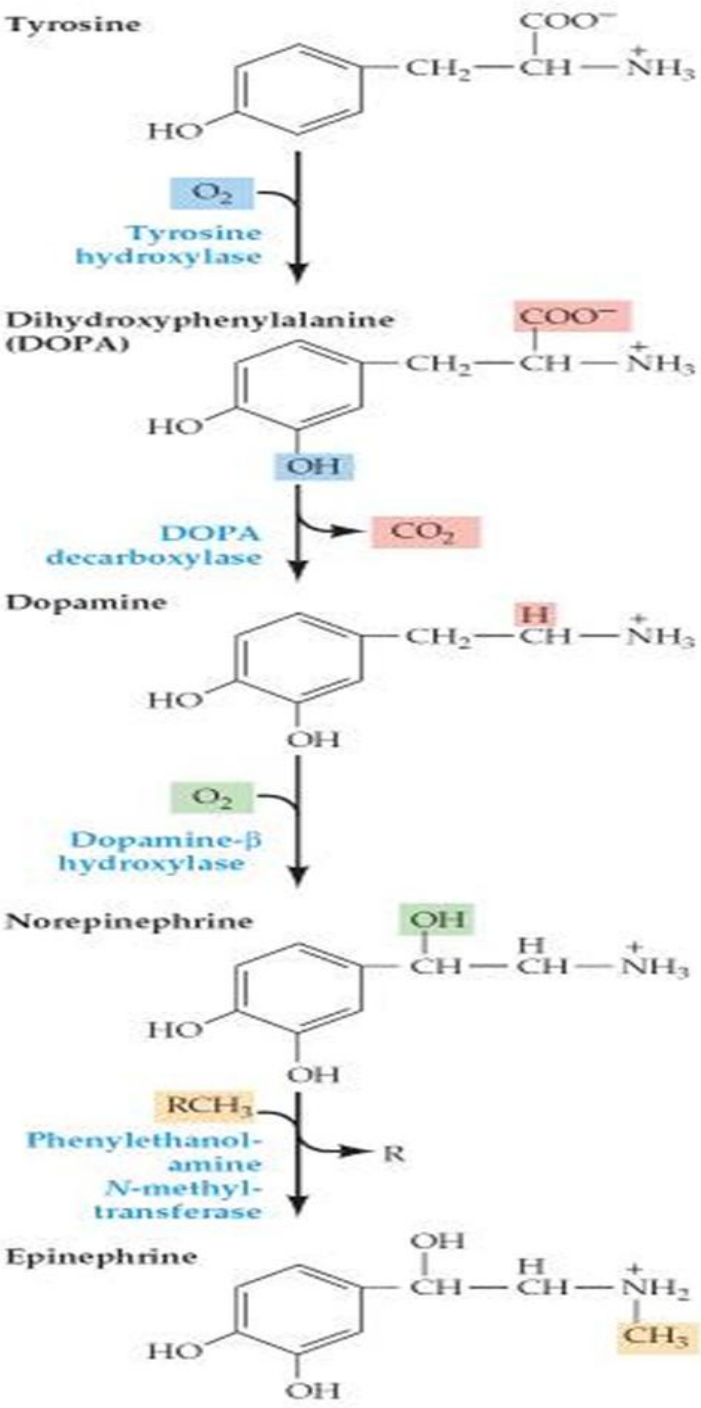

Tyrosine
Tyrosine hydroxylase
Dihydroxyphenylalanine (DOPA)
DOPA decarboxylase
Dopamine
Dopamine-β hydroxylase
Norepinephrine
Phenylethanol-amine N-methyl-transferase
Epinephrine

Fig. 13

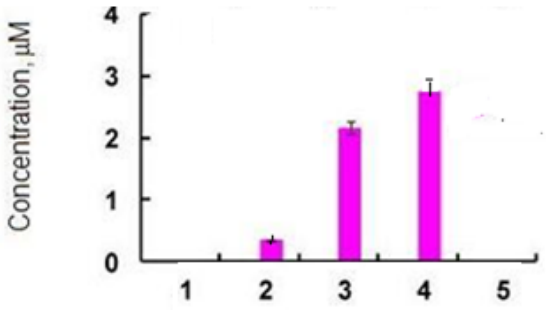
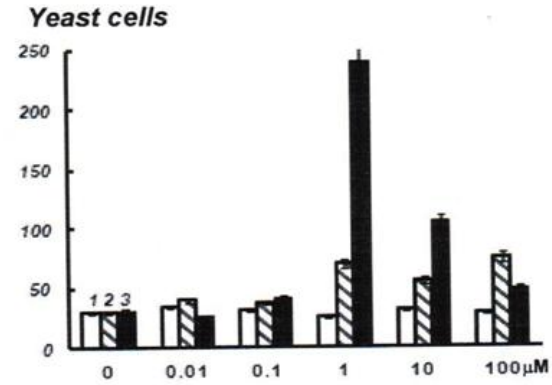


Fig .14

A



B

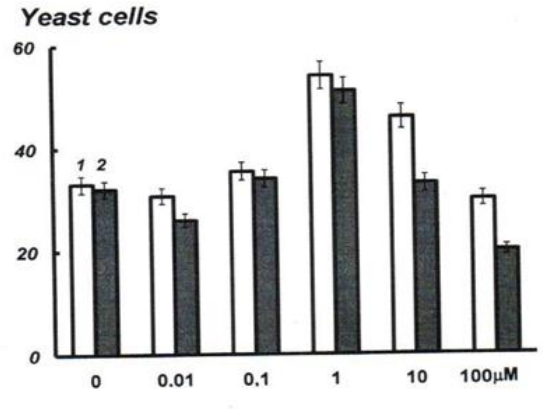


Fig. 15

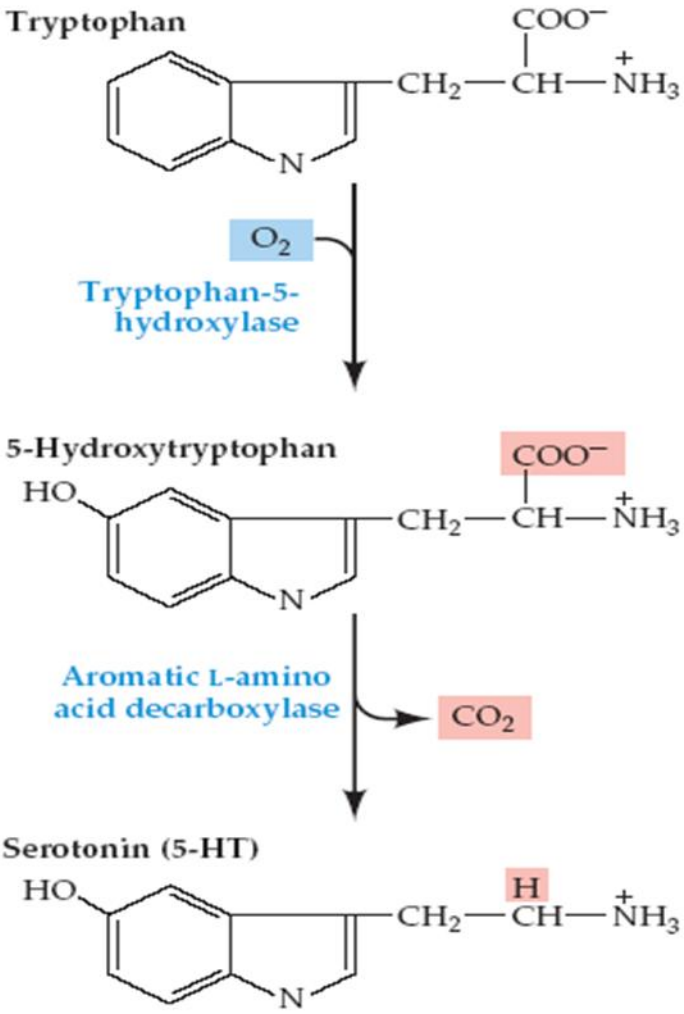

Tryptophan
COO⁻
CH₂—CH—NH₃
N
O₂
Tryptophan-5-hydroxylase
5-Hydroxytryptophan
HO
COO⁻
CH₂—CH—NH₃
N
Aromatic L-amino acid decarboxylase
CO₂
Serotonin (5-HT)
HO
H
CH₂—CH—NH₃
N

Fig. 16

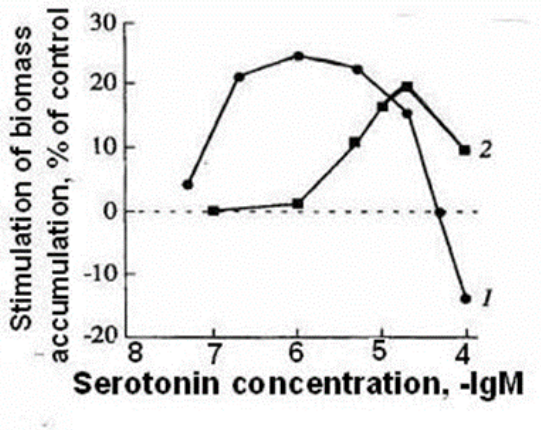

Stimulation of biomass accumulation, % of control
30
20
10
0
-10
-20
8
7
6
5
4
Serotonin concentration, -lgM
1
2

Fig. 17

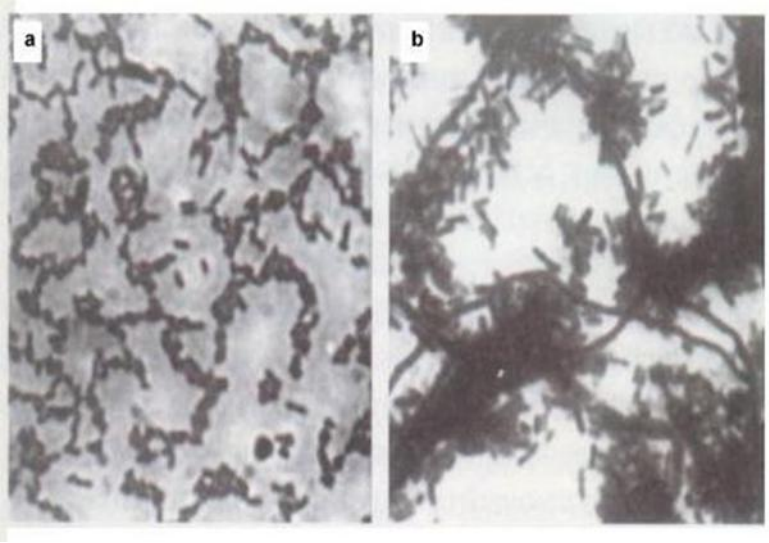


Fig. 18

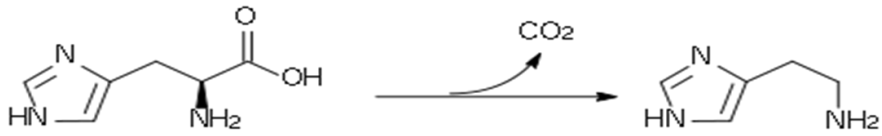


Fig. 19

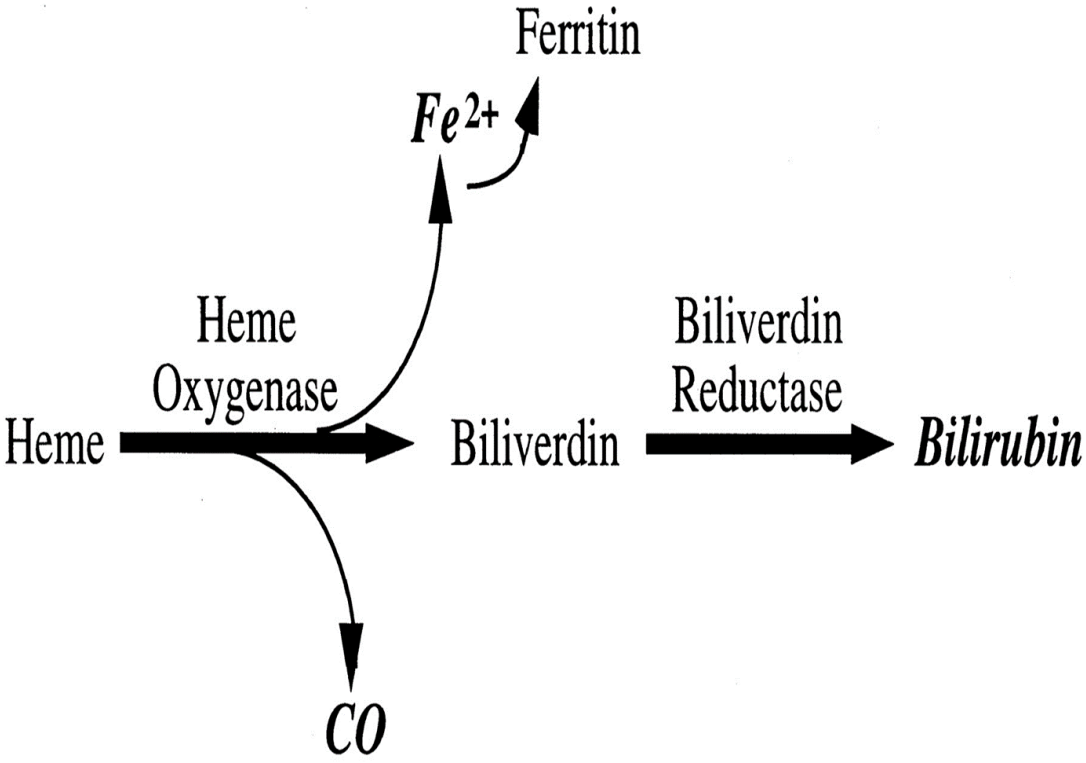


Fig. 20

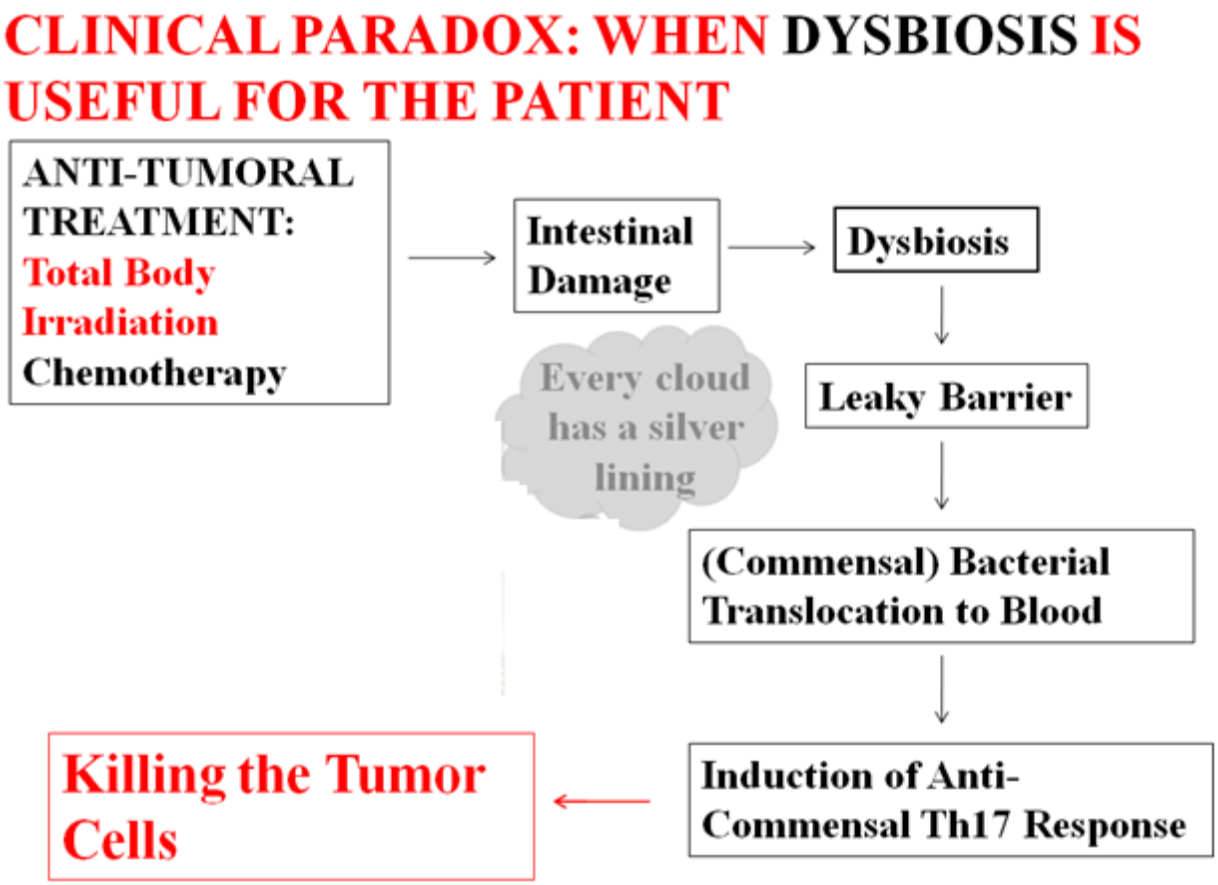

CLINICAL PARADOX: WHEN DYSBIOSIS IS USEFUL FOR THE PATIENT
ANTI-TUMORAL TREATMENT:
Total Body Irradiation
Chemotherapy
Intestinal Damage
Dysbiosis
Every cloud has a silver lining
Leaky Barrier
(Commensal) Bacterial Translocation to Blood
Induction of Anti-Commensal Th17 Response
Killing the Tumor Cells

Fig. 21