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Poly(3-hydroxybutyrate) and Human Microbiota (Review)

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Abstract—Natural polyhydroxyalkanoates (**PHAs**) and their synthetic analogs are widely used in medicine, including the production of biodegradable medical devices (prostheses, patches, stents, and plugs) intended for regenerative intestinal surgery. The possibility of biosynthesis and biodegradation of PHAs, primarily, their most common representative, poly(3-hydroxybutyrate) (**PHB**), by different symbiotic and infectious human and animal bacteria, particularly, multiple bacteria of intestinal microbiota, as well as the physiological role of biopolymer in bacterial cells, is discussed in detail in the review. The review also focuses on the problem of endogenous PHB in humans and animals. The assumption that microbiota bacteria can be a source of endogenous PHB is also discussed. In addition, the use of PHAs in regenerative intestine surgery is considered.

Keywords: polyhydroxyalkanoates, poly(3-hydroxybutyrate), biopolymer, oligomers, microbiota, transfection, translocation, type IV secretory system, intestine

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INTRODUCTION

Since the beginning of the 21th century, medical products and dosage forms based on biodegradable polyesters of hydroxycarboxylic acids (polyhydroxyalkanoates, **PHAs**) have been actively introduced into medical practices. The following polyhydroxycarboxylic acids are especially actively used in clinical practice and scientific research: poly(2-hydroxypropanoic) (polylactic, **PLA**, polylactides), poly(2-hydroxyacetic) (polyglycolic, **PGA**, polyglycolides), poly(6-hydroxycaprolactone) (**PCL**), poly(3-hydroxybutyric) (**PHB**), poly(3-hydroxyvaleric), etc., as well as their copolymers, for example, polylactide-co-glycolides (**PLGA**s) and polymers similar in structure, such as poly-*p*-dioxanone. Based on PHAs, a huge range of different medical devices are already used in medicine or are in the process of development: biodegradable suture threads, biodegradable fixing screws, pins, twines, staples, and plates, periodontal membranes, surgical mesh endoprostheses, wound and burn coverings, surgical patches to close the intestinal and pericardial defects, endoprosthesis plugs for coloproctology and hernioplasty, vessel prostheses, cardiovascular endoprosthesis stents, artificial heart valves, frame conductors for nerve regeneration, fillers, scaffolds to fill the tissue defects, and other products. New dosage forms of a number of drug substances, both low-molecular and, for example, therapeutic proteins that give new properties to medications (prolonged effect, targeted delivery, decreased toxicity, increased stability), are in development and are already used in pharmaceutics based on PHAs. Such active use of PHAs and their adoption in medical practice is caused by a unique combination of their properties: the ability to biodegrade in an organism without the formation of toxic products; biocompatibility with human organs and tissues; optimal mechanical properties (relatively high strength, plasticity); other physicochemical properties (thermoplasticity, specific diffusion properties); and the opportunity to use the efficient technological processes in their production. The development of medical products for surgical restoration of gastrointestinal tract (**GIT**) tissues (prostheses, patches, stents, plugs) is one of the most promising areas of the use of PHAs in medicine [1–5].

Meanwhile, the main properties of synthetic poly(2-hydroxyalkanoates) (PLA, PGA, and their copolymers PLGA), including the capacity for biodegradation and biocompatibility (which turned out to be very suitable for biomedical use), result from the fact that these polymers are synthetic analogs of natural PHAs. Synthetic PHAs are, to varying degrees, biomimetic materials. Therefore, the study of natural PHAs (including PHB) as biomaterials for the regeneration of different tissues and organs (primarily, the intestines) is of paramount importance.

Poly(3-hydroxybutyrate), the main polymer of a homologous series of the family of poly(3-hydroxyalkanoates), is the best known microbiological polyester, which is a promising alternative to biodegradable

synthetic thermoplastics [1–5] and is actively used in regenerative medicine and tissue engineering [6–10], along with other promising biomaterials: polysaccharide hydrogels [11, 12], biofunctional proteins [13, 14], electrically conductive polymers [15], polyplexes [16], biodegradable metals [17], etc. It is interesting that some PHB properties that distinguish it from the synthetic analogs can promote its use in GIT surgery. Thus, the biodegradation rate of PHB and its copolymers is much lower than the biodegradation rate of synthetic PLA, PGA, and their copolymers; with the effect of an aggressive medium (including bacteria in GIT), this makes the PHA use for regeneration of GIT organs more relevant [4, 18]. The polymer biodegradation mainly occurs due to the phagocytic activity of specialized cells (macrophages (foreign body giant cells) and osteoclasts), i.e., specialized cellular biodegradation of these biopolymers takes place [19–22]. The greater biocompatibility of PHB and its copolymers as compared with synthetic PHAs due to the absence of the effect of acidification of surrounding tissues by the polymer biodegradation products is also of great importance. When the tissue reaction of PHB and synthetic PLA, PGA polyesters, and their copolymers were compared, it was demonstrated in a number of works that the tissue reaction is mild or moderate for PHB, while severe chronic inflammatory reaction is not infrequently observed for PLA, PGA, or PLGA [4, 23–28].

However, despite the bacterial origin of natural PHAs, the problem of the role of this biopolymer in bacteria from the intestinal or other human organ (oral cavity, lungs, skin, and etc.) microbiota remains almost unstudied, although it is of great practical importance due to the effect of microbiota bacteria on this polymer biomaterial or, on the contrary, the biopolymer effect on human microbiota bacteria. Meanwhile, besides the applied studies of medical devices based on PHAs, there are other good reasons to conduct basic research on this problem. At present, there is a lot of data in favor of the fact that PHB, as a natural ancestor of almost all PHAs used in biomedicine (both synthetic and obtained biotechnologically), also plays an important role in the symbiosis of microbiota bacteria in the mammalian organism as an endogenous biopolymer [29]. Moreover, PHB is not only of bacterial origin; the presence of endogenous PHB was demonstrated in the organism of mammals and other eukaryotes [29, 30].

Therefore, the possibility of PHAs biosynthesis and biodegradation by different symbiotic and infectious human and animal bacteria (primarily, several bacteria of the intestine microbiota) and the physiological role that this biopolymer can play in these bacteria are discussed in detail in the review; examples of the use of PHAs for GIR regenerative surgery are also considered.

BIOSYNTHESIS OF POLY(3-HYDROXYBUTYRATE) BY SYMBIOTIC EUKARYOTIC BACTERIA

Natural PHAs represent polyesters of 3-hydroxyalkanoic acids; poly(3-hydroxybutyrate) is a linear polyester of 3-hydroxybutyric acid. The biopolymer includes only the R form of 3-hydroxybutyric acid, due to which it is a partially crystalline polyester in isolated and purified form (the PHB crystallinity is 55–80%) [31]. Depending on the length of alkane side radical, the following natural PHAs are distinguished: poly(3-hydroxybutyrate), poly(3-hydroxyvalerate), poly(3-hydroxyhexanoate), poly(3-hydroxyoctanoate), etc. During bacterial biosynthesis, it is not homopolymers of other PHAs but their copolymers with 3-hydroxybutyrate that are the most frequently obtained: poly(3-hydroxybutyrate)-co-3-oxyvalerate (**PHBV**), poly(3-hydroxybutyrate)-co-3-hydroxyhexanoate (**PHBHx**), poly(3-hydroxybutyrate)-co-3-hydroxyoctanoate (**PHBO**), etc. All of them are quite different in their physicochemical properties, such as crystallinity, melting temperature, glass transition temperature, hydrophobicity, plasticity, elastic modulus, and others [32–34].

At present, it is considered that there are three PHB types in nature that differ in molecular weight and functionally: high-molecular reserve PHB, which consists of more than 1000 3-hydroxybutyrate elements (reserve PHB, **rPHB**); low-molecular hydrophobic PHB with a chain length of 100–200 monomers (oligo-PHB, **oPHB**); and conjugated or complex-forming PHB (**cPHB**), which consists of no more than 30 3-hydroxybutyric acid residues, is relatively hydrophilic, is covalently bound to proteins, and generates complexes with other biopolymers. Low-molecular oPHB (especially if this biopolymer forms complexes with other biopolymers) is sometimes referred to in the literature as complex-forming PHB and is designated as cPHB. The reserve PHB is present in many prokaryotes (*Eubacteria* and *Archaea*), while oPHB and cPHB, as will be shown below, can be found in all prokaryotes and even in eukaryotes [35].

Hundreds of bacterium species have the ability to synthesize PHA as a reserve substance: gram-negative and gram-positive are written together as bacteria, some archaea and types of some cyanobacteria. Except for a few phototrophic microorganisms, the *Clostridium* and *Syntrophomonas* are the only strict anaerobes in which cells PHB was detected. It is interesting that enterobacteria (as, e.g., *Escherichia coli*), as a rule, do not synthesize PHB as a reserve substance [36–38]. Bacteria that are capable of biosynthesis accumulate PHB in the cytoplasm as discrete inclusions (granules, usually 100–800 nm in diameter) as a pool of carbon and chemical energy in conditions of nitrogen starvation. The granules are surrounded by a monolayer lipid membrane, in which biosynthesis and degradation/mobilization enzymes (intracellular PHB-

depolymerases) are localized [35, 39]. For most microorganisms, the accumulated PHB serves as a source of carbon and energy at their lack. The role of PHB as a reserve material was highlighted in detail in a review by Anderson and Dawes [37]. PHB and other PHAs are an ideal reserve material, since they do increase osmotic pressure in the cell due to hydrophobicity and high molecular weight [40]. Metabolic pathways of PHB biosynthesis and utilization in bacteria were considered in detail in a number of reviews [29, 37, 38, 41].

The main biosynthesis stages are β-ketotiolase enzyme catalyzes the formation of a carbon–carbon bond between two acetyl-CoA residues by Claisen condensation (acetyl-CoA molecules come from glycolysis through the pyruvate formation); NADPH-dependent acetoacetyl-CoA reductase then converts acetoacetyl-CoA into 3-hydroxybutyryl-CoA. At the next stage, 3-hydroxybutyryl-CoA molecules bind to PHB polymerase, which performs polymer synthesis on the granule surface. PHA synthases are divided into four groups depending on the substrate specificity and subunit composition: classes I, II, III, and IV. PHA synthases of the following three classes, including class I (found in the *Ralstonia eutropha*), class III (found in the *Allochromatium vinosum*), and class IV (found in the *Bacillus megaterium*), have a substrate specificity relative to short-chain monomers, 3-hydroxycarboxylic acids (C3–C5), while the PHA synthase of class II (found in the *Pseudomonas aeruginosa*) is able to carry out a reaction with long-chain 3-hydroxycarboxylic acids as a substrate (C6–C14) [42]. Simultaneously with the synthesis of PHB, its continuous decomposition to monomers by the PHB-depolymerase enzyme also occurs; these processes are in equilibrium and are regulated in the cell. In addition, many microorganisms excrete extracellular PHA depolymerases (the *PhaZ* gene), through which they can hydrolyze PHB till water-soluble monomers and oligomers; to date, more than 300 known microorganisms related to different taxonomic groups that are able to PHB degradation in vitro [43, 44].

As well as many free-living bacteria, a number of symbiotic bacteria of the GIT microbiota in mammals and other animals are able to biosynthesize and accumulate this reserve biopolymer. Information about the ability of GIT microbiota bacteria and other symbiotic bacteria of humans and animals to synthesize PHB is collected in Table 1. Thus, bacteria from the *Clostridium* genus are capable of synthesizing and accumulating reserve high-molecular PHB, as well as, e.g., the known PHB producers (soil *Azotobacter* sp. bacteria). *Clostridium* sp. bacteria are a natural component of intestinal microbiota in healthy individuals [45–48]. The ability of the *Clostridium* sp. to synthesize and store rPHB in PHA granules was discovered as early as 1973 [49], but these bacteria are not very efficient as PHB producers (accumulate only till 15% polymer); in addition, various species of this genus of intestinal bacteria can be pathogenic: *C. difficile*, *C. acetobutylicum*, and *C. propionicum*. They were subsequently used only as sources for the genes encoding a number of PHB synthesis biochemical pathways for metabolic engineering and enzymatic synthesis in vitro for this polymer [50, 51]. It should be noted that *Clostridium* sp. bacteria play a huge role, both as natural members of normal intestinal microbiota and in the pathogenesis of a number of diseases (diabetes, various chronic inflammatory GIT diseases, rectal cancer, rheumatoid arthritis, obesity and even autism), including infectious diseases of the intestines [47, 48, 52, 53]. Intestinal *Clostridium* sp. bacteria was also shown to have a significant effect on human immunity in many works, and their special role in the development of human immune system cells was registered. Preparations based on bacteria of this genus were proposed as a probiotic [54, 55].

However, aside from *Clostridium* bacteria, other bacteria of the microbiota of humans and various animals (insects, fishes) from the *Bacillus* and *Burkholderia* genera are capable of PHB synthesis [56–59]. The PHB-synthase enzyme and, appropriately, the *phaC* gene were also found in these bacteria [58]. The possibility of PHB biosynthesis by lactobacilli from the *Lactobacillus*, *Lactococcus*, *Pediococcus*, and *Streptococcus* genera and the accumulation by these bacteria of 6–35% biopolymer from dry biomass was demonstrated [59]. Bacteria of the *Lactobacillus* genus demonstrated the greatest capacity for PHB biosynthesis, although there is no evidence of the presence of the *phaC* PHB-synthase gene in these bacteria. It is interesting that bacteria from the *Ralstonia* genus (to which belongs *Ralstonia eutropha*, a known producer of PHB and other PHAs [60]) are also natural components of the mammalian microbiota. *R. pickettii* were found in the normal microbiota of human lungs and oral cavity [61]. In addition, intestinal bacteria (for example, *Escherichia coli*) that are unable to synthesize high-molecular rPHB can synthesize low-molecular oPHB or cPHB [35, 62, 63]. It was demonstrated that PHB synthesis does not occur in the conditionally pathogenic *Mycobacterium smegmatis* bacterium, a close relative of the causative agent of tuberculosis (although there are some lipophilic inclusions in the cells), but it was observed in these bacteria when transformed with a plasmid carrying the *phaA, phaB,* and *phaC* genes [64]. It should be noted that efficient PHB production was demonstrated not only by individual producer strains but also by consortia consisting of many different species of bacteria; PHB synthesis was required for the existence of the entire symbiotic community [65–67].

The important role of PHB biosynthesis in symbiotic relationships of the microbiota bacteria and the host organism was demonstrated on the example of a comparison of PHB biosynthesis regulation in free-living and symbiotic bacteria of the *Burkholderia* genus from the *Riptortus pedestris* bean beetle intes-

Table 1. PHB biosynthesis in symbiotic and infectious human and animal bacteria

Bacteria (genus, species)	Genes of PHA synthesis and cleavage enzymes	Ability to PHA biosynthesis and biodegradation	Role of bacteria in animal organism	Shown or assumed PHB function
Agrobacterium (*A. tumefaciens* or *Rhizobium radiobacter*)	*PhaC* (class III PHA-synthase), *PhaZ* (UniProt, Protein NCBI*) [157]	PHB biodegradation (indirectly) [212]	Causative agent of nosocomial infections (rarely) [158, 159]	Source of carbon and energy (indirectly) [212]
Clostridium (*C. difficile*, *C. acetobutylicum*, *C. propionicum, C. tetanomorphum, C. botulinum, C. beijerinckii, C. clostridioforme, C. coccoides*)	*PhaR, PhaC* (class III PHA-synthase), *PhaZ* (UniProt, Protein NCBI*)	PHB biosynthesis and accumulation in granules (up to 15% of the cell volume) [49]	Microbiota component of intestine and other organs, infectious agent, development of immunity [45–48, 52–55, 213]	Reserve substance [49]
Escherichia coli	*PhaR, PhaC* are absent (UniProt, Protein NCBI*). *YdcS* ABC transporter binding protein, has the ability to synthesize cPHB [214]	Reserve high-molecular PHB is not synthesized [36, 37, 39]. Synthesis of low-molecular oPHB and cPHB [30, 35, 62, 63, 100]	Microbiota component of intestine and other organs, infectious agent [215]	Complexes with different proteins (cPHB) [84, 101, 103, 120, 121] Improvement of the adaptation of bacteria to symbiosis (cPHB) [62, 63]. Suppression of the growth of pathogenic *E. coli* [76, 77]
Ralstonia (*Ralstonia* sp., *R. pickettii, R. insidiosa*)	*PhaA, PhaB, PhaR, PhaC* (class I PHA-synthase), *PhaZ* (UniProt, Protein NCBI*) [35]	PHB biosynthesis PHB biodegradation (*R. pickettii*) [216]. PHA biosynthesis (*R. eutropha*) [36, 60]	Lung microbiota component, causative agent of nosocomial infections in humans [61, 142]	Reserve substance, source of energy [36, 60, 216]
Bacillus (*B. subtilis, B. cereus, B. globisporus, B. thuringiensis, B. pasteurii, B. lentus, B. megaterium*)	*phaA, phaB, phaC, phaR phaP, phaQ* (class IV PHA-synthase), *PhaZ* (UniProt, Protein NCBI*) [35, 186]	PHB biosynthesis (up to 23.6% dry weight) [57]	Intestine microbiota component in humans, other mammals, fishes and insects, causative agent of infections in humans [217, 218]	Reserve substance [57]
Burkholderia (*B. mallei, B. thailandensis, B. pseudomallei, B. cepacia, B. cenocepacia*)	*phaA, phaB, phaC* (class I PHA-synthase), *phaP*, *PhaZ* (UniProt, Protein NCBI*) [59]	PHB biosynthesis and accumulation in granules (up to 10% from volume cell) [58, 59]	Intestine microbiota component and causative agent of infections in humans [218, 219] and insects [58, 59]	Reserve substance, plays an important role in symbiotic relations [59]
Vibrio (*V. cholerae, V. parahaemolyticus, V. vulnificus, V. cincinnatiensis, V. campbellii*)	*phaB, phaC* (class I PHA-synthase) (UniProt, Protein NCBI*)	PHB biosynthesis (in related marine bacteria *Vibrio* sp.) [220]	Causative agents of infections in humans, fishes, crustaceans, and mollusks [221]	PHB feeding suppresses the *V. campbellii* infectious activity in crustaceans [75]
Legionella (L. pneumophila)	*phaB, phaC* (class I PHA-synthase), *PhaZ* (UniProt, Protein NCBI*)	rPHB biosynthesis (up to 16% dry weight) [181, 182, 222]	Causative agent of infections (Legionnaires' disease), obligate intracellular parasites of lung macrophages [177, 178]	Reserve substance, is used in stressful conditions [181, 182, 222] and for the infectious activity [184, 185]

Table 1. (Contd.)

Bacteria (genus, species)	Genes of PHA synthesis and cleavage enzymes	Ability to PHA biosynthesis and biodegradation	Role of bacteria in animal organism	Shown or assumed PHB function
Pseudomonas (*P. fluorescens, P. micrococcus, P. putida, P. aeruginosa, P. (Stenotrophomonas) maltophilia)*	*phaB, phaC* (class II PHA-synthase), *PhaZ, PhaF* (UniProt, Protein NCBI*) [35, 42]	Biosynthesis of rPHB and different its copolymers (up to 69% dry weight), PHB accumulation in the cell granules in the *P. aeruginosa* [223]	Intestine microbiota component in human and other mammals [218], causative agent of nosocomial infections [224]	Reserve substance [223]
Mycobacterium (*M. tuberculosis, M. bovis, M. kansasii, M. smegmatis*)	*phaC* (class I PHA-synthase) (UniProt, Protein NCBI*)	PHB biosynthesis in the *M. smegmatis* does not occur, but there are lipophilic inclusions in the cells [64]	Causative agent of infections (tuberculosis), facultative intracellular parasites [225, 226]	Unknown
Acinetobacter (*A. baumannii*)	*phaA, phaB, phaC* (class III PHA-synthase) (UniProt, Protein NCBI*)	Demonstrated for soil bacteria, including the *A. oleivorans* [227, 228]	Intestine microbiota component of human and other mammals [213, 218], causative agent of nosocomial infections [224]	Unknown
Sphingomonas (S. paucimobilis)	*phaA, phaB, phaR, phaC* (class I PHA-synthase), *PhaZ, PhaF* (UniProt, Protein NCBI*)	Demonstrated for soil *S. sanxanigenens* bacteria [229]	Intestine and lung microbiota component of humans and other mammals [142, 218], causative agent of nosocomial infections [230].	Unknown
Fusobacterium (F. alocis, F. nucleatum)	*phaC* (class III PHA-synthase) (UniProt, Protein NCBI*)	Unknown	Intestine microbiota component of humans and other mammals [218], involvement of parodontosis pathogenesis [139].	Unknown
Neisseria (N. meningitidis, N. gonorrhoeae)	*phaB, phaR, phaC* (UniProt, Protein NCBI*) [186]	Unknown	Intestine microbiota component of humans and other mammals, causative agent of infections [218]	Unknown
Streptomyces (S. purpurogeneiscleroticus, S. aureofaciens)	*phaA, phaB, phaC*, *phaZ* (UniProt, Protein NCBI*)	rPHB biosynthesis (soil bacteria) [231]. cPHB was detected [84]	Usual component of intestine microbiota of humans and other mammals [218], causative agent of intestinal infections [232]	Reserve substance (rPHB) [231]. Functional component of cationic channel (cPHB) [84]
Haemophilus (*H. influenzae, H. haemolyticus*)	No (UniProt, Protein NCBI*)	cPHB in complex with polyphosphate and NTHi P5 protein [85]	Lung microbiota component, causative agent of nosocomial infections [233]	Functional component of cationic channel (cPHB) [85]

Table 1. (Contd.)

Bacteria (genus, species)	Genes of PHA synthesis and cleavage enzymes	Ability to PHA biosynthesis and biodegradation	Role of bacteria in animal organism	Shown or assumed PHB function
Bordetella (B. bronchiseptica, B. parapertussis, (Haemophilus) pertussis)	*phaC* (class I PHA-synthase), *PhaR, PhaZ* [186]	Unknown	Causative agents on infections in humans [234]	Unknown
Rickettsia (R. rickettsii, R. prowazekii, R. typhi, R. sibirica, R. prowazekii)	*phaC* (class I PHA-synthase), *phaR, PhaZ* (UniProt, Protein NCBI*)	Unknown	Causative agents on infections in humans, obligate intracellular parasites [235]	Unknown

* UniProt – http://www.uniprot.org, Protein NCBI – https://www.ncbi.nlm.nih.gov/protein.

tine. It was demonstrated that the number of granules containing the polymer is much higher in symbiotic bacteria, and the more intensive PHB biosynthesis in them is associated with the large amount of regulatory phasin proteins (the *PhaP* gene) on the granule surface. The ability of mutant bacteria with knocked-out genes of PHB synthesis and phasins to populate and reproduce in the beetle intestine was much weaker, which led to decreases in the body sizes and the weight of the beetles themselves. However, the most interesting thing is that the ability to biosynthesize PHB significantly increased the stress resistance of bacteria, while the survival of mutant bacteria with switched-off PHB biosynthesis genes in conditions of various types of stress (the effect of high temperature, depleted growth medium, high osmotic pressure) was sharply reduced. Analyzing these data, the authors suggested that PHB synthesis allows symbiotic bacteria to survive in the beetle intestines in the stress conditions induced by the host organism immune system in order to regulate the number of these bacteria [58]. In this regard, it should be noted that PHB synthesis is one of the evolutionary mechanisms for microorganism adaptation to extreme environmental conditions. In the evolutionary process, the ability to synthesize and accumulate PHB played an important role in the development by microorganisms of new ecological niches on Earth with extremely low or high temperatures, high salinity, acidity, or alkalinity of water reservoirs, since this reserve biopolymer is a universal energy accumulator, while the ability to metabolize it allows microorganisms to survive in stressful periods of existence [68–71]. It was demonstrated that PHB biosynthesis by bacteria sharply increases their resistance to various stressful effects: high temperature, osmotic pressure, UV radiation, oxidative stress, and etc. [72]. Artificial stressful conditions are created in the producer strain during biotechnological production of this biopolymer to stimulate the process of its biosynthesis [73].

It was also demonstrated with other organisms (*Apostichopus japonicus* sea cucumbers) that the PHB biosynthesis is of great importance for microbiota. Metagenomic analysis of the composition of GIT microbiota of sea cucumbers demonstrated that bacteria from the *Rhodobacterales* order prevailed in large individuals; this correlated with a larger portion of PHB biosynthesis genes (*PhaA*, *PhaB*, *PhaC*). Apparently, PHB synthesis modulated the GIT microbiota in sea cucumbers, contributing to an increase in animal size of multiple times [74]. A work in which the ability of histamine to regulate the synthesis of low-molecular cPHB in *E.coli* was demonstrated deserves attention. Histamine plays an important role as a means of bacterial communication with the host organism and the regulator of gastrointestinal tract immune system, allowing bacteria to be considered "native" for the host organism; therefore, the effect of histamine on the cPHB synthesis can indicate the involvement of this biopolymer in the processes of adaptation and coexistence with the host organism [62, 63]. Moreover, the efficiency of PHB in the fight against infectious diseases in animals was demonstrated: the use of PHB powder as additives to feeds protected the *Artemia nauplii* crustaceans from the infectious disease caused by pathogenic *Vibrio campbellii* bacteria. The efficiency of PHB was 100 times larger than that of 3-hydrobutyric acid monomer [75]; in addition, PHB has the ability to suppress pathogenic bacteria, not only *Vibrio* sp. but also *E. coli* and *Salmonella* sp. [76, 77]. The studies in which PHB was used as prebiotics indirectly indicate a significant role of PHB for microbiota. Its effect on microbiota and on the host organism was then studied. Unfortunately, no such biomedical studies were conducted on humans, but studies on the farm animals were conducted [76]. It was shown that the feeding of broiler chickens with PHB powder or biomass of the *Rhodobacler sphaeroides* PHB producer strain (containing approximately 25% PHB of the cell dry weight) improved the meat productivity of chickens due to stimulation of the growth of the microbiota bacteria of the animal GIT [77]. The feed-

ing of the *Dicentrarchus labrax* European sea bass with feed containing 2 and 5% PHB powder for 6 weeks led to a significant increase in fish weight; this was associated with a significant change in the composition of the fish intestine microbiota (probably, due to its stimulation) [78]. The feeding of the *Acipenser baerii* Siberian sturgeon fries with *Artemia nauplii* crustaceans (preliminary fed with the PHB powder) showed that the biopolymer changes the lipid composition of the fry body; this indicate changes in the lipid metabolism in fished. This was also associated with a change in the microbiota composition in the sturgeon fry intestine [79].

The PHB copolymer with 3-hydroxyvalerate is poorly exposed to the digestion in the gastrointestinal tract of pigs. However, preliminary treatment of PHBV with NaOH significantly improved the PHBV cleavage and led to the cleavage of 37% PHBV in the pig GIT. In the sheep GIT, PHBV cleaved much better: more than 40% polymer cleaved without the treatment, while more than 85% after the treatment with NaOH. The continuous (3 weeks) feeding of sheep and pigs with food containing up to 20% PHBV powder had no harmful effect on the animals [80, 81]. Bacterial depolymerases, which many bacterial species of human and animal microbiota also possess, play the main role in the cleavage of PHB and its copolymers in GIT [44, 76]. In addition to bacterial PHB depolymerases, other microbial enzymes are also capable of nonspecific cleavage of PHB and its copolymers; thus, β-mannanase (the enzyme cleaving mannan polysaccharides) was shown to have a high affinity to PHB granules [82]. It was also demonstrated that the biodegradation products of some natural PHAs (e.g., 3-hydroxyoctanoate) have antimicrobial activity relative to a number of infectious gram-negative and gram-positive bacteria and that they inhibit the production of metabolites associated with their pathogenic activity [83].

Short-chain, nonreserve oligo-PHB was also found in some infectious bacteria, and oPHB was found in the composition of a complex with polyphosphates in the *Streptomyces lividans* potassium channels [84] and in a complex with the NThiP5 protein in the *Haemophilus influenzae* [85].

The role of PHB in the symbiosis of human and animal microbiota bacteria can be better understood on the example of the role of PHB in symbiotic bacteria of other eukaryotes (for example, plants). Thus, PHB synthesis plays an important role in the symbiosis of nitrogen fixing bacteria with plants of the legume family. In these plants, nitrogen fixation occurs in nodules, in which nitrogen fixing bacteria are in a close symbiosis with plant cells in special, highly organized tissues. It should be noted that many nodule bacteria (e.g., from the *Rhizobium* and *Sinorhizobium* genera) are capable of PHB synthesis [86]. The bacteria, which coexisting in nodules in symbiosis with plant cells, infect the root meristem cells (penetrating inside) and pass in a special form (bacteroides), in which many physiological functions of bacteria are suppressed (particularly, the enzyme expression and activity), and metabolic pathways are reoriented to the implementation of the main (atmospheric nitrogen fixation, including PHB biosynthesis); this was demonstrated both by the methods of proteomic analysis and the study of gene expression [87–90]. In soybean (*Glycine max* L.) nodules, the *Rhizobium sp.* bacteroides synthesize a significant amount of PHB due to the high activity of PHB synthase and other enzymes that synthesize the polymer, while PHB synthesis in chickpea (*Cicer arietinum* L.) nodules is almost not carried out by bacteroids (which is associated with the low activity of the enzymes that carry it out) [91]. In another work using transmission electron microscopy (**TEM**), active PHB accumulation was demonstrated in *Sinorhizobium* sp. bacteroids of the *Vigna unguiculata* cow peas and *Leucaena leucocephala* nodules (accompanied by the expression of the phasing protein genes (*phaP1*, *phaP2*, *phaP3*) associated with bacterium PHA-granules) [90]. The phasins (*phaP1* and *phaP2*) in PHB biosynthesis, atmospheric nitrogen fixation, and simultaneously the ability to the production of nodules in Lucerne (*Medicago truncatula* and *Medicago sativa*) were shown to play an important role for symbiotic nitrogen fixing *Sinorhizobium meliloti* bacterium [92]. Active PHB accumulation, which was also detected by TEM, was observed in the *Rhizobia* sp. bacteroids of the albition nodules (*Samanea saman*). It is interesting that the nodules were infected not only with bacteria but also with hyphae of the rhizosphere fungi of the tree roots. The detection of the coexistence of fungi and nitrogen fixing PHB-synthesizing bacteria in nodules complements the study of Giles et al. [93] on the translocation of the *Azotobacter sp.* bacteria in the fungus hyphae [94]. Moreover, it was demonstrated that some proteins of the PHB granule membrane (for example, the *PhaR* phasing) regulates not only PHB biosynthesis in symbiotic *Bradyrhizobium diazoefficiens* bacteria; it is also involved in regulation of their symbiotic relationships with soybean, the host plant (*Glycine max* L.), which was particularly expressed by an increase in the plant biomass. This important role of phasins in the regulation of symbiotic relationships of bacteria with host organisms and in PHB biosynthesis was already demonstrated above on the example of relationships of the *Burkholderia* sp. bacteria and the bean beetle. This can indicate an association of PHB biosynthesis with symbiotic relationships of bacteria and multicellular eukaryotes [59, 95]. Other researchers also confirmed the importance of PHB accumulation for the process of plant infection, nodule development, the growth and reproduction of the *Sinorhizobium meliloti* symbiotic bacteria in the Lucerne nodules, and atmospheric nitrogen fixation by them; i.e., they confirmed the important role of PHB in symbiotic relationships of bacteria and

plants, although infection of the roots of legume plants and the development of nodules by nitrogen fixing bacteria (which are incapable of PHB synthesis) is possible [92, 96]. On the other hand, it was demonstrated that the host plant also significantly affects the symbiotic bacteria of nodules, regulating PHB synthesis by them. Thus, the gene of the *GmNMNa* protein with unknown functions, which was localized in nucleolus and mitochondria of the plant cells, was detected in the soybean. Suppression of the expression of this gene led to oppression of nodule development, a decrease in the number of the *Bradyrhizobium japonicum* bacteroids in infected plant cells, and decreased PHB synthesis and accumulation in bacteroids [97]. The mechanism of such a complex interaction of symbiotic bacteria and the host plant, which is associated with PHB synthesis, was considered in detail in the work by Ratcliff et al. It is known that the legume plants suppress the reproduction of the *Rhizobium* sp. bacteria that do not sufficiently fix atmospheric nitrogen [98]. However, symbiotic bacteria learn how to resist this pressure from the host plant by means of the synthesis and secretion of rhizobitoxin (Rtx) ethylene oxide inhibitor; this allowed them to fully reproduce and function in the nodules. Rhizobitoxin producers are capable of active PHB synthesis and accumulation in cells, but they are much worse at fixing atmospheric nitrogen [99].

MICROBIOTA AND ENDOGENOUS POLY-3-HYDROXYBUTYRATE IN EUKARYOTIC TISSUES

The finding of PHB in membrane fractions of different gram-negative and gram-positive bacteria incapable of its synthesis and having no PHB synthase [100], as well as the detection of this polyester in various tissues of eukaryotes and even in higher members (mammals), was one of the most interesting stages in the history of PHA research. It should be noted immediately that it is not high-molecular reserve polymer, the synthesis of which is typical for a number of bacteria, but the so-called short chain complex-forming cPHB and low-molecular oPHB was found [35]. While specialized PHB biosynthesis enzymes are available only in prokaryotes, this biopolymer was found in organisms of almost all types by a group led by the American professor R.N. Reusch [101–104]. Short- and medium-chain PHBs were found in many different mammalian organs and tissues (including in humans), i.e., in the blood plasma, heart, kidneys, liver, vessels (aorta), nerves, lipoprotein particles, platelets, etc. As in bacteria, it was demonstrated that oPHB is located in eukaryotic cell membranes in the form of a PHB–polyphosphate–calcium complex [101, 103]. The identity of detected oligo-PHB as a hydroxybutyric acid polyester was confirmed by means of ^{1}H-NMR spectroscopy [102, 103], while its molecular weight (MW = 12200 corresponds to a length of approximately 140 elements of 3-hydroxybutyric acid) was determined by means of chromatography and electrospray mass spectroscopy [30].

Using the test on determination of crotonic acid and antibodies to PHB, it was shown that the cPHB concentration varies from 3–4 μg/g in the nerve tissues and brain to 12 μg/g in the blood plasma [102, 105]. The oPHB concentration in human blood plasma can change in a rather wide range, from 0.6 to 18.2 mg/L at an averaged value of 3.5 mg/L, while albumin is the main PHB-binding protein [102]. It should be noted that the intermediate product of PHB degradation (D-3-hydroxybutyric acid) is a ketone body and is normally contained in the mammalian blood and tissues in concentrations of 0.3–1.3 mM and in much higher concentrations in pathological conditions [106, 107].

PHB (including oPHB and cPHB) was detected not only in animals but also in plant tissues: in rice stems [108], sugar beet leaves and stems [109], flax stems [110], and maize [111]. Moreover, the question of the presence of PHB in plant and fungal tissues is closely associated with the production of transgenic plants containing the PHB synthesis genes (*PhaC* PHB-synthase) that are capable of PHB synthesis [110–112] according to the traditional technology of agrobacterial plant transformation [113], since PHB was found in control samples of wild-type plants [110, 111]. In the discussion, the authors either accept the absence of PHB synthase in eukaryotes and bacteria that do not synthesize reserve PHB but assume that there is some alternative biochemical pathway of its synthesis and suggest schemes of possible biochemical reactions of PHB synthesis [109], or they do not explain the presence of PHB in wild-type plant tissues [110, 114].

Analysis of the protein UniProt and Protein NCBI databases demonstrated that the PHB-synthase protein (PHA-polymerase, *PhaC*) in both databases was detected in eukaryotes of only five species: the mollusk *Crassostrea gigas* (UniProt: K1QX58; NCBI: EKC26101.1), the Chinese hamster *Cricetulus griseus* (UniProt: A0A0L0DUY1; NCBI: ERE46547.1), actinia *Nematostella vectensis* (UniProt: A7TBP6; NCBI: EDO26560.1), an uncharacterized protein in the *Ricinus communis* castor-oil plant (UniProt: B9T9C3B; NCBI: EEF27538.1), and the sponge *Amphimedon queenslandica* (UniProt: I1EHL3; NCBI: XP_011407756.1). In the UniProt database (but not Protein NCBI), PHB synthase was also identified in the *Reticulomyxa filosa* foraminifera (UniProt: X6PA37) and another unicellular algae *Thecamonas trahens* (UniProt: A0A061HTL5). Such an exotic representation of PHB-synthase enzyme in the eukaryote superkingdom suggests that it may be falsely identified in the animal and plant species listed above, or (which is much more interesting) it may indirectly

indicate the phenomenon of horizontal gene transfer [115, 116].

The point is that the possibility of introduction of the genes of enzymes responsible for PHB synthesis by means of agrobacterial transformation and the fact of PHB synthesis and accumulation in the tissues of such transgenic plants indicates a very real possibility of such processes in nature itself. Modern genetic engineering is in fact based on the principles of horizontal gene transfer, though there was no clear understanding until recently that this kind of genetic engineering is widespread in nature. Horizontal gene transfer between phylogenetically distant taxa (e.g., between bacteria and plants) is extremely interesting and one of the most intensively studied phenomena in modern science. Most often, they speak about horizontal gene transfer as one of the key driving evolution factors in prokaryotes and, as it becomes more and more obvious, in eukaryotes. However, this phenomenon can be also manifested on an incommensurably smaller scale as quite routine processes in the interaction of organisms from different taxa with each other, e.g., infectious parasite bacteria and the cells of certain tissues of a multicellular animal or host plant. An agrobacterium itself is a real, natural genetic engineer, while humans only use its unique abilities. The possibility to transfer a variety of plant genes, including the genes for great length in the plant cell nucleus, is an important advantage of agrobacterial transformation as compared with other genetic engineering methods [115, 116]. It should be noted that, in addition to the ability to transfer genes to eukaryotes, the *Agrobacterium* genus bacteria can synthesize PHB, while its genome contains the genes of all enzymes required for this process. However, it should not be forgotten that the fixation of a particular gene in eukaryotic genome is primarily caused by evolutionary advantages that the gene can give to this species; if there is no such advantage, this gene can be subsequently lost at some stage of evolution, even with successful horizontal gene transfer from the bacterium to eukaryotic organism.

However, despite all of the above, the question of the source of PHB origin in mammalian organisms remains open, since, despite the significant progress in decoding genomes of various organisms, no PHA-synthase gene has been found in mammals to date (except for the detection of the PHB-synthase gene in the Chinese hamster).

One of the main tasks of this analytic review was an attempt to understand this difficult question. For this, we list all theoretically possible PHB sources in the mammalian (including human) organism in order to understand the likelihood of their existence:

(1) endogenous synthesis in human cells by means of yet-unknown metabolic pathways;

(2) synthesis by human microbiota bacteria and entry into human blood and tissues by absorption through the intestinal mucosa and subsequent migration in the blood;

(3) synthesis in certain human tissues inside mRNA cells carrying the PHB-synthase gene; human microbiota bacteria are a source of it.

The discoverers of PHB in humans are supporters of the first theory (endogenous biopolymer synthesis in human cells by means of yet-unknown biochemical mechanisms) [101, 102]. One of the most interesting studies on the detection of cPHB and the study of its possible role in mammals was conducted by the scientific group under the leadership of Professor Zakharian from New Jersey Medical School (United States) in collaboration with other scientific groups from United States, Canada, and Germany. These researchers demonstrated that cPHB binds to one of the proteins from the group of melastatin receptors (*TRPM8*) of mammals and humans, which leads to a change in its functioning [117, 118]. *TRPM8* is a membrane calcium channel functioning as a temperature sensor of neurons of the mammalian peripheral nervous system. It was demonstrated by mass spectrometry and enzyme-linked immunosorbent assay that this protein is covalently bound to the short-chain PHB with a chain length of 1–26 monomers (cPHB) in many (more than 25) binding sites through serine residues in extracellular and transmembrane domains. This protein was shown to be associated not only with cPHB but also with much more long-chain oPHB [35]. PHB bound to the transmembrane domains is located in the membrane in a complex with polyphosphate. Moreover, the *TRPM8* function as a temperature sensor depends on whether this protein is bound to PHB or not; i.e., *TRPM8* must be modified by PHB for normal functioning, and the authors proposed that such PHB functionality is associated with a change in the conformation state of PHB when passing through the glass transition temperature of this polymer, which is approximately 10°C. Coexpression of the *PhaZ7* PHB depolymerase (besides *TRPM8*) in cells or modification of the protein binding sites with PHB by genetic engineering led to a violation in the function of this membrane receptor as a temperature sensor [118, 199]. The authors conclude that the binding of the protein *TRPM8* receptor channel with cPHB is a posttranslational modification of this protein required for its normal functioning [117, 118]. However, it should be noted that the study was conducted on an experimental artificial model (a culture of embryonic cells of human kidneys and rat neurons transfected with the TRPM8 genome). In addition, the methods used in the study to prove the presence of PHB in these cells, including genetic engineering (the coexpression of cells with PHB-depolymerase), enzyme-linked immunosorbent assay (with antibodies to PHB), mass spectrometry (MALDI TOF, LC/MS/MS), and staining with Nile red dye cannot be considered direct methods for the analysis of the presence of PHB in the cells (despite their high complexity and workability)

and can give nonspecific reactions. In addition to the role as a reserve substance and energy depot in bacteria, such specific PHB functions were found by this group of scientists, both for prokaryotic and eukaryotic organisms. The specific role of PHB is apparently coupled with the regulation of different proteins due to the production by short-chain cPHB and oPHB of both noncovalent and covalent bonds with other biopolymers (proteins, nonorganic polyphosphates, and DNA) [30, 84, 85, 100–102, 120, 121].

Attention should be also paid to the possibility of successful transfection of mammalian cells themselves (embryonic cells of human kidneys and rat neurons by the gene of bacterial PHB-depolymerase enzyme *PhaZ7* DNA with subsequent synthesis of functional enzyme. This can indirectly indicate the possibility of mammalian cell transfection with the PHB-synthase gene. Research on cells transfected with *TRPM8* can indicate a possible nonspecific mechanism of covalent protein modification by cPHB oligomer. The works of the same scientific group, in which it was demonstrated that *TRPM8* is also a testosterone receptor and its expression is increased in tumor cells (indicating the involvement of this protein in oncogenesis), can indicate such nonspecificity [122].

A number of works devoted to the search for and study of low-molecular PHB in mammalian mitochondria also deserve attention, since the evolutionary origin of mitochondria is also associated with intracellular symbiosis with bacteria [123]. Thus, small amounts of cPHB were isolated from mitochondria isolated from healthy bovine hearts [103]. Scientists from the group of Pavlov studied the physiological role of cPHB in mammalian mitochondria and demonstrated that cPHB is involved in an increase in the flow of calcium ions into the mitochondrion due to a possible increase in the permeability of the internal mitochondrion membrane for these ions and a change in the viscosity of the membrane lipid bilayer [124, 125].

The possibility of some alternative physiological role of PHB in mammalian organisms is also confirmed by the works of some other researchers, who demonstrated that PHB oligomers and its copolymers (with a chain length of 20–25 monomers) are not toxic for the cells (to a concentration of 20 μg/mL) and have biological activity. Thus, PHB oligomers and its copolymers with 4-hydroxybutyrate and 3-hydroxyhexanoate stimulated proliferation, suppressed apoptosis, released calcium into the cytoplasm and intercellular contacts of B-cells of the mouse pancreas [126]. While the PHB biodegradation product (3-hydroxybutyrate, 3-HB) is a natural metabolite in mammalian organisms (the so-called ketone body) and had a pronounced versatile biological activity [106, 107].

The theories of endogenous PHB synthesis in mammalian cells by means of yet-unknown biochemical mechanisms are mainly associated with the functional role of cPHB in mammalian organisms postulated by the authors as a polymer modulating the function of ion channels and pumps in the membranes of the cells and mitochondria [127]. This role of cPHB makes it necessary to consider the presence of PHB in the organism as vital from the earliest stage of embryonic development; therefore, the researchers assume PHB synthesis in the cell by means of known or yet unknown enzymatic systems of the eukaryotic cell. However, it is extremely difficult to imagine that, despite the complete decoding of even such complex genome as human genome, it is possible to detect PHB synthase or any enzymes related to PHB synthase (nucleotide sequences); they should already have been found. However, since PHB synthase has still not been found in humans, we can only talk about nonspecific PHB synthesis by other enzymes. However, in human tissues, these researchers detected not only cPHB with a length of up to 30 monomers but also oPHB consisting of 100–200 monomers. It is extremely difficult to perform the synthesis of a homopolymer consisting of more than 100 residues of 3-hydroxybutyric acid by means of any nonspecific enzymatic system; at least, this assumption requires strong evidence of a real possibility of such synthesis. In addition, enzymatic systems for other biopolymers similar in structure to PHB (polyprenols and dolicholes) were found long ago, and biochemical mechanisms of their synthesis were decoded in detail in mammals, although all metabolites of these biochemical processes are also present both in prokaryotes and in eukaryotes [128]. Polyphosphate, the synthesis of which is performed by the same specific enzyme both in prokaryotes and in eukaryotes, can serve as an example [129]. Almost the same can be said about all biopolymers of prokaryotes and eukaryotes: DNA, RNA, proteins, polysaccharides, and lipids. We cannot find another example of the synthesis of such long-chain polyesters performed by a nonspecific enzyme or a complex of enzymes in the presence of an evolutionarily more ancient specific enzyme, which performs its synthesis in other organisms. Therefore, this theory is not convincing.

On the other hand, polyprenols (in bacteria, plants, and fungi) and dolicholes (in bacteria and animals) are a striking example of other biopolymers that are quite similar to PHB in chemical structure and are widely used in nature to perform different goals, like oPHB and cPHB. Polyprenylation (a covalent binding to polyprenols) plays an important role in the post-translational modification of a number of proteins to anchor them in the membrane [88]. All organisms (both eukaryotes and prokaryotes) have biochemical mechanisms for the synthesis of these biopolymers and their conjugation with proteins. The appropriate enzymes were also identified; this may indirectly indicate the possibility of the existence of endogenous pathways of PHB synthesis in mammals. Appropriately, there are grounds to believe that the functional-

ity of cPHB and oPHB in eukaryotes can be similar to the functionality of polyprenols and dolicholes.

Can microbiota be a source of PHB in human organisms? Indeed, the theory of its synthesis by some intestinal microbiota bacteria and biopolymer suction into the blood through the intestinal mucosa with subsequent penetration of other tissues may be the simplest explanation for the presence of PHB in mammalian tissues. However, is a hydrophobic polymer such as PHB capable of suction through the intestinal mucosa to the blood and migration in the blood flow? By the way, this question is also very important in order to use medical devices based on PHB for regenerative intestinal surgery. It is known that high-molecular PHB is insoluble in water, but its oligomers are not so hydrophobic; this makes it possible to assume the possibility of their suction through the intestinal mucosa. Thus, it was demonstrated that soluble PHB oligomers with a chain length of up to seven 3-HB residues were found in water after hydrolytic PHB decomposition and water extraction of the polymer degradation products [130]. However, it was later demonstrated that PHB oligomers consisting of approximately 25 3-HB residues conjugated with lipoic acid were also soluble in water [131]. However, PHB oligomers with a chain length of less than five monomers are able to penetrate the intestinal mucosa [132]. After oral administration of PHB tetramer (2-keto-butan-4-ol-KTX 0204 ether) to rats at a dose 300 mg/kg, the 3-HB concentration in the blood increased to more than 1.0 mM after 30 min and remained increased for 3 h, after which it returned to the physiological values (approximately 0.1 mM); this indicated rapid and active suction of these short PHB oligomers in the gastrointestinal tract. Short PHB oligomers in the blood plasma and tissue homogenates are rapidly cleaved to 3-hydroxybutyrate [132, 133]. However, some researchers suggest that cPHB with 30 3-HB residues can also be sucked into the bloodstream by the same mechanism as cholesterol, while the cPHB circulation in the blood occurs during its binding to albumin or in the composition of low-density lipoproteins, but the authors do not provide direct evidence of this theory [134]; therefore, it is quite difficult to explain the detection of cPHB, and even more difficult for oPHB with a chain length of more than 100 monomers of 3-HB residues only by the possibility of suction through the intestinal mucosa.

But how can PHB be in blood or human tissues in this case? A hint can be seen in the works of researchers from the group already mentioned above led by Professor Reusch, in which the role of low-molecular PHB in mammalian and human organisms was studied [105, 135]. A range of data indicating the involvement of PHB in pathological processes is given in them. Thus, an eightfold increase in the cPHB 3 concentration in the blood and other organs involved in diabetes pathophysiology (kidneys, eyes, sciatic nerve and aorta) was demonstrated on a streptozotocin model of diabetes in rats; this can indicate the involvement of cPHB in the pathogenesis of diabetes, and the correlation between the cPHB concentration and atherogenic lipid profile in the blood plasma is observed [105]. Theoretically, it was also suggested that the increased viscosity caused by the presence of cPHB and its interaction with extracellular matrix biopolymers can lead to an increase in intraocular pressure, which in turn leads to glaucoma. Here, the authors also suggest that cPHB can be synthesized nonspecifically, and its synthesis can be associated with the biosynthesis of cholesterol, which causes its role in the pathogenesis of diabetes and glaucoma [135].

However, there is a much more intriguing association of PHB with the pathogenesis of diabetes and concomitant diseases (not suggested by these authors) that can be traced here. At present, the direction on the study of the role of intestinal microbiota in the pathogenesis of type 2 diabetes is actively developed. Thus, a clear association between dysbiosis and type 2 diabetes was demonstrated. The authors of these works (published in *Nature*) showed an increase in the number of the *Clostridium clostridioforme* bacteria in the intestines with type 2 diabetes [136, 137]. It was shown that the permeability of the intestinal mucosa is increased with this type of diabetes, and translocation of bacteria from the intestinal lumen to the system blood flow is observed (i.e., bacteria of different species are present in the blood of diabetes patients). Bacteria found in the blood were identified as obligatory anaerobes from the *Clostridium coccoides* group and *Atopobium* cluster, as well as facultative anaerobes from the *Streptococcus* and *Enterobacteriaceae*. Moreover, the bacterial DNA was detected in the system blood flow in individuals before the beginning of diabetes clinical manifestations in them and in a larger number in those who suffered from obesity. Thus, the translocation phenomenon can be used as a diagnostic tool for the earliest stages of diabetes or even predisposition to its development [137, 138]. How is this phenomenon of the translocation of intestinal bacteria related to PHB? It was found that this was the most direct relation, if we take into account that the *Clostridium* genus bacteria from the intestinal microbiota are capable of synthesizing the reserve high-molecular PHB (as shown above) [49]. Other bacteria from the GIT microbiota of humans and different animals are also capable of synthesizing both high-molecular PHB and low-molecular cPHB and oPHB (which was already described in an earlier part of the review).

Different species of aerobic bacteria of human microbiota were shown to have the ability to translocate through the intestinal and oral cavity mucosa. Thus, the *Fusobacterium nucleatum*, another gram-negative, anaerobic bacteria of the oral cavity having PHB synthase, is able to attach to epithelial and endothelial cells of the oral mucosa and then to penetrate them by means of a special *FadA* protein, which binds to the vascular endothelial cadherin. It is interesting

that the joint incubation of the *F. nucleatum* with bacteria of other species (e.g., with *E. coli*) increased the endothelium permeability for all bacteria; this explains the detection of this bacterium during the infectious processes caused by other pathogens. The translocation of these bacteria from the oral cavity mucosa into atherosclerotic plaques of coronary vessels was also shown to be possible [139, 140]. Twenty-seven different species (strains) of bacteria, of which 11 (from the *Acinetobacter, Burkholderia, Pseudomonas, Fusobacterium, Neisseria, Sphingomonas, Ralstonia* genera, including 5 of the 15 most numerous species) have PHA synthase and/or are capable of PHA synthesis, were detected in atherosclerotic plaques in patients who underwent coronary balloon angioplasty and simultaneous suffered from periodontitis. This can indicate a significant role for PHB biosynthesis in bacterial translocation from the mucosa into the blood or at all larger portions of PHB-synthesizing bacteria in human microbiota [140]. By means of fluorescent in situ hybridization and TEM, the presence of bacteria of different species, normally in the cytoplasm of the gallbladder epithelial cells in healthy pigs [141] and in the cytoplasm of the lung alveoli epithelial cells in healthy mice [142], was demonstrated. More and more arguments are emerging in favor of the fact that human microbiota bacteria can penetrate into the vascular bed, not only during the infectious process but in healthy individuals as well. The presence of bacterial cells in the blood of healthy individuals was demonstrated by TEM [143]. The presence of ribonomal RNA of eight bacterial species in the blood of healthy individuals was established by polymerase chain reaction [144]. The translocation of bacteria from the intestines into the blood vessel was also detected by TEM in healthy rats and in the lymph in healthy dogs [145, 146]. Besides the detection of bacteria in the blood in healthy individuals with a predisposition to diabetes and suffering from obesity, this marker of the presence of bacteria as a lipopolysaccharide-binding protein was also detected in healthy elderly people (60–89) without clinical manifestations of acute and chronic diseases associated (as suggested by the authors of the article) with their sedentary lifestyle [147]. It should be noted that those researchers who detected bacteria in patients with type 2 diabetes also detected bacteria in a small amount in healthy individuals and in the control group, and they identified these bacteria as the *Clostridium coccoides* and *Streptococcus* group [138]. As demonstrated by Professor Tedeschi et al. in *Nature* in 1969, Mycoplasma-like L-forms of the *Listeria* sp. bacteria were detected in the blood of healthy individuals. Moreover, it was demonstrated that their presence in blood is associated with an atypical erythrocyte metabolism, particularly, with the absorption of nucleotides and amino acids by them [148]. The ability of the *Clostridium* genus bacteria to produce L-forms was also demonstrated [149, 150]. Moreover, the last 2–3 years have seen the appearance of papers that actually recognize the presence of bacteria in the blood of healthy individuals and even papers in which the presence of the blood microbiota of healthy individuals is recognized and its composition is studied [151, 152]. In an article published in 2017, a bioinformatic study of the composition and phylogeny of bacteria of the blood microbiota was conducted in healthy individuals based on extensive data on the sequencing of 16S ribosomal RNA in their blood obtained in the course of the International Project of the National Institute of Health (NIH), "Human Microbiota" (the NIH's Human Microbiome project). It was demonstrated that there are 43 bacterium species in the blood of healthy individuals from the genera *Enterococcus*, *Streptococcus*, *Cardiobacterium*, *Psychrobacter*, *Bacteroides*, and etc. [152].

The scientific direction for the detection of bacteria in different tissues of healthy individuals and clarification of their normal role is being actively developed at present, but it is a real breakthrough in medicine, since it is associated with the hardest way to overcome the established paradigm, according to which the presence of bacteria somewhere in human tissues necessarily indicates an infectious or different kind of pathological process. Though the ideas on the important role of microbiota in normal human physiology, not only in the process of digestion but also for normal functioning of the immune, humoral, and nervous systems, are actively expanding in Russia and around the world, a new vision of the human as a symbiotic superorganism has been revealed [153]. The detection of bacteria in the blood of a control group of healthy patients would uniquely indicate the incorrect selection of the control group in this study, which, according to old views, would require anti-infectious therapy for patients from this control group. The presence of bacteria in norm in the urine of healthy individuals was accepted at the 114th Congress of American Microbiology Society in 2014. This was followed by publication in a prestigious European Urology journal [56, 154]. The possibility of complete acceptance by the world scientific community of the view that bacteria are normally present in some small amount in the blood of healthy individuals, but not in patients with noninfectious diseases (which is already generally accepted [151]), does not seem as unbelievable and unacceptable now as it recently did. The International Project of the National Institute of Health, Human Microbiota (NIH's Human Microbiome project), in the course of which extensive data on the study of the composition of microbiota in different human tissues in normal conditions were obtained, made a huge contribution to the change in the views of the world scientific community on this question [155, 156].

Thus, PHB synthesis is apparently possible in certain human tissues inside cells on PHB synthase with the mRNA of human microbiota bacteria carrying the gene of this enzyme. Significant opportunities for plant agrobacterial transformation with the PHB-syn-

thesis enzyme genes were described above. In light of this, the presence of PHB synthase and, appropriately, its gene in bacteria of the very same *Agrobacterium* genus (which are widely used to obtain the transgenic plants, for example, in the *Agrobacterium tumefaciens* CCNWGS0286 (UniProt: B9JEJ2; Protein NCBI: EHH09041.1; ACM26413.1)) is interesting [157]. The most striking thing is that *Agrobacterium tumefaciens* (also known as the *Rhizobium radiobacter*) can cause infections in humans (catheter infections in individuals with weakened immunity, particularly, in HIV-infected people). *Agrobacterium tumefaciens* affects prosthetic joints and prosthetic valves and causes sepsis, peritonitis, and urinary tract infections. Thus, a clinical case of peritonitis caused by infection with this bacterium during a surgical operation was described [158, 159]. This means that these bacteria can theoretically transfect human cells, e.g., the intestinal mucosal cells, with the PHB-synthesis genes (in addition to the genes of other bacterial proteins). Moreover, there are direct confirmations of agrobacterial transformation (transfection) of human cells. The successful genetic transfection of mammalian cells, including human cells (cell cultures of cervical cancer (*HeLa*), human embryonic kidney cells (*HEK 29*), rat adrenal pheochromocytoma (*PC12*), and monkey fibroblasts (*COS-1*)) by means of *Agrobacterium tumefaciens* was demonstrated in a number of works [160–162]. With this technology, it was possible to obtain mammalian transgenic cells with built-in genes of different proteins (neomycin phosphotransferase and green fluorescent protein), and the successful expression of these proteins in transfected cells was also demonstrated. The transfection of mammalian cells was performed according to the same mechanism as the plant cell transfection. The similarity of the process of bacterium attachment to the mammalian cell membrane with plant protoplast cells was demonstrated. In addition, the genetic construction synthesized in vitro from the proteins (*VirD2* and *VirE2*) included in the *Agrobacterium tumefaciens* T4SS secretory system was used to deliver DNA in the mammalian cell nucleus (*HeLa* cell culture), which also confirmed the mammalian cell transfection according to the usual mechanism of agrobacterial plant transformation [163, 164]. To check the efficiency of agrobacterial transformation of mammalian cells in vivo, Russian scientists reported an experimental mouse infection (by introducing bacteria into the blood) with *Agrobacterium tumefaciens* bacteria carrying genetic constructions for the transfection of mouse cells and the subsequent expression of green fluorescent protein in the animal tissue. It was shown that, despite the successful transfection of mammalian cells in vitro (the *HEK293* culture of embryonic human liver cells), the successful genetic transfection of the mouse cells in vivo was not realized in any animal tissues, although the same genetic construction was successfully used to transfect tobacco leaf tissues [165]. Successful agrobacterial transformation (transfection) was demonstrated not only for the mammalian cells but also for the cells of other animals (e.g., arachnids). Thus, the successful transfection in vitro of *Rhipicephalus microplus* and *Ixodes scapularis* tick cells with *SALP15* saliva factor and green fluorescent protein genes by means of *Agrobacterium tumefaciens* was demonstrated. Moreover, unlike mammals, successful agrobacterial transformation (transfection) of tissues of live tick larvae in vivo was demonstrated [162].

However, *Agrobacterium tumefaciens* is not the only bacterium species possessing the T4SS IV type secretory system. This system is used by symbiotic and infectious human bacteria of different genera: *Enterococcus*, *Bifidobacterium, Lactococcus, Clostridium*, *Streptococcus*, *Staphylococcus*, *Streptomyces*, *Actinomyces*, *Bacillus*, *Mycobacterium, Helicobacter, Legionella, Listeria, Escherichia*, etc.; its structure, mechanisms of functioning, role, and significance for bacteria were described in a number of reviews [166, 167]. It was demonstrated that, using the T4SS IV type secretory system, these bacteria successfully transfer their genes in mammalian cells: gastric mucosal cells [168, 169], Chinese hamster ovary cells (*CHO K1*) [170], human endothelial cells [171], *HeLa*, *HepG2*, *COS-1*, *J774*, and peritoneal macrophages [172].

However, in this review, we are especially interested in the T4SS secretory system in those bacterium species that, on the one hand, have the ability to synthesize PHB and, on the other, are components of human microflora or are infectious agents in humans, i.e., have the potential ability to horizontally transfer bacterial genes in the cells of different human tissues and organs, primarily, in the cells of GIT mucosal epithelium. *Clostridium* genus bacteria (which are capable of rPHB synthesis) have the T4SS secretory system; among them, there are both infectious species and species that are a natural component of human intestinal microbiota, the role of which have been already described in detail above [173, 174]. *Bacillus* genus bacteria capable of PHB synthesis (which are also ordinary members of human intestinal microbiota and are able to cause infectious diseases) also have many elements of the type IV secretory system [175]. The aforementioned pathogenic insect bacteria from the *Burkholderia* genus (capable of the PHB synthesis) also have the T4SS system, which is homologous to the similar *Agrobacterium tumefaciens* system, and are able to transfect eukaryotic cells by bacterial genes [176]. However, the role of the type IV secretory system in infectious *Legionella pneumophila* bacteria, which cause such dangerous infectious lung diseases as Legionnaires' disease, is especially interesting. These bacteria are obligatory intracellular parasites of free-living amoebae in water reservoirs and human lung macrophages. When a person is infected, *L. pneumophila* reproduce inside neutrophils, macrophages, and dendritic cells. Moreover, these bacteria, actively using the T4SS system, introduce into the host cell

more than 300 different bacterial enzymes and bioactive factors [177, 178]. Due to the presence of the secretory T4SS system, *L. pneumophila* is apparently also able to transfect the host cells with its genes, since bacteria can take eukaryotic host genes; as a result, many *L. pneumophila* genes have a eukaryotic origin due to horizontal gene transfer [177, 179, 180], although we found no direct confirmation of transfection in vitro of mammalian cells by this bacterium. Meanwhile, their capacity for the biosynthesis of reserve high-molecular PHB (which they use as a source of energy under stressful conditions) plays an important role in the survival of these parasitic bacteria [181–183]. It was demonstrated that PHB utilization genes such as *bdhA* (3-hydroxybutyrate dehydrogenase enzyme) are closely related to the ability of these bacteria to replicate inside amoeba and lung macrophages, i.e., their infectious activity [184, 185]. Some researchers suggest that the genes required for PHA biosynthesis (*phaA*, *phaB*, and *phaC*) were actively transmitted in the process of evolution from one species of infectious bacteria or microbiota bacteria to another by means of horizontal gene transfer.

It should be noted that horizontal gene transfer between symbiotic or parasitic bacteria and their host (multicellular animal) is especially widespread. Many researchers indicate the leading role of the animal intestinal microbiota and the important role of the type IV secretory system of symbiotic and infectious bacteria in the horizontal gene transfer between bacteria and eukaryotes in the process of evolution [178, 180, 186]. They suggest that this is a quite common phenomenon and that it plays an important role in the pathogenesis of many diseases, e.g., tumors [187]. The clear illustration of the scale of such horizontal gene transfer between the parasite bacterium and the host animal is an example of the transfer of an almost complete genome of the intracellular parasite *Wolbachia* sp. bacterium in the genome of their host (the *Drosophila ananassae* fly) [188].

Thus, proceeding from the above, the functions of natural PHAs (primarily, PHB) in human and animal microbiota bacteria, both those proven and those still requiring proof, can be summarized in Table 1.

Analysis of the scientific literature indicates that many typical members of human GIT microbiota and known causative agents of human infectious diseases (including obligatory intracellular parasites) simultaneously have the type IV secretory system and enzymes for PHB synthesis (Table 1); this significantly increases the probability of genetic transfection of cells of the intestinal epithelium, stomach, oral cavity, lungs, and other organs with the triad of the *PhaA*, *PhaB*, and *PhaC* genes required for PHB synthesis. It can even be assumed that such transfection is directed locally in some intestinal mucosa region, e.g., in the caecum and appendix, where PHB synthesis can be performed in amounts sufficient to reach those concentrations in the blood that were previously identified by some researchers. Of course, this is only an assumption that is still awaiting experimental confirmation.

Thus, the function of the microbiota bacterium PHB cannot be exhausted by the use of this biopolymer as a reserve substance and a source of energy in stressful conditions. In light of the strongest effect of the host animal's immune system on its symbiotic and infectious bacteria, it is exactly the presence of such an efficient mechanism of energy accumulation that probably gives bacteria synthesizing PHAs huge advantages for adaptation to stressful conditions. However, the detection of PHB in the tissues and cells of the animal itself necessitates the assumption of the presence of some other additional functions of this biopolymer. In rare works (e.g., in a wonderful review of Madison and Huisman [29] devoted to PHA biosynthesis), the question of such assumed additional functions of PHB and its copolymers (including low-molecular PHB) has already been posed and the paradoxes associated with this question have already been mentioned, but no possible special role of this biopolymer was expressed in symbiotic relations of bacteria and eukaryotes. Meanwhile, the significant role of PHB for symbiotic relations of microbiota bacteria with the host organism and regulation of the animal immunity (which was demonstrated in many studies) can indicate exactly the use of this biopolymer by bacteria as a signal molecule, both for communication with each other and for "communication" with the host organism immune system cells. This probably explains the high biocompatibility of PHB as a biomedical material, mainly, its cellular biodegradation in the animal tissues and its bioactivity (in some cases). After all, this biopolymer is not only a natural product of human microbiota (i.e., the immune system knows it from the earliest period of human life), but it can be used as a bioactive macromolecule for the interaction of bacteria with the cells of the immune system, intestinal mucosa, and other tissues, causing a particular physiological response in them. The detection of PHB-synthesizing bacteria in the blood and different tissues in healthy samples and in different pathologies, their capacity for the transfection of mammalian cells by bacterial genes, and the presence of the type IV (and other types) secretory system in these bacteria can explain not only the presence of PHB in animal tissues not possessing prokaryotic PHA-synthase; it can also indicate the signaling or regulatory function of PHB for the microbiota bacteria. The specific, complex, supramolecular PHB structure also indicates this presumable function of the biopolymer. Thus, the field of science affected by us still has gaps and requires extensive and thorough study.

USE OF POLYOXYALKANOATES FOR REGENERATIVE INTESTINAL SURGERY

As shown above, PHB has different natural properties as a reserve or, likely, as a regulatory biopolymer in

prokaryotes and eukaryotes, and it plays a large role in human intestinal microbiota bacteria. Returning to the beginning of the review, PHB, other natural PHAs, and their synthetic analogs are polymers with a medical purpose that can be used for regenerative intestinal surgery. There are important peculiarities of the use of biodegradable medical products for GIT surgery when compared with the use of these biomaterials in surgery on other organs. Thus, when implanted in the intestinal wall, PHAs are affected not only by the cells of the tissues surrounding the intestine (e.g., macrophages or foreign body giant cells (FBGCs)), but also by the aggressive environment of the intestinal contents and, more importantly, the intestinal microbiota bacteria, at least because of the presence of specific PHA-depolymerases in many microbiota bacterium species. We will further consider examples of the use of PHAs as materials for the regenerative intestinal surgery.

Chronic inflammatory diseases of the large and small intestine, such as peptic ulcer of the duodenum, nonspecific ulcerative colitis, ischemic colitis, and Crohn's disease, as well as surgery on the intestine with local tissue removal and their complications, can cause perforated ulcers and fistulas. In the event of such dangerous conditions (especially in the presence of bacterial inflammation), a common practice of treatment is carried out; it suggests surgical removal of the aperture with subsequent antibacterial chemotherapy with broad-spectrum antibiotics [189]. If it is necessary to close the gastrointestinal tract defect, the historically established standard approach to treatment, which consists of the suturing of a wound with the installation of Graham's patch from the omentum, is frequently used in practice at present [190, 191]. However, a severe pathological state such as "short bowel syndrome" arises as a consequence of extensive surgical intervention on the small intestine due to the mentioned inflammatory diseases, innate pathologies, traumatic injuries, volvulus, and ischemic necrosis. A decrease in intestinal length in adults to 100 cm leads to a violation in the suction of nutrients and liquid and a deficiency of calcium, magnesium, zinc, ferrum, B_{12} vitamin, and fat-soluble vitamins; this is accompanied by diarrhea, dehydration, and progressive nonassimilation of nutrients. Intestinal transplantation is conducted for treatment of the short bowel syndrome, but with multiple technical difficulties, a high frequency of the transplant rejection, and long-term immunosuppressive chemotherapy [192].

PHB and its copolymers, as well as their composites with other polymers, were used to develop intestinal and esophageal patches and prostheses for regenerative surgery in these GIT departments. It was demonstrated that the products had a high biocompatibility and were exposed to biodegradation and substitution with the esophagus and intestinal wall tissue [193, 194]. The development of biodegradable prostheses based on PHAs for regenerative surgery of the intestinal wall, especially its mucous, is conducted since the 1990s. Tubular scaffolds with a porous wall were obtained at early stages of the development of this direction for the attachment and growth of the cells on them [195–197]. Tubular scaffolds with a wall of nonbraided fiber from different synthetic PHAs (PLA, PGA, PLGA, and PCL) were implanted intraperitoneally in the rats, and their biodegradation and biocompatibility were studied. It was demonstrated that scaffolds from PGA and PLGA had the highest biodegradation rate in vivo (until complete resorption for 3 months). The tissue reaction to implantation of the devices of all these polymers was characterized to varying degrees by pronounced acute and chronic inflammatory reaction and fibrosis [198]. To study the regenerative potential of tubular scaffolds with a porous wall, the devices were implanted in the rats subcutaneously, and the suspension of the mucosa tissues of the small intestine was introduced in the lumen of scaffolds in 5 weeks, and the study on the growth of tissues was conducted for 4 weeks. It was demonstrated that newly formed small intestinal mucosa consisting of enterocytes and goblet cells developed in the scaffold lumen on the internal surface of its wall, and villi-like structures were generated [199–201]. In similar work by Japanese researchers, tubular scaffolds braided from PGA filaments were used to obtain a tissue-engineering construction by their filling with a collagen gel and to cultivate human esophagus epithelium cells on them. The regenerative potential of the obtained constructions was studied during intramuscular implantation. The migration of fibroblasts and neovascularization in the prosthesis collagen gel was demonstrated, while implanted epithelial cells developed 15 cellular layers and a basal membrane [197]. Despite the unsuccessful use of reticular endoprostheses braided from PLGA filaments, mucosa recovery was observed in the remaining two rabbits (three of five rabbits died during the first week after the implantation due to peritonitis) [202]. However, the successful transplantation of tubular prosthesis on the basis of nonwoven mats out of PGA was demonstrated in another work, in which part of a dog esophagus was replaced. For this, prostheses were seeded with fibroblasts and keratinocytes and were placed in the abdominal cavity to develop the tissue-engineering construction in situ. The development of the mucosa and muscular membrane also functioned after esophageal substitution, while the prosthesis not seeded with cells was rejected [203].

The successful use of a tissue-engineering construction based on a surgical mesh from PGA with a PLA cover as a patch of the stomach wall was demonstrated on the model of the stomach wall defect on rats. The surgical mesh was seeded by embryonic epithelial cells, and the patch fusion with the stomach wall tissues and the development of the mucous and smooth muscle membranes on it were demonstrated [204]. The absence of acute and chronic inflamma-

tory reaction to the implantation in order to maintain the rat small intestine lumen was demonstrated for tubular scaffolds obtained by electrospinning from PLGA composite with gelatin. Good growth of the intestinal mucosa epithelium cells on fibrous polymer matrixes was also demonstrated [205]. Japanese researchers developed a tissue-engineering construction based on a porous, tubular prosthesis of PGA seeded with embryonic epithelial intestine cells. In rats, the anastomosis of a tubular prosthesis with rat jejunum was surgically generated to analyze the regenerative potential of the construction [206], and the development of mucosa with villous epithelium on a polymer scaffold and its integration with the intestine wall tissues were demonstrated. Porous scaffolds based on the PLGA of a complex microarchitecture (modeling the small intestine mucosa villi) were developed for tissue engineering of the intestinal mucosa; active growth of the intestine epithelial cells was demonstrated in both the commercial Caco-2 line and those isolated from human intestinal mucosa on the obtained scaffolds. The development of villus-like, polymer-cellular hybrid structures, the differentiation of cultivated cells in functional cells of the intestinal mucosa (enterocytes, goblet cells, and Paneth cells), and mucus production were demonstrated [207].

With improvement of the endoscopic suturing technique, new opportunities for the application of developments made in the field of tissue engineering arise for the regeneration of gastrointestinal tract tissues; they already demonstrated good results and proved to be safe and reliable. Thus, Takeshita et al. successfully used bioengineering material, a culture of fibroblasts on a matrix of PCL obtained by 3D printing, to close the intestinal wall defect [208]. Cerna et al. published a report about several successfully conducted operations in which partially biodegrading stents out of poly-*p*-dioxanone and polyurethane were used to close leakages of the colon anastomosis and to screen the wound from the intestinal content, as well as in patients with esophagal perforation [209]. A positive result was obtained in 87% patients with gastrointestinal tract defects with a combination of fibrin glue and vicryl patch based on PLGA during the operation [210]. Thus, the active introduction of PHA biomaterials and tissue engineering constructions on their basis occurs in the field of GIT regenerative surgery. PHB and its copolymers have some advantages as biomaterials for GIT regenerative surgery, not only due to their greater resistance to the aggressive environment of the intestine content but primarily as polymers obtained by means of microbiological biosynthesis, which allows the synthesis of polymers of a given chemical composition and to control their physicochemical properties [73, 211].

However, the possible effect of the intestinal microbiota bacteria on these polymeric devices (and, on the contrary, the effect of polymeric material of these devices on symbiotic bacteria) is not always taken into account when PHAs are used to regenerate intestinal tissues. Thus, those natural roles and functions that PHB performs in human intestinal microbiota bacteria should be taken into account in the development of medical devices based not only on PHB and other natural PHAs, but also their biomimetic analogs (synthetic PHAs, especially the products based on these polymers for the intestine surgery).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals performed by any of the authors.

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