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Features of the intestinal microbiota functional status in early-aged children with rotavirus infection

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Abstract

The aim is to assess the intestinal microflora functional and structural disorders in early-aged children in the dynamics of rotavirus infection by studying intestinal metabolites in faeces – short-chain fatty acids (SCFA).

Materials and methods. The study included 60 breastfed children aged 1-24 months with rotavirus infection (the study group) and 30 healthy children, representative by age and sex (the comparison group). Determination of SCFA (acetate, propionate, butyrate) in faeces was carried out in all children of the study group three times in the dynamics of the disease (on the 3rd, 5th and 10th day) and in healthy children once.

Results. The total concentration of SCFA in children with rotavirus infection was 3 and 2.2 times lower than in healthy children in the acute period of the disease ($p<0.01$ on the 3rd and 5th day, respectively), increasing on the 10th day ($p<0.05$), however, not reaching the normal level ($p<0.01$). The decrease in the total pool of SCFA occurred due to all volatile

acids (C2, C3, C4), the concentrations of which were lower than in healthy children ($p<0.01$). Violation of the volatile acids ratio in their general pool was observed from the first days of rotavirus gastroenteritis in the form of an increase in the C2 relative concentration ($p<0.05$) and a decrease in C3 and C4 profiles ($p<0.05$). Correspondingly, a decrease in anaerobic index was noted. It was equal to 0.04 [0.01; 0.11] on the 3rd and 5th day of the disease, constituting only 1/5 of the healthy children values ($p<0,01$), increasing on the 10th day to 0,09 [0,02; 0.17], however, remaining twice as lower than in children of the comparison group ($p<0,01$).

Conclusions. There is a violation of the intestinal microflora functional condition in early-aged children from the first days of rotavirus infection, which is expressed by depletion of the total pool of SCFA and concentrations of each of them, as well as structural disorders of intestinal microbiocinosis in the form of reducing its anaerobiosis. These changes are most pronounced during the first five days of rotavirus gastroenteritis and last up to 10th day of illness.

Key words: rotavirus infection; early-aged children; gut microbiota; short-chain fatty acids.

Acute gastroenteritis remains the major cause of morbidity and mortality among early-aged children around the world. It is a reason for about 1.34 million, or 15% of all cases of deaths of children under five years annually [1]. Viral infections prevail among the pathogens of acute gastroenteritis in recent years, evoking 70-80% of diarrhea diseases in children [2, 3]. Among the viral agents Rotavirus infection (RVI) occupies a leading place, remaining the most common cause of infectious diarrhea [1, 4].

Rotavirus (RV) infects a small intestine, an important area of microbiota colonization. The complex system of interaction between the RV and intestinal microflora has become the subject of studying many researchers at the present stage [1, 4, 5]. It is proved that there are stable violations of both taxonomic composition and the variety of intestinal microbiom in children after infection by rotavirus, which can burden the course of the disease and promote the development of functional disorders of the gastrointestinal tract during the reconvalescence period [1, 4, 6, 7]. Since short-chain fatty acids (SCFA) are the main metabolites of intestinal bacteria, the deviation of their level and spectrum from the normalcy may reflect the degree of the intestinal microflora disorders in children with the RVI. Each SCFA is synthesized during bacterial fermentation of the substrate by microorganisms of a

certain type, which allows assessing the functional activity of specific representatives of the intestinal microflora.

Short-chain (volatile) fatty acids are monocarboxylic acids with a chain length of up to 6 carbon atoms. Acetic (C2), propionic (C3) and butyric (C4) acids are the most common, accounting for 90-95% of those present in the colon. The main source of SCFA is carbohydrates, but the amino acids, such as valine, leucine and isoleucine, the breakdown products of proteins, can be converted to isobutyrate, isovalerate and isocapronic acid (known as branched chain SCFA). They make a small (5%) contribution to the total production of SCFA [1, 8, 9].

Acetate (C2) is the main SCFA in the colon, which makes more than half of all volatile acids, and about 80% of them in early-aged children [10]. There are 2 main ways of its formation with intestinal microbiota. The bulk of C2 is synthesized by the majority of intestinal bacteria (anaerobic and aerobic) from undigested oligosaccharides. Approximately one third of C2 is a product of acetogenic bacteria metabolism that synthesizes it from H2 and CO2 (or formic acid) by Wood-Ljungdahl pathway [8, 9].

Colon bacteria form propionate (C3) in three ways: succinate, acrylate and propanediol. The succinate pathway is dominant; it is basic for some Firmicutes and Bacteroidetes, which use succinate as a substrate. The synthesis of C3 from lactate by lactoyl-CoA dehydratase and subsequent enzymatic reactions (acrylate pathway) is limited by some members of the families Veillonellaceae and Lachnospiraceae. Propanodiol method of conversion of deoxysaccharides to propionate is present in proteobacteria and members of the family Lachnospiraceae [8, 9, 11].

The main route of butyrate formation (C4) in the intestine is the butyryl-CoA pathway, in which it is converted to butyrate in a one-step enzymatic reaction. It is characteristic of most C4 producers, namely Faecalibacterium, Eubacterium and Roseburia. The butyrate kinase pathway for the conversion of butyryl-CoA to butyrate is not common in the intestinal microbiota and is limited to some species of Coprococcus [9].

The final products of microbial metabolism of carbohydrates have important physiological effects on the organism. For example, intestinal epithelial cells use them as a source of energy. It has been proven that SCFA make up about 10% of the calories, that human body needs for normal functioning [12]. Butyrate is the most important source of energy among all volatile acids. In addition, it provides 70% of the energy supply of colonocytes [2]. It helps to improve the integrity of intestinal epitheliocytes by providing tight contacts, cell proliferation and increasing mucin production by goblet cells [12, 13]. C4 has

an anti-inflammatory effect, stimulating both intestinal epitheliocytes and antigen-presenting cells to produce cytokines TGF-β, IL-10, IL-18, and initiating the differentiation of naive T cells into T-regulatory cells [12]. C2 and C3 can also be used as a source of energy by colonocytes, however, less than C4. They provide anti-inflammatory action by inhibiting the production of pro-inflammatory cytokines (by stimulation of Toll-like receptors). C3 like C4, can initiate the differentiation of T cells into T-regulatory cells. SCFA that have not been metabolized by intestinal epitheliocytes are absorbed and transported through the portal vein to the liver, where they are included in gluconeogenesis, lipogenesis and cholesterogenesis [12, 13]. Only 2-4% of volatile acids are excreted in the feces [2, 10]. One of the important functions of SCFA is the preservation of fluid in the intestinal lumen by increasing the absorption of water and electrolytes, as SCFA stimulate the absorption of Na +, secretion of K + and bicarbonate into the intestinal lumen [2].

Thus, RVI in children causes gastrointestinal damage with a probable violation of the intestinal microbiome. An indicator of the functional activity of gastrointestinal microorganisms is the level of certain SCFA in the faeces. Therefore, the study of this indicator is relevant for the disclosure of the pathophysiological mechanisms of diarrheal syndrome in RVI.

The aim is to assess the intestinal microflora functional and structural disorders in early-aged children in the dynamics of rotavirus infection by studying intestinal metabolites in faeces – short-chain fatty acids (SCFA).

Materials and methods: 60 breastfed children aged 1-24 months with acute intestinal infection of rotavirus etiology (63.3% – boys, 36.7% – girls) were included in an open prospective study according to the following criteria: laboratory confirmed rotavirus infection, hospitalization no later than the 3rd day of the disease, absence of pathogenic bacterial microflora in faeces, absence of congenital or chronic gastrointestinal pathology, congenital or acquired immunodeficiency, availability of the informed parental consent for their child participation in the study. All children were hospitalized in the intestinal department of the Municipal Institution "Zaporizhzhia Regional Infectious Clinical Hospital" of the Zaporizhzhia Regional Council. Children aged 12-24 months made up the majority in the age structure of patients – 46.7% (28), children aged 6-12 months – 30% (18), children 1-6 months – 23.3% (14).

Rotavirus infection was confirmed by determination of rotavirus antigen in faeces with immunochromatographic method (using CITO TEST ROTA test systems; Pharmasco, Ukraine).

The comparison group consisted of 30 healthy children, representative by age and sex. Due to the direct dependence of concentrations and spectrum of SCFA on age, the comparison group was formed according to the study group in proportion to the number of children in each of the subgroups: 1-6 months, 6-12 months, 12-24 months to avoid distortion results and conclusions about the degree of violation of the metabolic activity of the microflora in relation to healthy children.

The main intestinal microflora metabolites – SCFA (C2, C3 and C4) were determined by liquid chromatography in all children in the dynamics of RVI: at the beginning of the disease (on the 3rd day), during the heat (on the 5th day) and during convalescence (on the 10th day), as well as healthy children in the comparison group once to achieve the purpose of the study.

Chromatographic analysis was conducted at the Training and Laboratory Center of Zaporizhzhia State Medical University at the Department of Physical and Colloidal Chemistry (headed by Doctor of Pharmaceutical Sciences, Professor Kaplaushenko A. G). On the first stage, the supernatant was prepared by mixing a sample of 1 g of faeces with 1 ml of a solution consisting of 0.1 N hydrochloric acid and isopropyl alcohol in a ratio of 1:3. Then the resulting homogenized mixture was centrifuged. Next, we analyzed the content of SCFA in the obtained supernatants using a highly efficient liquid chromatographic system with mass spectrometric detection (HPLC-MS), consisting of a degasser (Agilent Technologies, Japan), a binary pump (Agilent Technologies, Germany), an autosampler (Agilent Technologies, Germany), a column thermostat (Agilent Technologies, Germany), diode-array detector (Agilent Technologies, Germany), Open LAB CDS software.

The following indicators were determined in the samples:

- Absolute concentrations of acetic (C2), propionic (C3) and butyric acids (C4), μmol/l.

- Total concentration of SCFA: Cn = C2 + C3 + C4, μmol / l.

- Relative concentrations of acetic, propionic and butyric acids (R_{C2-C4},%): R_{C2} = C2 / (C2 + C3 + C4); R_{C3} = C3 / (C2 + C3 + C4); R_{C4} = C4 / (C2 + C3 + C4).

- Anaerobic index: AI = (C3 + C4) / C2.

Statistical analysis of the obtained data was performed in the program "STATISTICA for Windows 13" (StatSoftInc., №JPZ804I382130ARCN10-J). The Shapiro-Wilk test was used to determine the nature of the data distribution (the null hypothesis about the normality of the distribution was rejected at $p < 0.05$). Non-parametric methods were used due to the deviation of quantitative values from the normal distribution. Quantitative values were

presented as median (Me) and interquartile range (IQR: Q25-Q75). The Mann-Whitney test was used to assess the significance of differences between quantitative traits in two independent groups, the Kraskell-Wallace test was used in several independent groups, and the Wilcoxon test was used in two dependent groups. The difference was considered statistically significant at $p<0.05$. Spearman's correlation coefficient (r) was determined to assess the strength and direction of the relationship between quantitative values.

Results

Evaluation of the metabolic activity of the intestinal microflora by liquid chromatography showed a significant decrease in the total amount of SCFA in the feces in the early stages of RVI in children relative to normal values. Thus, the total level of volatile acids on the third day of the disease was only 1/3 of their level in healthy children – 324.48 [251.63; 590.39] μmol / l, against 978.60 [681.83; 1286.05] μmol / l, respectively ($p<0.01$). Therefore, in the first days of RVI there was a decrease in the integrated enzymatic activity of both luminal and parietal intestinal microflora, which was expressed in the depletion of the total pool of SCFA (Fig. 1).

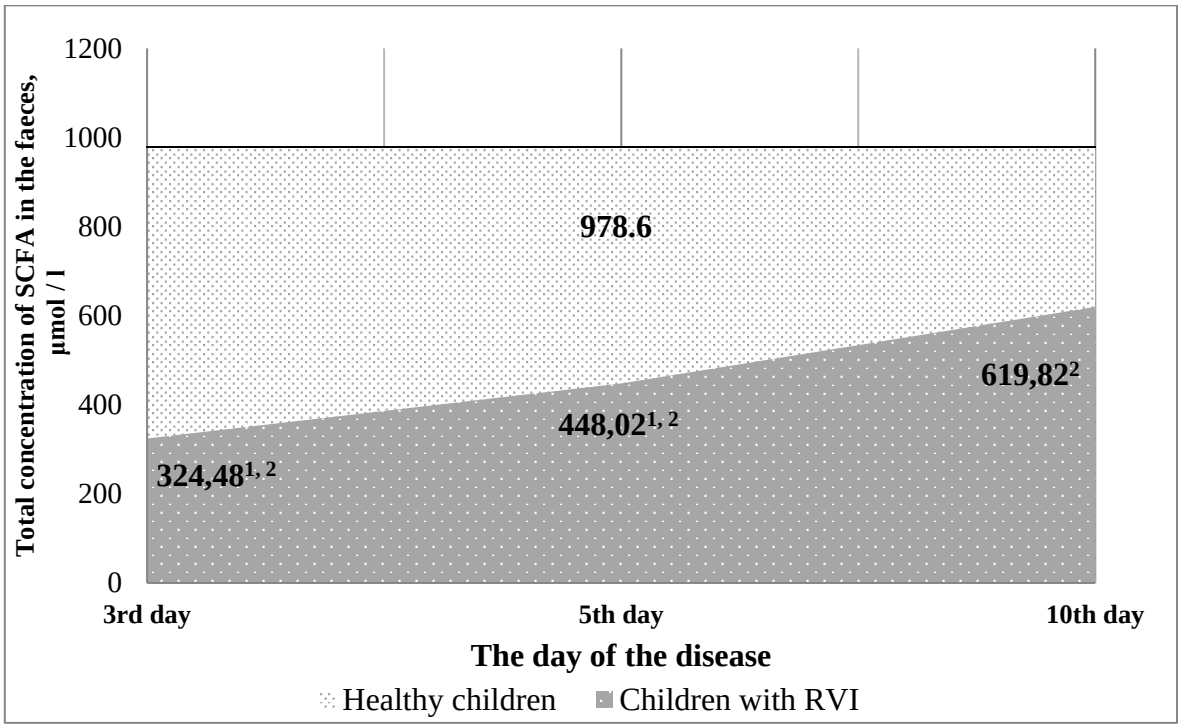

Figure 1. Dynamic changes in the total concentration of SCFA in children with RVI, n = 60

Note: [1] - $p<0.01$ the difference is significant compared to the 10th day by Wilcoxon criterion; [2] - $p<0.01$ the difference is significant compared with the healthy children by the Mann-Whitney criterion

The decrease in the total number of intestinal metabolites was due to all studied SCFA, because the absolute concentrations of each of them were significantly lower than in healthy children (p<0,01) (Table 1).

Table 1

Concentrations of each SCFA in children in the dynamics of rotavirus infection (n=60), Me [Q25; Q75]

Concentration of each SCFA, μmol / l	The day of RVI			Healthy children (n = 30)
	III	V	X	
Acetic	315,34 [233,59; 574,37][1, 2]	396,02 [209,18; 611,68] [1, 2]	569,36 [308,14; 749,00] [2]	861,17 [606,26; 993,61]
Propionic	13,16 [2,55; 51,18][1,2]	9,46 [3,39; 53,98][1, 2]	33,02 [12,25; 79,96] [2]	97,49 [74,73; 183,85]
Butyric	0,00 [0,00; 0,58][2]	0,00 [0,00; 1,87][2]	0,25 [0,00; 2,58] [2]	32,99 [14,43; 53,21]
Anaerobic index	0,04 [0,01; 0,11][1, 2]	0,04 [0,01; 0,10] [1, 2]	0,09 [0,02; 0,17][2]	0,20 [0,11; 0,34]

Note: [1] - p<0.05 the difference is significant compared to the 10th day by Wilcoxon criterion; [2] - p<0.01 the difference is significant compared with the healthy children by the Mann-Whitney criterion

Thus, the level of C2, which is the main product of most intestinal bacteria (both obligate anaerobes and aerobes) was 315.34 [233.59; 574.37] μmol / l, approaching the lower limit of normal values in only 25% of patients (p<0.01 relative to healthy children).

The concentration of C3, that is produced by obligate anaerobes such as Veilonella, Propionibacterium, Bacteroides, Fusobacterium, Clostridium, Gaffkya, etc., was sharply reduced at the beginning of the disease and was less than 1/7 of healthy children C3 level (p<0.01). Since according to the literature the dominant route of propionate production is the succinate pathway, used by Bacteroidetes, Propionibacterium shermanii and Veillonella spp., a significant decrease in this metabolite in the first days of RVI indicates inhibition of these types of the intestinal microbiome. These findings are confirmed by the results of studies [1, 4, 7], from which it is known that there is a decrease in the number of Bacteroides – one of the main microorganisms of the intestinal microbiocinosis in rotavirus diarrhea.

It should be noted that among all metabolites of intestinal saccharolytic microflora the concentration of butyric acid in faeces decreased most significantly. The levels of C4 were minimal, equal to 0.00 [0.00; 0.58] μmol / l, and in 71.8% of children of the study group was not determined at all during the first days of RVI, while in healthy children its level was 32.99

[14.43; 53.21] μmol / l ($p<0.01$). A significant decrease in butyrate in patients of the study group reflects the significant depression and deficiency of its producers from the first days of RVI. This statement is supported by data from a number of studies in which deficiency of Roseburia and Ruminococcus (Clostridium XIVa cluster), Faecalibacterium prausnitzii – the main producers of C4 in the intestines, was discovered in patients with rotavirus diarrhea [14].

Analysis of volatile fatty acids profile showed significant changes in the relative concentrations of each of them compared with normal values in the early stages of RVI. Thus, the ratio of C2: C3: C4 in healthy children of the appropriate age was 83.4: 13.3: 3.3, respectively (Fig. 2), while patients with RVI had a statistically significant increase in the part of C2 – 96.6 % ($p<0.01$) and a significant decrease in the relative concentrations of C3 and C4 of SCFA total pool ($p<0.01$). The percentage of C3 in the total SCFA pool was 4.2 times lower than in healthy children and was only 3.2 %. Levels of C4 in children's coprofiltrates were so insignificant in comparison with other volatile acids that the share of this metabolite in the total amount of SCFA was equal to 0 %. According to these changes in the SCFA profile, there was a statistically significant decrease in anaerobic index (AI), which is defined as the ratio of the sum of all SCFA concentrations, except the least reduced C2, to the concentration of C2 and reflects the degree of environment anaerobiosis, since C2 is produced by obligate anaerobes, most facultative anaerobes and some aerobes, while only severe anaerobes produce C3 and C4. Thus, children with RVI had 5 times lower values of the structural index than healthy children in the comparison group – 0.04 [0.01; 0.11], vs. 0.20 [0.11; 0.34], respectively ($p<0.01$) at the beginning of the disease.

A less pronounced decrease in absolute concentrations of C2 compared with C3 and C4, as well as an increase in its share in the total pool of SCFA with a significant decrease in propionate and butyrate profiles is associated with inhibition of obligate anaerobes, which form the epithelial layer of the intestinal microbiota, and the relative increase of representatives of the aerobic (optional-anaerobic) microflora, including conditionally pathogenic microorganisms, such as E. Coli, Staphylococcus, Proteus, etc., in the intestinal microbiocynosis structure starting from the first days of RVI. These conclusions are confirmed by the results of a study [7], where using the cultural method it was revealed excessive growth of Proteus spp, Bacteroides fragilis – opportunistic bacteria with a decrease in symbiotic Bacteroides thetaiataomicron, Lactobacillus spp. and Faecalibacterium prausnitzii.

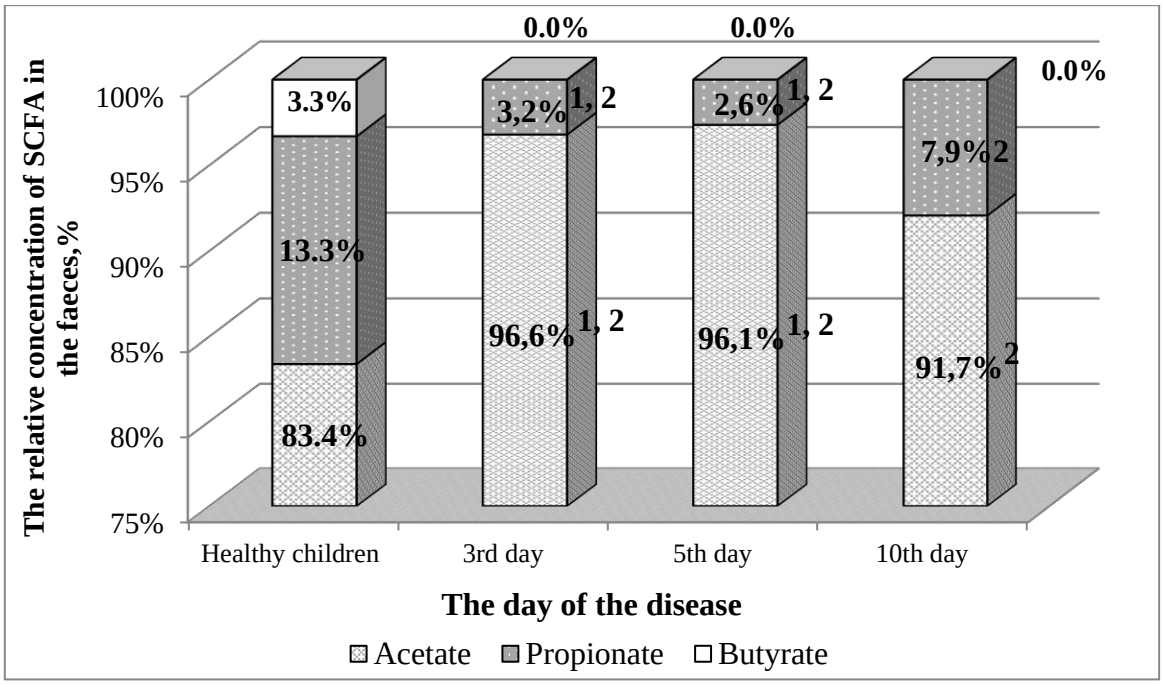

Figure 2. Dynamic changes in relative concentrations of SCFA in children with RVI (n = 60), compared with healthy children (n = 30)

Note: [1] - $p<0.05$ the difference is significant compared to the 10th day by Wilcoxon criterion; [2] - $p<0.01$ the difference is significant compared with the healthy children by the Mann-Whitney criterion

Determination of intestinal metabolites in the dynamics – in the in the height of the disease (on the 5th day) and in the convalescent period of RVI (on the 10th day) showed a very slow recovery of integrated enzymatic activity of intestinal bacteria and normalization of its microbial infrastructure (Table 1). Thus, in the midst of the disease, we noted an increase in the total pool of volatile acids only 1.4 times relative to the first days of the disease ($p>0.05$) (Fig. 1). A significant increase in the total amount of SCFA was observed only on the 10th day, when it increased to 619.82 [344.60; 769.93] μmol / l, exceeding 1.9 and 1.4 times the values that were noted on the 3rd and 5th days of the disease, respectively ($p<0.01$ by Wilcoxon criterion), but remaining statistically lower than normal ($p<0.05$). Only in 25% of patients the total concentration of SCFA reached the lower limit of normal values in the convalescent period of RVI, which proves the presence of a long-term deficiency of metabolic activity of the colon microflora in RVI.

Analysis of each SCFA concentration in the dynamics of rotavirus gastroenteritis showed that the level of the main metabolite of saccharolytic intestinal bacteria – acetate, increased gradually and slowly, and only on the 10th day of the disease was significantly higher than in the acute period ($p<0.01$ relative to the 3rd and 5th days by Wilcoxon

criterion), remaining below the lower age limit in 75% of children in the study group (p<0.01 relative to healthy children) (Fig. 3).

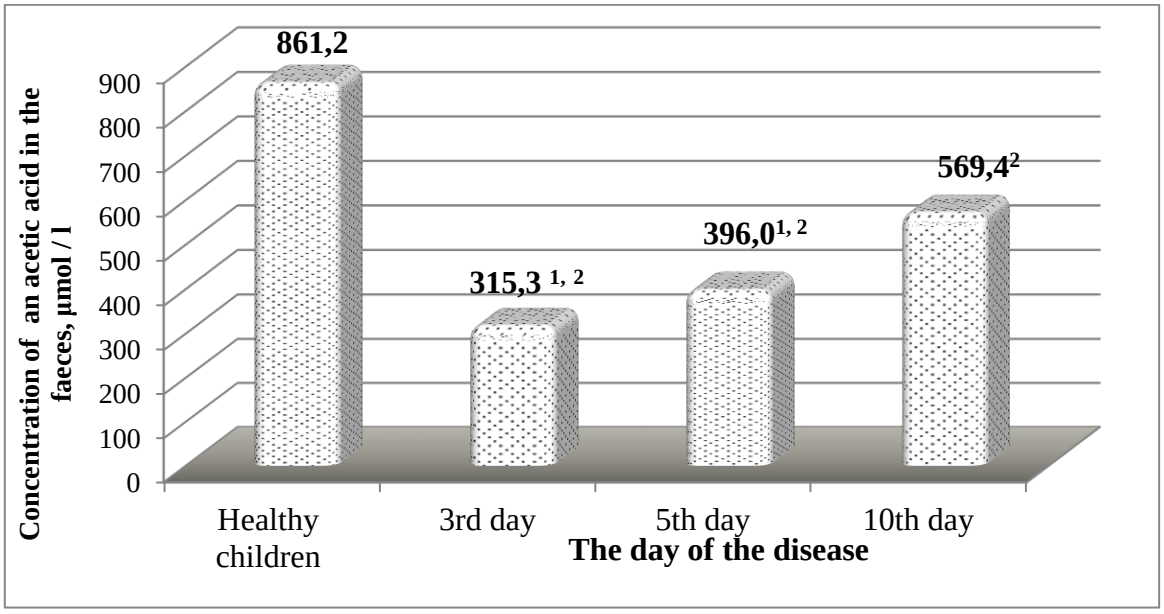

Figure 3. Dynamic changes in an acetic acid concentration in children with RVI (n = 60), compared with healthy children (n = 30)

Note: [1] - p<0.01 the difference is significant compared to the 10th day by Wilcoxon criterion; [2] - p<0.01 the difference is significant compared with the healthy children by the Mann-Whitney criterion

The result of determining of C3 fecal concentration in the dynamics showed the preservation of a sharp deficit of this metabolite in the midst of the disease (Fig. 4), when its amount reached a minimum value for the entire time of RVI – 9,46 [3,39; 53.98] µmol / l, which was only 1/10 of normal value (p<0,01), indicating a sharp suppression of C3 producers during the entire acute period of RVI (up to 5th day of illness). Only on the 10th day we noted a significant increase in the propionate amount to 33.02 [12,25; 79.96] µmol / l (p<0.05 relative to the 3rd and 5th days by Wilcoxon criterion), but its level remained almost three times lower than in healthy children of the appropriate age (p<0.01).

The most insignificant dynamic changes among all intestinal metabolites were observed in the concentration of butyrate during the whole period of RVI (Table 1), which in the first days of the disease and on the 5th and 10th days remained at a minimum level – 0, 00 [0.00; 0.58] µmol / l, 0.00 [0.00; 1.87] µmol / l and 0.25 [0.00; 2.58] µmol / l, respectively, with only a tendency to a slight increase in the dynamics (p>0.05 by Wilcoxon criterion). At all stages of the disease, C4 levels were sharply reduced relative to healthy children (p<0.01).

The obtained data indicate the most pronounced and long-term deficiency of functional activity of obligate anaerobes, namely Fusobacterium, Eubacterium, Coprococcus, Bacteroides, Megasphaera, Clostridium, Peptococcus in children of the first two years of life with RVI.

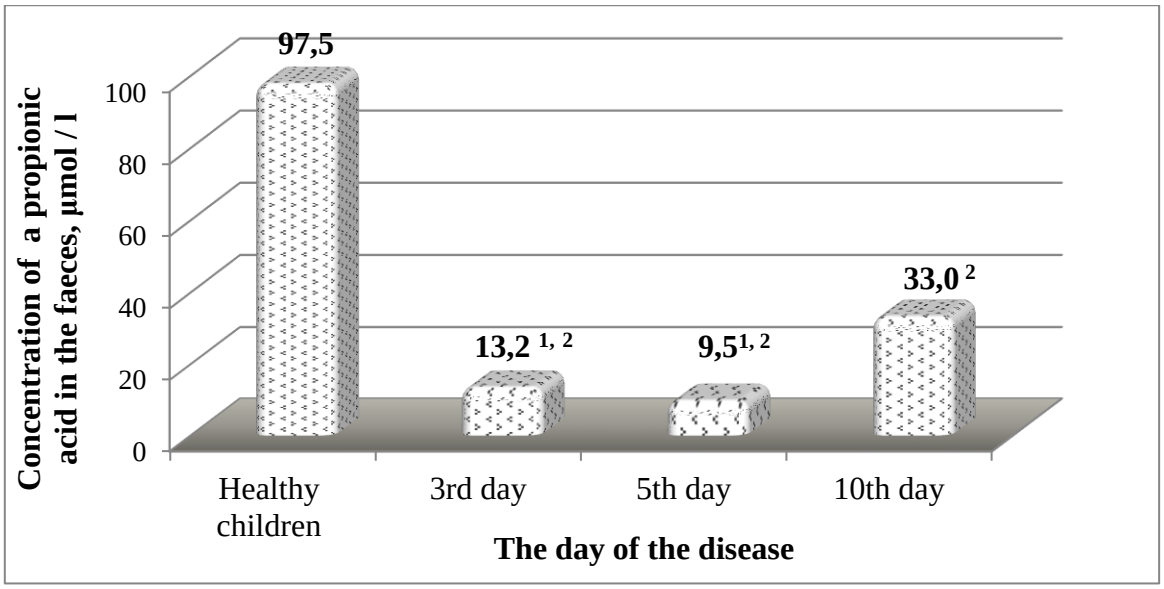

Figure 4. Dynamic changes in the concentration of a propionic acid in children with RVI (n = 60), compared with healthy children (n = 30)

Note: [1] - $p<0.01$ the difference is significant compared to the 10th day by Wilcoxon criterion; [2] - $p<0.01$ the difference is significant compared with the healthy children by the Mann-Whitney criterion

The results of the assessment of SCFA profiles and AI in the dynamics of RVI showed that the violation of the colon microbiota infrastructure, which was expressed in a statistically significant decrease in relative concentrations of propionate and butyrate (metabolites of anaerobic bacterial populations) and an increase in the proportion of acetate in the total pool of SCFA (and, accordingly, a decrease in AI relative to healthy children), was observed not only in the first days of illness, but continued throughout the acute period of RVI (up to the 5th day inclusive) (Fig. 2). Thus, we did not note a statistically significant difference in the relative concentrations of C2, C3, C4 in the dynamics on the 5th day of the disease ($p>0.05$ by Wilcoxon criterion), and AI remained almost at the input level, amounting to 0.04 [0.01; 0.10] ($p>0.05$). Only on the 10th day was a significant increase in the share of C3 in the total pool of SCFA to 7.88 [1.81; 13.83] % ($p<0.05$ relative to the 3rd and 5th days) with a decrease in the relative concentration of C2 to 91.68 [85.67; 98.19] % ($p<0.05$ relative to the 3rd and 5th days). However, complete recovery of the intestinal microbiome infrastructure did not occur

at this time of the disease, as the ratio of SCFA in their total pool remained different from normal ($p<0.01$ comparing the relative concentrations of C2, C3 and C4 in children with RVI and healthy children), and AI values were more than twice lower than normal ($p<0.01$). It indicates the long-term inhibition of propionic and butyric fermentation, which are characteristic for obligate anaerobic colon bacteria of the genera bacteroids, propionibacteria, eubacteria, fusobacteria, clostridia against the background of increased activity of aerobes populations – representatives of the facultative and residual microflora, that produce more acetate.

Thus, changes in the functional state of the intestinal microbiome in children were detected during the entire period of RVI, which was expressed in the depletion of the total number of saccharolytic microflora metabolites and were most pronounced in the acute period of the disease and persisted for a long time (up to the 10th day of the disease inclusive). Such disorders can be explained by an imbalance between saccharolytic and proteolytic intestinal bacteria with increased activity of the second one. Thus, the study [4] showed that the structure of the intestinal microbiota was changed in favor of proteolytic bacteria in children with RVI. This is also evidenced by data [14] on intestinal hypercolonization by representatives of the type Proteobacteria (Hyphomicrobium, Klebsiella and Rhodobacter) and [7] overgrowth of Proteus spp. (type Proteobacteria), incapable of lactose fermentation, in RVI in children.

It should be borne in mind, that since acetate, propinate and butyrate provide energy to intestinal epithelial cells, stimulate their proliferation, mucin synthesis by goblet cells, help maintain close contacts of epitheliocytes, inhibit the synthesis of proinflammatory and stimulate anti-inflammatory cytokines in the intestine, reducing the integral activity of saccharolytic intestinal bacteria, and, accordingly, reducing the number of their major metabolites (C2, C3 and C4) may contribute to the inhibition of reparative processes of the intestinal mucosa, violation of its barrier function, maintenance of inflammation due to disturbance of proinflammatory and anti-inflammatory cytokines homeostasis in children with RVI.

It is known that one of the pathogenetic components of RVI is a violation of carbohydrate metabolism in the small intestine in the form of insufficient fermentation of disaccharides and impaired absorption of monosaccharides by enterocytes with their subsequent accumulation in the intestinal lumen. Oligosaccharides accumulated in the intestinal lumen, in turn, can cause the formation of osmotic diarrhea [15, 16]. At the same time, according to some researchers, the occurrence of osmotic diarrhea is possible only in the

case of impaired fermentation of sugars that accumulate in the intestinal lumen by saccharolytic microbiota [3], which has high metabolic activity against them – during fermentation 50-60 g of carbohydrates per day – 600 mmol of SCFA in the intestine [8]. 80.6% of fecal bacteria in healthy people produce the enzyme β-galactosidase, which indicates its high activity in the colon [17]. Thus, insufficient fermentation of oligosaccharides by the intestinal microbiota, which is manifested by the depletion of the total pool of metabolites (SCFA), leads to the accumulation of undigested sugars in the intestinal lumen.The long-term (up the 10th day inclusive) decrease in total saccharolytic activity of bacteria in children with rotavirus gastroenteritis, revealed during this research, may explain the presence of laboratory signs of long-term carbohydrate malabsorption syndrome – throughout the acute period of the disease and the period of convalescence (up to the middle of the second week), which was described in our previous study [15].

Conclusions:

1. There is a decrease in the enzymatic activity of saccharolytic bacteria in early-aged children with rotavirus infection from the first days of the disease, expressed in the depletion of the total pool of SCFA (acetate, propionate, butyrate), which is more pronounced in the acute period – from the 3rd to 5th day, inclusive, and is manifested by three and twice lower values of the total concentration of volatile acids relative to healthy children ($p<0.01$) with some increase in the convalescent period ($p<0.01$ relative to the 3rd and 5th days), however, without complete normalization ($p<0.01$ relative to healthy children on the 10th day of the disease).

2. Against the background of a significant decrease in the concentrations of each SCFA (acetate, popionate, butyrate) in the dynamics of the disease ($p<0.01$ relative to healthy children on the 3rd, 5th and 10th days), propionate and butyrate levels are most significantly reduced, which even in the convalescent period make up only 1/3 and 1/128 of the C3 and C4 levels of healthy children, respectively, reflecting a deep deficit of their producers – severe anaerobes such as bacteroids, clostridia, fusobacteria, propionibacteria, fecalibacteria.

3. Violation of the intestinal microflora infrastructure in the form of functional deficiency of obligate anaerobes that form the parietal (mucous) layer of microorganisms, against the background of the predominance of facultative anaerobic and aerobic bacteria - representatives of transient and residual microflora, are observed during the entire period of rotavirus gastroenteritis, and to a greater extent in the acute period – from 3rd to 5th days. It is expressed in a decrease in AI 5 times compared with healthy children in the acute period of

the disease ($p<0.01$) and the lack of its normalization on the 10th day of RVI ($p<0.01$ relative to the comparison group).

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Conflicts of interest: authors have no conflict of interest to declare.

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