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Pathomorphologic Changes in Acute Intestinal Infections in Infants with Premorbid Diseases

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Abstract: The significance of determining specific pathomorphological changes and morphometric indices of lymphoid structures of the intestinal wall in children who died from acute intestinal infections in assessing the level and activity of the local intestinal immune system against acute infection depending on the age of children and premorbid diseases was studied.

Keywords: acute intestinal infection, premorbid disease, lymphoid tissue, immune system, morphological changes.

Relevance. From the moment a child is born, the digestive system plays a key role in re-establishing communication with the external environment. In this process, various foreign, toxic, poorly digestible substances ingested with food affect the body through the mucous membrane of the gastrointestinal tract. Therefore, in the mucosa and submucosa of the digestive system there are widely branched lymphatic vessels, lymphoid bundles and grouped lymphoid structures. They enter the peripheral organs of the immune system and perform the function of primary immune defence and border barrier (M.R.Sapin, 1989; K.A.Zufarov, 1976-1997; G.R.Tokhtaev, 1983-1996; A.Y.Yuldashev, 1980-2003). . Thus, the lymphoid structures of the digestive system carry out a dual immune defence based on their morphofunctional properties. Firstly, it is considered as a biological filter for antigenic substances coming with food, and secondly, tonsils, lymphoid nodes of the intestinal wall and lymphoid structures located in this place group, even within the epithelial layer, carry out local immune reaction against foreign antigens (Sapin M.R., Borzak E.I., 1982; Aruin L.I., Shatalov O.L., 1982; Petrov R.V., 1983; Yuldashev A.Y., 1996).

When studying the structure and functioning of the lymphoid structures of the organs of the digestive system, it is necessary to consider the microflora of the small and large intestine. In addition to the normal microflora, which is constantly present in the intestinal cavity, there are also pathogenic bacteria. At the same time, normal microflora opposes pathogenic bacteria, absorbs vitamins in the intestinal wall and, in turn, permanently activates lymphoid tissue (Zmushko B.I., 2001).

The aim of the studies:

Determination of pathomorphological changes in premorbid disease of intestinal mucosa and lymphoid tissue in children under one year of age.

MATERIALS AND METHODS

The material of the work was autopsy data of children who died from various types of acute intestinal infections in the period of five years, i.e. 2018-2022, at the age of one year in the Bukhara Regional Pathological and Anatomical Dispensary. Bureau. The anamnesis of the deceased children was studied and clinical and laboratory data was collected. Specifically, the child's birth weight, body weight at the time of death, maternal diseases during pregnancy, and premorbid diseases present in the child were recorded. Premorbid conditions included: 1) premature birth; 2) hypotrophy; 3) rickets; 4) anaemia; 5) calculated congenital malformations, and mixed or artificial feeding of the child under one year of age. Initially, the children selected for this study who died of AII before one year of age were distributed as follows (Table 1).

Dissemination of paediatric autopsy material **Table 1.**

Group No.	Age (months)	A number	Percentage
1	0-30 days	8	12,5
2	1-3 months	26	40,6
3	4-6 months	17	26,5
4	7-12 months	13	20,4
Total:		64	100

In the study of infant weight and physical development coefficient data, we used the normative indicators presented in the methodological manual "Infancy and Breastfeeding" approved by the European Office of the World Health Organisation (WHO) (2000). According to this manual, the average weight of newborns is 3,200 g for boys and 3,000 g for girls. If the weight of children under one year of age grows perfectly, then 600 g are added each month of the first half of the year and 500 g each month of the second half of the year to obtain the normal weight of children indicated in the table.

In order to make it easier to understand the degree of constant weight gain of children in mathematical formulae and numbers, a physical development coefficient has been developed, which is defined as follows, i.e. Q = weight found/ideal weight. Example: 3200 г. The body weight of a 6-month-old baby was 4400 g, in fact it should be as follows. 3200+3600 (6x600)=6800 g. The coefficient is Q=4400/6800=0,65. If you multiply this figure by 100, you will get what percentage of the ideal indicator is the weight of the child, that is, 65%. That is, the child lacks 35 per cent of weight.

It is known that normal growth of a child's body weight continues with normal development of all internal organs, including the organs of the gastrointestinal tract. If a child has a 35 per cent body weight deficit, as in the above example, the gastrointestinal system may lag behind in development and even die from various acute intestinal infections from infancy. In our material such cases were observed in 84.6 per cent of cases.

In all our cases, autopsy was performed within 0.5-1 hour after the death of the children. First, 5.0-10.0 ml of blood from the heart and large vessels was taken from the child, plasma was collected from it on a centrifuge and immunological studies were performed. For bacteriological examination, a piece of distal small intestine and a piece of colon were separated

and ligated on both sides.

The ileum and a piece of colon of equal length were removed, the inner part was opened and rolled up with thin paper, tied with a rope around the loin and frozen in 10% neutral formalin. solution for 3 days. Each twisted intestine was divided into two parts, dehydrated in alcohol, chloroform and poured into paraffin. Histological sections 5-8 µm thick were prepared from it and stained with hematoxylin-eosin, SHIK reaction and Van-Gieson methods. Histological and histochemical sections were examined under a light microscope and data-rich areas were visualised. In order to quantify the intestinal lymphoid tissue, 10 slices were prepared from paraffin blocks of ileum and colon, the number of lymphoid follicles was counted, and the average number of them located towards one line of the intestinal wall was determined. The relative percentage of reticular cells, blast cells, large, medium and small lymphocytes, as well as degenerated cells and macrophages in lymphoid follicles were counted. Quantitative parameters were statistically processed and the arithmetic mean, standard error and reliability index were determined.

RESULTS AND DISCUSSION

Analysis of clinical and anamnestic data of autopsies of those who died from acute intestinal infection showed that out of 64 cases: 8 (12.5%) were aged 0-30 days, 26 (40.6%) were aged 1-3 months. 17 (26 Established 0.5%) died after 4-6 months, 13 (20.4%) died after 7-12 months (Table 1). In most cases it was observed that this infection developed in children on the basis of diseases weakening the defence system of the organism, i.e. premorbid diseases. Premature births were found to be 44.8 per cent, hypotrophy 34.5 per cent, rickets 48.3 per cent, anaemia 33.6 per cent and congenital malformations 12.6 per cent. At the same time, 83.6 per cent of children were on mixed or artificial feeding, especially during the 1-3 month period. It was found that the average body weight indices in all age periods of children who died of OIA were significantly lower than the norm (Table 4). This situation can be confirmed by calculating the coefficient of physical development of children, i.e. this indicator was 25% for babies aged 0-30 days, 17% for 1-3 months, 12% for 4-6 months, and 19% for 7-12 months.

It was found that acute intestinal infection was predominantly caused by opportunistic microorganisms due to the presence of the above premorbid diseases in all groups of children: prematurity, hypotrophy, rickets, anaemia and congenital malformations. Cases of dysentery, salmonellosis and staphylococcal enterocolitis were found only in mixed or artificially fed children. Although the causative agents of acute intestinal infections are different, it has been noted that they often have similar pathogenesis and morphogenesis clinically and morphologically and appear in the development of intestinal syndrome, exicosis and toxicosis.

In the etiology of acute intestinal infections, opportunistic microorganisms predominate, that is, in enterocolitis, the most common pathogens are intestinal infection (28.4 %), Proteus (23.7 %), Klebsiella (12.6 %), Enterobacter (4.3 %), Citrobacter (3.6 %). These were found to be caused by (Table 5). In addition, participation of pathogenic microorganisms was found in a number of cases of AKI: shigella - in 3.5% of cases, salmonella - in 12.4%, staphylococci - in 5.6%. In 7.9% of cases, the etiology of AII was not determined.

In all groups of children who died from AII, immunological parameters were found to be low: immunoglobulin A - 0.27±0.06 g/l, G - 5.4±0.8 g/l on average. In children aged 1-3 months, due to a large number of premorbid diseases, these indices were even lower, IgA - 0.11±0.02,

IgG - 1.86±0.4 g/l (Table 6). In these cases, blood levels of cortisol (5487.62±34.5 nmol/l) and immunoglobulin E (143.65±12.34 ki/l) were significantly elevated. It was noted that as the children grew older, immunological indices slightly increased, which is certainly associated with the improvement of the body's immune system. In enterocolitis caused by pathogenic microorganisms, a sharp increase in the amount of immunoglobulins M and G in the blood was found, in some cases, i.e. goitre and salmonellosis, IgM - 5.6, IgG - 15.8 g/l. In the period of 7-12 years, when artificial feeding of children prevailed, in their blood there was observed a violation of the synthesis of immunoglobulins against Gram-negative microorganisms. It was noticed that the amount of IgG decreased to 2.81 g/l, and IgM increased to 1.94 g/l.

The analysis of cellular immunity indicators revealed that the number of T-lymphocytes varied depending on the clinical and morphological features of AII. In enterocolitis caused by opportunistic microorganisms, it was found that the number of all types of T-lymphocytes decreased in the blood. In acute haemorrhagic enterocolitis, the number of T-helpers decreased to 15%, and the number of T-suppressors increased to 17%. Such a condition indicates a severe paralysis of cellular immunity and the development of acute immune failure.

Table 2

Body weight indicators of children who died from AII, by months(M±n)

Months	At birth weight, g	Weight at death, g	Ideal body weight, g	Physical development coefficient
0-30 days, n=8	2360±26	2210±24	2960	0,75 (25%)
1-3 months, n=26	2840±38	3856±42	4640	0,83 (17%)
4-6 months, n=17	3120±41	5924±54	6710	0,88 (12%)
7-12 months, n=13	3186±52	7842±58	9640	0,81 (19%)
Average, n=64	2876±42	4958±48	5987	0,83 (17%)

Table 3

Frequency of occurrence of AII triggers, in percentange

Conditionally pathogenic bacteria		*Pathogenic bacteria*	
Name of the bacteria	Percentage	Percentage	Name of the bacteria
E. coli	28,4%	12,4%	Salmonella
Proteus	23,7%	5,6%	St. aureus
Klebsiella	12,6%	3,5%	Shigella
Enterobacter	4,3%	7,9%	Unknown
Citrobacter	3,6%	-	-
Total:	72,6%	27,4%	
	100%		

Table 4

Immunological indices of blood of children who died of AII

Months	Cortisol Nmoll	Immunoglobulins, g/L				Lymphocytes, %			
		A	M	G	E, КИ/л E, KI/l	T	Tx	Tc	B
0-30 days	1236,6±12,7	0,16±0,02	0,43±0,2	3,2±0,3	23,5	12,6	8,7	4,2	4,3
1-3	2143,5±23,7	0,26±0,07	0,67±0,3	4,86±0,5	43,7	22,3	16,3	5,8	5,2
4-6	3245,6±15,2	0,28±0,09	1,34±0,4	2,81±0,3	55,8	28,7	18,2	10,5	6,3
7-12	5487,6±16,4	0,32±0,08	1,21±0,3	6,2±0,6	123,4	31,4	22,1	9,3	7,8
Average	3028,2±18,3	0,26±0,07	0,91±0,4	4,27±0,6	61,6	23,7	16,3	7,5	5,9
p	0,001	0,01	0,01	0,01					

The results of microscopic examination showed that although the mucous membranes of the ileum in children who died of pulmonary pneumopathy unrelated to intestinal diseases and various traumas had the same histotopographic structure, their thickness and length were different. It was noted that lymphoid follicles were located everywhere in the private layer of the mucosa with connective tissue, the surface of which was covered with single-layer cylindrical epithelium.

Microscopic examination of the ileum of children of the first 3 months shows that the intestinal mucosa in most children is not fully developed. Papillae in it are rarely located, they are short, and the covering epithelium consists mainly of hyperchromic enterocytes, among which there are very few bocaloid cells (Fig. 1). The private connective tissue layer was found to be poorly organised and composed of a young lymphohistiocytic-cell infiltrate and thin-walled blood vessels. In these sections, the lymphoid tissue of the ileum wall was found to be incompletely formed. Lymphocytes and plasma cells are scattered in the private layer of the mucosa. In some areas of the ileum mucosa it is found that lymphoid cells accumulate in one place and form primary lymphoid follicles (Fig. 2). Such lymphoid clusters consist of sparsely arranged small and medium-sized lymphocytes, with reticular cells sparsely and irregularly arranged in the stroma. It was found that postcapillary venules with dilated cavity occupy peripheral parts of lymphoid cells, and lymphocytes migrate through their walls. No papillae appear on the surface of the areas of the mucosa where such lymphoid follicles appear, but it is determined that the covering epithelium is uniformly covered with tubercles.

Microscopic examination of the ileum of children 4-6 months old revealed the following changes: papillae elongated on the surface of the mucosa and various meshes appeared. It was found that the lining epithelium of the nipples consists mainly of hyperchromic enterocytes and a small number of bocaloid cells located between them. The crypts of the mucosa were found to consist of numerous hyperchromic enterocytes of different sizes. Even during this period of postnatal ontogenesis, it was found that the private layer of the ileum mucosa was not fully developed as connective tissue, but consisted only of an infiltrate of lymphohistiocytic cells. It revealed that lymphoid cells were predominantly scattered, only in some areas they formed small primary lymphoid follicles (Figure 3). The lymphoid follicles are located in the submucosal layer, their base is widely spread, their borders are indistinct, and the germinal centre is not defined. In some children of this age, the ileum mucosa was underdeveloped.

It was found that the mucous membranes were short, underdeveloped, their crypts were shallow, of different sizes, there were few bocaloid cells, and those that were present were vacuolised and dystrophic. It was noted that the special layer of the mucous membrane was hypoplastic, lymphoid cells were not determined, blood vessels were few, connective tissue fibres were sparsely and irregularly arranged. The submucosal layer is not formed, crypts are located directly in the layer of smooth muscle cells (Fig. 4).

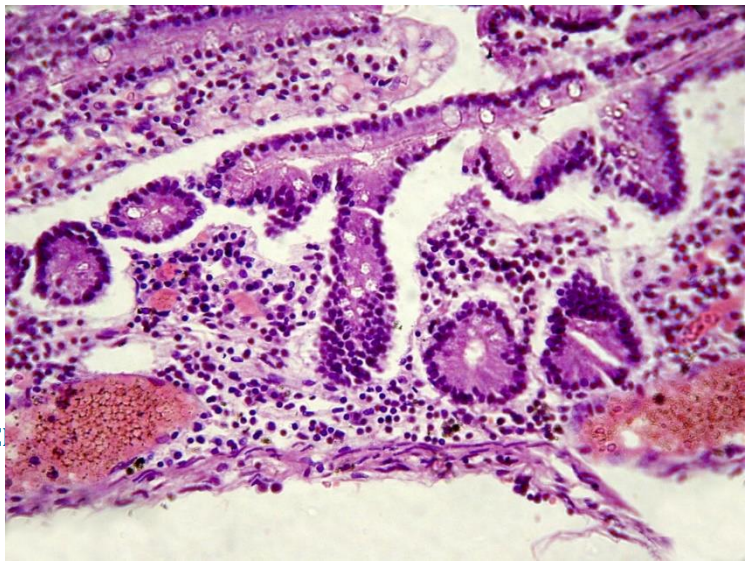

Figure 1: The ileum of a child 1.5 months old. Formation of crypts and suckers on the mucosa. Staining: haematoxylin and eosin. X: ca.10, vol.20.

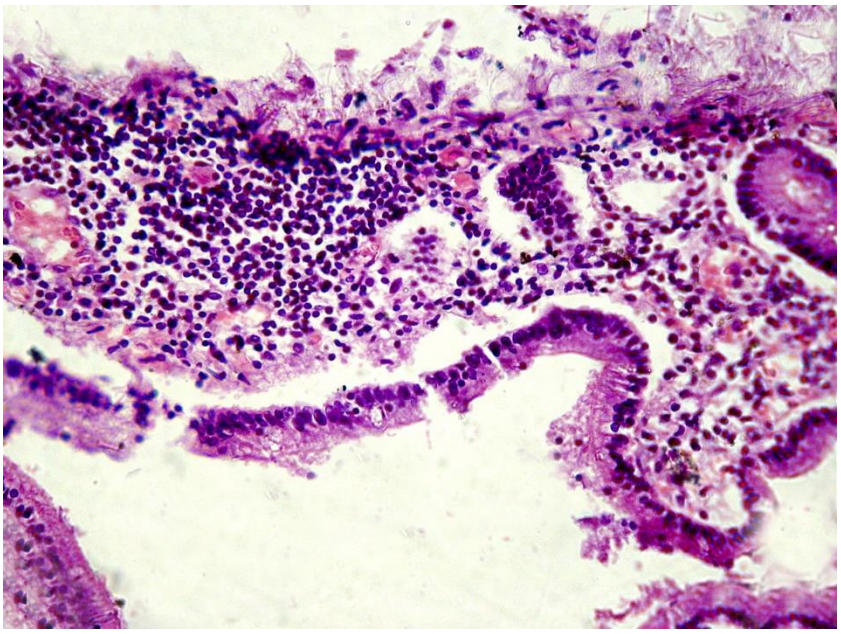

Figure 2. The ileum in a child 1.5 months old. Appearance of primary lymphoid follicle in the private layer of the mucosa. Staining: haematoxylin and eosin. X: ca.10, ob.20.

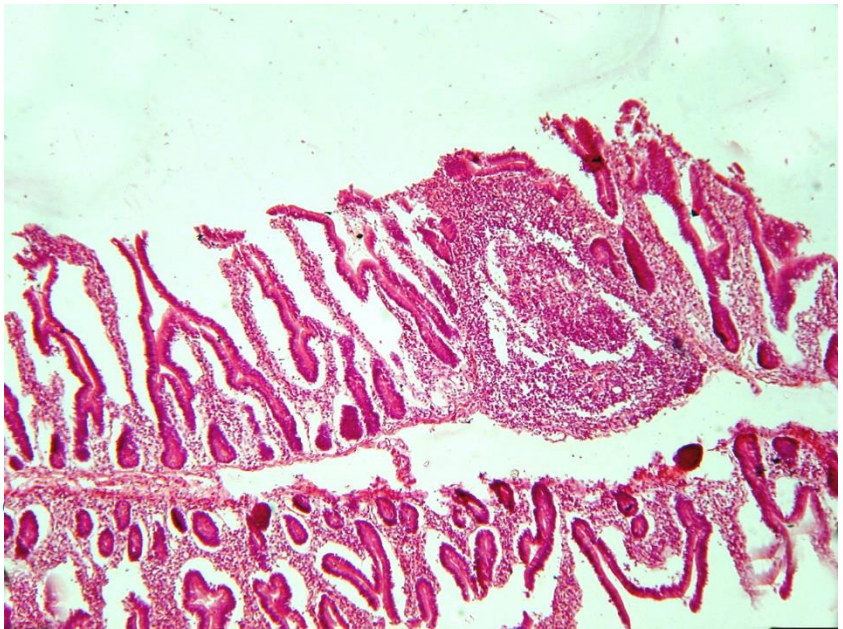

Figure 3: The ileum of a 6-month-old child. Nipple elongation and primary lymphoid follicle formation. staining: haematoxylin and eosin. X: ca.10, ob.10.

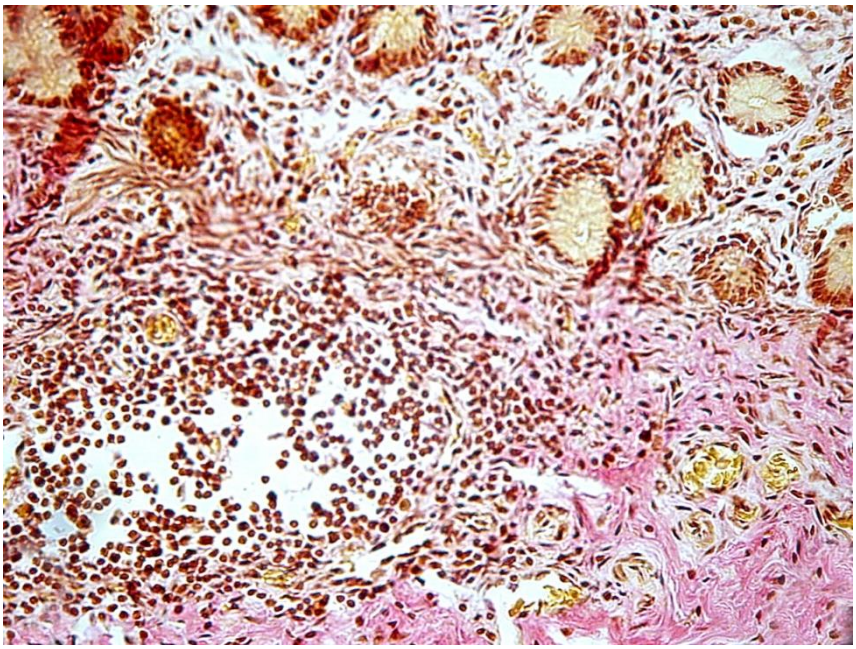

Figure 4: The ileum of a 6-month-old child. Absence of submucosal layer, overgrowth of bocaloid cells in crypts. Staining: Van Gieson, X: ca.10.vol.20.

Examination of the ileum of children who died of diseases not related to the intestine during the period of 7-12 months revealed that during this period the intestinal mucosa had developed almost completely, and all its structural units were close to normal. . The suckers of the mucous membrane are long in comparison with the previous periods, some of them branched, their private layer in all parts consists of young connective tissue. The covering epithelium has a uniform cylindrical form, between the hyperchromic enterocytes a sufficient number of bocaloid cells is established. However, already during this period of childhood, it was observed that the lymphoid tissue of the ileum consisted of individual follicles (Fig. 5). Grouped lymphoid follicles were found to appear in small numbers only in the distal intestine. Although

the lymphoid follicles have a single structure, they have a centre of cell proliferation and have developed into secondary follicles, indicating repeated exposure of the intestinal mucosa to exogenous antigens and the development of secondary immunity. In some children of this age, lymphoid follicles appeared on the ileum wall, even among the inner bundles of the muscle layer (Fig. 6). The area of such lymphoid follicles is relatively wider, the cluster of lymphoid cells is sparser, reticular stroma is less developed in the centre, and lymphocyte infiltration of surrounding tissues is observed in the peripheral parts.

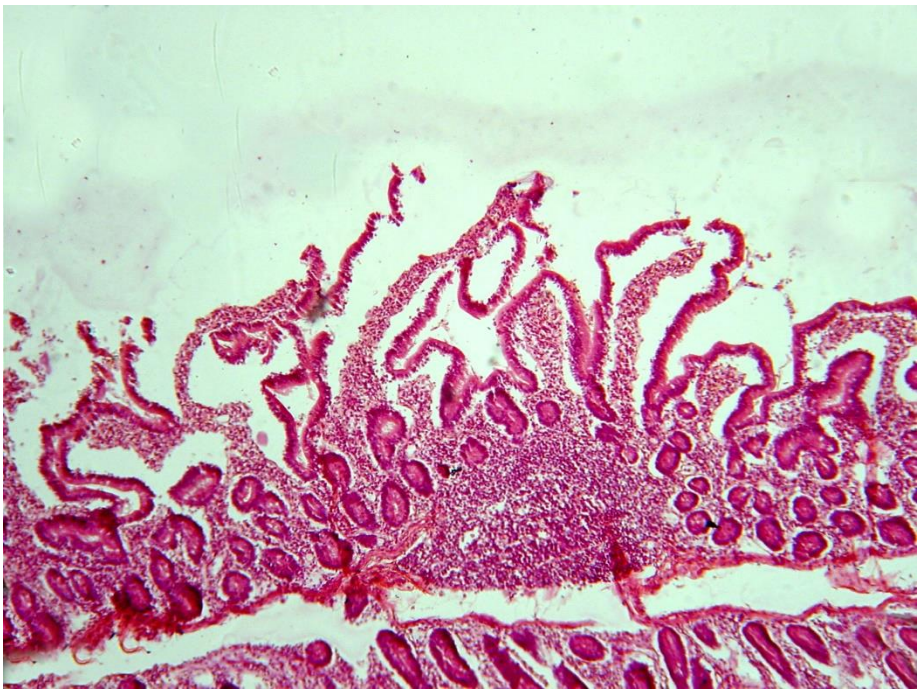

Figure 5. The ileum of an 8.5 month old child. Individual arrangement of lymphoid follicles. Staining: haematoxylin and eosin. X: ca.10, ob.10.

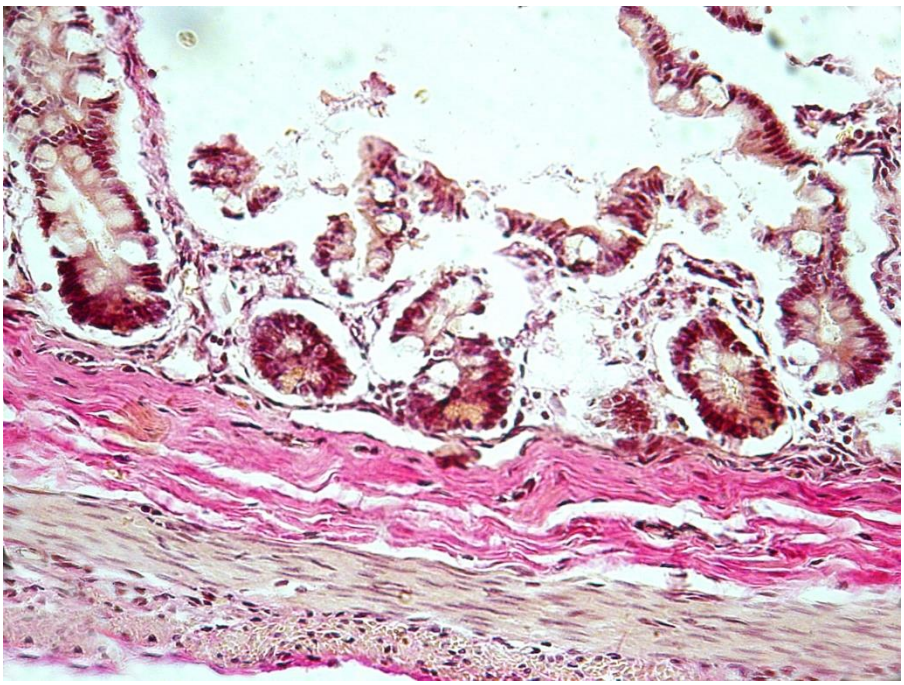

Figure 6. The ileum in a 9-month-old child. Appearance of lymphoid follicle between the muscular layer. Staining: Van Gieson. X: ca.10, vol.20.

Morphometrically, the number of lymphoid follicles of the iliac wall was found to be different depending on the age of children. On average, 34.8±5.2 in newborns, 38.5±6.3 in children 1-3 months, 75.4±9.4 in children 4-6 months, and 78.3±8.5 in children 7-12 months. It can be seen that the number of lymphoid follicles in the ileum wall increases sharply during the growth period from birth to one year of age, and when the child reaches adulthood, their number increases more than twice as much as in infants. In infants and children up to 6 months of age, lymphoid follicles are of different sizes, the distance between them is also different, and the germinal zone is identified in only 65%. In the second 6-month period all lymphoid follicles have practically the same structure and composition, i.e. their central part is lighter in colour, comparatively larger in size and consists of a germinal area consisting of reticular cells and lymphoblasts. Their base, facing the muscular layer, is somewhat broader, the upper side of the mucous membrane being thinned like the tip of an egg. It was found that the germinal area of all lymphoid follicles is not uniform in size, i.e. large ones have tubercles on the surface of the mucous membrane, and the surface of small ones is depressed into the intestinal wall. It was observed that small lymphocytes appeared densely around the germinal area, while in the peripheral areas lymphocytes were sparsely located and infiltrated into the surrounding tissues. In the superficial area of the mucosa, lymphocytes were found to proliferate between cells of the covering epithelial layer and to form a symbiosis of cells with the epithelium.

Calculation of morphometric indices of the cells that make up the lymphoid follicles showed that the relative percentage of cells varies sharply depending on the period of life of children up to one year of age. In young children, it was found that most of the cellular composition of lymphoid follicles is made up of small lymphocytes, i.e. 87.8±8.6% (Table 2). Reticular cells (2.1±0.2%) and lymphoblasts (0.9±0.2%), which form the basis of lymphoid follicles, were in a functionally passive state and accounted for only 3%. Large (1.2±0.3%) and medium-sized lymphocytes (4.6±0.7%) were also found in the germinal region. Decaying and dying cells (2.8±0.4%) and macrophages phagocytising them (0.6±0.2%) occupied the peripheral areas of the lymphoid follicles. The above-mentioned quantitative composition of cells of lymphoid follicles of the ileum of young children indicates that they are in a dormant state without antigen exposure.

It has been established that the composition of cells of lymphoid nodules of the ileum in children who died from traumas in the period from one to three months did not change significantly in comparison with infants. It was found that the number of small lymphocytes decreased by only 3%, reticular cells - by 2.8±0.3%, large lymphocytes - by 1.4±0.3%, medium lymphocytes - by 6.4±0.8%. It was found that the number of dying cells and macrophages in peripheral sections of lymphoid follicles of the ileum in children of this age increased twofold (Table 4). If we analyse the above-mentioned quantitative changes in the lymphoid follicles of the ileum of children of the first months of the postnatal period, it should be noted that in the lymphoid nodes In the immune system of the intestine in response to antigenic influences entering the intestine in the first months of the process of adaptation of the newborn's organism to the external environment, an increase in the number of macrophages, large blast lymphocytes is considered natural.

Mean number and cellular composition of iliac lymphoid follicles in children under one year of age, in per cent ***Table 4***

№	Cellular composition	Cell counts by month, in per cent, M±m, (min-max)			
		0-30 кун n=7	1-3ой n=9	4-6 ой n=8	7-12 ой n=10
1	Number of lymphoid follicles	34,8±5,2	38,5±6,3	75,4±9,4	78,3±8
2	Reticular cell	2,1±0,2 (1-6)	2,8±0,4* (1,5-6)	4,2±0,5** (2-7,4)	6,0±0,7 (4,2-8,5)
3	Blast cells	0,9±0,1 (0-3)	0,7±0,1* (0-4)	0,5±0,08 (0-3)	0,6±0,07 (0-4)
4	Large lymphocytes	1,2±0,3 (0-2,4)	1,4±0,3 (0-2,5)	2,1±0,4** (0-3,6)	3,4±0,5** (1-5,2)
5	Medium lymphocytes	4,6±0,7 (1-7)	6,4±0,8* (2-8)	10,3±2,1 (5-12)	12,5±2,4 (6-13)
6	Small lymphocytes	87,8±4,6 (56-98)	84,6±4,2 (54-94)	77,8±3,6** (65-82)	70,6±3,8 (52-81)
7	Macrophages	0,6±0,1 (0-1,4)	1,1±0,4* (0-1,6)	2,7±0,6 (1,2-4,4)	3,5±0,7** (1,5-6,5)
8	Degenerative cells	2,8±0,4 (1-4,2)	3,0±0,5* (1,2-6)	3,5±0,6** (2-6)	5,4±0,8** (2-8,5)
	Total	100	100	100	100

* - level of reliability R>0.01 compared to the previous line

** - level of reliability R>0.001 compared to the first line

Significant qualitative and quantitative changes in the composition of cells of lymphoid nodes of the ileum became apparent after the sixth month of life of the child. Histological studies showed that the number of lymphoid nodes increased sharply, their size increased, and they appeared more grouped. It is known that in the second half of a young person's life the state of own immune system in the organism of breastfed children histotopographically and morphofunctionally improves. In our material, this situation was confirmed by the following changes. The size of the germinal field in the centre of lymphoid follicles increased, reticular cells, lymphoblasts and large lymphocytes in it were qualitatively activated, and their number also increased significantly compared to the period of infancy (Table 1). The dense ring of small lymphocytes located around the germinal field also thinned and thinned, although the number of small lymphocytes in its contents decreased by 17% compared to infants, they were found to be

in a morphofunctionally activated state. There was an increase in the level of infiltration of small lymphocytes into the surrounding tissue, including the covering epithelial layer, as well as an increase in the number of lymphocytes that have established symbiotic relations with the epithelium. During the development of hyperplasia and hyperfunction in the lymphoid tissue due to the development of immune reaction the number of decaying and dying cells increases (5.2±0.8%), they are phagocytised and transformed into determinants transmitting immunological information. It was found that by the 12th month of the child's life, compared to the period of infancy, the number of macrophages in the periphery of lymphoid follicles increased 6 times.

CONCLUSIONS

1. In most cases, acute intestinal infections in children under one year of age are caused by opportunistic microorganisms, primarily due to premorbid conditions.

2. It has been established that the peculiarities of pathomorphological changes in lymphoid structures of the mucous membrane in acute intestinal infection depend on the age of children and premorbid pathology.

3. The number of lymphoid follicles in the wall of the ileum sharply increases during the growth period from birth to one year of age, and by the time the child reaches adulthood their number increases more than twice as much as in infants.

4. During the first 6 months in babies, lymphoid follicles are of different sizes, the distance between them is not uniform, and the germinal zone is identified in only 65 per cent. In the second 6-month period, all lymphoid follicles have virtually the same structure and composition.

5. Children who died from trauma in the period from one to three months have no significant changes in the composition of cells of lymphoid nodes of the ileum compared to infants.

6. Significant qualitative and quantitative changes in the composition of cells of lymphoid nodes of the ileum are clearly noticeable after the sixth month of life of children.

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