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#### ABSTRACT

The article presents the results of scientific research on the study of the location and distribution of lymphoid formations, their cellular composition in various areas adjacent to the sphincters of the rectum in rats at various stages of postnatal ontogenesis.

**KEY WORDS:** cell, lymphocytes, immune system, mucous membrane, lymphoid nodules, rectum, reproductive period, small intestine, ontogenesis.

#### **INTRODUCTION**

The fact that in the mucous membrane of the digestive tract there is an increase in the number of lymphoid formations towards its distal part is of great importance in the body's immune defense [1,7]. The density of single lymphoid nodules is maximal in the cecum and minimal in the ascending colon and rectum [2]. The macroscopic structure of Peyer's patches of the rat small intestine and changes in the intestine under the influence of chronic irradiation were studied [6,8].

According to a number of authors [4, 5], the spleen in different periods of postnatal ontogenesis is represented by well-defined lymph nodules. In animals of the reproductive period, nodules with germinal centers predominate among the lymph nodules. In senile rats, involutive changes prevail [3].

But in the literature, the microscopic structure of lymphoid formations in the zone of sphincters of the rat rectum at different stages of postnatal development has not been sufficiently illuminated.

The aim of this work - is to study the peculiarities of the location and distribution of lymphoid formations, their cellular composition in various areas adjacent to the sphincters of the rectum in rats at various stages of postnatal ontogenesis.

#### MATERIALS AND METHODS

The material for the study was 85 anal canal preparations taken together with the anus in newborn rats, 6, 11, 16 and 22 days old, 3 months, 6 months, 12 months, and 24 months. the age of the rats.

The animals were slaughtered under ether anesthesia; after opening the pelvic cavity, the rectum with the anus was removed. Sections  $6-8 \mu m$  thick were stained with hematoxylin-eosin and van Gieson's method.

The number of lymphoid formations and their cellular composition were counted along the anal canal sections. For the quantitative analysis of lymphoid cells, a grid with 36 nodal points was inserted into the eyepiece of the microscope. The shape and location of lymphoid accumulations were



revealed, the distance between lymphocytes in the clusters was studied depending on age.

## **RESULTS AND DISCUSSION**

In a newborn rat in the pre-sphincter part of the anal canal of the rectum, lymphoid formations are presented in the form of chains and clusters of lymphocytes. In the lamina propria of the mucous membrane, rare, short 1-2 row chains of small lymphocytes are detected. In each row of these chains, up to 4-5 lymphocytes are found. In the submucosa, there are accumulations of lymphocytes, they have a rounded and oval shape, not clearly delimited from the surrounding tissue. The distance between the accumulations of lymphocytes is on average 71.8 + 5.7 microns. The quantitative content of cells in accumulations of lymphocytes averaged 4.8 + 0.6.

By the age of 6 days in the submucosa, the number of accumulations of lymphocytes slightly increases. The distance between clusters decreases by 8.7%. The content of cells in accumulations of lymphocytes is on average - 6.5 + 0.7. In the presphincter zone of 6 day old rat pups, lymphoid formations are represented by diffusely located lymphocytes, chains of lymphocytes from 1-3 rows of cells, clusters of round or oval lymphocytes with a fuzzy border from the surrounding tissue. In most cases, accumulations of lymphocytes are found at the base of the crypts. From 11 days of age, the distance between the clusters decreases, and there are more cells in them.

On the 11th day of development in the lamina propria of the mucous membrane, the content of cells increases to 6-7 in each row of lymphocyte chains. In the submucosa, the distance between the accumulations of lymphocytes is reduced by 4.8%. In clusters of lymphocytes, the quantitative content of cells averaged 9.5 + 0.9.

By the 16th day of life in the submucosa of the pre-sphincter zone, the number of accumulations of lymphocytes slightly increases. In the presphincter region, the distance between the accumulations of lymphocytes increases by 13.4%. The density of the location of lymphocytes in the clusters increases. In clusters of lymphocytes, the cell content averaged 11.3 + 1.0. On the 22nd day of development, chains of small lymphocytes are detected in the lamina propria of the mucous membrane, the number of cell rows in them reaches 3. No change in the distance between the accumulations of lymphocytes was found in the submucosa of the pre-sphincter zone. In clusters of lymphocytes, an average of 14.2 + 1.0 lymphocytes are detected. In the pre-sphincter part, the intraorgan vessels of the submucosa are surrounded by lymphocytes.

At the age of 3 months in rats, 1-3 row chains of small lymphocytes are detected in the lamina propria of the mucous membrane. In each row

of these chains, up to 5-7 lymphocytes are found. In the submucosa, there are accumulations of lymphocytes, they have a round and oval shape, but are not clearly delimited from the surrounding tissue. By the age of 3 months in rats in the submucosa, the distance between the accumulations of lymphocytes averages  $54.4 \pm 3.1 \mu m$ . The content of cells in accumulations of lymphocytes averaged  $15.2 \pm 1.3$ . At 6 months of age in the submucosa, the number of accumulations of lymphocytes slightly increases. The distance between clusters is reduced by 4.0%. The content of cells in accumulations of lymphocytes averages  $17.6 \pm 1.1$ .

At 12 months of age in the pre-sphincter zone, the content of cells increases to 6-9 in each row of lymphocyte chains in the lamina propria of the mucous membrane. In the submucosa, the distance between the accumulations of lymphocytes increases by 13.0%. In clusters of lymphocytes, the quantitative content of cells averaged  $18.8 \pm 1.2$ . By the age of 24 months, chains of small lymphocytes are detected in the lamina propria of the mucous membrane, the number of cell rows in them reaches 5. In the submucosa, the distance between the accumulations of lymphocytes increases by 5.0%. In the accumulations of lymphocytes, the cell content averaged  $19.7 \pm 1.0$ .

From 3 months of age in the pre-sphincter region, lymphoid nodules of round, oval and triangular shape are revealed, they do not have a clear border from the surrounding tissue. The number of cells in lymphoid nodules averages  $25.0 \pm 1.9$ . By the age of 6 months, lymphoid nodules are not clearly demarcated from the surrounding tissue, their structural changes are expressed in a slight increase in the cellular composition. The number of cells in lymphoid nodules averages  $28.5 \pm 1.6$ .

At 12 months of age, clear boundaries from the surrounding tissue are formed in the lymphoid nodules. In them, an average of  $31.1 \pm 1.6$ lymphocytes are detected. At 24 months of age, the lymphoid nodules are round, oval and triangular in shape, clearly delimited from the surrounding tissue (Fig. 1). The number of cells in lymphoid nodules averages  $35.5 \pm 2.0$ .

In the pre-sphincter region, single small lymphocytes are located around the capillaries of the anal canal, and around the arterioles and venules there are 1-3 rows of lymphocyte chains; around the capillaries, lymphocytes are located more densely than around venules and arterioles.

In a newborn rat, short 1-2 row chains of small lymphocytes lie in the lamina propria of the mucous membrane of the transitional zone. Up to 3-5 small lymphocytes are detected in each row. These chains of lymphocytes lie at a smaller distance from each other than in the pre-sphincter region. In the submucosa, the distance between the accumulations of lymphocytes is  $65.9 \pm 4.8 \ \mu m$ .



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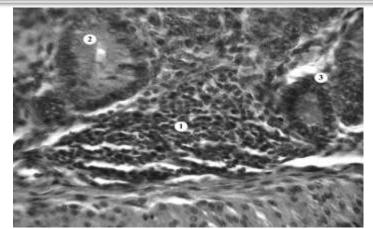


Fig. 1. Lymphoid nodule in the pre-sphincter part in a 24-month-old rat. 1. Lymphoid nodule. 2. Mucous membrane 3. Single-layer columnar epithelium. Staining with hematoxylin and eosin. About. 40x approx. 7.

The number of lymphocytes in the clusters averaged 5.1  $\pm$  0.6. In clusters, large single lymphocytes are detected. The content of medium lymphocytes is  $1.5 \pm 0.2$ , small -  $2.7 \pm 0.2$ . By the 6th day of development in the submucosa, the distance between the accumulations of lymphocytes decreases by 11.0%. The number of lymphocytes in the clusters averaged 7.5  $\pm$  0.8. On the 11th day of life in the submucosa, the number of lymphocytes in the clusters averages  $11.0 \pm 1.1$ . By the age of 16 days in the submucosa, the distance between the accumulations of lymphocytes is reduced by 10.0%. The content of lymphocytes in clusters averages 12.4  $\pm$  1.1. At 22 days of age, the length of chains of small lymphocytes in the lamina propria of the mucous membrane increases. The number of cell rows increases in them

In each row, up to 7-8 small lymphocytes are detected. In the submucosa, the distance between the accumulations of lymphocytes decreases by 5.0%. In the clusters, an average of  $14.9 \pm 1.0$  lymphocytes are found. In the mucous membrane under and above the muscle plates, single lymphocytes and accumulations of lymphocytes are detected, they have an oval and round shape. Under the anal pillars are clusters of oval-elongated lymphocytes and 1-2 row chains of lymphocytes, which are distributed parallel to the mucous membrane of the anal canal.

In the transition zone around arterioles and venules there are 1-2 rows of lymphocyte chains, but around the capillaries they are more dense than around venules and arterioles.

At 3 months of age, rats have 1-3 row chains of small lymphocytes in their own lamina of the mucous membrane of the transition zone, up to 4-6 small lymphocytes are detected in each row. These chains of lymphocytes lie at a closer distance from each other than in the pre-sphincter region. In the submucosa, the distance between the accumulations of lymphocytes is  $43.9 \pm 2.6 \ \mu m$ . By the age of 6 months in the submucosa, the distance between the accumulations of lymphocytes increases by 8.0%. In the clusters, an average of  $16.7 \pm 1.1$  lymphocytes are detected. At 12 months of age, in the submucosa, the distance between the accumulations of lymphocytes increases by 10.0%. The content of lymphocytes in the clusters averaged 17.7  $\pm$  1.1. By the age of 24 months, the length of the chains of small lymphocytes in the lamina propria of the mucous membrane increases. The number of cell rows increases in them. In each row, up to 7-8 small lymphocytes are detected. In the submucosa, the distance between the accumulations of lymphocytes increases by 10.0%. In the clusters, an average of  $19.3 \pm 1.0$  lymphocytes are detected.

In the transition zone, single and diffuse accumulations of lymphocytes are detected, located under and above the muscle plates of the mucous membrane, they have an oval and rounded shape. Under the anal pillars of the canal, accumulations of oval-elongated lymphocytes and 1-2 row chains of lymphocytes are revealed, which are evenly distributed and parallel to the anal canal. In the submucosa, lymphoid accumulations lie in the form of a 1-3 row chain of lymphocytes, they are located closer to the muscle plate of the mucous membrane. Arterioles and venules are surrounded by 1-2 row chains of lymphocytes.

In the lamina propria of the mucous membrane in the areas adjacent to the anal columns, oval-shaped accumulations of lymphocytes were revealed. They are located at a small distance from the epithelial cover. In the distal part of the transition zone, clusters of oval or round lymphocytes are located. They do not have a clear border from the surrounding tissue. In this section, in most cases, small single lymphocytes lie next to the capillaries and venules.

Lymphoid accumulations in the form of 1-3 row chains are located along the length of the anal canal. From 6 months of age in the transitional zone



of the organ, lymphoid nodules of a round and oval shape were revealed, they are indistinctly delimited from the surrounding tissue and lie in the lamina propria of the mucous membrane and submucosa.

By the age of 6 months in this zone, the lymphoid nodules have clear boundaries from the surrounding tissue and lie in the submucosa of the anal canal (Fig. 2). The number of cells in lymphoid nodules averages  $23.3 \pm 1.6$ . By the age of 12

months, the number of cells in the lymphoid nodules averages  $25.5 \pm 1.3$ . At 24 months of age, the number of cells in the lymphoid nodules averages  $26.7 \pm 1.4$ .

In the transition zone, the capillaries are surrounded by 1-3 small lymphocytes. Around the arterioles and venules are located 1-2 rows of chains of lymphocytes, but around the capillaries they are more dense than around the venules and arterioles.

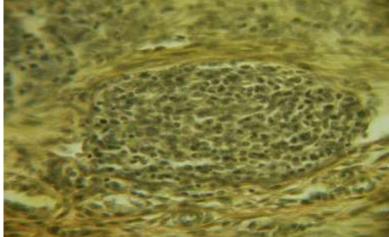


Fig. 2. Lymphoid nodule of the transition zone in a 6-month-old rat. Coloring according to van Gieson. Ob. 7x approx. 40.

In the internal sphincter, diffuse lymphocytes adjoin the epithelial cover. In the areas of the submucosa adjacent to the deepening of the mucous membrane, there are accumulations of lymphocytes in the form of chains. In this section, small single lymphocytes are found that lie under the transition of a single-layer columnar epithelium into a stratified squamous non-keratinizing epithelium.

In a newborn rat in the intersphincter zone, lymphoid formations are represented by accumulations of lymphocytes. In the submucosa, the distance between the accumulations of lymphocytes is  $61.1 \pm 4.8 \ \mu\text{m}$ . The number of lymphocytes in clusters is, on average,  $7.1 \pm 0.7$ .

On the 6th day of development, the distance between the accumulations of lymphocytes decreases by 13.7%. The number of lymphocytes in the clusters is  $9.5 \pm 1.0$ . On the 11th day of life, the content of lymphocytes in the clusters averaged  $12.2 \pm 1.0$ . By the 16th day, the distance between the accumulations

of lymphocytes is reduced by 13.0%. In clusters, an average of  $15.5 \pm 1.2$  lymphocytes are found. At 22 days of age, the distance between the accumulations of lymphocytes decreases by 7.0%. The content of lymphocytes in the clusters averaged  $17.4 \pm 1.3$ .

In the intersphincter zone in the areas adjacent to the anal columns, accumulations of lymphocytes in the form of 1-3 row chains were revealed (Fig. 3). Small lymphocytes were found around the venules at the base of the stratified squamous epithelium. They are located at a small distance from the epithelial cover and lie between bundles of fibrous structures of connective tissue in the back of the internal sphincter.

In the intersphincter zone, lymphoid formations are represented by accumulations of lymphocytes. In rats of 3 months of age in the submucosa, the distance between the accumulations of lymphocytes is  $38.0 \pm 2.4 \ \mu\text{m}$ . The number of lymphocytes in clusters averages  $19.2 \pm 1.4$ .



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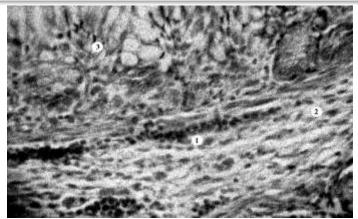


Fig. 3. Accumulations of lymphocytes in the form of a chain in the intersphincter zone in a 22 day old rat. 1. Accumulation of lymphocytes. 2. Submucosal base. 3. Unilamellar columnar epithelium. Staining with hematoxylin and eosin. Ob. 40 x about 7.

Accumulations of lymphocytes lie in the form of oval or round chains at the level of the posterior part of the inner and above the anterior part of the external sphincters of the anal canal. In the intersphincter zone in the areas adjacent to the anal columns, oval-shaped accumulations of lymphocytes were found (Fig. 4), and in the form of 1-3 row chains.

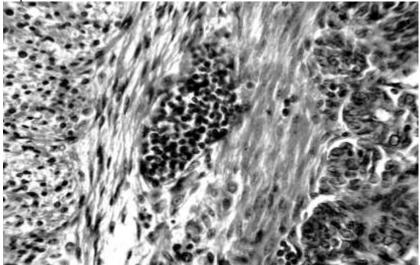


Fig. 4. Clusters of oval-shaped lymphocytes in the intersphincter zone in a 6-month-old rat. Staining with hematoxylin and eosin. Ob. 40 x approx. 7.

By the age of 6 months, the distance between the accumulations of lymphocytes increases by 9.0%. The number of lymphocytes in the clusters averaged 20.2  $\pm$  1.5. At 12 months of age, the distance between the accumulations of lymphocytes increases by 15.0%. The number of lymphocytes in the clusters averaged 21.7  $\pm$  1.5. By the age of 24 months, the distance between the accumulations of lymphocytes increases by 4.0%. The content of lymphocytes in the clusters averaged 22.5  $\pm$  1.3. On the border with the internal sphincter, there are accumulations of lymphocytes, the number of cells in them ranges from 4 to 10.

#### CONCLUSIONS

Thus, the results of the study showed that lymphoid formations in the pre-sphincter region and

the transition zone are presented in the form of chains and clusters of lymphocytes. From 3 months, lymphoid nodules form in the pre-sphincter region. In the transition zone, they are detected later by the 6th month. In the internal sphincter, diffuse lymphocytes are detected. In the intersphincter zone, small accumulations of lymphocytes were noted.

In the formation of the local immune system of the rat anal canal, stages can be traced. At the initial stages, diffuse lymphoid tissue is revealed. With age, diffuse lymphoid tissue thickens in the form of lymphoid nodules. In the intersphincter zone, differences in the structure of the epithelium, lymphoid formations and fibrous structures of the connective tissue were revealed. This is due to the fact that this zone is located on the border between the anal canal and the external environment.



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