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# INTRAORGAN CHANGES OF RAT SPLEEN VESSELS IN EARLY POSTNATAL ONTOGENESIS.

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#### Abstract:

The aim is to study intraorgan changes in spleen vessels in white rats in early postnatal ontogenesis.

**Materials and methods of research.** The experiment was carried out on 90 male mongrel rats in the newborn and at 6, 11, 16 and 21 days of age. To study the histotopography of the processes, polyclonal antibodies to CD3 and CD20 (Ventana, Switzerland) were used at a dilution of 1:100, carried out on histological glasses with an adhesive coating (Ventana, Switzerland). In the preparations, the spleen structures were examined morphometrically using an ocular micrometer. The diameter of the periarterial lymphatic couplings of the spleen, lymph nodes and their germinal centers, the width of the mantle, marginal and periarterial zones of lymph nodes, the relative area of connective tissue elements and white pulp (relative to the total area of the cut) were measured.

**Results.** The spleen of newborn baby rats is functionally immature, lymph nodes are not fully formed, it is difficult to distinguish all zones in them and germinative centers are not determined. When performing IHC with CD 20 markers, it was found that the number of LF without breeding centers in the spleen of the control group of rats reaches a maximum by 6 days of age, and with breeding centers by 11 days of age.

**Conclusions.** It has been established that, starting from the age of nine months, involutive changes are observed, characterized by an increase in the number of connective tissue elements in the spleen, a decrease in germinal centers in lymph nodes, the total number of lymphocytes in lymph nodes without reproduction centers, periarterial lymphatic couplings.

Keywords: immune system, spleen, lymphoid follicles, early postnatal ontogenesis.

**The relevance of the problem.** The spleen is considered to be the largest peripheral organ of immunogenesis, which largely determines the immune status: the state of innate and acquired immunity, its humoral and cellular links, the quality and quantity of lymphoid cells in humans and animals [6, 23].

At the suckling age, rats showed signs of intensive growth and morphofunctional development of the spleen, which consisted in a gradual increase in organometric parameters of the organ and morphometric parameters of the white pulp. By the end of the suckling age (21 days after birth), a qualitative transformation of the spleen parenchyma took place in the form of the formation of mature secondary lymph nodes and zones of periarterial lymphatic vaginas, which was a sign of the onset of functional maturity of the immune apparatus of the organ [11,17,28].

With age, lymphoid nodes with a germinative center are found in isolated cases, their sizes also gradually decrease. The content of connective tissue stroma increases in the spleen, and this process continues in the elderly and, especially, in old age [24, 32].

Thus, the decrease in the overall immune function of the spleen during ontogenesis is largely associated with the suppression of the humoral immune response, that is, with the suppression of B-cell immunity. On the other hand, a certain role is played by a certain weakening of the immune response of the cellular type, due to a reduction in the T-cell pool of spleen lymphocytes as well. In general, the detected changes have a corresponding effect on the state of both B- and T-cell immunity [1,5,21,33].

The aim of the study was to study structural changes in the vascular morphology of the spleen in white rats at the early stages of postnatal ontogenesis.

# Materials and methods of research.

The experiment was carried out on 90 male mongrel rats. To identify morphological and morphometric parameters of the spleen structure in postnatal ontogenesis, groups of animals were formed in the newborn and at 6, 11, 16 and 21 days of age.

For histological examination, tissue samples were fixed in 10% neutral formalin, after histological wiring on an automatic carousel type STP 120 by Thermo Fisher Scientific (TFS, USA), poured into paraffin wax at the Histo Star Thermo Fisher filling station (TFS, USA). Sections with a thickness of 3-4 microns were obtained on a rotary microtome NM 325 (TFS, USA).

Immunohistochemical preparations were manufactured in the Ventana Bench MarkXT autostainer (Switzerland). To study the histotopography of the processes, polyclonal antibodies to CD 3 and CD 20 (Ventana, Switzerland) were used at a dilution of 1:100, carried out on histological glasses with an adhesive coating (Ventana, Switzerland). Immunohistochemical reactions were carried out in accordance with the protocol of the antibody manufacturer. The sections were finished with Mayer's hematoxylin.

Finished histological preparations were studied under the CX40 trinocular microscope (Soptop, China), which has a digital image transmission system of the OD400UHW10 4 Mp microscope with a built-in morphometric program.

# The results of the study and their discussion.

The spleen of newborn baby rats is covered with a capsule on top, which consists of connective tissue. From it, trabeculae containing arteries and veins go deep into the spleen.

The thickness of the capsule at the gate averaged  $-4.70\pm0.15$ , at the front end it averaged  $6.2\pm0.3$  microns, at the rear end it averaged  $5.5\pm0.14$  microns. The diameter of the trabecula in the proximal part averaged  $11.8\pm0.12$ , and in the distal part it averaged  $8.7\pm0.6$  microns. The depth of the trabecula averaged  $12.12\pm0.17$ 

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microns. The relative area of the white pulp was on average  $-24.6 \pm 0.6\%$ , and the red pulp was on average  $-75.4 \pm 0.6\%$ .

The study of the trabecular vessels of newborn rats (Fig.1) showed that the thickness of the venous wall was on average  $10.6\pm 0.7$  microns, the inner diameter of the vein was on average  $19.8\pm 0.2$  microns, the thickness of the arteriole wall was on average  $14.3\pm0.1$ , the inner diameter of the arteriole was on average  $-16.2\pm0.3$ . The thickness of the capillary the walls of the trabecular vessel averaged  $4.2\pm0.1$ , the internal diameter of the capillary averaged  $5.5\pm0.4$  microns. The wall thickness of the venule of the pulpary vessel was on average  $8.4\pm0.3$  microns, the inner diameter of the venule was on average  $15.1\pm0.6$ , the wall thickness of the arteriole was on average  $10.9\pm0.3$ , and its inner diameter was on average  $11.2\pm0.9$  microns. The wall thickness of the capillary of the pulp vessel averaged  $4.0\pm0.1$ , and the inner diameter was  $5.2\pm0.4$ .

The wall thickness of the lymphoid follicle venule is on average  $5.3 \pm 0.3$ , the inner diameter is on average  $14.8 \pm 0.2$ . The wall thickness of the lymphoid follicle arteriole was on average  $5.9 \pm 0.5$ , and the inner diameter of the LF arteriole is on average  $11.5 \pm 0.7$  microns. The wall thickness of the capillary of the lymphoid

follicle averaged  $3.0\pm0.1$ , and the inner diameter was  $4.8\pm0.3$ .

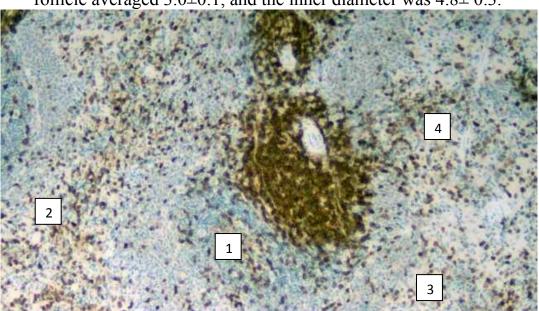


Fig.1. Spleen of a 6-day-old rat. IHH coloring on CD 3

1- white pulp, 2- lymph node, 3- trabeculae, 4 - spleen capsule. Approx. 10 x 20 vol.

The study of the trabecular vessels of 6-day-old rats showed that the thickness of the venous wall averaged  $11.6\pm0.8$  microns, the inner diameter of the venule was on average  $20.7\pm0.6$  microns, the thickness of the arteriole wall averaged  $14.9\pm0.22$ , the inner diameter of the artery averaged  $-17.1\pm0.5$ . The thickness of the capillary wall was on average  $4.4\pm0.2$ , the inner diameter of the capillary averaged  $5.7\pm0.3$  microns. The wall thickness of the venule of the pulpary vessel is on average  $8.4\pm0.13$  microns, the inner diameter of the venule was on average  $15.1\pm0.6$ , the wall thickness of the arteriole was on average  $11.6\pm0.19$ , and its inner diameter was on average  $12.4\pm0.5$  microns. The thickness of the capillary wall of the pulp vessel was

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on average 4.2  $\pm$  0.4, the inner diameter of the capillary was on average 5.5  $\pm$  0.3 microns.

The wall thickness of the lymphoid follicle venule is on average  $5.8 \pm 0.6$ , the inner diameter is on average  $14.9 \pm 0.3$ . The wall thickness of the lymphoid follicle arteriole was on average  $6.3 \pm 0.3$ , and the inner diameter of the LF arteriole is on average  $12.1 \pm 0.5$  microns. The wall thickness of the capillary of the LF vessel averaged  $3.2 \pm 0.2$ , and the inner diameter was  $5.2 \pm 0.3$ .

The study of the trabecular vessels of 11-day-old rats showed that the thickness of the venous wall was on average  $13.1\pm0.5$  microns, the inner diameter of the vein was on average  $22.8\pm0.5$  microns, the thickness of the arteriole wall was on average  $16.8\pm0.5$ , the inner diameter of the arteriole was on average  $-20.1\pm0.12$ . The thickness of the capillary wall was on average  $4.8\pm0.1$ , the inner diameter of the capillary averaged  $5.9\pm0.17$  microns.

The wall thickness of the venule of the pulpary vessel averaged  $10.3\pm0.4$  microns, the inner diameter of the venule averaged  $19.4\pm0.7$ , the wall thickness of the arteriole was on average  $12.8\pm0.15$ , and its inner diameter was on average  $13.8\pm0.42$  microns. The thickness of the capillary wall of the pulp vessel averaged  $4.6\pm0.1$ , the inner diameter of the capillary averaged  $5.7\pm0.2$  microns.

The wall thickness of the lymphoid follicle venule is on average  $7.5 \pm 0.13$ , the inner diameter is on average  $17.5 \pm 0.17$ . The wall thickness of the lymphoid follicle arteriole was on average  $6.8 \pm 0.6$ , and the inner diameter of the LF arteriole was on average  $12.4 \pm 0.54$  microns. The thickness of the capillary wall of the LF was on average  $4.0 \pm 0.1$ , the inner diameter of the capillary was on average  $5.5 \pm 0.2$  microns.

The study of the trabecular vessel of 16-day-old rats showed that the thickness of the venous wall was on average  $14.4\pm0.9$  microns, the inner diameter of the venule was on average  $23.7\pm0.43$  microns, the thickness of the arteriole wall was on average  $17.8\pm0.3$  microns, the inner diameter was on average  $-21.7\pm0.52$ . The thickness of the capillary wall was on average  $5.4\pm0.2$ , the inner diameter of the capillary averaged  $6.3\pm0.5$  microns.

The wall thickness of the venule of the pulpary vessel averaged  $10.6 \pm 0.6$  microns, the inner diameter of the venule averaged  $21.0\pm 0.8$ , the wall thickness of the arteriole is on average  $13.3 \pm 0.4$ , and its inner diameter was on average  $15.6 \pm 0.7$  microns. The thickness of the capillary wall was on average  $5.2 \pm 0.2$ , the inner diameter of the capillary was on average  $6.0 \pm 0.5$  microns.

The wall thickness of the lymphoid follicle venule is on average  $7.9 \pm 0.12$ , the inner diameter is on average  $17.8 \pm 0.5$ . The wall thickness of the lymphoid follicle arteriole was on average  $7.2 \pm 0.3$ , and the inner diameter of the LF arteriole is on average  $13.1 \pm 0.5$  microns. The capillary wall thickness averaged  $4.2\pm0.2$ , the internal diameter of the capillary averaged  $6.0\pm0.4$  microns.

The study of the trabecular vessels of 21-day-old rats showed that the thickness of the venous wall averaged  $15.7\pm0.22$  microns, the inner diameter of the vein was on average  $26.04\pm0.72$  microns, the thickness of the arteriole wall averaged  $19.04\pm0.3$ , the inner diameter of the artery averaged  $-23.8\pm0.4$ . The thickness of the capillary

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wall was on average  $5.8 \pm 0.3$ , the inner diameter of the capillary averaged  $6.8 \pm 0.7$  microns.

The wall thickness of the venule of the pulpary vessel averaged  $11.5\pm 0.8$  microns, the inner diameter of the venule averaged  $21.6\pm 0.24$ , the wall thickness of the arteriole was on average  $14.3\pm 0.8$ , and its inner diameter was on average  $18.2\pm 0.23$  microns. The thickness of the capillary wall averaged  $5.5\pm 0.5$ , the inner diameter of the capillary averaged  $6.5\pm 0.1$  microns.

The wall thickness of the lymphoid follicle venule is on average  $8.9 \pm 0.3$ , the inner diameter is on average  $18.2 \pm 0.7$ . The wall thickness of the lymphoid follicle arteriole was on average  $8.1 \pm 0.4$ , and the inner diameter of the arteriole is on average  $14.3 \pm 0.4$  microns. The capillary wall thickness averaged  $4.5\pm0.5$ , the internal diameter of the capillary averaged  $6.3\pm0.1$  microns.

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