EVALUATION OF MORPHOMETRIC PARAMETERS OF SPLEEN DEVELOPMENT IN RATS DURING EARLY POSTNATAL ONTOGENESIS

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Abstract. *This article examines the characteristics of morphometric changes in the main structures of the spleen in white rats during early postnatal ontogenesis in the course of development. It reveals the patterns of formation of various structural and functional zones of this organ at different stages of early postnatal ontogenesis. It has been established that during postnatal ontogenesis, the morphological and morphometric parameters of the functional zones of the spleen in white rats undergo significant changes, which are reflected in different age-related aspects.*

Keywords*: spleen, white pulp, lymphoid follicle, germinal center, periarterial cuff, red pulp.*

Introduction. The immune response is one of the body's adaptation mechanisms and plays an important role in maintaining its antigenic homeostasis [2,5,7,9]. The spleen is a vital organ of the immune system that performs a wide range of functions, including immunological defense, blood filtration and storage, and the destruction of aged erythrocytes. The spleen is a parenchymal organ composed of pulp and connective tissue stroma, which includes the capsule, trabeculae, and reticular framework [6,9, 12]. The parenchyma of the spleen consists of two functional zones: the red and white pulp, which differ in structure, composition, and function [2, 3,9,13,14,15,16]. Morphologists are increasingly interested in studying the structure of the spleen, largely due to the rapid development of immunology [1, 3, 6, 8,12]. The multifaceted role of the spleen in the body is regulated by a complex control system, which remains insufficiently studied. In order to understand the mechanisms underlying various immunodeficiency and autoimmune conditions, it is important to study the morphogenesis of the spleen at different stages of postnatal ontogenesis [10,11,17]. Currently, the study of the spleen's lymphoid structures, which are responsible for the effectiveness of both cellular and humoral immune responses—innate and acquired—is a pressing issue [1,4,5,9,11].

Material and methods of research. To determine the structural and functional features of spleen development during early postnatal ontogenesis, rats aged 1, 3, 7, 14, 21, and 30 days after birth were used. For this purpose, adult male and female rats weighing 150–170 g were selected and kept under quarantine for two weeks. After ruling out somatic and infectious diseases, the animals were placed under standard laboratory conditions and allowed to mate. The gestational period was monitored based on the morphology of vaginal smears. Pregnancy and delivery in most animals proceeded without complications. On days 1, 3, 7, 14, 21, and 30 after birth, the rats were euthanized by decapitation under light ether anesthesia. The spleen was weighed and measured to assess changes in mass and volume over time.

For light microscopy, spleen samples were fixed in 10% formalin, processed, and embedded in paraffin. The paraffin sections were deparaffinized and stained with hematoxylin and eosin. The resulting histological specimens were digitized using the Hamamatsu NanoZoomer whole-slide scanning system (REF C13140-21, S/N000198, HAMAMATSU PHOTONICS, 431-3196 JAPAN). Morphometric analysis of the obtained images was performed using the NDP.view2 software.

Results and discussion. To investigate the dynamics of quantitative changes in the spleen during postnatal ontogenesis, we analyzed the following key criteria:

- Determination of the body mass of the animals and spleen mass with calculation of the weight index;

• Measurement of the absolute area of the spleen, as well as the areas of the red pulp, white pulp, periarterial zone, and stromal components;

• Counting the number of lymphoid follicles and determining their average diameter and the diameter of the germinal centers during the course of postnatal spleen development.

The average values of body mass and spleen mass are presented in Table 1. As shown in the table, in newborn rats, the spleen weighs 11.0 ± 0.24 mg, while the average body mass is 5.6 ± 0.03 g. The weight index, or the ratio of spleen mass to body mass, is 509 at this stage.

Subsequently, the spleen mass increases rapidly: by day 3, it is 1.3 times greater; by day 7, 2.8 times; and by day 14, it is 6.4 times higher compared to that of newborn rats. Meanwhile, the body mass of the rats increases at a much slower rate. By day 7, the body mass is only 1.6 times that of newborns, and by day 14, it has increased just threefold.

This trend continues in the later stages of the study. By day 30, the spleen mass has increased more than 20-fold compared to that of newborns, whereas the overall body mass has increased only 8.7 times.

Table 1

The ratio of body mass to spleen mass in first-generation rats during early postnatal ontogenesis

Age (days)	Body mass (g)	Spleen mass (mg)	Weight index
1	5,6± 0,03	11,0±0,24	2,0+
3	6,0± 0,11	14,5±0,77	2,4
7	8,9± 0.05	31,2±1,11	3,5
14	18,5± 0,27	70,4±3,47	3,8
21	31,4± 0,99	135,0±3,39	4,3
30	48,9± 1,41	229,6±4,31	4,7

Thus, the rate of spleen mass increase exceeds the rate of overall body mass growth in the animals. This is likely associated with the processes of formation and growth of lymphoid follicles, the enhanced development of the vascular system, and the organ's increasing capacity to store significant volumes of circulating blood. The higher rate of spleen mass growth is also due to the stimulating influence of antigens from the external environment on the developing immune system during postnatal ontogenesis. Finally, the role of genetic determinants in the development of immune organs cannot be ruled out.

The increase in spleen mass is accompanied by specific dynamics in the changes of the organ's absolute area and the areas of its various structural and functional zones. The average values of these parameters are presented in Table 2.

Table 2

Absolute area of the structural and functional zones of the rat spleen during early postnatal ontogenesis (in mm² and %)

Age (days)	Total spleen area (mm²)	Red pulp (mm²)	White pulp (mm²)		Connective tissue (mm²)
			general	Periarterial zone (mm²)	
1	1,68±0,08	1,54±0,07 92%	-		0.13±0,006 8%
3	2,3±0,1	1,97±0,09 86%	0,09±0,004 4%		0,23±0,01 10%
7	3,6±0,1	3,04±0,1	0,2±0,01	0,01±0,0005	0,36±0,01

		84,4%	5,6%		10%
14	5,8±0,2	4,45±0,2 76,8%	0,65±0,03 11,3%	0,06±0,003	0,63±0,03 10,9%
21	11,3±0,5	8,58±0,4 76%	1,46±0,07 13%	0,13±0,006	1,24±0,06 11%
30	16,2±0,8	11,82±0,5 73%	2,26±0,1 14%	0,24±0,01	2,1±0,1 13%

As shown in the table, the absolute area of the spleen in newborn animals is 1.68 mm². Of this, 92% is occupied by red pulp, while stromal components account for 8% of the organ's total area.

By day 3 after birth, the spleen area exceeds that of newborns by 1.3 times. During this period, the red pulp still dominates the parenchyma. However, initial clusters of lymphoid tissue begin to appear, accounting for about 4% of the total spleen area.

By day 7 postnatally, the spleen area has increased more than 2.1 times compared to the neonatal period. At this stage, the formation of white pulp becomes evident, seen as lymphoid cell aggregates around the central artery, comprising 5.6% of the total organ area. As a result, the relative proportion of red pulp slightly decreases to 84.4%, although its absolute area continues to grow compared to earlier stages.

By days 21–30 of postnatal development, the absolute spleen area increases 8–10 times compared to that of newborns. During this time, the areas of red and white pulp, as well as stromal components, progressively increase. At the same time, a stable proportion among these structural components is established. Red pulp constitutes 73–76%, white pulp 13–14%, and connective tissue elements 10–13% of the total spleen area.

Thus, postnatal spleen development is accompanied by an increase in both the total area of the organ and the areas of its individual structural-functional components. Differentiation of white pulp begins on day 7 after birth and reaches its peak of development by days 21–30. By this period, the stable proportion of red and white pulp is established, and the formation of the organ's connective tissue stroma is completed.

The formation of white pulp is accompanied by the differentiation of T- and B-dependent zones. As morphological studies have shown, the periarterial or thymus-dependent zone begins to form only on day 7 of postnatal life. At this stage, its area remains relatively small—only 0.01 mm², which corresponds to 2% of the entire white pulp area. The T-dependent zone of the spleen reaches its maximum development by days 21–30 of postnatal ontogenesis, reaching 0.24 mm² or 10.9% of the white pulp area. Thus, the differentiation of T- and B-dependent zones of the spleen occurs simultaneously with the formation of white pulp and is completed by days 21–30.

The number and diameter of splenic lymphoid follicles during postnatal development also show specific dynamics, increasing in parallel with the growth of their absolute area. The average results of these measurements are presented in Table 3.

Table 3

Average number and diameter of splenic lymphoid follicles in rats during early postnatal ontogenesis (M±m, µm)

Age (days)	**Average number of lymphoid follicles (M±m)**			**Average follicle diameter (µm)**	**Average GC diameter (µm)**	**PALS width (µm)**
	without GC	with GC	general			
7	7,3±0,3	-	15,3±0,7	111,7±5	-	17±0,8
14	10,8±0,5	-	18,8±0,9	182,0±9	-	24±1,2

21	11,3±0,5	2,7± 0,1	24,0±1,2	244,8+12	33,3±1,6	38±1,9
30	16,2±0,8	6,3±0,3	31,5±1,5	388,5±18	74,3±3,7	45±2,2

Legend:

- *GC – Germinal Center*
- *PALS – Periarteriolar Lymphoid Sheath*

As noted above, fully formed white pulp is absent during the first 3 days after birth. Therefore, the quantitative assessment and measurement of lymphoid follicle diameters were conducted starting from day 7 of the postnatal period. On postnatal day 7, the number of lymphoid follicles was 7.3 ± 0.3, while their average diameter was 111.7 ± 5 µm. Subsequently, the rate of increase in follicle diameter slightly outpaced the rate of follicle formation. By day 21, the follicle diameter had increased nearly 2.2 times compared to day 7, reaching 244.8 ± 12 µm, whereas the number of follicles increased only 1.5 times, reaching 24.0 ± 1.2. By day 30, the number of follicles increased only 1.2 times, while the diameter increased 1.6 times.

Thus, the increase in the number and diameter of the white pulp follicles of the spleen during postnatal development correlates with other growth parameters and reaches its peak between postnatal days 21 and 30.

Conclusion. Thus, during the early period of postnatal ontogenesis in rats, signs of intensive growth and morphofunctional development of the spleen are observed, manifested in the gradual increase of organometric indicators and morphometric parameters of the white pulp. During this period, the spleen undergoes significant structural changes affecting both the stroma and the parenchyma of the organ. In the first three days, erythro- and thrombocytopoiesis predominate in the spleen, while the lymphoid apparatus is still in a rudimentary stage.

Beginning on the 7th day after birth, the white pulp develops intensively, accompanied by progressive complexity of the organ's vascular system, a decrease in erythro- and thrombocytopoiesis, and an increase in lymphocytopoiesis and antibody production in the spleen. By the end of the suckling period, a qualitative transformation of the spleen parenchyma occurs, marked by the formation of mature secondary lymphoid follicles and periarteriolar lymphoid sheath zones, which signifies the onset of functional maturity of the organ’s immune apparatus.

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