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THE ALGORITHM FOR DETERMINING THE FUNCTIONAL IMMUNOMORPHOLOGY IN RABBITS SPLEEN

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The study focused on determining the functional immunomorphology of rabbit's spleen. The subject of the study was spleen of clinically healthy adult rabbits the california breed at 6-8 months of age, of both sexes. The material was fixed in formalin and poured in paraffin for histological and morphometric studies. The histological preparations are dyeing to hematoxylin and eosin according to Van-Gison`s technology and investigating in a light microscope for study the structure of cells and tissues. A number of quantitative indicators were identified: the relative area of stroma was equal 5,87±0,69 %, the white pulp – 17,68±4,40 %; the ratio of stroma to parenchyma was 33,20 %; the diameter of lymphoid nodule was 240,01±72,32 micrometers; the diameter of light center – 122,43±49,24 micrometers; the germinative-follicular` index was 57,70 %; the width of lymphoid sheaths near the vessels – 39,24± 26,03 micrometers; the lymphoid coefficient – 3,12; the Kernohan` index for the central artery in lymphoid nodule was equal to 1,44±0,34 and 1,26±0,59 for arteries of lymphoid sheaths near the vessels. The results are used for investigating the influence of various factors, immunomodulators and rabbits breeding for produce safeness feed.

Key words: spleen, morphology, immunology, relative area, white pulp, lymphoid nodule, diameter, Kernohan` index.

Introduction.

Currently, it is known that the function of adaptive immunity that defines the cellular and humoral homoeostasis provides the immune system. Its morphological basis is the lymphoid tissue which organized in functional creation; the largest of it is the spleen. It belongs to the peripheral organs of immunogenesis and performs a variety of functions which differ in species specificity. In humans, one of the main functions of the spleen is the formation generalized immune response to a variety of factors. It is direct involvement in maintaining immune homoeostasis and, consequently, the required level of adaptive capacity of the organism. However, the relationship of immunological processes with the structural organization of the spleen has been studied insufficiently [1].

The immunocompetent compartment of the parenchyma of the spleen is presented its white pulp, which is formed by two main B - and T-dependent zones. There is the region of localization predominantly B - and T-cells. It is, accordingly, lymphoid nodules and lymphoid sheaths near the vessels [1, 2].

The study of reactive changes of the spleen, in particular, structural elements of the white pulp, dedicated the numerous scientific studies. They are studying the spleen of human, various farm and laboratory animals. Many of paper about spleen, usually, have descriptive characterizing. The morphometric methods are used for generalizing and statistical processing of the results of modern exploration. A certain number of works are devoted to such an important issue as the consideration of splenic functional immunomorphology [3-5].

However, the studies by various authors are used different and, sometimes, overlapping the morphometric criteria for assessment of

morphological and functional status of the spleen. This status is based on various measurements of tissue and cellular components of the body.

In addition, the relationship about dynamics of quantitative parameters of different structural components of the spleen tissue is often impossible to trace. This situation complicates the interpretation of results, especially in comparative terms, and does not allow convincing enough to judge about morphofunctional changes in the organ. Therefore, a standardization of the methods of morphometric studies of the spleen is developing a unified algorithm for obtaining quantitative data and a clear approach to the assessment of their dynamics in terms of ontogeny and in different pathological conditions of existence of the organism. This algorithm was developed by V. P. Volkov (2015) [1].

At the Department of anatomy and histology of the Zhytomyr National Agroecological University in the framework of the research topic "Development, morphology and histochemistry of animal`s organs in norm and pathology" (state registration № 0113V000900) pays great attention to the study of the morphology of the spleen. Accordingly, the question of the morphology of the spleen of rabbits has already been widely covered [2], however, in the context of the new algorithm needs will be clarifying. This determined the aim of our article. The rabbits as laboratory animals are used for a long time. It is acquiring the great importance in the study of the spleen as people and rabbits belong to the same group of mammals with the spleen of protection type. In this case, the immune function is dominated over deposition of blood in this groups of mammals [2].

For the study the selection of the spleen was made of adult rabbits of the california breed (6-8 months), both sexes (ratio of females: males was 1:1) in the phase of morphological and functional maturity of the body.

For histological studies, the pieces of material fixed in 10-12 % chilled neutral formalin solution, followed by pouring in paraffin. The paraffin

sections were produced on a sledge scrotom SS- 2 of a thickness have not been exceeding 10 microns. The morphology of cells and tissues were studied under the light microscope after dyeing to hematoxylin and eosin according to Van-Gison`s technology [4]. The morphometric studies (the determination of the relative squares, the diameters, the lumen of blood vessels) was performed by the program "Master of Morphology". The quantitative indicators were processed using the software "Statistic 6.0".

All experimental parts of the studies were established in accordance to the requirements of international principles of the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986) and the relevant law of Ukraine "On the protection of animals from cruel treatment" (№ 3446-IV dated by 21.02.2006, c. Kyiv).

The parameters were calculated as a result of researches. There are characterizing the morphological and functional status of the spleen. The changes of these parameters allow interpreting the immunological status of the organism.

Firstly, the overview of the ratios of the tissue elements of the spleen was calculated. This is a relative area of stroma 5,87±0,69 % (Fig. 1) and the relative areas of white pulp – 17,68±4,40 %. The decrease in the relative area of the white pulp indicates the reduction of lymphoid tissue and immune protection.

An integral indicator that characterizes the ratio immunoactive component of the parenchyma of the spleen (white pulp) is the ratio of stroma to parenchyma which is 33,20 %. This indicator is determining to assess the degree of sclerosis of the tissues of the spleen.

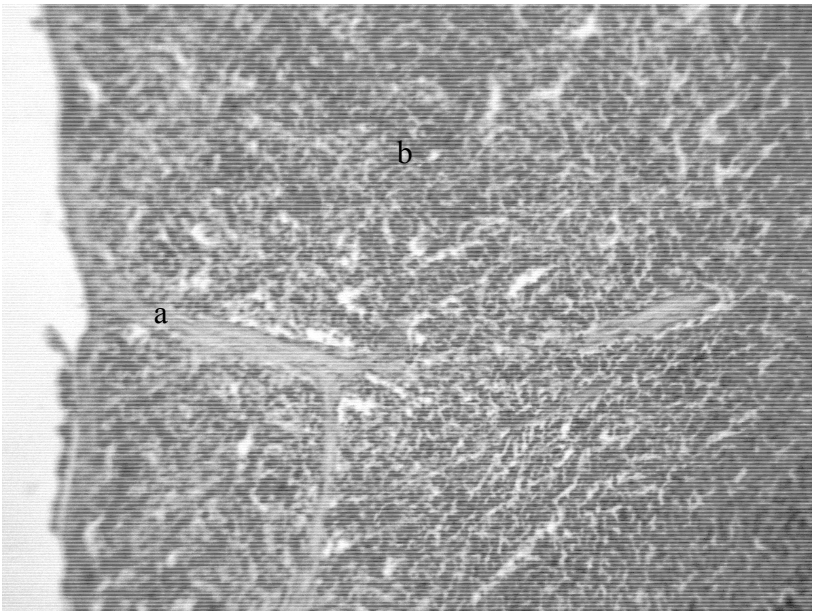

Fig. 1. The stroma (a) and the parenchyma (b) of the rabbit`s spleen. The hematoxylin and eosin. Micrograph. × 56.

The next group of indicators characterizes the morphological and functional state of white pulp. The first of these is the diameter of the lymphoid nodule (Fig. 2), which provides a quantitative characteristic of each specific nodule separately, objectively and adequately reflecting its size. Scilicet the number of group in this lymphoid tissue tells about the degree of hyper - or hypotrophy of this tissue structure [1]. It should be noted that many researchers as a criterion for the size of the various structural elements of the spleen tissue used the size of their area without diameter. It is impractical because the basis of calculation of the area takes the value of the diameter of one or another of the corresponding geometric shapes. Therefore, such a morphometric measure the diameter reflects the morphological and functional the status of the various tissue structures of the spleen adequately and accurately. The procedure of the research process significantly simplifies. That did not relate to these measurements but are important for organizing the further unnecessary calculations. The diameter of the lymphoid nodule of the spleen of rabbits was 240,01±72,32 micrometers.

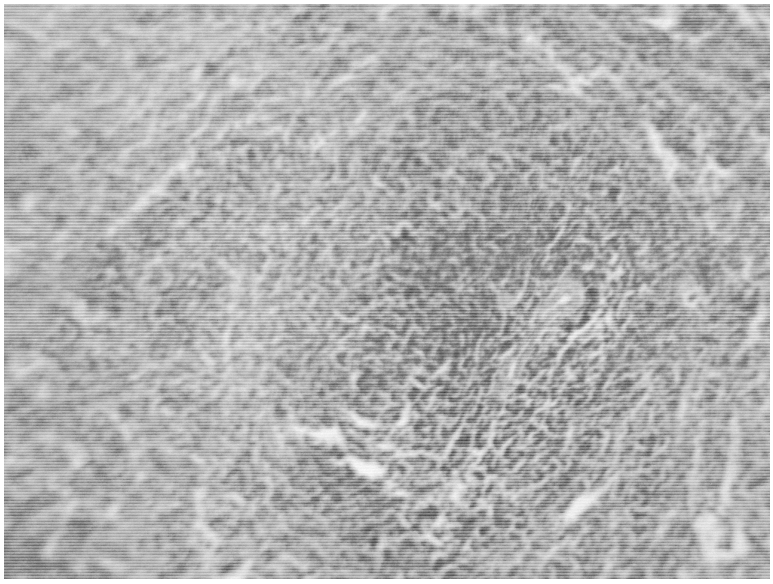

Fig. 2 . The lymphoid nodule with the central artery of the rabbit`s spleen. The hematoxylin and eosin. Micrograph. × 56.

The integral connection reflects the follicular coefficient which showing the total volume of functioning lymphoid system of the spleen. It is defined as the product of the relative area of the white pulp and lymphoid nodule diameter, divided by 20 [1]. It is 212,17 micrometers for the rabbit`s spleen.

The certain information about the functional state of lymphoid nodules gives also such indicator as the diameter of their light (germ) centers which serve as a place of the proliferation of B-cells and their differentiation into plasma cells. It is helpful to define a second ratio which called germinative-follicular` index. The diameter of the light center of the rabbit`s spleen is amounted to 122,43±49,24 micrometers. The germinative-follicular` index was 57,70 % (the ratio of the diameters germinative center to the overall diameter of a lymphoid nodule). All described quantitative indicators are characterizing the development of a B-cellular population of white pulp of the spleen. For T-cell lymphoid component is important to describe the width of the lymphoid sheaths near the vessels. It is equal to 39,24±of 26,03 micrometers.

The lymphoid coefficient shows the ratio of the diameter of lymphoid nodule to the width of lymphoid sheaths near the vessels (Fig. 3). It shows the ratio of the volume of the zones of localization of B - and T-cells. This coefficient amounts to 3,12 for the rabbit`s spleen.

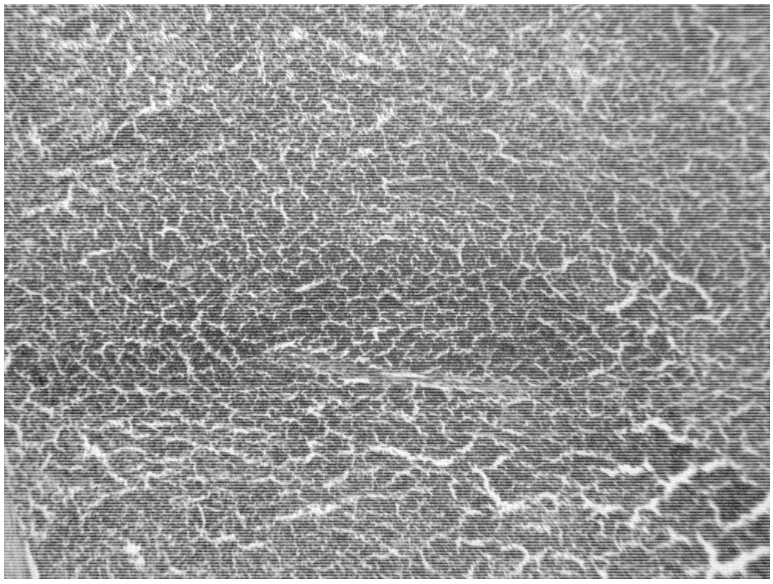

Fig. 3. The lymphoid sheaths near the vessels of the rabbit`s spleen. The hematoxylin and eosin. Micrograph. × 56.

The determination of the microcirculation in the white pulp of the spleen is important for physiology organ`s activities. The Kernohan` index is determined for this. It displays the throughput of the microvessels. We are calculated the Kernohan` index for the central artery of lymphoid nodule and arteries of lymphoid sheaths near the vessels. The Kernohan` index of the vessels in the white pulp of the spleen of the rabbit was equal to 1,44±0,34 and 1,26±0,59, respectively.

We are carrying the immunomodulation now. The first results of the application of the preparation the gamavit are indicating an increase in the relative area of white pulp on 1,75 %. This results in combination the stress load increases only on 0,3 %.

Conclusions.

We are determined the main indicators of the functional immunomorphology of rabbit's spleen according to the proposed algorithm. We are expecting the prospects for further research in the study of the processes of immunomodulation, the effects of environmental factors, the stress, the unbalanced diet and changes established indicators.

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