

CHAPTER «VETERINARY SCIENCES»

MORPHOGENESIS OF THE PARENCHYMA AND INTRANODE LYMPHATIC BED OF LYMPH NODES OF MUSCOVY DUCK

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Abstract. Age-related transformations of muscovy duck lymph nodes parenchyma, including its zonal specialization, are directly related to the process of development and reduction of the lymph sinus system, which determines the mechanism of entry and distribution within the lymphoid tissue of nodes of antigens and other foreign components of lymph. The lymph nodes of the muscovy ducks are «intravascular» uptake of lymphoid tissue. As a result, unlike mammals, there are virtually no capsular trabecules in the lymph nodes in birds, and due to the fact that the number of lymphatic vessels is often limited to one afferent and, accordingly, efferent vessel, between which, within the parenchyma of the node, central lymph sinus is located, characteristic subdivision of the lymphoid parenchyma for cortical and medullary substance is absent. The general pattern of quantitative correlation of the two main tissue components, connective-tissue stroma and lymphoid parenchyma of the cervicothoracic lymph nodes of muscovy ducks is a tendency to increase the percent area of the lymphoid parenchyma against the background of a decrease in the corresponding index of connective-tissue stroma.

Formation of the complete complex of morphological features of immunologic reactivity in the lymph nodes of the muscovy ducks occurs during the first three months of postnatal ontogenesis: in the first stage (up to

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25 days of age) the cortical and medullary substance of the nodes is formed, in the second stage (up to 60 days of age), diffusive structural and functional areas of cortical and medullary substances (deep cortical units, medullary cords), in the third stage (up to 90 days of age), lymphatic follicles, the quantitative indicators of which continue to grow with age.

The lymphoid parenchyma of the lymph nodes of the muscovy ducks has a pronounced zonal structure with the concentration of its main medullary zones around the central sinus, resulting in the cortical substance located inside the nodes, and the medullary substance is located at their periphery under the capsule, along the external lymph basin. The definitive histoarchitecture of the parenchyma of the lymph nodes of the muscovy ducks is characterized by a spherical spatial configuration of its main structural and functional zones (deep cortical units and lymphatic follicles) with a mosaic orientation principle.

The lymphatic bed of the lymph nodes consists of two lymph basins: the external (subcapsular or accentric lymph sinus) and the internal (central lymph sinus), which are connected by numerous intermediate sinuses. The dynamics of the relative capacity of the intranode lymphatic bed is marked. The first period (from birth up to 30 days of age) is the period of intensive growth of the capacity of the lymphatic bed, which is accompanied by a gradual reduction of the central sinus and compensatory development of cortical and medullary intermediate sinuses. The second period (up to 180 days of age) is the period of relative stabilization of the corresponding indicator with a tendency to decrease, mainly due to cortical sinuses due to the formation in the parenchyma of nodes of numerical lymphatic follicles. The third period (up to 240 days of age) is the period of marked decrease in the capacity of the lymphatic bed due to the reduction of the area of both cortical and medullary sinuses.

1. Introduction

In recent decades, veterinary morphology has paid particular attention to the study of the morphogenesis patterns of organs of the immune system of productive mammals and birds [16; 17; 22; 23]. Objective and highly informative interpretation of the results of morphological studies of the organs of immunogenesis of productive animals, today, is impossible without taking into account the modern aspects of their morpho-functional organization, the

main aspects of which are the provisions on the structural and functional heterogeneity of lymphocytes and the zonal nature of localization of the population of lymphoid cells in parenchyma of lymphoid organs [6; 14; 15].

The patterns of morphogenesis and age-related zonal specialization of the parenchyma of peripheral lymphoid organs have been most thoroughly studied in humans, laboratory animals and in some species of productive mammals [3; 4; 7–15; 20; 24]. It has been found that one of the most perfect forms of development of secondary lymphoid formations in mammals is lymph nodes (LNs), the morphological transformations of which in the early stages of ontogenesis are highly probable structural markers of the state and features of the development of immunologic reactivity. At the same time, the peculiarities of morphogenesis of the parenchyma of LNs in birds remain almost unclear to this day, despite the fact that in the conditions of intensive duck breeding, the biological preparations with antigenic properties are widely used, the effectiveness of which on the body of a bird is determined primarily by the level of immunologic reactivity. To date, the question of the features of intra-node lymphodynamics in LNs in birds, the presence in the parenchyma of nodes of signs of structural and functional differentiation of the parenchyma are debatable. There is practically no information on the regularities of age-related changes in the parenchyma of LNs of muscovy ducks during early postnatal ontogenesis and on the peculiarities of formation and development during this period of signs of the functional state of lymphoid tissues of LNs.

2. Analysis of recent researches and publications

The common opinion of many researchers is that the parenchyma of LNs of a bird is formed by the reticular tissue, which contains cells of the lymphoid row, but the data on the structural and functional organization of the parenchyma and the peculiarities of its location in individual functional zones, especially in age aspect, are ambiguous and debatable [5; 11; 16; 18]. Also, the questions regarding the structure of the lymph sinus system and the nature of the intranode lymphodynamics in LNs of a bird remain, which according to some authors are also controversial.

According to Berezina E.A. [5; 6], who studied the duck lymph nodes, found that in this species of bird lymph nodes are of two types: cervical and lumbar, but the presence of lumbar LNs is not always detected by the

author, as evidenced by data from other researchers [16; 17], and in the works of V.V. Melnyk [23] it is noted that there are no lumbar LNs of ducks and geese. When studying the structure of LNs of ducks Berezina E.A. [6] noted that each of the nodes usually has one afferent and one efferent lymphatic vessels, but in some lumbar LNs sometimes several afferent lymphatic vessels are observed. The acontoid of LNs consists of connective tissue that forms a capsule and underdeveloped trabecules. Organ parenchyma is represented by reticular tissue. The parenchyma of LNs is represented by the cortical and medullary substance and the secondary lymphatic follicles. The boundaries between the cortical and medullary substance are not clear, and secondary lymphatic follicles, as the author notes, can be rounded or of round and round multiangular shape and with a chaotic nature of their location on the entire surface of the parenchyma of the lymph nodes.

In the study of LNs in ducks and geese, V.V. Melnyk [23] found that these representatives of birds consisted of paired cervicothoracic lymph nodes located under the skin in the lower neck prior to entering the diaphragm cavity. The author also established the fact that 19% of the birds (geese) studied had two lymph nodes connected by a constriction mainly on the left side. According to the author, the microscopic structure of the cervicothoracic lymph nodes is similar to that of mammalian organs, that is, they are formed by a connective-tissue capsule, parenchyma and sinus.

E.A. Berezina [5] divided parenchyma of LNs in ducks into a dense, compact and spongy substances. The compact substance consists of rounded uptakes of mainly small and medium lymphocytes and spongy is of anastomosing lymphoid tissue, but most of the parenchyma of the nodes is represented by diffusive lymphoid tissue [24]. The localization, distribution and correlation between compact and spongy substances are not constant, and as the author notes, the birds under study are characterized by zonal formation of individual structural and functional zones, which is not mandatory for mammalian lymph nodes, but the boundaries between contactand spongy substances are unclear, uneven, and wavy.

In the study of the LNs sinus system in ducks, the authors [6; 19] note that the existence of the central sinus is observed only during the formation of the lymph node, in the first 1-2 months of life of ducklings, which is reduced and not detected at all in an adult bird. According to J.Jolly [17], only the central sinus, around which the cortical nodes are located, is pres-

ent in the bird's LNs sinus system, and the medullary cords are located at the periphery of the organs.

V.V. Melnyk in the work [23] proves that the intrabode lymph basin of LNs in geese and ducks is also represented only by the central sinus. The author divides the parenchyma of nodes into a cortical substance consisting of lymphatic follicles and diffusive lymphoid tissue located at the periphery of the nodes, as well as into a medullary substance that forms unclear cords of diffusive lymphoid tissue and is localized between the bendings of the central sinus, in the central part of organs.

In the works of many authors, devoted to the regularities of the structure of mammalian LNs, it is proved that they consist of parenchyma, connective-tissue acontoid and sinus system [6–8; 15; 21].

The mammalian parenchyma of LN consists of lymphoid tissue, which is represented by the reticular tissue in the cords of which the lymphocytes are located at different stages of development, and is divided into cortical and medullary substances. Cortical substance is represented by lymphatic follicles, interfollicular cortex (interfollicular zone) and paracortical (T-dependent zone), which in most mammal species is located at the periphery of nodes. Medullary substance is represented by medullary or lymph cords located in the central part of the organs.

Recently, a number of works by many authors is devoted to aspects of the morpho-functional organization of the parenchyma of LNs in which the lymphoid tissue is divided into separate structural and functional segments or compartments [11–13; 28]. Each individual segment includes paracortical zones or deep cortical units (T-dependent zones) and lymph nodes and medullary cords (B-dependent zones) that have a specific arrangement from the capsule nodes to the portal thickening [2; 21; 27].

It is noteworthy that the data available to date regarding the aspects of structural and functional specialization of secondary lymphoid organs obtained in the study of lymphoid organs and tissues in higher mammals have helped the authors to convincingly prove the leading role of immunologic reactivity in protecting the organism of higher mammals from foreign antigenic components. At the same time, aspects of morphogenesis and morpho-functional transformations of peripheral lymphoid organs in birds are less well known, especially lymph nodes, and the available literature is controversial in many cases. To date, there is no consensus on the

nature of the intranode lymphodynamics in the structure of the intranode lymphatic bed in birds, the dominant is the provision on the absence of evident features of structural and functional differentiation of parenchyma of LNs and, respectively, the presence compartments and segments, which are morpho-functional integrated units of common to mammals.

Particularly small are the data regarding age-related transformations of LNs in birds, which are characteristic only of the waterfowl representatives of this class of vertebrates. The lack of basic information on the principles of peripheral lymphoid organs in birds is a deterrent to the widespread use of modern immuno- and cytochemical methods for deeper studies of the specifics of their structural and functional organization. This fact will become of particular importance for the morphological substantiation of the efficiency and expediency of the use of technologies of intensive artificial antigenic stimulation of the organs of immunity of birds, which is most directly connected with the use of «intensive» vaccination schedule of young birds in the conditions of the poultry industry.

3. Material and methods of research

The study was conducted on the basis of the department of morphological studies of the Scientific Research Centre of Biosafety and Environmental Control of Agro-Industrial Complex of the Dnipro State Agrarian and Economic University

The material of the study were cervicothoracic LNs of clinically healthy muscovy ducks. The birds were raised under vivarium conditions, feeding was carried out according to existing standards, preventive vaccinations and antiparasitic treatments were not carried out.

The removal of animals from the experiment was carried out in accordance with the rules of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes» [9] by euthanasia with the gradual anesthetic overdose.

The cervicothoracic LNs from muscovy ducks of 1; 10; 20; 60; 90; 180; 240 days of age (5 heads of each age) were selected for morphological studies by anatomical preparation. To determine the features of the architecture of the intranode lymphatic bed of LN, indirect and direct injection of pre-node lymphatic vessels in the neck and head area with a gelatin-based dispersion of black ink was performed.

Wax-based total medial histo sections (5–10 µm thick for preparations for examination and histological examinations, 3–5 µm for cytological examinations) were made using the MC-2 sliding microtome. The resulting histo sections were stained with Ehrlich's hematoxylin and eosin, azure-II-eosin by conventional methods.

Features of localization within the parenchyma of organs and syntopia of functional zones of LNs (interfollicular cortex, paracortical zone or complex of deep cortical units, lymph nodes, medullary cords) were revealed using the method of impregnation of frozen sections with silver by Foote [11; 12], which provides the accurate visualization of the corresponding sites according to the peculiar architectonics of meshwork of reticulin fiber. Frozen sections of LNs were made on the MC-25 microtome cryostat at a temperature of 15-18°C with a thickness of 10-20 µm.

Histological examination was performed using Olympus CH-20, CX-41 light microscopes (eyelens 10×; lens 10×; 40×; 100×) and a microscope of biological stereoscopic MBS-10 (eyelens 8×, lens 4×, 7×). Quantitative morphological analysis of the structural components was performed by the method of “point counting” using the Avtandilov's test system [1] (3 measurements on 5 preparations of each group).

4. Results of the research

The cervicothoracic LNs of muscovy ducks represent a local uptake of lymphoid tissue in the lumen of the lymphatic vessel, resulting in them having one afferent and one efferent lymphatic vessels. The connective-tissue acontoid of LNs is represented by a capsule, which is formed as a result of thickening of the lymphatic vessel in the place of lymphatic uptakes due to the development of loose fibrous connective tissue on the basis of their internal and middle membranes and not numerous, short, underdeveloped capsular trabecules, which deepen only to the «superficial layers» of parenchyma close to the node's capsule. Microscopic examinations of the intranode lumens revealed that the lymphatic bed of LNs consists of two lymph basins: the external (subcapsular or accentric lymph sinus) and the internal (central lymph sinus), which are connected by numerous intermediate sinuses.

Age peculiarities of histoarchitecture and histological changes of the parenchyma of LNs of muscovy ducks are due primarily to the peculiarities

of the structure of the intranode microcirculatory bed and the development of separate structural and functional zones of the parenchyma of organs.

The parenchyma of LNs of neonatal ducks is characterized by a relatively low degree of differentiation and is represented by diffusive lymphoid tissue without marked signs of its division into separate functional zones, the percent area (PA) of which is $50.72 \pm 0.44\%$. The intranode lymphatic bed is represented by only two lymph sinuses: the central one, which is located in the central part of the organ and occupies a larger percent area ($13.91 \pm 0.57\%$) and a discrete accentric sinus adjacent to the capsule of the node and having a much smaller percent area ($5.99 \pm 0.53\%$), and is accordingly at its periphery.

The architectonics of the reticulate acontoid of the parenchyma of LN of neonatal ducks is characterized by the formation of bundles of reticulin fibers, which are located mainly at the periphery of the node, and closer to its center, i.e., to meshworks of thin reticulin fiber, the holes of which are small and uneven, directed mainly to the central lymph sinus.

In LNs of ducklings of 10 day of age, histoarchitecture of the reticular acontoid, the structure of the intranode lymphatic bed and its total capacity do not change significantly. Characteristic changes are observed in the parenchyma of LNs, where there is a decrease in PA of cortical substance (up to $19.21 \pm 0.51\%$), with the density of located cells in it increases markedly, with a simultaneous significant increase in PA of medullary substance (up to $42.43 \pm 0, 84\%$), without significant changes in lymphocyte density.

In LNs of ducklings of 20 days of age in the area of medullary substance of the lymph node, the formation of intermediate sinuses is observed for the first time, PA of which is $4.36 \pm 0.24\%$, PA of the accentric lymph sinus is significantly increased (up to $9.30 \pm 0.36\%$), as a result, the capacity of the lymphatic bed increases, but the tendency of decrease in PA of the central lymph sinus remains (by 0.75%).

The dynamics of structural and functional transformations of the parenchyma of LNs in ducklings of 20 days of age compared to ducklings of 10 days of age has the opposite character. Due to the formation of intermediate sinuses of medullary substance, its PA decreases (up to $31.52 \pm 0.29\%$), whereas PA of the cortical substance increases on the contrary (up to $20.50 \pm 0.52\%$) against the background of the decrease of the central lymph sinus.

Architectonics of the reticular acontoid of LNs of ducklings of 20 days of age is characterized by the presence of relatively uniform reticulin fiber meshworks in the cortical nodes, but the fibers of these meshworks become more flexuose, lose their characteristic direction relative to the central lymph sinus, and acquire the nature of chaos, in particular, it is for medullary substance of the meshworks. In the medullary substance of LNs together with reticulin fibers, which are located parallel to the eccentric lymph sinus, the fibers with perpendicular direction relative to it are more clearly detected, which, when connected together, also form relatively uniform meshworks, but their cells, unlike the cortical substance meshwork, are thinner due to the fact that the reticulin fibers are diluted.

Up to 60 days of age, muscovy ducks have characteristic structural and functional transformations of the parenchyma of LNs, namely, the boundaries between the cortical and medullary substances become clearer, with PA of lymphoid tissue enlarges in both functional zones, as well as formation of the antenodal lymphoid tissue on the basis of interfollicular cortex. The so-called antenodes localize mainly on the border with the internal lymph basin, are single uptakes of lymphoid cells, mostly rounded, which do not have a characteristic connective-tissue capsule.

Against the background of an increase in the parenchyma of LNs, there is a significant decrease in the total capacity of the intranode lymphatic bed by 7.93%, due to the decrease in the eccentric lymph sinus (to $5.85 \pm 0.13\%$), which becomes more discrete, and of the intermediate sinuses of medullary substance (to $5.31 \pm 0.07\%$).

Moreover, it is characteristic that the relative number of intermediate sinuses of medullary substance does not change, and the length and width of their lumen is significantly reduced. The relative number of intermediate sinuses of the cortical substance also does not change, but their PA on the contrary increases, mainly due to the increase in the width of their lumen.

The architectonics of the reticulin fibers of the parenchyma of LNs in muscovy ducks of 60 days of age is characterized by a «honeycomb» structure, with the formation of uniform reticulin fibers, but with some differences in each functional zone of nodes.

In the cortical substance, the reticulin fibers of parenchyma of LNs become more dense, arranged without a specific orientation, but thicker fibers can be distinguished, with a perpendicular direction relative to the

eccentric lymph sinus. For the first time, between the thick fibers, thin reticulin fibers are found in the area of the cortical substance, with a parallel arrangement relative to the eccentric sinus. Anastomosing thick and thin reticulin fibers between them form a thick medium-hole meshwork.

In medullary substance of LNs, the architectonics of the reticulate acanoid becomes more homogeneous with the formation of reticulin fibers, which acquire a «wool-like» structure. Reticulin fibers thin out, become more wavy and, intertwined, form a thick, small-hole meshwork.

In muscovy ducks of 90 days of age, histological changes of the parenchyma of LNs are characterized by the formation of separate structural and functional zones: interfollicular cortex with paracortical area, lymphatic follicles with and without germinal centers and medullary cords.

The interfollicular cortex is formed in the central part of the nodes, at the site of the central lymph sinus, in the form of a thin strip that repeats its contours and extends into the thickness of the parenchyma of LNs, forming the periphery of the intermediate lymph sinuses of the cortical substance. Lymphatic follicles are formed on the basis of the interfollicular cortex, they are localized both on the border of the rudiment of the central lymph sinus and in the «thickness» of the lymphoid parenchyma. The paracortex circuits in LNs of ducks have the form of a chain consisting of separate rounded deep cortical units (DCUs) located around the rudiment of the central lymph sinus, separated from it by the intermediate lymph sinuses of the cortical substance and sections of the interfollicular cortex with the lymphatic follicles. From the eccentric lymph sinus, DCUs are separated by medullary cords, which are, respectively, located at the periphery of the nodes, and the intermediate sinuses of medullary substance.

Starting from 90 days of age of muscovy ducks, the structural and functional zones of the parenchyma of LNs form identical histoarchitectural structural and functional units, or compartments, that are not clearly separated from each other, due to the complex relief of the rudiment of the central lymph sinus and relatively underdeveloped intermediate sinuses of cortical and medullary substances, causing the segments of the parenchyma to have a multi-level localization pattern in relation to the central axis of the node. The basis of each compartment of LNs in muscovy ducks is a roundish area of the paracortex, or a deep cortical unit, which is covered by «cortex» in the form of interfollicular cortex with an intersection of the

lymph nodes, from the rudiment of the central lymph sinus and intermediate sinuses of the cortical substance; from the external (eccentric) lymph sinus it is covered by a complex of medullary cords with intermediate sinuses of medullary substance.

The total capacity of intranode lymphatic bed of LNs of muscovy ducks of 90 days of age, compared to the previous period, is significantly increased (up to $20.70 \pm 3.17\%$), due to the increase in PA of all the lymph sinuses without exceptions. Along with the increase of PA of lymph sinuses, there is an increase in the number of intermediate sinuses of the cortical substance, against the background of the decrease in most of them the width and length of their lumen.

Since 90 days of age, each structural and functional zone of the parenchyma of LNs has a characteristic architectonics of the reticulate acontoid.

In the area of follicular lymphoid tissue, the structure of the reticulate acontoid depends on the presence of germinal centers in the lymphatic follicles. In lymphatic follicles without germinal centers, the reticulin fibers are relatively thick with homogeneous histoarchitecture form uniform medium-hole meshworks. In lymphatic follicles with germinal centers, the structure of the reticulate acontoid is more sparse and the number of reticulin fibers is smaller. The meshworks of reticulin fibers have large holes, which are mainly transversely in the central area and longitudinally in the mantle. On the periphery of the lymphatic follicles with the germinal centers, the formation of a characteristic seal of the reticulate acontoid, that is the formation of «reticular boxes», is noted, but in LNs of ducks of 90 days of age they are weakly expressed.

The architectonics of the reticulate acontoid of medullary cords of LNs of muscovy ducks of 90 days of age is characterized by a homogeneous «wool-like» architectonics of the reticular fibers, which are relatively thin and, anastomosing with each other, form a dense meshwork of reticulin fibers with narrow small-hole cells.

Up to 180 days of age in LNs of muscovy ducks, among the structural and functional zones, the most developed zone is the zone of medullary cords, which in this period increases to $28.05 \pm 0.51\%$ against the background of a slight decrease in PA of interfollicular cortex and paracortical zone (up to $17.91 \pm 0.80\%$) and a significant decrease in PA of follicular lymphoid tissue. It is noteworthy that the decrease in the number of fol-

licular LT occurs primarily by reducing their absolute size by 1.90% and 1.56%. PA of lymphatic follicles with germinal centers thus also decreases to $13.07 \pm 0.80\%$, and in lymphatic follicles without germinal centers it increases on the contrary to $6.94 \pm 0.40\%$. It is characteristic that an increase in the number of lymphatic follicles without germinal centers is noted in the course of the intermediate lymph sinuses of the cortical substance, which are located closer to the central part of the organs, the relative number of which also increases during this period.

Changes in intranode lymphatic bed in LNs of muscovy ducks of 180 days of age are not characterized by a significant increase in its total capacity, only by 0.57%, due to an increase in PA of eccentric lymph sinus (up to $7.57 \pm 0.30\%$) and in PA of intermediate lymph sinuses of cortical substances (up to $6.80 \pm 0.8\%$). PA of intermediate lymph sinuses of medullary substance during this period remains almost unchanged.

The histoarchitecture of the reticulate acotoid of LNs of muscovy ducks of 180 days of age does not change significantly. There is only a slight thickening of the reticulin fibers in zone of medullary cortices, which are located closer to eccentric lymph sinus.

Structural and functional transformations of the parenchyma of LNs of ducks of 240 days of age are associated with a further decrease in PA of nodular lymphoid tissue, but in this period, the decrease occurs as a result of a decrease in PA of lymphatic follicles without germinal centers and lymphatic follicles with germinal centers up to $7.97 \pm 23\%$ and $3.58 \pm 0.24\%$ respectively, and by reducing their absolute size by 6.48% and 0.42% respectively. PA of interfollicular cortex with paracortical zone remains almost unchanged against the background of an increase in PA of medullary cortex, resulting in corresponding figures of 30.33% – 30.83%, etc.

No significant changes in the architectonics of the reticulate acotoid of parenchyma in LNs of muscovy ducks of 240 days of age are detected. Only further thinning and loss of flexuosity of the reticulin fibers are observed in the bright centers of the lymphatic follicles with the germinal centers, and the decay of reticulin fibers into small fragments in some of them.

The tendency to decrease the total capacity of intranode lymphatic bed due to the decrease in PA of all lymph sinuses remains, but there is a decrease in the thickness and length of the lumen of lymph sinuses, as well as a decrease in their relative capacity.

In general, for the whole study period, from the moment of birth to the onset of puberty, the parenchyma of LNs of muscovy ducks is characterized by varying degrees of manifestation of the morphological features of zonal specialization, which increases with age in parallel with the development and transformation of the system of intranode lymph sinuses.

Transformation of the system of intranode lymphatic bed of LNs of muscovy ducks is primarily due to the reduction of the central lymph sinus against the formation of intermediate cortical and medullary sinuses, and the dynamics of its total capacity is closely related to the process of formation in parenchyma of LN of individual structural and functional zones.

It is noteworthy that in the period from birth to ducklings of 20 days of age, lymphatic bed of LNs is represented only by two lymph sinuses: accentric and central, the dynamics of which is characterized by a gradual decrease in PA of central lymph sinus on the background of an increase in the corresponding index of accentric lymph sinus.

Up to ducklings of 30 days of age, there is a complete reduction of the central lymph sinus in LNs with the simultaneous formation of intermediate cortical and medullary sinuses, and on the background of an increase of PA of accentric lymph sinus, resulting in a significant increase in the total capacity of the intranode lymphatic bed.

During the period up to ducks of 90 days of age, as the signs of zonal specialization of parenchyma of LNs are formed, a relative stabilization of the parameters of PA of lymph sinus is observed, followed by a decrease in the total capacity of intranode lymphatic bed of LNs of ducks of 150-180 days of age due to the intensive development on the basis of interfollicular cortex of lymphatic follicles.

Before puberty, there is a significant reduction in the total capacity of intranode lymphatic bed in LNs of ducks of 210-240 days of age, mainly due to the decrease in the intermediate cortical and medullary sinuses.

Morphological features of structural and functional specialization of the parenchyma of LNs are detected at first in ducklings of 20-25 days of age, against the background of intensive formation of the system of intermediate medullary and cortical sinuses.

It is noteworthy that during the first three months of life the companions of the parenchyma of the ducklings consist exclusively of «diffusive» structural and functional zones: cortical and medullary substances.

Structural and functional transformations of the compartments of LNs of muscovy ducks during early postnatal ontogenesis are primarily due to the formation of lymphatic follicles based on the interfollicular cortex, with their predominant location on the lateral surfaces of DCUs by the rudiment of central lymph sinus, with gradual enlargement of PA and absolute sizes of the lymphatic follicles and their «infusion» along the intermediate cortical sinuses in the direction of accentric lymph sinus, encompassing the entire surface of DCU, with the subsequent development of lymphatic follicles with germinal centers mainly, also on the basis of medullary cords.

Lymphatic follicles both with and without germinal centers in the composition of the compartments of parenchyma of ducks are detected at first at 90 days of age, their formation occurs on the basis of interfollicular cortex on the lateral surfaces of DCU. Intensive development of the lymphatic follicles with their infusion along the intermediate cortical sinuses in the direction from the central to the peripheral lymphatic basins, with simultaneous coverage of DCU on all sides is observed in LNs of ducks of 120 days of age, and the maximum development of lymphatic follicles and formation of them on the basis of interfollicular cortex and medullary cords is noted in LN of muscovy ducks of 150-180 days of age.

The absolute sizes of the lymphatic follicles depend on the presence of germinal centers in them, in this regard, the larger diameter is in the lymphatic follicles with germinal centers. Generally, the minimum absolute sizes of lymphatic follicles, both with and without germinal centers, are observed in LNs of 90-days of age birds, which increase to 150 days of age, acquiring maximum values for the entire period of study. From 180 days of age to puberty, the diameter of lymphatic follicles decreases, and at 240 days of age compared to 90 days of age they have almost the same rates.

Thus, the definitive histoarchitecture of the compartments of parenchyma of LNs of muscovy ducks is a set of relatively spherical large «diffusive» deep cortical units, surrounded by lymphatic follicles of smaller diameter, most of which have germinal centers, located between the rudimental central sinus and peripheral lymph basin from which the above-mentioned formations are separated by a narrow strip of medullary cords.

Histological changes in the architectonics of the reticulate acaninoid of parenchyma of LNs of muscovy ducks are also interrelated with the development of the morphological features and structural and functional spe-

cialization and are characterized by the gradual formation of reticulin fibers meshworks characteristic of each individual functional zone of LNs.

In this regard, the formation of bundles of reticulin fibers on the periphery of LNs and uneven small-hole meshworks of the reticulin fibers in the central area of the organs is observed in neonatal ducklings. Beginning at the age of 5 days, the architectonics of the reticulate accontoid of parenchyma of LNs is characterized by the formation of irregular reticulin fibers meshwork in both the central and peripheral zones of the nodes. With aging, during the first three months of life, when parenchyma of LNs is represented only by «diffusive» cortical and medullary substances, reticulin fibers at the periphery of the nodes are slightly tightened and characterized by a thinner structure, and the reticulin fibers, which are located closer to the center, thicken, slightly desaturate, and become more flexuose. It is characteristic that most fibers that had a direction relative to the central lymph sinus, as it is reduced, and from the ducklings of 30 days of age observed their chaotic location in both diffuse zones of parenchyma of LNs.

From the moment of formation of separate structural and functional zones of the parenchyma of LNs, the reticulate accontoid of interfollicular cortex with the paracortical zone is characterized by a «honeycomb» structure, «wool-like» in the medullary cords, and the structure of reticulin fibers of the follicular LT is somewhat similar to the structure of interfollicular cortex and paracortical zone, but in lymphatic follicles without the germinal centers, the argyrophilic fibers are thinner and more elongated, and in the lymphatic follicles with the germinal centers the formation of characteristic reticular «boxes» is observed. With aging, thickening of the reticulin fibers with the simultaneous thinning of the meshworks formed by them are observed in all structural and functional zones, especially in the zones of medullary cords, and vice versa, thinning, but also by the desaturation of the reticulin fibers meshworks in the germinal centers of lymphatic follicles, are revealed.

5. Analysis of the results of experimental studies

The data obtained by us show that age-related transformations of parenchyma of LNs in birds, including its zonal specialization, are directly related to the process of development and reduction of the lymph sinus system and, above all, the central sinus, which determines the mechanism of entry

and distribution of nodes of antigens and other foreign components of the lymph within the lymphoid tissue.

The results of the analysis of the peculiarities of the morpho-functional status of the parenchyma of LNs of neonatal ducklings indicate a relatively low level of development and specialization in comparison with mammals, which according to some researchers may indicate a lower level of development of the factors of immunologic reactivity in the bird during the postnatal adaptation.

It should also be noted that the lymph nodes in muscovy ducks are «intra-vascular» uptakes of lymphoid tissue, which according to some authors is a characteristic feature of the structure of these organs in the representatives of the class of birds in general. As a result, unlike mammals, there are virtually no capsular trabecules in LNs in birds, and due to the fact that the number of lymphatic vessels is often limited to one afferent and, accordingly, efferent vessel, between which, within the parenchyma of the node, central lymph sinus is located, characteristic subdivision of the lymphoid parenchyma for cortical and medullary substance is also absent.

When examining the literature on the presence, number and topography of LNs in birds, we have found that the data on these issues are quite controversial, but there is a common belief among many authors that lymph nodes are found only in waterfowl birds, namely, geese, ducks, swans, teals and pintails, which are represented by two pairs of cervical, thoracic, or cervicothoracic and lumbar [5; 16; 18].

In our studies it is proved that muscovy ducks show a clearly formed pair of cervicothoracic lymph nodes localized near the thyroid, which confirms the data of V.A. Florensov [24] according to only the location of LNs in the cervical area, but when macroscopic studies of the kidneys were performed, after a pre-injection of a gelatin-based dispersion of black ink to lymphatic vessels in the lumbar region, we could assume the existence of the lumbar nodes in muscovy ducks, but they were not morphologically formed, in the form of a diffusive lymphoid tissue uptake among the kidney tissue. Also, in our work, the fact of the existence of more than one lymph node on one side of the neck in muscovy ducks is not confirmed, as it is noted in the works of Melnyk V.V. [23], revealing two lymph nodes connected by a constriction in geese, mainly on the left side of the neck, and in ducks, which is noted in the works of Berezina E.A. [5], but according to

the data of E.A. Berezina, unlike the data of Melnyk V.V., two lymph nodes are isolated from each other, and one of them is smaller.

It should be noted that the study of morphogenesis of organs of hematopoiesis and immune protection in mammals and birds, the morpho-functional status of which, first of all, determines the state of natural resistance of nonspecific and immunologic reactivity of the organism, is impossible without taking into account the morphological aspects of their growth and development, particularly, without the features of their dynamics and morphometric indicators.

To date, it is known that the most pronounced structural transformations of the parenchyma of peripheral lymphoid organs in mammals and birds, which are associated with the functions of formation of immunologic reactivity, occur in the early stages of postnatal ontogenesis [17; 21; 22; 27], but in more detail, morphological aspects development of immunologic reactivity among vertebrates has been studied in mammals [2; 4; 7; 14; 15].

Thus, according to our data, the general pattern of quantitative correlation of the two main tissue components, connective-tissue stroma and lymphoid parenchyma of the cervicothoracic LNs of muscovy ducks is a tendency to increase PA of the lymphoid parenchyma against the background of a decrease in the corresponding index of connective-tissue stroma. But it is worth noting that in the period of early postnatal adaptation, namely, up to ducks of 30 days of age of the dynamics of parenchymal and stromal correlation of LNs of ducklings is characterized by full periodicity. The intense growth of PA of lymphoid parenchyma in the background of decreased stroma of LNs occurs in the first 10 days of life of ducklings. In the period of up to 20 days of age, there is an inverse trend of changes in the parenchymal and stromal correlation: an increase of PA of the connective-tissue stroma on the background of a decrease in the amount of lymphoid parenchyma. In the period of up to 30 days of age, there is a decrease in both the lymphoid parenchyma and the connective-tissue stroma.

It should be noted that the quantitative changes of the two main tissue components of LNs of muscovy ducks are associated with low qualitative transformations of the parenchyma of these organs, and the frequency of the dynamics of the parenchymal and stromal correlation, probably related to the process of formation of separate structural and functional zones of

parenchyma of the lymph nodes and with features, during this period, qualitative and quantitative changes in the intranode lymphatic bed.

Thus, in the period from birth to 10 days of age, as it was noted earlier, in LNs of ducklings there is an increase in the amount of lymphoid parenchyma, which in our opinion is directly related to its qualitative transformations, namely the formation of two diffusive zones: cortical and medullary substances. During the period of characteristic decrease in the lymphoid parenchyma of the lymph nodes, from 20 to 30 days of age ducklings, there is a significant restructuring of the intranode lymphatic bed, which is primarily associated with the reduction of the central lymph sinus and the formation of intermediate cortical and medullary sinuses.

In muscovy ducks of 90 days of age of, as well as in ducklings of 10 days of age, there is an intense increase in PA of lymphoid parenchyma, which in our opinion is a reflection of quantitative changes in the tissue components of LN against the qualitative transformations of the parenchyma of these organs, namely the formation in this period of complex of immunocompetence, that is, individual structural and functional areas including lymphatic follicles with germinal centers.

It should be noted that the analysis of the results of our studies on the quantitative and qualitative transformation of the parenchyma of the cervico-thoracic LNs of muscovy ducks shows that significant changes in the parenchyma of these organs occur in the first months of life, before the onset of puberty and physiological maturity of ducks, which is a general biological biofeature of development of lymphoid organs in vertebrates, but still at a later date than in the corresponding mammalian organs [7; 11; 15; 17].

The peculiarities of postnatal morphogenesis of parenchymal and stromal correlation of LNs of muscovy ducks, established by us, may indicate that the leading role in immunity belongs to factors of nonspecific reactivity in combination with maternal humoral factors in the period of postnatal adaptation.

To date, it has been finally established that the histo- and cytoarchitectonics of mammalian lymphoid parenchyma are determined by the peculiarities of the structure of the intranode circulatory and lymphatic microcirculatory beds and, accordingly, by the nature of hemo- and lymphodynamics of the organs [7; 21]. According to the results of immunocytochemical studies, the basic provisions on the regularities of formation of zonal architectonics of lymphoid tissue of LNs of mammals are formulated, depending on the

specific relationship of parenchyma of organs with extra- and intra-organ vessels and lymph sinuses, the main of which are the following: provision on «concentration» of the main cellular zones in the direction of afferent lymphatic vessels; a discrete structure of the deep cortex of the nodes, similar to the segmentation of the intranode arteries and veins and sections of their microcirculatory bed; exclusively parasinusoidal localization of lymphatic follicles [28]. In the works of P.M. Havrylin, N.M. Tishkina and M.O. Leshchova [13–15] it is pointed out to the end-to-end type of localization of lymphatic follicles within segments of mammalian parenchyma of LNs, as well as the mosaic character of the histoarchitecture.

The results obtained indicate that the parenchyma of LNs in muscovy ducks also has a zonal structure, but the architectonics of the intranode lymphatic bed, as well as the nature of the localization of the structural and functional zones of their parenchyma have a number of features unlike the corresponding organs of mammals.

Aspects of the structural and functional organization of the parenchyma and the intranode lymphatic bed of LNs in birds have been investigated to a much lesser extent than in mammals, and are very controversial. According to V.V. Melnyk [23], histoarchitecture of the parenchyma of LNs in geese and ducks is generally similar to that of mammals, with the concentration of cortical substance at the periphery of the nodes under their capsule. In the works of other authors, the leading role in the intranode lymphodynamics of the so-called internal or central sinus is emphasized, it is also indicated that the cortical substance with lymph nodes «concentrates» along the central sinus, and medullary cords are close to the capsule of the node [5; 16].

In general, in most works it is emphasized that the parenchyma of LNs of birds has a somewhat primitive structure in comparison with the corresponding organs in mammals, and therefore the authors deny the existence of a distribution of the parenchyma of these organs into separate structural and functional zones [5; 16; 18]. The objection may be due to the fact that the structural and functional zones in the parenchyma of LNs in birds are located on a different principle than in mammals, and most likely due to another principle of lymphodynamics, namely the presence of the central sinus, its reduction, which causes more intensive passage of lymph through the center of the nodes.

As a result of our research, it has been established that lymph nodes of muscovy ducks represent a local uptake of the lymphoid parenchyma in the

lumen of the lymphatic vessel, as a result of which they typically have one afferent and, accordingly, one efferent lymphatic vessel. The walls of the lymphatic vessel at the site of the lymphoid uptakes are thickened by the development of loose fibrous connective tissue on the basis of their external and middle membranes, which is confirmed in the works of Melnyk V.V. and Khomych V.T. [21; 23]. The capsular trabecules in the cervicothoracic LNs of muscovy ducks are not numerous, short, poorly developed, which extend only to the «surface», close to the capsule of the node, «layers» of the parenchyma.

During the studies of intra-node lymphatic spaces, contrasted with black ink, it was found that the lymphatic bed of cervicothoracic LNs in ducks consists of two parts: internal and external lymphatic basins, which are connected by intermediate lymph sinuses.

The internal lymphatic basin in LNs in ducks is represented by the so-called central sinus, which is a continuation in the parenchyma of the lumen node of the lymphatic vessel, on the basis of which the lymph nodes develop. It should be noted that the vascular form of the central sinus is only found in ducklings of 1-25 days of age. With aging, the parenchyma of the node stops in its lumen, resulting in it sharply narrows and takes the form of a flexuose gap, which is confirmed in the works of Berezina E.A. [5; 6].

The external lymphatic basin in the cervicothoracic LNs in ducks is a lymphatic gap located between the capsule node and its lymphoid parenchyma, similar to the eccentric (portal) sinuses of mammalian LNs [24]. The external lymphatic basin directly communicates with the lumens of afferent and efferent lymphatic vessels at their inlet and outlet sites, and is mediated via the intermediate lymph sinuses with the central sinus cavity.

6. Conclusions

The cervicothoracic LNs of muscovy ducks is an uptake of lymphoid tissue in the lumen of a lymphatic vessel between two lymphatic basins: the inner (central sinus or its rudiment) and the external (discrete eccentric sinus), which are connected by numerous intermediate sinuses.

The lymphoid parenchyma of LNs of the muscovy ducks has a pronounced zonal structure with the concentration of its main medullary zones around the central sinus, resulting in the cortical substance located inside the nodes, and the medullary substance is located at their periphery under the capsule, along the external lymph basin. The definitive histoarchitec-

ture of the parenchyma of LNs of the muscovy ducks is characterized by a spherical spatial configuration of its main structural and functional zones (deep cortical units and lymphatic follicles) with a mosaic orientation principle. As a result, the lymphoid tissue of the nodes is divided into several identical compartments consisting of deep cortical units surrounded on all sides by an interfollicular cortex and numerous lymphatic follicles.

The formation of the complex of morphological features in LNs in muscovy ducks, which indicates the maturity of their parenchyma in relation to the function of immunologic reactivity, occurs during the first three months of postnatal ontogenesis. In the first stage (up to 25 days of age), the cortical and medullary substances of the nodes are formed, in the second (up to 60 days of age) there are diffusive structural and functional zones of the cortical and medullary substances (deep cortical units, medullary cords), in the third (up to 90 days of age) there are lymphatic follicles, the quantitative indicators of which continue to rise to 150 days of age. In general, the maximum relative area of medullary cords is observed in ducks of 60 days of age, interfollicular cortex with deep cord is in 90 days of age, lymphatic follicles are in 150 days of age.

The dynamics of the relative capacity of the intranode lymphatic bed is periodic. The first period (from birth up to 30 days of age) is the period of intensive growth of the capacity of the lymphatic bed, which is accompanied by a gradual reduction of the central sinus and compensatory development of cortical and medullary intermediate sinuses. The second period (up to 180 days of age) is the period of relative stabilization of the corresponding indicator with a tendency to decrease, mainly due to cortical sinuses due to the formation in the parenchyma of nodes of numerical lymphatic follicles. The third period (up to 240 days of age) is the period of marked decrease in the capacity of the lymphatic bed due to the reduction of the area of both cortical and medullary sinuses.

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