

The Evolution and Structural Organization of the Organs of Vertebrate Immune System

Sergey B. Seleznev¹, Evgeny V. Kulikov¹, Galina A. Vetoshkina²,
Yury A. Vatnikov¹, Elena D. Sotnikova¹, Elena A. Krotova¹, Sergey A. Yagnikov¹,
Marina N. Yakunina³

¹Department of Veterinary Medicine, Peoples' Friendship University of Russia (RUDN University), 6, Miklukho-Maklaya Street, Moscow - 117198, Russian Federation, ²Department of Animal Anatomy, Moscow State Academy of Veterinary Medicine and Biotechnology Named K.I. Skryabin, 23, Academician Skryabin Street, Moscow - 109472, Russian Federation, ³Veterinary Clinic "Biocontrol", 24/10, Kashirskoye Shosse, Moscow - 115478, Russian Federation

Abstract

Aim: This study aimed to define the main ways of evolution of the organs of the immune system in vertebrate animals that include the segregation, i.e., the separation of the organs of immune system from the hematopoietic system, on the basis of which they were formed; differentiation, i.e., the division of initially integrated lymphoid structures into diffuse clumps, lymphoid nodules, and the germinal centers; transformation, i.e., the transfiguration of rudimentary organs into organs of the immune system; and polymerization, i.e., the increase in the number of peripheral organs of immunogenesis in phylogenesis. **Materials and Methods:** This is a comparative analysis of the morphology of the organs of immune system in those orders of birds and mammals which have passed in their development in different periods of time in terms of the fossil record. Statistical processing of the obtained digital data was conducted by individually tailored programs. **Results and Conclusions:** Conducted research demonstrates the historical trend of immune system in terms of morphological progress, which is manifested in the alternation of relatively fast flowing aromorphoses with longer periods of idioadaptations.

Key words: Differentiation, evolutionary morphology of the organs, immune system of animals and birds, polymerization, segregation, transformation

INTRODUCTION

The immune system, formed on the basis of the hematopoietic system, has "absorbed all the achievements" of the evolution of multicellular organisms, and along with metabolic, endocrine, and conditioned reflex forms, created the fourth form of the reflection of the living matter and the fourth regulating system of vertebrates.^[1,2] It is represented by the organs, which provide protection of the organism against genetically foreign cells or structures. However, their structural organization, as a rule, was usually considered as part of other body systems, and therefore the thymus was attributed to the internal secretion glands; tonsils, Peyer's patches, diverticulum, and the appendix were relegated to the digestive system; gland of the third eyelid to accessory organs of an eye; while lymphatic nodes to the lymphatic system. Thus, all these indicated organs have not been studied

as the elements of a single body's immune system, which "not only (provides) a pass to the external world, but also guarantees against internal change."^[3] However, a number of issues in the morphology of the immune system still remain unresolved and speculative. First of all, this concerns the historical and comparative morphological aspects of the study of the concerned system, that is, those system directions, which undeservedly are beyond the proper attention of researchers.^[4-6]

It is known that evolutionary morphology, using the method of triple parallelism in combination with historic and systemic

Address for correspondence:

Elena D. Sotnikova, RUDN University,
6, Miklukho-Maklaya Street, Moscow - 117198, Russian
Federation. E-mail: sotnikova_ed@rudn.university

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approaches, allows identifying the possible ways of evolution of animals' organs and tissues, as well as gives the opportunity to trace their transformation in the phylogenesis.^[7] It should be noted that the existing studies of the immune system are mostly fragmentary and often contradictory.

MATERIALS AND METHODS

The research scheme envisaged the study of the morphology of the organs of immune system in those orders of birds and mammals which have passed in their development of different periods of time in terms of the fossil record.

For the comparative analysis of the obtained results, the following representatives of the bird class were chosen: Fowl-like birds (*Galliformes*), where age of the discovered fossil ancestral form is about 50 million years; pigeons (*Columbiformes*), whose ancestral forms appeared about 40 million years ago; and anseriformes, which appeared about 30 million years ago. The mammal species were selected similarly: Gnawing animals (*Rodentia*), whose fossils of ancestral forms are found in the fossil record about 80 million years ago; predaceous (*Carnivora*), whose ancestral forms appeared about 60 million years ago; and double-toothed rodents (*Lagomorpha*), which originated about 45 million years ago.^[8]

The research targets included the following vertebrate animals: White leghorn chickens (*Gallus domesticus*), blue rock pigeons (*Columba livia*), Peking ducks (*Anas domesticus*), Wistar laboratory rats (*Rattus norvegicus*), domestic dogs (*Canis familiaris*), and rabbits (*Oryctolagus cuniculus*). These types of animals were obtained from vivarium of the Peoples' Friendship University of Russia, as well as from the state and private farms of the Moscow Region, unharmed in terms of infectious and invasive diseases.

The research material included central (thymus, cloacal bursa) and peripheral (gland of the third eyelid, palatine tonsils, ileum and cecal lymphoid patches, lymphoid diverticulum, appendix, spleen, cervicothoracic and mandibular lymph nodes) organs of the immune system, obtained from clinically healthy species.

To study the age variability of the organs of the immune system and to compare the obtained results between different types of animals, material was sampled at certain stages of postnatal (postembryonic) ontogeny: Neonatal, juvenile (preweaning period), puberty, morphological and functional maturity, and gerontology periods of development.

Each of these stages is characterized by certain specific features and has a different duration in animals. Therefore, to increase the objectivity of the research results, the material was sampled, as a rule, in the middle of a certain stage of postnatal (postembryonic) ontogenesis in the amount of five specimens for each age group of animals.

To solve the set tasks, we used the range of macro- and micro-morphology and immunology methods, including macro- and micro-preparation followed by a characterization; a macroscopic morphometry taking into account weight as well as linear indicators and calculation on their basis of organs' cross-sectional areas; light microscopy with subsequent stereometric analysis of the structures studied; histochemical studies to identify the localization of glycoproteins and lipids; immunological methodologies for the determination of bactericidal and lysozyme activities of blood serum.

Statistical processing of the obtained digital data was conducted by individually tailored programs employing a personal computer.

RESULTS AND DISCUSSION

A comparative approach to the organization of the immune system in animal species of different classes of vertebrates showed that this system develops on the basis of the hematopoiesis system. Therefore, the myxines mostly reveal only hematopoietic organs ("bone marrow" and spleen). As for the more highly organized stone eels, they are the first organisms in the phylogenetic series, who are characterized by the emergence of the central organ of immune system, namely thymus.^[9] The segregation (from Latin segregation - separation) of the immune system from hematopoiesis starts exactly from this time point onward. At that time, in every class, this trend is manifested in different ways. In fish, the parenchyma of the spleen exudes white (lymphoid) pulp, the bone marrow of amphibians generates lymphoid stem cells, while in reptiles, thymus serves the organ for proliferation and maturation of T-lymphocytes.^[1]

A second central organ of immune system, namely cloacal bursa (*Fabriciana*), in which population of B-lymphocytes is differentiated from stem cells, appears only in birds. Cloacal bursa is a luminal organ, which has a few longitudinal folds in it containing 1-2 rows of lymphoid cells, whose parenchyma is differentiated into three layers: Cortical, boundary, and medullary layers, which is the exit site of mature lymphocytes. According to our data, the relative area occupied by the cortical layer reaches maximal values in the juvenile period (chickens – 39.2 + 1.9%, pigeons – 30.2 + 2.4%, and ducks – 33.1 + 5.5%) and is reflected in the increased bactericidal activity of blood serum during this period by 5-10%. As for the medullary layer, its relative area in the histological sections reaches maximum by the age of puberty (chickens – 29.5 + 1.9%, pigeons – 34.3 + 1.4%, and ducks – 28.3 + 1.8%). This is associated, primarily, with the age involution of the cloacal bursa [Figures 1 and 2].

Thanks to cloacal bursa and also the fact that red marrow of birds is not involved in lymphization, birds' central link of the immune system completely separated from the

hematogenesis. Similar trend is evident also with regard to the spleen of birds, whose parenchyma is differentiated into the red pulp, white pulp, and marginal (border) zone.

According to our data, the red pulp in the histological sections of the birds' spleen has a maximum only during the neonatal period (chickens – 52.9 + 2.2%, pigeons – 47.8 + 3.1%, ducks – 53.3 + 5.3%), while by the period of morphological and functional maturity, it reduces by 2.1 folds (chickens – 22.3 + 2.0%, pigeons – 29.4 + 2.0%, and ducks – 20.5 + 2.4%).

As for the white pulp composed of lymphadenoids, in the neonatal period, it has a minimum value (chickens – 16.0 + 2.6%, pigeons – 16.5 + 2.3%, and ducks – 13.4 + 2.7%) and reaches a maximum by the period of maturity, increasing by 3.1 folds (chickens – 41.7 + 0.8%, pigeons – 43.1 + 1.7%, and ducks – 53.8 + 3.2%). Thus, the birds' spleen in postembryonic ontogeny is characterized by a decrease in the relative areas occupied by the red pulp due

to the development of lymphoid elements in the white pulp [Figures 3 and 4].

In this case, we can assume that there is a reduction in the number of functions in the birds' spleen that according to Severtsov can be seen as an evolutionary transformation of the organ toward the segregation of the immune system from hematopoiesis.^[7]

Unlike birds, mammals still have the organs of the immune system, which retain “mixed structures” involving both lympho- and myelo-poiesis. A typical example is red bone marrow of mammals, which is not the only organ involved in hematogenesis, but also the organ of the immune system because B-lymphocytes' population matures and differentiates exactly here. As shown by our studies, its relative weight in mammals (3.5-5.0%) is almost 2 times more than that in birds (1.7-2.5%). Similar trend is noted with regard to the mammal spleen. According to our data, the red pulp of the mammal spleen, with a maximum value

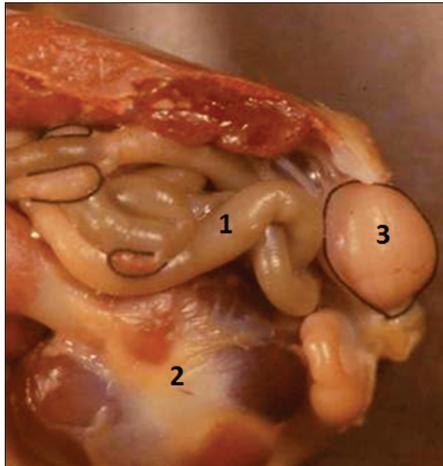


Figure 1: Chicken cloacal bursae: 1 - intestines; 2 - muscular section of the stomach; 3 - cloacal bursae

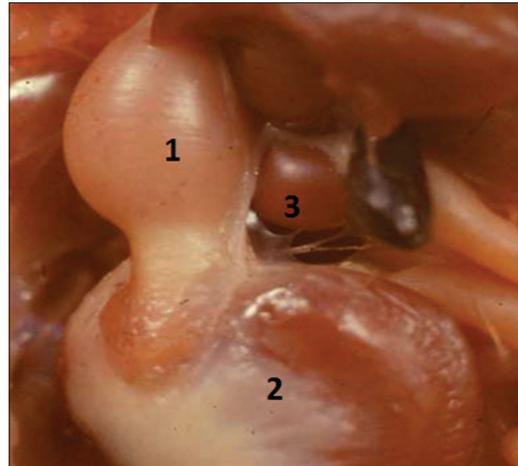


Figure 3: Chicken spleen: 1 - glandular section of the stomach; 2 - muscular section of the stomach; 3 - spleen



Figure 2: Micromorphology of the chicken cloacal bursae. Hematoxylin and eosin, 7 × 10: 1 - lymphoid cells; 2 - bursae wall; 3 - lumina

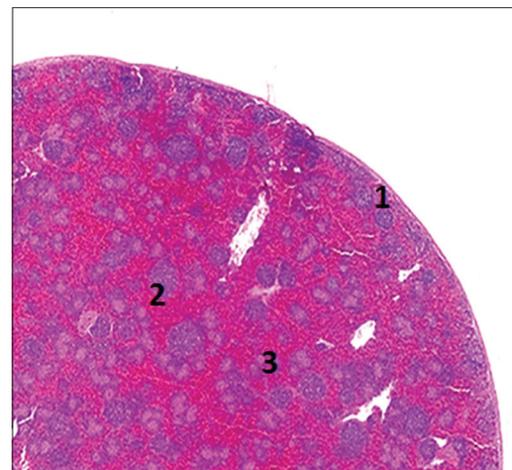


Figure 4: Micromorphology of the chicken spleen. Hematoxylin and eosin, 7 × 10: 1 - capsule; 2 - white pulp; 3 - red pulp

in neonatal period (rats – 32.4 + 2.5%, dogs – 35.9 + 1.9%, and rabbits – 48.3 + 2.9%), unlike birds, is not reduced further and remains at the same level (rats – 30.0 + 1.4%, dogs – 30.1 + 2.7%, and rabbits – 40.0 + 1.2%). White pulp of the mammal spleen, like in birds, has minimal value in neonatal period (rats – 17.7 + 4.2%, dogs – 13.3 + 2.7%, and rabbits – 14.9 + 1.6%) and reaches maximum for the period of morphological and functional maturity of the body, increasing by 3.3 times (rats – 55.5 + 2.8%, dogs – 49.7 + 1.8%, and rabbits – 47.0 + 1.9%). Thus, the spleen in mammals in postnatal ontogeny, similarly as the red bone marrow, is not only an organ of the immune system but also the organ of hematopoiesis that is consistent with the data of other researchers.^[1] Summing up, it should be noted that the immune system of mammals, unlike birds, yet is not fully separated from the hematopoietic system as it incorporates red bone marrow and spleen.

Differentiation (from Latin *differentia*) processes occur simultaneously with the segregation of the immune system. These processes primarily affect the lymphoid parenchyma of organs of immunogenesis. As a consequence, lymphadenoids, originally unified, are divided into more or less separate related parts; this makes the immune system more effective because it leads to the specialization of its specific parts.^[9]

Lymphadenoids in the lamprey are found only in the form of diffuse clumps in gills' area (rudiment of the thymus) as well as in the pronephros, the intestines, and the spleen. As for fish and amphibians, their thymus acquires the features of a compact body and their parenchyma is differentiated into cortical and medullary zones, while peripheral lymphadenoids, represented by diffuse clumps, are situated focally in the throat, intestines, spleen, and lymphatics. Further progress in the differentiation of lymphadenoids is associated with the class of reptiles. In cortical area of their thymus, except of lymphoid elements, epithelial cells are found, while in brain area, macrophages, eosinophils, and monocytes are found. As for the lymphadenoids of peripheral organs, they manifest lymphoid nodules at antigenic stimulation.^[1,3]

The parenchyma of the thymus in birds and mammals, unlike reptiles, is differentiated into four layers. Two layers are isolated in the cortical zone: Subcapsular and cortical layers, and the other two layers in brain, namely, medullary and perivascular spaces, which are the ultimate transport routes for thymus-derived lymphocytes. Proliferation of T-lymphocytes occurs in subcapsular layer, while their maturation occurs in cortical layer. The exit point of mature T-lymphocytes is medullary layer. According to our data, in the neonatal period, cortical layer is the one which is developed most intensively (chickens – 48.5 + 3.3%, pigeons 26.5 + 2.2%, ducks – 40.0 + 5.5%, rats – 54.0 + 3.0%, dogs 52.9 + 1.1%, and rabbits – 52.0 + 2.7%), while in juvenile (preweaning) period, subcapsular layer is developed most intensively (chickens – 14.2 + 2.6%, pigeons – 14.8 + 2.3%, ducks – 22.5



Figure 5: The chicken thymus lobules (1) in the neck area



Figure 6: Micromorphology of the chicken thymus lobule. Hematoxylin and eosin, 7 × 10: 1 - cortical zone; 2 - cerebral zone; 3 - trabeculae

+ 2.7%, rats – 10.8 + 0.6%, dogs – 9.9 + 0.4%, and rabbits – 21.7 + 2.2%). This is also reflected in the level of humoral factors of immunity and leads to increased bactericidal properties of blood serum in juvenile (preweaning) period by 5-10%. As for the medullary layer, the relative area occupied by it reaches its maximum by the period of morphological and functional maturity of the body (chickens – 43.6 + 2.8%, pigeons – 40.7 + 3.6%, ducks – 23.7 + 3.2%, rats – 30.0 + 2.2%, dogs – 17.7 + 4.2%, and rabbits – 35.1 + 1.7%) that is caused in the first place by age-related involution of the organ, similarly to that for cloacal bursae [Figures 5 and 6].

In addition, unique thymic cells, which are concentric clumps of elongated and spindle-shaped cells with a large nucleus and weak acidophilic cytoplasm, are detected in the medullary layer of thymus in birds and mammals. As shown by our study, the number of Hassall's bodies in the studied orders of birds and mammals increases with age. In the brain area of newborns, there are 2 to 5 thymic cells, while their number increases to 100-150 during gerontological period.

As for the peripheral organs of the immune system of birds and mammals, among them, the greatest interest is caused by lymph nodes that are located on the pathways of lymph and represents lymphadenoids, whose shape and structure are essentially different in birds and mammals. According to our data, in fowl-like birds (*G. domesticus*), which appeared about 50 million years ago, in the course of lymphatic vessels, only diffuse lymphadenoids are detected; in *Columbiformes* (*C. livia*), whose ancestors appeared about 40 million years ago, solitary lymphoid nodules are identified among the clumps; while in anseriformes (*Anas domesticus*), which appeared about 30 million years ago, a group of lymphoid nodules with germinal centers are surrounded by a connective tissue capsule. According to our research, each lymph node of the ducks includes just one major afferent lymphatic vessel and one efferent vessel. Lymphatic sinuses, lined with endothelium, exit into the lymph node from both vessels. The space between the sinuses is filled with lymphadenoids which are differentiated into diffuse clumps and nodules. A similar structure of the lymph nodes is presented in egg-laying mammals.^[8] As shown by our study, diffuse clumps of lymph nodes are found in the cervicothoracic lymph node of ducks during the neonatal period. At that time, their relative area equals to $13.2 \pm 2.4\%$. The formation of lymphoid nodules occurs in the juvenile period ($10.1 \pm 0.7\%$), while the emergence of proper germinal centers is noted in puberty and reaches maximum by the period of morphological and functional maturity of the body ($16.4 \pm 1.2\%$). Thus, cervicothoracic lymph nodes of ducks are characterized by certain formation stages: The appearance of diffuse lymphadenoids is noted in the neonatal period, lymphoid nodules in the juvenile period, and germinal centers, which reach maximum development by the period of maturity of the body in the puberty period.

In mammals, the lymph nodes reach their full development and control lymph flowing not only from the internal organs, but also from locomotor apparatus. The most detail attention was focused on the study of the morphology of the mandibular lymph nodes, which are located in the maxillary space under the skin. Each lymph node includes several incoming afferent lymphatic vessels and several outgoing efferent vessels, though the number of latter is usually lower. According to our data, diffuse clumps of lymph nodes are found in the mandibular lymph node in the neonatal period (rats – $35.0 \pm 2.7\%$, dogs $46.8 \pm 1.0\%$, and rabbits – $38.9 \pm 2.4\%$), lymphoid nodules in the preweaning period (rats – $15.5 \pm 2.1\%$, dogs $12.3 \pm 2.3\%$, and rabbits – $17.5 \pm 2.7\%$), while germinal centers reach maximum at puberty (rats – $30.9 \pm 1.5\%$, dogs – $28.4 \pm 1.9\%$, and rabbits – $20.1 \pm 3.6\%$). Thus, the mandibular lymph nodes of mammals, similarly as cervicothoracic lymph nodes of ducks, are characterized by certain formation stages.

The appearance of new organs and the disappearance of vestigial organs that have lost their functionality are among the most difficult morphological problems of evolution theory.^[7] New organs usually arise within already existing

bodies and body parts.^[9] In this case, considering the possible evolution ways of organs of immunogenesis, we can talk about the transformation (from the Latin transformation - transformation) of vestigial organs (gills, yolk-sac, etc.) in the organs of the immune system.

According to the embryological studies, the epithelial rudiment of the thymus develops from the third and fourth visceral furrow, while fate of a bursa of Fabricius from epithelial outgrowth of the cloaca, which in most mammals is subjected to reduction.^[1] As for the peripheral organs of the immune system, the tonsils develop from the second pair of visceral furrows, lymphoid diverticulum from the rudiment of the yolk sac, the appendix from the distal portion of the cecum,^[9] while Garder's gland from the third eyelid, which also undergoes reduction in part of the mammals. Among the noted organs, a lymphoid diverticulum of birds is of particular interest. The formation of its structural organization occurs in the neonatal period.

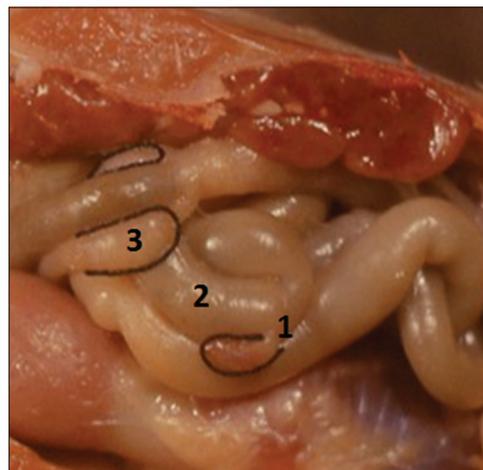


Figure 7: The lymphoid apparatus of the digestive tract: 1 - lymphoid diverticulum; 2 - empty intestine; 3 - cecal lymphoid plaque

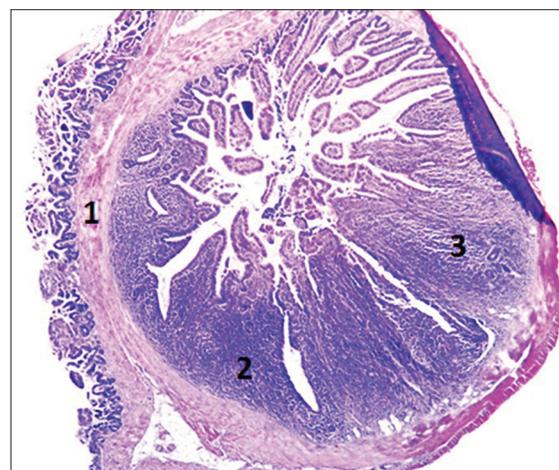


Figure 8: Micromorphology of lymphoid diverticulum. Hematoxylin and eosin, 7×10 : 1 - capsule; 2 - lymphoid nodules; 3 - diffuse clumps

In birds, lymphoid diverticulum (diverticulum of Mekele) is located almost in the middle of the empty intestine on the side opposite to the attachment of mesentery. It is an abdominal bag-shaped organ, which is connected with the intestine cavity through short duct. As shown by our experiments, it is found in fowl-like birds (*G. domesticus*), which appeared about 50 million years ago, and in anseriformes (*Anas domestica*), originated about 30 million years ago [Figures 7 and 8].

In the newborn chick (duckling), the diverticulum has no clear boundaries and is filled with yolk, which serves food for the chicken. In the course of the yolk consumption, it is reduced in size and by the end of the neonatal period takes a rounded shape with a length of 0.5-1.0 cm and a diameter of 0.1-0.3 cm. The wall of lymphoid diverticulum consists of mucous, muscular, and serous membranes. The mucous membrane is covered with a single layer of cylindrical epithelium, which forms numerous crypts while penetrating into the lymphoid structures. According to our data, the relative area occupied by the crypts is maximal in the juvenile period (chickens – 47.1 + 2.7% and ducks – 43.4 + 3.8%), while it is reduced by the maturity period by 2.3 times. As for the lymphadenoids, diffuse clumps are found in lymphoid diverticulum in the juvenile period (chickens – 16.2 + 2.6% and ducks – 13.7 + 1.9%), while the formation of lymphoid nodules reaches maximum at puberty, accounting for 13.9 + 2.1% in chickens and 16.7 + 3.2% in ducks. The emergence of germinal centers in the latter occurs in puberty and reaches maximum by the period of morphological and functional maturity of the body, accounting for 16.7 + 0.6% in chickens and 13.4 + 1.2% in ducks. Thus, the relative space occupied by lymphadenoids in lymphoid diverticulum of birds increases with age.

In mammals, lymphoid diverticulum is located in the atrium of the ileum into the cecum, though it is found only in double-toothed rodents (*O. cuniculus*) whose ancestral forms appeared about 45 million years ago. In the rabbit, it has an oval shape and a large cavity with an inlet opening, which is connected with the ileum, and outlet opening ingrown from above to the right wall of the cecum. The wall of lymphoid diverticulum consists of mucous, muscular, and serous membranes. The mucous membrane is covered with a single layer of cylindrical epithelium, which, penetrating into the lymphadenoids, forms hard-branched crypts. The relative area occupied by the crypts in histological sections of the lymphoid diverticulum has a maximum value in the neonatal period (34.1 + 2.2%), while it is reduced by 1.6 times (20.8 + 1.4%) in the maturity period. As for the lymphadenoids, diffuse clumps are found in lymphoid diverticulum in the neonatal period (14.1 + 1.2%), while formation of lymphoid nodules in them reaches maximum at puberty (21.4 + 2.3%). The emergence of the germinal centers in them occurs in the preweaning period and reaches maximum by the period of morphological and functional maturity of the body (26.3 + 2.3%).

Summing up the consideration of the structural organization of lymphoid diverticulum, it should be noted that this body organ is unique as it serves a vivid example of the organ's evolution. While in birds, during the neonatal period, it performs essentially a trophic function, further it starts functioning as a peripheral organ of the immune system, synthesizing immunoglobulins. In this case, we can assume that the organ changes its function that according to Severtsov can be qualified as its evolutionary transformation.^[7]

Differentiation and transformation in the immune system are accompanied by simultaneous polymerization of the organs (from the Greek polys - multitudinous and meristos - divisible), i.e., an increase in the phylogeny of the number of equivalent homologous formations in the organism. This principle, formulated by Dogel,^[10] provides a plurality of elements of the given biological system and increases the reliability of its operation due to interchangeable components [Figures 9 and 10].

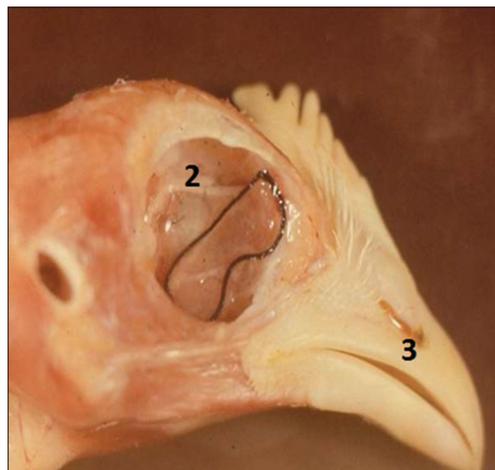


Figure 9: The gland of the third eyelid: 1 - the lobe of the gland of the third eyelid; 2 - orbit; 3 - the initial section of digestive and respiratory organs



Figure 10: Micromorphology of the gland of the third eyelid. Hematoxylin and eosin 7 × 10: 1 - capsule; 2 - lymphoid nodules; 3 - diffuse clumps

Polymerization is most characteristic to peripheral immune system. While peripheral part of the immune system in myxines is presented only by the lymphadenoids of the spleen, pronephros, and intestine, in fish and amphibians, lymph nodes appear along the lymphatic vessels, while in reptiles, tonsils are found in the pharynx area.^[1] As for birds and mammals, they are characterized by developed Peyer's patches, which are located on the border of small and large intestines. Besides, the birds have also gland of the third eyelid, while mammals have appendix.

CONCLUSION

The comparative analysis of organ and tissue structures of the immune system of vertebrates clearly demonstrates the historical trend of this system in terms of morphological progress, which is manifested in the alternation of relatively fast flowing aromorphoses (the appearance of the thymus gland in lampreys, cloacal bursae in birds, tonsils in reptiles, etc.), with longer periods of idioadaptations (formation of the lymph nodes' structure, as well as Peyer's patches in amphibians, reptiles, birds, and mammals).

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