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**AĞ SIÇOVULLARIN NAZİK BAĞIRSAQLARINDA OLAN PEYER DÜYÜNLƏRİ İLƏ
BÖYÜK PİYLIYIN SÜDLÜ LƏKƏLƏRİNİN OXŞARLIQ VƏ FƏRQLƏRİ****Yu.P.Kostilenko, V.H.Hrin, O.S.Maksimenko, V.P.Bilaş, N.L.Svintsiska,
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Xülasə. Məqalədə şərh edilən tədqiqat işinin məqsədi ağ siçovullarda nazik bağırsaqların Peyer düyünləri ilə böyük piyliyin südlü (ağ) ləkələrinin strukturunu tədqiq və müqayisə etmək məqsədilə aparılmış tədqiqat işi haqqında məlumat verilmişdir. Bundan ötrü eksperimental şəraitdə 20 baş erkək ağ siçovul üzərində tədqiqat aparılmışdır (siçovulların kütləsi 278,08 qramdan 346,47 qrama qədər olmuşdur). Tədqiqat materiallarını Peyer düyünləri vizuallaşdırılmış nazik bağısaq preparatları və böyük piyliyin total preparatları təşkil etmişdir. Preparatlar 10%-li formalin məhlulunda fiksasiya edilmiş və hematoksilin-eozinlə boyadılmışdır. Peyer düyünləri və böyük piyliyin südlü ləkələri ölçü baxımından bir-birinə uyğun deyil. Südlü ləkələr böyük piyliyin damar-piy qövsü üzrə yerləşmiş limfa düyünlərinin strukturuna uyğun gələn müəyyən miqdarda diskret formada yerləşmiş törəmələrdir. Peyer düyünləri südlü ləkələrdən fərqli olaraq topalar şəklində yerləşirlər. Bu baxımdan südlü ləkələr nazik bağırsağın yalnız limfoid vəziləri ilə müqayisə edilə bilər. Onların oxşar cəhətləri yalnız strukturundan və limfoid komponentinin hüceyrəvi tərkibi ilə əlaqədardır, fərqlər isə limfoid elementlərinin sıx morfofunksional əlaqədə olduğu toxuma strukturlarından asılıdır.

Açar sözlər: böyük piylik, südlü ləkə, Peyer düyünləri, mədə, onikibarmaq bağırsaq, seroz qışa

Ключевые слова: большой сальник, млечные пятна, Пейеровы бляшки, желудок, двенадцатиперстная кишка, слизистая оболочка

Key words: greater omentum, milky spots, Peyer's patches, stomach, duodenum, tissue, membrane mucosa

**SIMILARITIES AND DIFFERENCES BETWEEN PEYER'S PATCHES OF THE SMALL
INTESTINE AND MILKY SPOTS OF THE GREATER OMENTUM OF WHITE RATS****Yu.P.Kostylenko, V.H.Hryn, O.S.Maksymenko, V.P.Bilash,
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The article presents the results of a study aimed at comparing the basic structural organization of Peyer's patches in the small intestine with milky spots in the greater omentum of white rats. The experiment involved 20 white male rats of reproductive age, weighing between 278.08 and 346.47 grams. The materials included preparations of the small intestine with visualized Peyer's patches and preparations of the greater omentum fixed in a 10% solution of neutral formalin. Additionally, total preparations of the greater omentum were stained with hematoxylin-eosin. Peyer's patches in the small intestine of white rats appear as raised whitish nodules of various shapes and sizes, forming a collection of structurally similar lymphoid nodules. These are integrated into a single morphofunctional community through separate modular associations of the blood microcirculatory channel. Milky spots in the greater omentum can only be visualized through total staining with basophilic dyes. It has been established that these spots are located on both sides along the vascular-fatty arcades, appearing as small lymphoid nodules of various shapes and sizes.

Milky spots in the greater omentum and Peyer's patches in the small intestine of white male rats do not belong to commensurate formations. Milky spots represent a discrete form of dispersal along the vascular-fatty arcades of the greater omentum, comprising a certain number of unified lymphoid nodules in structure. In contrast, Peyer's patches are a consolidated collection of similar formations.

Peyer's patches (aggregated lymphoid nodules) and milky spots of serous membranes belong to the peripheral part of the immune system, where, according to existing concepts, antigen-dependent specialization and proliferation of immunocompetent cells occur as a result of antigenic activation of committed lymphocytes by resident (fixed) macrophages [1-4].

It has been established that Peyer's patches are a structured association of lymphoid tissue with the epithelium covering the digestive tract, along with the tonsils and solitary nodules of the small intestine. All of these are considered outposts at the boundary with the external environment, being concentrated in those sections where mucous membranes are continuously exposed to antigens due to attempts by pathogenic microorganisms to penetrate the body's internal environment. At this point, not only do they initiate immune reactions, but they also transfer them from a local level to a systemic one (the phenomenon of "mucosal immune solidarity") [5]. Notably, Peyer's patches, as dense aggregates of lymphoid nodules, are located in the distal part of the small intestine and appendix, which directly correlates with the gradient of quantitative increase in the microbiota in the gastrointestinal tract [6].

A completely different location is occupied by the so-called milky spots, which in the abdominal cavity are predominantly found in the greater omentum. They are described in the publications as whitish nodules that are usually located near blood vessels, being associated with them and islets of white adipose tissue [1, 7].

Their cellular composition is mainly represented by various types of lymphocytic elements, among which macrophages are found in large quantities. These cellular groupings are located in the compartments of loose fibrous connective tissue of the serous membrane directly beneath the mesothelium. A notable feature of milky spots is that in the areas where they come into contact with the mesothelium, the latter undergoes "disintegration" in some places, forming tiny openings called "stigmata," which serve as migration pathways for macrophages into the peritoneal fluid [8-9].

To date, the findings of numerous

experimental studies have established that milky spots have the ability to specifically respond to the entering of various foreign particles and microorganisms into the abdominal cavity, undergoing transformation in their shape, size and cellular composition [10-13]. Apparently, these lymphoid formations are designed for immune surveillance over the antigenic composition of the peritoneal fluid, which is commonly known as a filtrate of blood plasma.

Notably, publications report that the above-mentioned formations are homologous in terms of localization and architecture in both human and laboratory animal. This provides a basis for extrapolating the findings of experimental studies to the clinical field.

However, despite the significant interest of researchers in Peyer's patches and milky spots, attempts to conduct a comparative morphological analysis between them have not yet been undertaken. Filling this gap would provide the opportunity to significantly expand our understanding of the principles of structural organization of organs in the peripheral part of the immune system [14].

Purpose. The study aims to investigate and compare the fundamental principles of the structural organization of Peyer's patches in the small intestine with milky spots in the greater omentum of white rats.

Material and Methods. The experiment involved 20 mature male white rats, weighing 278.08 to 346.47 grams. The specimens used were preparations of the small intestine with visualized Peyer's patches and preparations of the greater omentum, fixed in 10% neutral buffered formalin, as well as total preparations of the greater omentum stained with hematoxylin-eosin. Prior to vivisection, all animals were kept in standard conditions at the experimental-biological clinic (vivarium) of Poltava State Medical University, in compliance with the rules for the care of experimental animals established by the Directive of the European Parliament and Council (2010/63/EU), the Order of the Ministry of Education and Science, Youth and Sports of Ukraine as of March 1, 2012, No. 249 "On Approval of the Procedure for Conducting Experiments by Research Institutions on Animals" and "General Ethical Principles of Experiments on Animals" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), (Minutes No. 198 as of October 21, 2021, from the meeting of the Biomedical Ethics Commission of Poltava State Medical University) [15-16]. After vivisection, which was carried out by

ether anesthesia overdose [17] in accordance with all norms and requirements for conducting acute experiments on animals, all animals underwent sequential laparotomy, providing a complete overview of the internal organs in their natural relationships. Prior further manipulations, initially, all contents of the abdominal cavity were subjected to gentle washing with warm 0.9% physiological saline (NaCl), and then irrigated with 10% neutral buffered formalin from a syringe. Subsequently, the segments of the small intestine with Peyer's patches and total preparations of the greater omentum were extracted, and some omenta were stained with hematoxylin-eosin. Some preparations of the small intestine with Peyer's patches underwent epoxy resin plastination, and for the study of the angioarchitecture of the lymphoid nodules, injection of the blood vessels of the abdominal organs was performed with 5% solution of gelatin, colored with filtered India ink, using a method validated in our practice, while maintaining the solution temperature within 37–40°C [18].

Morphological analysis of the findings of the study was carried out using the traditional anatomical and histological methods for obtaining serial paraffin slices of 4 µm thick (Microm HM 325), which were stained with hematoxylin-eosin.

The method of plastination of tissue complexes in epoxy resin was also used, followed by the manufacture of polished slices, which were stained with 1% methylene blue solution. The specimens were studied and documented using the Konus light microscope equipped with the Sigeta DCM-900 9.0MP digital microphotographic attachment with the Biorex 3 software adapted for these studies (serial number 5604). Morphometric characteristics of the tissue structures of the corresponding specimens were obtained using a system for visual analysis of histological specimens, as well the Sigeta X 1 mm/100 Div.x0.01 mm object micrometer, the scale of which (equal to 1 mm, where the smallest division corresponds to 10 µm) was applied on the corresponding micro image obtained at an equivalent magnification.

Results and Discussion.

1. Key features of the structural organization of Peyer's patches in the small intestine of white rats.

In contrast to humans, the abovementioned formations in white rats are visually accessible to the naked eye from the outer surface of the small intestine, appearing as whitish evaginations of various shapes and sizes (Figure 1B).

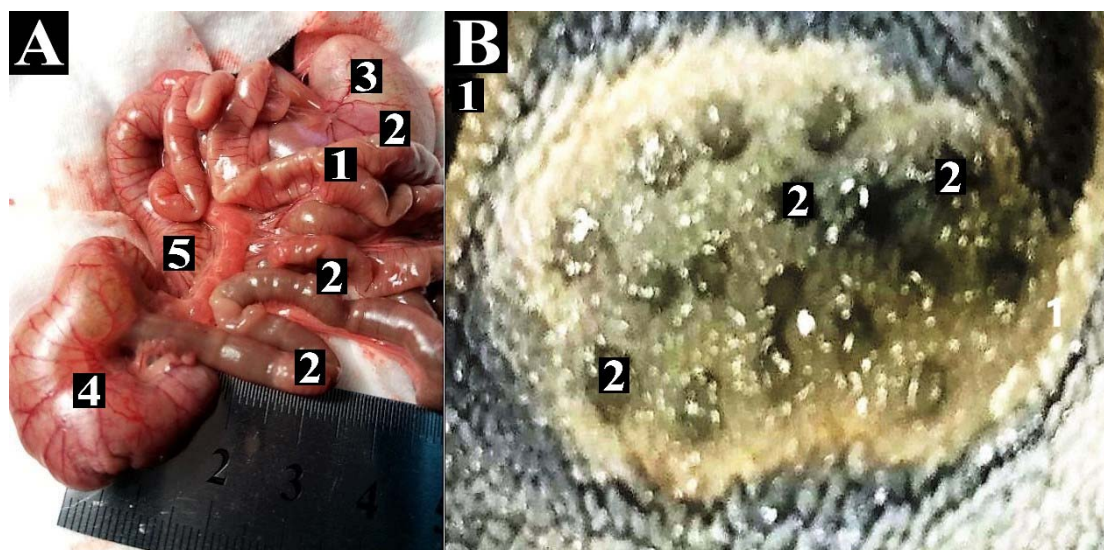


Fig. 1. The outer surface of the loops of the small intestine (A) and the relief of the mucous membrane in the distal part of the small intestine at the site of Peyer's patches localization (B). B - macrophoto. 9× magnification.

A: 1 – small intestine; 2 – Peyer's patches; 3 – stomach; 4 – cecum; 5 – colon;

B: 1 – folds of the mucous membrane bordering a Peyer's patch; 2 – solitary lymphoid nodules included in its composition.

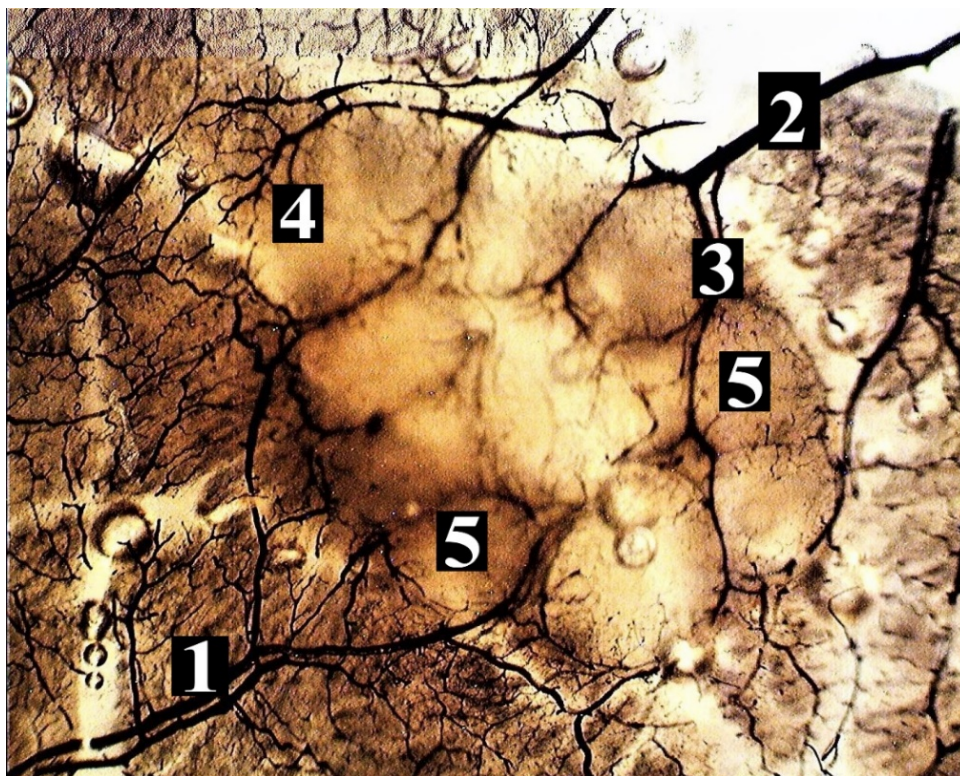


Fig. 2. Angioarchitecture of a Peyer's patch in the small intestine of a white rat. Epoxy resin plastination of the small intestine wall with injected blood vessels. Ocular lens 10 $\times$ magnification. Objective lens 4 $\times$ magnification. MBS-9.

1 – segmental arterial and venous vessels; 2 – nutritive blood vessels; 3 – perinodal microvessels; 4 – intranodal microvessels; 5 – solitary lymphoid nodules.

Verification of these structures as Peyer's patches is reinforced by the examination their mucous membrane in the ileal section of the small intestine (Figure 1B). Specifics regarding their quantity distribution, as well as the variations in their shapes and sizes, are omitted, as our main interest lies in the overarching principle of the structural organization of a Peyer's patch (averaged in terms of shape and size).

Our investigations visually confirm that a Peyer's patch is a specific aggregate of structurally similar but varied in shape and size lymphoid nodules, which are integrated into a unified morphofunctional entity through solitary modular associations of the microcirculatory blood flow (Figure 2).

This implies that a Peyer's patch can be viewed as a structure consisting of a specific number of uniform modular (structural-functional) units known as lymphoid nodules. Despite their small size (within the range of 0.5-0.8 mm), they embody a structured compact consolidation of lymphoid tissue with

epithelial structures of the mucous membrane of the small intestine. This consolidation is not limited to the presence of epithelial covering on the apical surface of the lymphoid nodule, known in the literature as follicle-associated epithelium [2]. We have found that intestinal villi, located in the Peyer's patch around each lymphoid nodule, as well as intestinal crypts (Lieberkühn's glands), play a role in this. Some of these crypts open their orifices on the surface of the follicle-associated epithelium, while others are located between the intestinal villi that surround the lymphoid nodules (Figure 3).

In our studies, we have noted a significant polymorphism of lymphoid nodules, which depends on the constantly changing factors of the antigenic state of the contents of the small intestine. However, even considering this morphofunctional variability, the characterization of lymphoid nodules, provided above, would be incomplete without examining their lymphoid component itself.

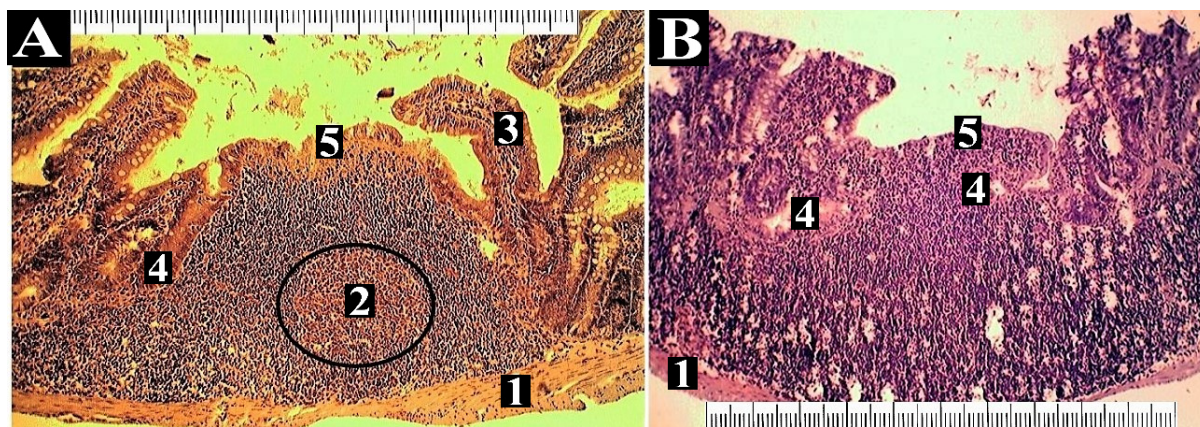


Fig. 3. Lymphoid nodules of Peyer's patches in the small intestine. Paraffin sections; H&E stain; ocular lens 10 $\times$ magnification; objective lens 10 $\times$ magnification. The smallest division of the scale is 10 μ m: 1 – muscular layer; 2 – germinal center; 3 – intestinal villi; 4 – intra-nodal crypts; 5 – apical (luminal) surface of the lymphoid nodules.

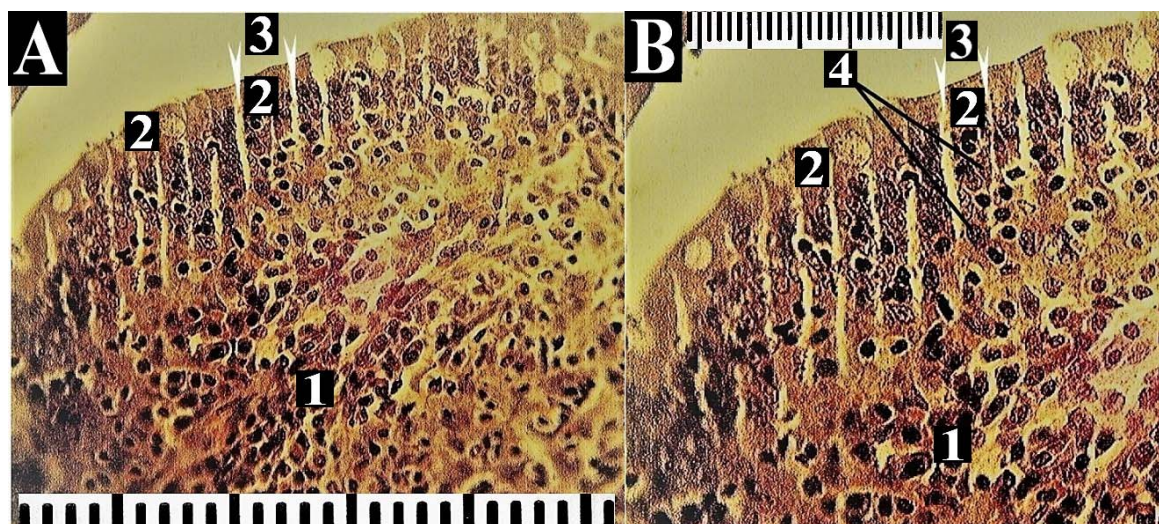


Fig. 4. Apical part of the lymphoid nodule in a Peyer's patch of the small intestine. Paraffin section; H&E stain. Ocular lens 10 $\times$ magnification. A – objective lens 40 $\times$ magnification (one division of the scale is 10 μ m), B – objective lens 100 $\times$ magnification: 1 – lymphoid elements; 2 – lymphoepithelial columns; 3 – intercellular spaces; 4 – dendritic cells.

We have found that it is characterized by a focal accumulation of all types of immune-competent cells, which are spatially organized in the form of convoluted cords, separated by clearly defined interstitial spaces (Figure 4).

Additionally, it should be noted that the replenishment of these structures with committed T- and B-lymphocytes, as well as monocytes, occurs through the blood vessels located in the vicinity of the lymphoid nodules. We omit the details related to the initial steps of the immunization process of these lymphoepithelial structures of the small intestine mucosa, as the general characteristics provided above are sufficient for the morphological comparison with the milky

spots of the white rat's greater omentum.

2. Key features of the structural organization of milky spots of the greater omentum of white rats

The findings of our studies show that the greater omentum is characterized by vascular-fatty arcades, which we have identified, and intermediate serous structures that conjoin them. However, upon visual examination of native total preparations of the greater omentum, no other loci are discernible that even remotely resemble the milky spots. It is only through total staining of these preparations with basophilic dyes that we gain the ability to visualize them on both sides along the course of the vascular-fatty arcades in the

form of small lymphoid nodules of various shapes and sizes (Figure 5). Importantly, all of them, distributed concentrically along the pathway of the blood vessels of the vascular-fatty arcades, are connected to them by microvessels of pre-capillary and post-capillary types. It is noteworthy that some of these nodules are located within the adipose tissue, while others are located outside its boundaries. Publication report that it is precisely the latter form that is typically attributed to milky spots [19]. On average, their sizes do not exceed 0.8 mm, which, as

mentioned above, coincides with the size of the lymphoid nodules of Peyer's patches.

The resemblance between them also extends to their cytoarchitecture, with the exception that milky spots are associated with a small number of adipocytes (Figure 6).

Furthermore, while the lymphoid nodules of Peyer's patches are closely integrated with the epithelial structures of the mucous membrane of the small intestine, the lymphoid tissue of milky spots is in morphofunctional relationship with the mesothelium of the greater omentum.

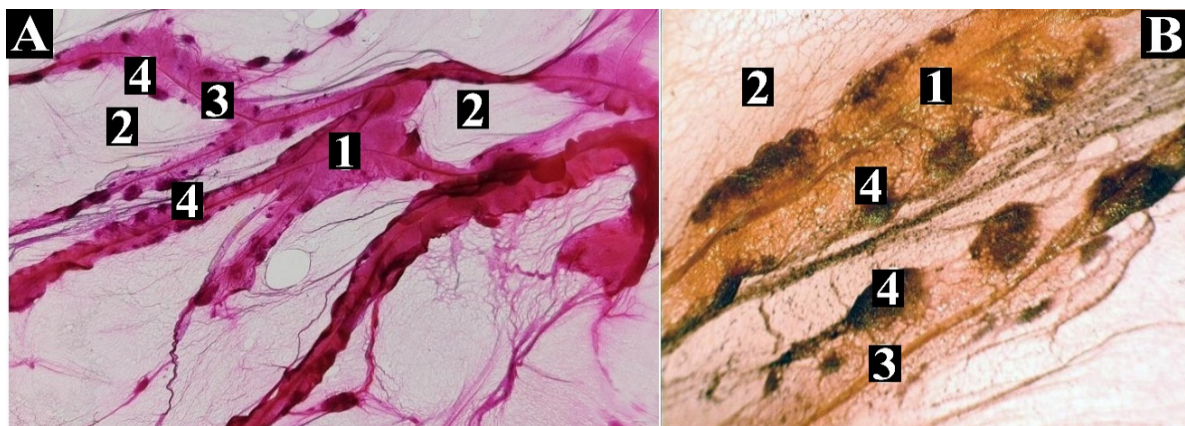


Fig. 5. Overall view of the internal structure of the vascular-fatty arcades in the greater omentum of white rats. Total preparation; H&E stain. A – macrophoto; 3 $\times$ magnification; B – ocular lens 10 $\times$ magnification; objective lens 2 $\times$ magnification. MBS-9: 1 – vascular-fatty arcades; 2 – intermediate serous structures; 3 – axial blood vessels; 4 – milky spots.

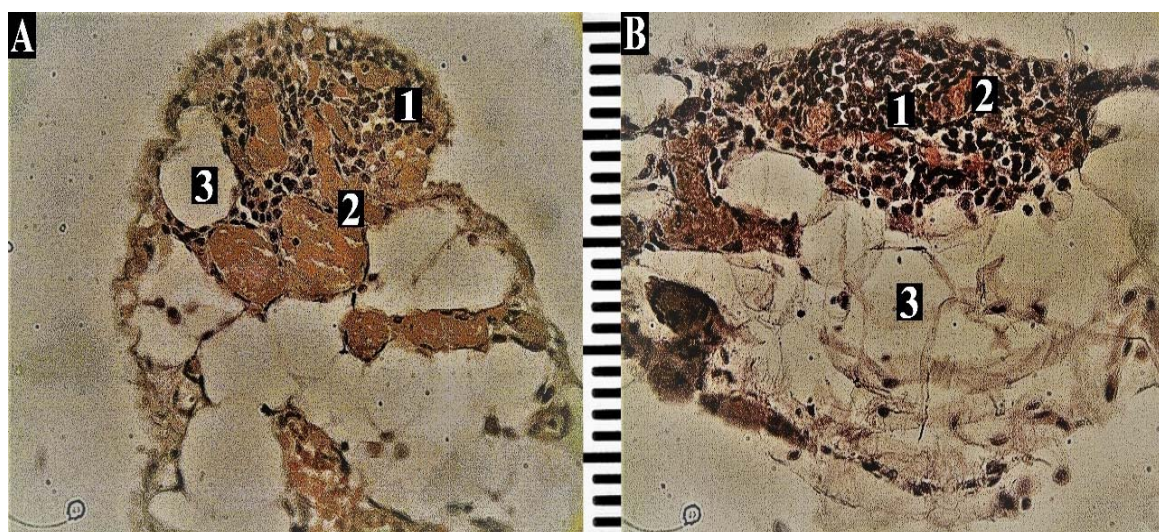


Fig. 6. Milky spots in the greater omentum of white rats. Paraffin sections; H&E stain; ocular lens 10 $\times$ magnification; objective lens 40 $\times$ magnification. The smallest division of the metric scale is 10 μ m. 1 – concentration of lymphoid elements in adipose lobules; 2 – venous microvessels; 3 – adipocytes.

Conclusions:

1. Milky spots of the greater omentum and Peyer's patches in the small intestine of male white rats do not belong to equivalent structures. Milky spots represent a discrete form of distribution along the pathway of the vascular-fatty arcades of the greater omentum, consisting of a specific number of structurally unified lymphoid nodules. In contrast, Peyer's patches are a consolidated (concentrated) aggregation of similar structures.

2. From this perspective, it is more appropriate to compare milky spots with solitary lymphoid nodules of the small intestine. The similarity between them lies solely in the structure and cellular composition of their lymphoid component, while the difference depends on the tissue structures with which lymphoid elements have a close morphofunctional relationship.

3. Considering Peyer's patches as a concentrated aggregation of structurally unified

lymphoid nodules allows us to extend this understanding and apply it to other peripheral organs of the immune system. The analysis of the publications reveals that all of them also represent one form or another of consolidation of lymphoid nodules as the most minimal accumulations of genetically predetermined immunocompetent cells in a specific location within the body, associated with various bordering tissue structures.

4. At this point, in lymph nodes they are associated with lymphatic vessels, in tonsils with the epithelium of secondary crypts, in the spleen with microvessels, and solitary and grouped lymphoid nodules are associated with intestinal epithelium. In contrast, milky spots are functionally linked to the mesothelium of serous membranes. However, unlike lymphoid nodules in other secondary immune organs, milky spots also have a morphogenetic relationship with adipose tissue, the functional essence of which remains unknown.

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СХОДСТВО И РАЗЛИЧИЕ МЕЖДУ ПЕЙЕРОВЫМИ БЛЯШКАМИ ТОНКОЙ КИШКИ И МЛЕЧНЫМИ ПЯТНАМИ БОЛЬШОГО САЛЬНИКА БЕЛЫХ КРЫС

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Резюме. Представлены результаты исследования, проведенного с целью изучить и сравнить основные принципы структурной организации пейеровых бляшек тонкой кишки с млечными пятнами большого сальника белых крыс. Для этого в эксперименте задействовано 20 белых крыс-самцов репродуктивного возраста, массой от 278,08 до 346,47 грамм. Материалом служили препараты тонкой кишки с визуализированными пейеровыми бляшками и препараты большого сальника, фиксированные в 10% растворе нейтрального формалина, а также тотальные препараты большого сальника, окрашенные гематоксилин-эозином. Исследование осуществлено с помощью традиционных гистологических методов получения серийных парафиновых срезов, толщиной 4 мкм (Microm HM 325), которые окрашивали гематоксилин-эозином. Изучение и документирование осуществлено с помощью МБС-9, а также в световом микроскопе «Konus», оснащенного цифровой микрофотонасадкой Sigeta DCM-900 9.0MP с адаптированной для данных исследований программой Biorex 3 (серийный номер 5604).

Пейеровы бляшки тонкой кишки у белых крыс представлены в виде приподнятых бугорков белесоватого цвета различной формы и размеров и являют собой определенную совокупность однотипных по строению, лимфоидных узелков, которые интегрированы в единую морфофункциональную общность посредством отдельных модульных ассоциаций кровеносного микроциркуляторного русла. Млечные пятна большого сальника можно визуализировать только путем тотальной окраски этих препаратов базофильными красителями, установлено что они располагаются с двух сторон по ходу сосудисто-жировых аркад в виде мелких лимфоидных узелков различной формы и величины. Таким образом, млечные пятна большого сальника и пейеровы бляшки тонкой кишки белых крыс-самцов не относятся к соразмерным образованиям, так как первые представляют собой дискретную форму рассредоточения по ходу сосудисто-жировых аркад большого сальника определенного количества унифицированных по структуре лимфоидных узелков, тогда как пейеровы бляшки являются консолидированной совокупностью подобных образований.

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