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REMODELING OF THE STRUCTURAL COMPONENTS OF THE SPLEEN CAPSULE IN RATS UNDER THE INFLUENCE OF A COMPLEX OF CHEMICAL FOOD ADDITIVES

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The spleen, although often underestimated, is a particularly important component of the immune system, which some scientists call the "forgotten" organ. Its importance and role, in our opinion, are undoubtedly necessary for maintaining and regulating the body's immunogenic and protective processes. The spleen plays a key role not only as a universal organ of hematopoiesis in the fetus and ensures the constancy of blood composition and properties, but also as a crucial organ in the process of antigen-dependent differentiation of T- and B-lymphocytes. In addition, the state of the spleen's connective tissue capsule, which is an integral part of the musculoskeletal system, requires further study to understand the pathophysiological mechanisms of the effect of a complex of chemical food additives on the biological structures of the immune system, which was the purpose of the study. In this study, using histological and morphometric methods of research, the overall average thickness of the spleen connective tissue capsule was determined. It was found that the introduction of a complex of food chemical additives to white experimental rats leads to thinning and destruction of the connective tissue capsule in the early stages and to a significant thickening of the capsule in the later stages due to hyperhydration of the amorphous substance and destruction and swelling of the fibrous component.

Key words: hematopoietic and immune defense organs, morphometry, spleen, lymph nodes, sinusoid, food additives, monosodium glutamate, sodium nitrite, Ponceau 4R, connective tissue capsule.

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РЕМОДЕЛЮВАННЯ СТРУКТУРНИХ КОМПОНЕНТІВ КАПСУЛИ СЕЛЕЗІНКИ ЩУРІВ ПІД ДІЄЮ КОМПЛЕКСУ ХІМІЧНИХ ХАРЧОВИХ ДОБАВОК

Селезінка, хоч і часто недооцінювана, є особливо важливою складовою імунної системи, яку деякі науковці називають «забутим» органом. Її значення та роль, на нашу думку, безперечно необхідні для підтримання та регуляції імуногенних та захисних процесів організму. Селезінка відіграє ключову роль не тільки як універсальний орган гематопоезу у плода і забезпечує сталість складу та властивостей крові, але й як вирішальний орган у процесі антигензалежної диференціації Т- і В-лімфоцитів. До того ж стан сполучнотканинної каспули селезінки, яка є складовою частиною опорно-скоротливого апарату, потребує додаткового вивчення для розуміння патофізіологічних механізмів впливу комплексу хімічних харчових добавок на біологічні структури імунної системи, що й стало метою дослідження. У роботі, за допомогою гістологічного та морфометричного методів дослідження, було проведено визначення загальних середніх показників товщини сполучнотканинної капсули селезінки. Встановлено, що введення комплексу харчових хімічних добавок білим експериментальним щурам призводить на ранніх етапах до стоншення та деструкції сполучнотканинної капсули а на пізніх термінах до значного потовщення капсули за рахунок гіпергідратації аморфної речовини та деструкції і набухання волокнистого компоненту.

Ключові слова: органи кровотворення та імунного захисту, морфометрія, селезінка, лімфоїдні вузлики, синусоїд, харчові добавки, глутамат натрію, нітрит натрію, Понсо 4R, сполучнотканинна капсула.

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Over the past century, the food industry has undergone significant transformations. These changes are mainly related to the mass production and distribution of products, which has become possible due to improved production methods and long-term storage. The main idea behind new food technologies was the introduction of new chemical synthetic food additives that not only improve the organoleptic properties of products but also facilitate longer storage, facilitate transportation and increase shelf life, which is beneficial for manufacturers, suppliers and large retail chains [6]. At the same time, excessive consumption of food additives can lead to morphological changes in internal organs, in particular in the organs of the immune system, which affects their functional activity and properties [8, 10]. Among the components of the immune system, the spleen deserves special attention, which, according to some reports, is an underestimated, "forgotten" organ [5]. The role of the spleen in the maintenance and regulation of immune processes is extremely important, not only in the context of its function as a universal organ of hematopoiesis in the fetus and maintaining the stability of blood composition, but also as a key organ in ensuring antigen-dependent differentiation of T and B lymphocytes [3]. Previous studies emphasize the significant impact of food supplements on the development of various pathological conditions of organs and systems [4]. However, these studies do not provide a complete picture of the impact, as they focus on the study of individual food additives. An analysis of modern food products has shown that the most common additives are monosodium

glutamate, sodium nitrite, and the synthetic dye Ponceau-4R, but there is not enough information about their complex effect on the spleen, especially in the dynamics [2]. Therefore, the study of the effect of a complex of chemical synthetic food additives on the morphofunctional features of the spleen cassula as an active participant in immune reactions is of great importance. That is why it became an important and urgent issue to analyze the role of these substances as a nonspecific antigenic factor that affects the functional state of the spleen, namely its capsule, in order to prevent the development of a pathophysiological cascade of events and ensure qualitative changes towards a harmless, healthy and safe food industry.

The purpose of the study was to determine the morphological and functional changes in the structural components of the spleen capsule of rats under the influence of a complex of chemical food additives.

Materials and methods. The study was conducted on 70 mature rats, which were divided into one control and six experimental groups. The control group received saline, and the experimental group received a complex of chemical food additives (monosodium glutamate, sodium nitrite, Ponceau 4R) at doses two times lower than the maximum permissible doses. After weeks 1, 4, 8, 12, 16 and 20 of the experimental study, the experimental animals were withdrawn from the experiment by an overdose of ether anesthesia. Guided by generally accepted methods, spleen biopsies were removed, sealed in paraffin and epoxy resin, and thin and semi-thin sections were made with subsequent staining. The obtained histologic sections were examined using a light microscope with a digital microphotographic attachment. The results were evaluated using the InStat software package. The experimental study was carried out in compliance with the requirements of humane treatment of experimental animals regulated by the relevant law of Ukraine and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Results of the study and their discussion. In our opinion, it is extremely important in the process of studying the structural reorganization of any parenchymal organ, of which the spleen is a direct representative, which reacts to the introduction of a complex of chemical synthetic food additives, to establish the processes that occur in the connective tissue capsule, which is woven into the spleen throughout the entire thickness to form trabeculae - a matrix or framework specific to this type of organ. This “soft” skeleton is a part of the spleen's musculoskeletal system and penetrates the complex structure and contains blood vessels (Fig. 1A).

During the experimental study, it was found that after 1 week of administration of a complex of chemical food additives to laboratory animals, moderate thinning of the connective tissue capsule was determined in the histological preparations of the capsule obtained by us. Its mean thickness, statistically significant at $p<0.05$, decreased by 1.54 times compared to the control values (Fig. 1B).

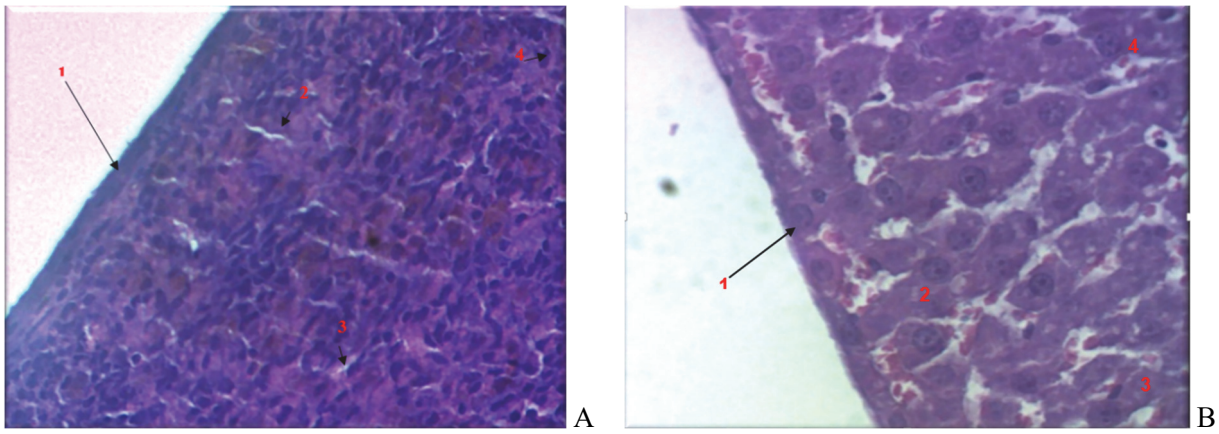
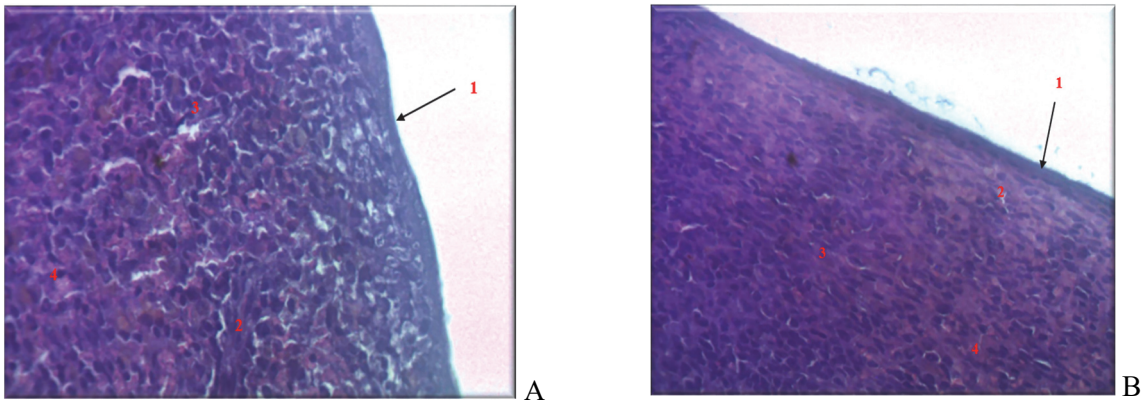


Fig. 1. Spleen of a white laboratory rat of the control group. Paraffin section (A). Spleen capsule of a white laboratory rat after 1 week of administration of a complex of food additives (B). Staining: hematoxylin and eosin. Collection: ca. 10, vol. 40. Designations: 1 – connective tissue capsule; 2 – trabeculae; 3 – marginal sinus; 4 – lymph node.

After 4 weeks of the experiment, the mean thickness of the connective tissue capsule, statistically significantly at $p<0.05$, decreased by 1.17 times compared with the control values, but compared with the previous observation period, thickening was observed and the index at $p<0.05$ increased by 1.31 times (Fig. 2A).

After 8 weeks of the experimental study, similar dynamics were observed with the control group and the 1st group - the mean thickness of the connective tissue capsule, statistically significant at $p<0.05$, decreased by 1.67 times compared to the control values and compared to the previous observation period, the indicator statistically significant at $p<0.05$ also decreased by 1.42 times (Fig. 2B).

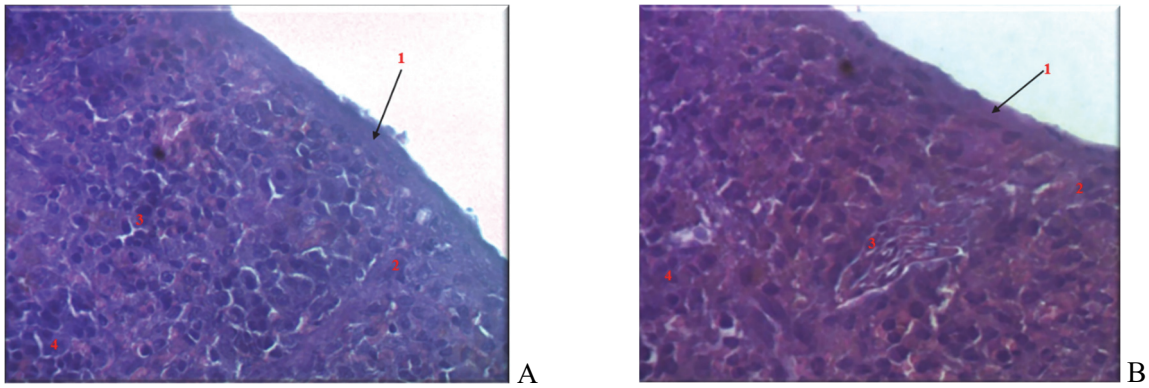
After the 12th week of the experimental study, a sharp thickening of the connective tissue capsule was observed - the mean thickness of the connective tissue capsule, statistically significant at $p<0.05$, increased by 1.33 times compared to the control values and by 2.22 times compared to the previous observation period at $p<0.05$ (Fig. 3A).



A B

Fig. 2. Spleen capsule of a white laboratory rat after 4 weeks of administration of a complex of food additives (A). Spleen capsule of a white laboratory rat after 8 weeks of administration of a complex of food additives (B) Paraffin section. Staining: hematoxylin and eosin. Collection: ca. 10, vol. 40. Designations: 1 – connective tissue capsule; 2 – trabeculae; 3 – marginal sinus; 4 – lymph node

After the 16th week of the experimental study, a slight oscillatory dynamics towards thinning of the connective tissue capsule was observed, so at $p<0.05$ the mean thickness of the connective tissue capsule increased by 1.18 times, but compared to the previous period at $p<0.05$ it decreased by 1.13 times (Fig. 3B).



A B

Fig. 3. Spleen capsule of a white laboratory rat after 12 weeks of administration of a complex of food additives (A). Spleen capsule of a white laboratory rat after 16 weeks of administration of a complex of food additives (B) Paraffin section. Staining: hematoxylin and eosin. Collection: ca. 10, vol. 40. Designations: 1 – connective tissue capsule; 2 – trabeculae; 3 – marginal sinus; 4 – lymph node

After the 20th week of the experimental study, at the final stage, a very significant thickening of the connective tissue capsule was observed. The mean thickness index, at $p<0.05$, increased by 2.03 times compared to the control group, and compared to the same index in the previous group, at $p<0.05$, increased by 1.72 times (Fig. 4A).

The dynamics of morphometric changes in the mean total thickness of the connective tissue capsule is shown in the table (Fig. 4B).

Analyzing the results obtained, it was found that the administration of a complex of food chemicals (monosodium glutamate, sodium nitrite, ponceau 4R) for 20 weeks causes both morphological and morphometric changes in the spleen capsule, which changed in the dynamics of the experiment. Thus, it was found that after 1 week of administration of a complex of food chemical additives to white laboratory rats, it leads to thinning of the connective tissue capsule due to the compressive effect of the spleen parenchyma, which was visualized as hyperhydrated and destruction of the fibrous component of the connective tissue capsule. After 4 weeks, the fibrous component of the connective tissue capsule and the active component are restored and the mean number of fibroblastic cells increases. After 8 weeks of the experiment, destructive changes in the connective tissue capsule were visualized, which were manifested by destructive changes in collagen and elastic fibers, while the average number of fibroblastic cells did not increase compared to the previous observation period. Starting from week 12, signs of capsular thickening due to hyperhydration of the amorphous substance gradually increased, progressing to week 20 of the study. In addition, at this stage, irreversible changes occurred in the form of destruction and swelling of the fibrous

component. These changes are most likely the result of prolonged negative effects of chemicals that deplete the defense mechanisms of spleen cell elements.

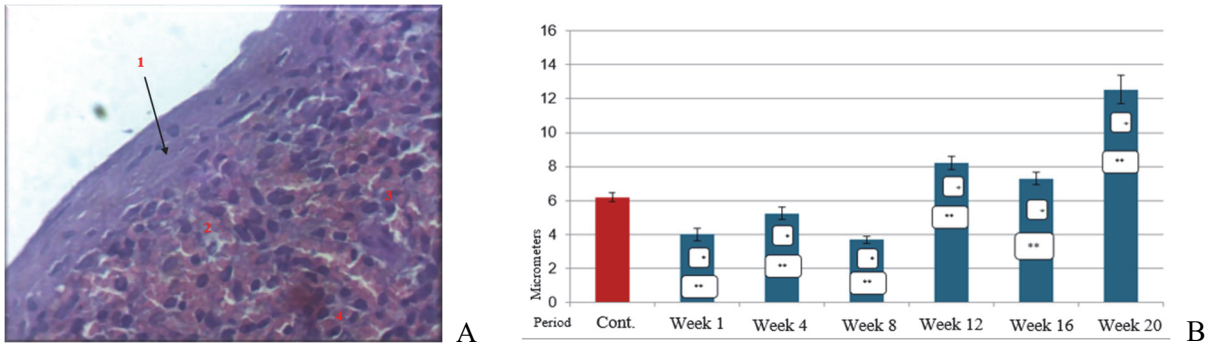


Fig. 4. Spleen capsule of a white laboratory rat after 20 weeks of administration of a complex of food additives Paraffin section. Staining: hematoxylin and eosin. Collection: ca. 10, vol. 40. Designations: 1 – connective tissue capsule; 2 – trabeculae; 3 – marginal sinus; 4 – lymph node (A). Morphometric changes in the average total thickness of the connective tissue capsule of the spleen of white laboratory rats in the dynamics of the experimental study (B). Note: * is a statistically significant difference at $p<0.05$ compared to the control values; ** is a statistically significant difference at $p<0.05$ compared to the previous observation period (B).

The negative damaging effects of the chemical food additives selected by us when used in combination have been proven by other scientists. In particular, changes in the mofro-functional properties of such organs as the liver [7], tongue [1], submandibular salivary glands [11], duodenum [12], lungs [9] and a number of others have been established. Taking into account the above, as well as the results of our own research, it can be argued that chemical food additives cause not only changes in the organs of the digestive system with which they come into direct contact, but also in other systems. Thus, we can assume that monosodium glutamate, monosodium nitrite, and ponceau 4R have a systemic negative impact on the body.

Conclusions

The administration of sodium glutamate, sodium nitrite and ponceau 4R in combination for 20 weeks of experimental study led to significant morphological and metric changes in the spleen capsule, which at the initial stages were characterized by hyperhydration of the fibrous component and an increase in the number of fibroblasts with gradual depletion of defense mechanisms and the appearance of signs of edema and destruction in the late stages of the experiment.

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