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ABSTRACT

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SUBMICROSCOPIC CHANGES IN THE PARENCHYMA OF THE SPLEEN UNDER THE ACTION OF EXTRACELLULAR DEHYDRATION

Introduction: Dehydration is a perilous condition affecting the body, leading to alterations in the structural and functional integrity of all organs and tissues. This study aimed to examine the changes in the spleen under conditions of mild, moderate, and severe extracellular dehydration.

Methods: Sixty adult male rats were divided into control and experimental groups. Extracellular dehydration was induced by demineralised diet and intraperitoneal injections of furosemide for 30, 60 and 90 days. Organometric parameters and ultrastructural changes of spleen were investigated.

Results: Dehydration caused a progressive decrease in the size of the spleen. Submicroscopically, initial cellular damage was observed after 30 days, including nuclear deformation and mitochondrial oedema. By day 60, apoptosis and cytolysis with extensive vacuolisation and accumulation of cellular detritus were predominant. Severe degeneration by day 90 included cell membrane destruction, white pulp depletion and significant vascular stasis and thrombosis.

Discussion: The results highlight the sensitivity of the spleen to dehydration-induced stress, with changes in lymphocyte populations and macrophage structures suggesting impaired immune function. Alterations in macrophage structure and lymphocyte density highlight dehydration's effect on immune function. Observed vascular responses, such as ischemia and microthrombi formation, reflect systemic impacts across organ systems. The findings align with studies on thyroid adaptations under dehydration, underscoring organ-specific resilience. Furthermore, oxidative stress-induced changes from nanoparticles mirror dehydration-induced spleen pathology, emphasizing the spleen's role as a biomarker for systemic stress. The obtained data obtained give an idea of the adaptability of the spleen to external factors.

Keywords: spleen, dehydration, rat, lymphocyte, reticular cell, endotheliocyte.

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СУБМІКРОСКОПІЧНІ ЗМІНИ В ПАРЕНХІМІ СЕЛЕЗІНКИ ПІД ДІЄЮ ПОЗАКЛІТИННОЇ ДЕГІДРАТАЦІЇ

Вступ: Зневоднення – це небезпечний стан, що впливає на організм і призводить до змін структурної та функціональної цілісності всіх органів і тканин. Метою цього дослідження було вивчення змін у селезінці за умов позаклітинної дегідратації легкого, середнього та тяжкого ступеню.

Методи: Шістдесят дорослих самців щурів були розділені на контрольну та експериментальну групи. Позаклітинну дегідратацію індукували демінералізованою дієтою та внутрішньочеревними ін'єкціями фуросеміду протягом 30, 60 та 90 днів. Досліджено органометричні параметри та ультраструктурні зміни селезінки.

Результати: Зневоднення спричинило прогресуюче зменшення розміру селезінки. Субмікроскопічно початкове пошкодження клітин спостерігалося через 30 днів, включно з деформацією ядра та мітохондріальним набряком. До 60-го дня переважали апоптоз та цитоліз з екстенсивною вакуолізацією та накопиченням клітинного детриту. Тяжка дегенерація до 90-го дня включала руйнування клітинної мембрани, виснаження білої пульпи та значний судинний застій і тромбоз.

Обговорення: Результати підкреслюють чутливість селезінки до стресу, викликаного зневодненням, зі змінами в популяціях лімфоцитів та структурах макрофагів, що свідчить про порушення імунної функції. Зміни в структурі макрофагів та щільності лімфоцитів вказують на вплив зневоднення на імунну функцію. Виявлені судинні реакції, такі як ішемія та утворення мікротромбів, відображають системний вплив на всі системи органів. Результати узгоджуються з дослідженнями адаптації щитовидної залози до зневоднення, підкреслюючи органоспецифічну стійкість. Крім того, зміни, викликані оксидативним стресом від наночастинок вказують на патологію селезінки, викликану зневодненням, що підтверджує роль селезінки як біомаркера системного стресу. Отримані дані дають уявлення про адаптивність селезінки до зовнішніх факторів.

Ключові слова: селезінка, зневоднення, щур, лімфоцит, ретикулярна клітина, ендотеліоцит.

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INTRODUCTION

Thirst is a physiological desire to drink water, an important nerve impulse, a protective mechanism of the body to avoid the development of dehydration [1]. A state of dehydration is extremely dangerous, it affects the function of all organs and tissues, and it directly affects the state of health and work capacityof a person [2]. According to modern classifications, general, intracellular and extracellular (hypovolemic) dehydration are distinguished [3]. Extracellular dehydration results from too much water loss while retaining electrolytes and can cause physiological disturbances including increase in the secretory activity of the adenohypophysis, hypofunction of the thyroid gland, and negatively affects

the development of compensatory and adaptive processes in the body [4]. Gonadal steroids in females can help protect against learning impairments under conditions of hypovolemic dehydration [5]. Estradiol has a homeostatic role in the control of fluid balance, by increasing water consumption. In ovariectomised animals, daily water consumption increases, and this can be reduced by treatment of these animals with estradiol [6]. Dopamine plays an important role in the mechanism of thirst and hunger/satiety, it affects the level of water and sodium in the body [1].

Studying the effect of dehydration on organs and tissues is important to understand the pathophysiology associated with excessive water loss and the impact this has on specific tissues. Of particular interest are the tissues of the immune system, including lymphoid organs such as the spleen, where antigen-dependent proliferation and differentiation of T- and B-lymphocytes, death of spent blood cells, and formation of some biologically active substances occur [7, 8, 9]. It has been shown that the spleen plays a role in the course of a stroke and is a factor in increasing secondary damage to the nervous system after a stroke. Splenectomy performed two weeks before ischemic and haemorrhagic stroke in mice and rats shows a decrease in infarct volumes [9]. It has been shown however that splenectomy has long-term negative consequences, therefore ligation of the main vessels in case of injuries is more recommended as a clinical treatment [10]. Moreover, induction of chronic stress with immunosuppression and induction of apoptosis of splenocytes is associated with increased susceptibility to various diseases [11] highlighting the physiological importance of the spleen.

Considering the importance of water for the optimal function of all tissues and organs in the body, an urgent task is to study the impact of its deficiency, in particular, at different degrees of dehydration. There is surprisingly little data on the effect of dehydration on the structural organization of the spleen parenchyma at the cellular level. There are studies of structural changes in other organs under the conditions of dehydration [12] and various experimental models of dehydration [13, 14].

The purpose of the study: to examine the organometric and submicroscopic changes of the spleen in mild, medium and severe extracellular dehydration.

MATERIALS AND METHODS

The study was conducted on 60 mature in the age range of 5 to 8 months white male rats weighing 130-235 g. Extracellular dehydration was modeled in experimental groups of animals (30 animals) by keeping them on a demineralized diet.

The diet consisted of boiled food and distilled water for 30 (Experimental group 1, 10 animals), 60 (Experimental group 2, 10 animals), or 90 days (Experimental group 3, 10 animals). In addition, during the experiment, the animals were injected intraperitoneally with Furosemide, its dose was 0.3 mg. For the calculation of the dosage for the laboratory rat, the formula by R. S. and Y. R. Rybolovlev was used, along with the corresponding calculations [15]. The control group of animals (30 animals) was on the standard drinking and food ration of the animal house for 30, 60, and 90 days, respectively. After 30 days, a mild degree of dehydration develops in the experimental groups, after 60 days – medium dehydration, and after 90 days – severe dehydration. According to the water deficit indicator, three degrees of dehydration were identified. In mild dehydration, the water deficit ranges from 2% to 5%; in moderate dehydration, it reaches 5% to 10%; and in severe dehydration, it exceeds 10% [16].

All experimental animals were kept in the animal facility of Sumy State University. The research was carried out in accordance with the provisions of the "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), in accordance with Council of Europe Directives 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from of cruel treatment", general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

At the end of the experiment [17], the spleen of each rat was collected. For this, an autopsy was performed on the ventral wall of the abdominal cavity, and the spleen was isolated. Their weight of each spleen was studied on unfixed material. The organ was weighed on a VLR-200 M analytical balance with an accuracy of ±0.01 mg. After that, the length, width, and thickness of the spleen were measured in millimeters. Statistical processing of quantitative data was performed on a computer using the Statistica v.10 program (StatSoft Inc., USA). Descriptive analysis of each sample was performed with calculation of arithmetic mean (M) and standard deviation (SD). Non-parametric Mann-Whitney U-test (Mann-Whitney U-test) was used to assess the reliability of the difference in values between the samples according to the studied indicators. The difference was considered significant at $p<0.05$.

All collection of material and production of preparations for the electron-microscopic research method were carried out according to generally accepted rules and methods. The volume of selected pieces of spleen for electron microscopic examination was 2 × 2 mm. They were fixed in glutaraldehyde, then in 1% osmium tetroxide. Then it was passed through alcohols of increasing concentration and poured into a mixture of epoxy resins and polymerized. Ultrathin

sections were prepared using an UMTP- 4 ultramicrotome. Studying and photographing the objects was carried out using a PEM-125 microscope at an accelerating voltage of 90 kV at magnifications of ×4050-10000.

RESULTS

The rats in Experimental Group 1 (mild degree of extracellular dehydration) showed a significant decrease in the mass of the spleen by 9.73% (p=0.003), and a decrease in the length of the spleen by 11.46% (p<0.001), the thickness by 3.95% (p=0.32), and the width by 6.6% (p=0.15).

According to the data of the organometric study, in the animals of the studied group 2 with a moderate degree of extracellular dehydration at the time of withdrawal from the experiment, there is a significant decrease in the mass of the spleen by 16.63% (p<0.001), the linear dimensions of the spleen due to the length of the spleen by 12.62% (p< 0.001), thickness – by 10.65% (p=0.013), width – by 9.2% (p=0.019).

Based on organometric research, in the animals of the studied group with a severe degree of extracellular dehydration at the time of withdrawal from the experiment, there is a significant decrease in the mass of the spleen by 24.79% (p<0.001), the linear dimensions of the spleen due to the length of the spleen by 25.81% (p<0.001), thickness – by 23.43% (p<0.001), width – by 22.66% (p<0.001) (**Table 1**).

Table 1. Spleen parameters during extracellular dehydration (M±SD)

Observation period, indicator	Mass, mg	Length, mm	Thickness, mm	Width, mm
		30 days		
Experimental group 1	532.42±27.88	40,64±1,27	5.10±0.26	8.07±0.67
Control group	589.79±45.51	45.90±1.46	5.31±0.61	8.64±1.01
p	0.003	<0.001	0.32	0.15
		60 days		
Experimental group 2	507.02±28.93	40.44±0.99	4.95±0.31	8.59±0.56
Control group	608.17±37.22	46.28±1.68	5.54±0.61	9.46±0.91
p	<0.001	<0.001	0.013	0.019
		90 days		
Experimental group 3	469.18±41.36	34.46±1.25	4.38±0.57	7.78±1.08
Control group	623.82±49.61	46.45±1.38	5.72±0.53	10.06±1.08
p	<0.001	<0.001	<0.001	<0.001

The the control group indicate that the structure of the spleen corresponds to the species norm. Most of the parenchyma of the spleen is occupied by the red pulp, which is formed by the splenic cords and venous sinuses of the spleen, contains elements of blood, since the death of spent erythrocytes and platelets occurs here. The white pulp occupies a smaller part, contains a supporting framework of reticular tissue filled with lymphocytes, plasma cells, macrophages, interdigitating and dendritic cells. The structure of cells in the spleen parenchyma of control group animals is typical (**Fig. 1**).

Ultrastructural examination of the parenchyma of the spleen of Experimental Group 1 (30 days dehydration), showed signs of cell damage in all areas of both the white and red pulp. There were many lymphoblasts, macrophages and plasma cells in the white pulp. The nucleus of the lymphoblasts was often reduced, deformed, contained one nucleolus, the chromatin was condensed, but euchromatin prevailed. The nuclear envelope of increased osmiophilicity was uneven, sometimes with areas of lysis, and the the perinuclear space was not expanded (Fig. 2). The cytoplasm of lymphoblasts was clear, sometimes containing vacuole-like structures and single organelles. The Golgi complex in most cells was represented by flat cisternae with bound vesicles. The mitochondrial matrix was swollen, often reduced.

Small and medium-sized lymphocytes had a slightly reduced nucleus, with condensed chromatin, located in large pits on the periphery of the nuclear envelope. The contour of the nuclear envelope was uneven, in places with a vague wavy contour, forming small protrusions and depressions. The cytoplasm of the small lymphocytes was most often visualized as a narrow rim and contained almost no organelles. The perinuclear space was often expanded (**Fig. 3**). In the cytoplasm of the medium lymphocytes there were single vacuole-like structures, which were represented by structureless amorphous masses. Most cells had an electron-dense cytoplasm. Mitochondria were often damaged, the matrix was heterogeneous. The Golgi complex and

tubules of the granular endoplasmic reticulum were not clearly visualized.

Cells undergoing mitosis were visualized in the parenchyma in a small amount, with a predominance in the white pulp. There were also many cells in various stages of apoptosis.The number of plasma cells and active macrophages was increased. Their structure is generally typical, but the nucleus was slightly reduced, eccentrically located, contained a clear nucleolus, and the nuclear envelope contained local damage and significant invaginations. The cytoplasm was filled with expanded deformed tubules of the granular endoplasmic reticulum. Mitochondria had a matrix containing dense osmiophilic inclusions. The nucleus of the macrophage was deformed, had an elongated oval shape with deeply condensed chromatin, and the nucleolus was well visualized.

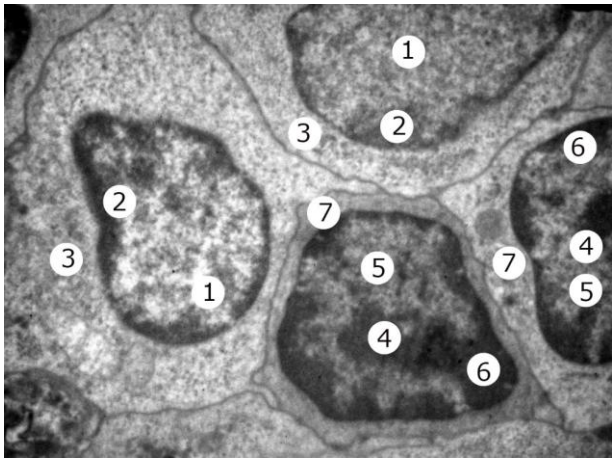


Figure 1. Electron micrograph image of the germinal center of the lymphoid nodule of the white pulp of the spleen of a control group rat. Magnification: ×8000 (It would be better to have a scale bar rather than a magnification). Designation: 1 – euchromatin and heterochromatin (2) in the lymphoblast nucleus; 3 – lymphoblast cytoplasm; 4 – nucleolus, euchromatin (5) and heterochromatin (6) in the nucleus of a lymphocyte; 7 – cytoplasm of the lymphocyte

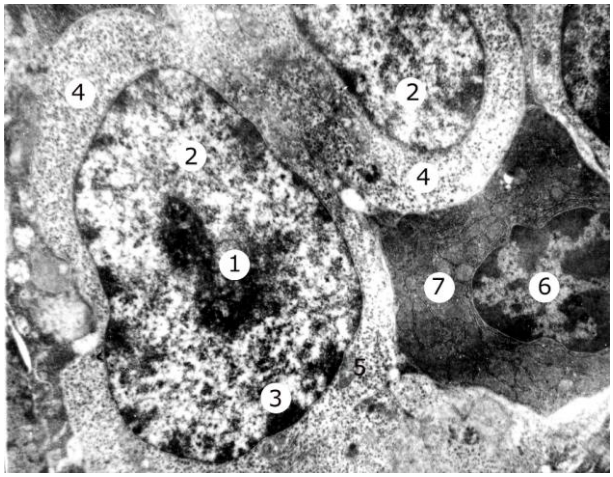


Figure 2. Ultrastructural organization of the white pulp of the spleen of an experimental animal. Extracellular dehydration, (30 days). Electronic photography. Magnification: ×8000. Designation: 1 – nucleolus, euchromatin (2) and heterochromatin (3) in the lymphoblast nucleus; 4 – lymphoblast cytoplasm; 5 – mitochondrion; 6 – plasma cell nucleus; 7 – expanded tubules of the granular endoplasmic reticulum in the cytoplasm of a plasma cell

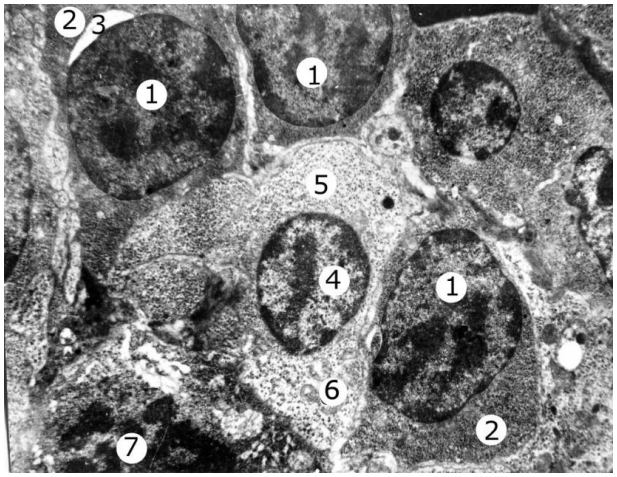


Figure 3. Ultrastructural organization of the white pulp of the spleen of an experimental animal. Extracellular dehydration, (30 days). Electronic photography. Mangification: ×6000. Designation: 1 – nucleus of a lymphocyte; 2 – osmiophilic condensed lymphocyte cytoplasm; 3 – expanded perinuclear space; 4 – reduced lymphocyte nucleus; 5 – lymphocyte cytoplasm; 6 – mitochondrion; 7 - karyolysis

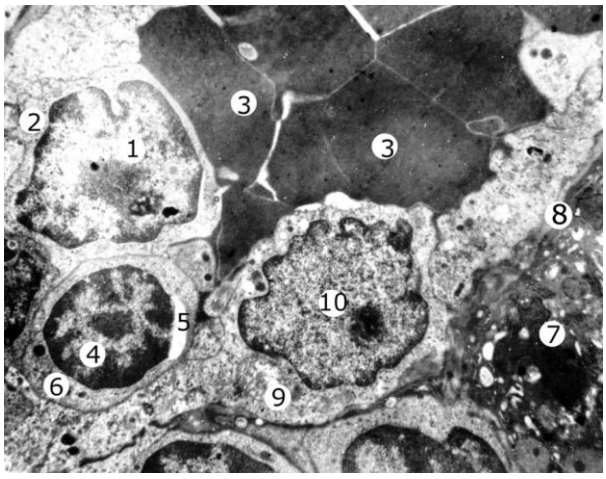


Figure 4. Ultrastructural organization of a fragment of the venous sinus of the spleen of an experimental rat. Extracellular dehydration, (60 days). Electronic photography. Magnification: ×6000. Designation: 1 – nucleus of a large lymphocyte; 2 – illuminated cytoplasm of a large lymphocyte; 3 – erythrocytes in the lumen of the venous sinus of the spleen; 4 – nucleus of the lymphocyte; 5 – perinuclear space; 6 – cytoplasm of lymphocyte; 7 – a cell in a state of karyolysis; 8 – basement membrane; 9 – swollen mitochondrion in the cytoplasm of an endothelial cell; 10 – nucleus of an endothelial cell

A large proportion of reticular cells had a deformed, "dark" and elongated nucleus, contained condensed chromatin, and the contour of the nuclear envelope was uneven. Cytoplasm contains vacuole-like structures, contacts between cells are not clearly traced.

Ultrastructural examination of the spleens of Experimental Group 2 (60 days dehydration), showed cellular disorders in all areas of the parenchyma. The nuclei of cells were most often deformed, shriveled, the perinuclear space was expanded, the cytoplasm contained organelles. Mitotic cells were rarely observed. Many cells appeared to be undergoing apoptosis, and necrosis was more characteristic for this period of study. The intercellular space was not expanded, but contained many vacuole-like structures. There were many areas that contained cellular detritus, including cell membranes, organelles, and chromatin. Large lymphocytes were deformed, their nuclei irregularly rounded, the nuclear envelope formed numerous protrusions and depressions, and the perinuclear space was well visualized in some areas (**Fig. 4**). The cytoplasm contained micropinocytotic vesicles and often contained vacuole-like structures.

Medium and small lymphocytes often had a pyknotically changed nucleus, reduced in size, and irregular in shape. In part of the cells, the cytoplasm was electron-dense, without organelles, and in some it is less electron dense containing swollen mitochondria. The phenomenon of cell death was quite common, that is the nucleus was in a state of karyolysis, the cytoplasm was clear, with signs of cytolysis. Presumably this is why the intercellular space contained areas of cellular detritus. The perinuclear space was not well-defined.

The number of plasma cells and active macrophages in the parenchyma of the spleen was increased at this time point. The nucleus of plasma cells was located eccentrically, had an elongated oval shape, with condensed chromatin. The cytoplasm was filled with pronounced expanded tubules of the granular endoplasmic reticulum (**Fig. 5**). The cytoplasm of active macrophages was completely filled with and chromosomal material of other cells.

Blood capillaries often had a narrowed and deformed space. Endotheliocyte nuclei were deformed and swollen, the cytoplasm contained single organelles, and a large number of pinocytous vesicles. The basal membrane was stratified and thickened.

Ultrastructural examination of the parenchyma of the spleen of an experimental group of animals that underwent a severe degree of extracellular dehydration (after 90 days) revealed deep destructive and degenerative changes. Against the background of depletion of the white pulp, numerous macrophages were identified. In most cells, the nuclei were deformed,

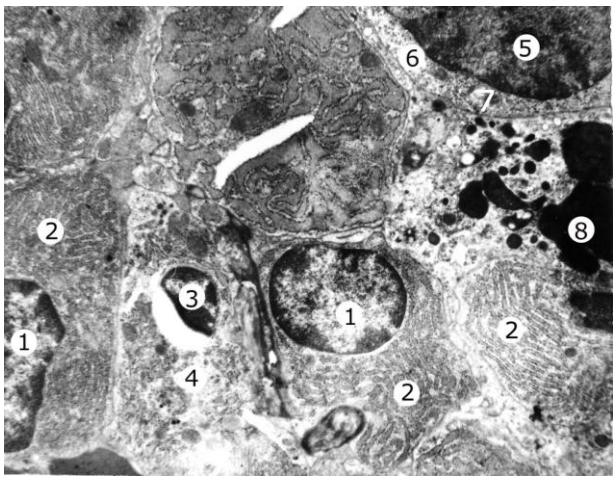


Figure 5. Ultrastructural organization of the spleen parenchyma of an experimental rat. Extracellular dehydration, (60 days). Electronic photography. Magnification: ×8000. Designation: 1 – plasma cell nucleus; 2 – tubules of the granular endoplasmic reticulum in the cytoplasm of a plasma cell; 3 – cell nucleus in a state of karyopyknosis; 4 – cytoplasm with signs of organelle lysis; 5 – the nucleus of a large lymphocyte; 6 – cytoplasm of a large lymphocyte; 7 – mitochondrion; 8 - erythrocyte fragments

shrivelled, and their cytoplasm was often vacuolated or contained damaged organelles. The intercellular space contained numerous polygonal vacuole-like structures and areas of continuous cellular destructuring, which is associated with the total destruction of membranes, nuclei, cytoplasmic organelles, the evacuation of cytoplasmic material into the intercellular space and, ultimately, the formation of vacuole-like structures. Many cells had vacuolated cytoplasm. Isolated areas of slightly changed cells were also observed.The red pulp in this period of the study was characterized by diffuse full blood and small areas of fibrosis, aggregation of blood cellular elements.

Reticular cells have a deformed, reduced, often "dark" nucleus, the nuclear envelope was thinned and formed numerous irregularities, the perinuclear space was pronounced, the cytoplasm was contained almost no organelles. In some cases, the cytoplasm was lightened and vacuolated, filled with fine granular material (**Fig. 6**). Mitochondria were swollen, enlarged and rounded in size. The cytoplasm is vacuolated and filled with fine granular material.

Both in the white and red pulp of the spleen of experimental animals with a severe degree of extracellular dehydration, a large number of active macrophages and plasma cells were preserved (**Fig. 7**). The nuclei of macrophages were often an irregular oval shape, located centrally, with a decreased nuclear-cytoplasmic ratio. The nucleolus was not always visualized, the chromatin was most often condensed (**Fig. 7**). The cytoplasm of macrophages was completely filled with fragments of other cells and hemosiderin.

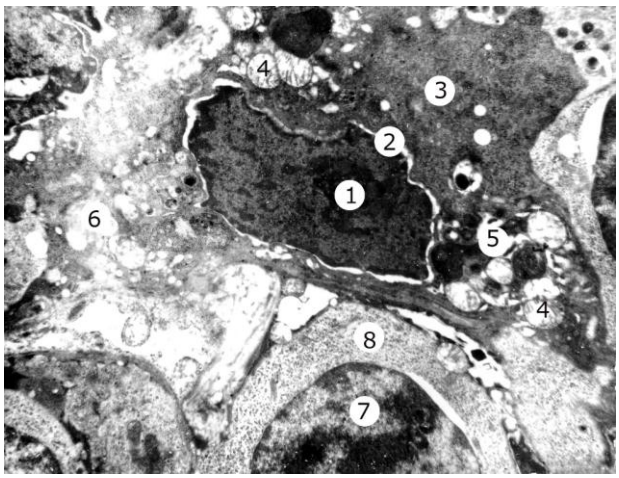


Figure 6. Ultrastructural organization of the white pulp of the spleen of an experimental animal. Extracellular dehydration, (90 day). Electronic photography. Magnification: ×10000. Designation: 1 – nucleus of a reticular cell; 2 – expanded perinuclear space; 3 – osmiophilic condensed cytoplasm of a reticular cell; 4 – swollen mitochondria; 5 – lysis of organelles; 6 – area of cellular detritus; 7 – lymphocyte nucleus; 8 – lymphocyte cytoplasm

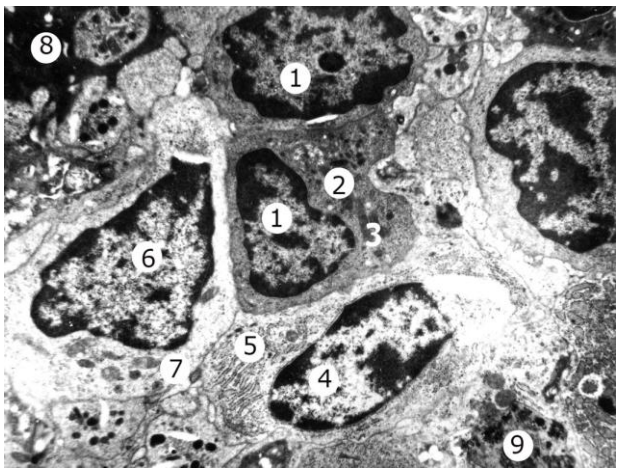


Figure 7. Ultrastructural organization of the red pulp of the spleen of an experimental animal. Extracellular dehydration, (90 day). Electronic photography. Magnification: ×6000. Designation: 1 – macrophage nucleus; 2 – fragments of hemosiderin in the macrophage cytoplasm; 3 –mitochondrion; 4 – plasma cell nucleus; 5 – tubules of the granular endoplasmic reticulum; 6 – lymphocyte nucleus; 7 – mitochondria in the lymphocyte cytoplasm 8 – cellular detritus; 9 – a cell in a state of karyolysis

Many macrophages with congested cytoplasm appeared to be in a state of pre-apoptosis and apoptosis.

Plasma cells had a reduced deformed nucleus, most often located eccentrically, the contour of the nuclear envelope was not clearly visualized throughout, with signs of membrane lysis (**Fig. 7**). The cytoplasm was filled with expanded tubules of the granular endoplasmic reticulum, single swollen mitochondria, ribosomes, and phagosomes.

The main sign of changes in the vessels of the haemomicrocirculatory channel was stasis and thrombosis in the vessels, violation of the rheological properties of blood, and haemorrhages in the parenchyma of the organ. The basal membrane of blood vessels was stratified and swollen. The lumen of blood capillaries was narrowed and deformed, contained deformed erythrocytes. Often, the formed elements of the blood in the lumen were located in a "coin column", completely covering the lumen of the vessel. Endotheliocyte nuclei were deformed, swollen, with increased nuclear-cytoplasmic ratio and the cytoplasm contained a large number of pinocytotic vesicles.

DISCUSSION

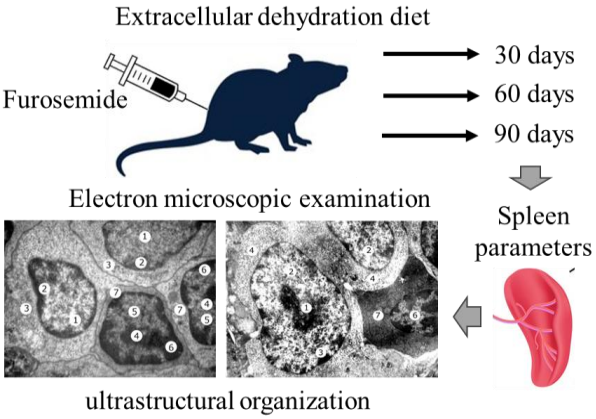
Disorders of sodium balance are manifested as disruption of extracellular fluid volume. Total body water is estimated to be 50%-60% of body weight, varying with age, gender and race, and resides in three main fluid compartments in the body: intracellular (67%), interstitial (25%) and intravascular (8%). Loss of water reduces the distribution space of Na+, thereby disturbing the Na+ and water ratio, leading to hypernatremia and hypertonicity. Because cell membranes are freely permeable to water, this results in osmotic movement of water from the larger intracellular compartment to the extracellular compartment [18].

In this study, as the extent of experimental extracellular dehydration increased, both organometric changes to the spleen and structural changes of all components of the parenchyma at the cellular level increase. After 30 days of extracellular dehydration, the first signs of cell damage are observed in all areas of both the white and red pulp of the spleen. After 60 days, the cell nuclei were deformed, shrivelled, the perinuclear space was expanded, the cytoplasm was lightened, and contained damaged organelles. After 90 days of extracellular dehydration, there was a significant decrease in the mass of the spleen by 24.79% ($p<0.001$), the spleen length by 25.81% ($p<0.001$), thickness – by 23.43% ($p<0.001$),and width – by 22.66 % ($p<0.001$). Submicroscopically, deep destructive-degenerative changes, signs of depletion of the white pulp, diffuse full blood in the red pulp, aggregation of blood cellular elements, stasis and thrombosis occur in the vessels of the hemomicrocirculatory channel, violations of the hemodynamics and rheological properties of the blood in the direction of increased viscosity, hemorrhages in organ parenchyma.

Alterations in the lymphocyte population density within the spleen could reflect modulations in the immune system, precipitated by disruptions in the body's hydration equilibrium.

Changed in macrophage structure, which serve a pivotal role in the body's defensive mechanisms, may constitute one of the grave consequences of dehydration.

During our review of literature, we also analysed the fundamental aspects associated with general dehydration. With general dehydration in rats, in the first days vasodilation occurs, but after 6 days vasocontraction of the vessel wall occurs. This is a protective and compensatory mechanism of the body [19].



As example, under the condition of a mild degree of general dehydration, the authors did not find any significant changes in the vocal folds in the experiment. Under conditions of medium and severe degree, their reliable and significant thinning was revealed. Rehydration restored the condition of the vocal folds only after mild and moderate dehydration [20].

In another study, the influence of dehydration, hypoxia and low temperature on the deep core body temperature of experimental animals was examined. Only the combination of all three factors led to a decrease in internal body temperature [21].

Fetissov S.O. and Meguid M.M. proved that the depth of changes in organs and tissues caused by dehydration is influenced by excess body weight and obesity as accompanying conditions. Under the action of intracellular dehydration in rats receiving a hyperosmotic solution as a drink for eight days, the loss of body weight was 15% in a group of non-obese animals and 10% in obese rats [22].

In the context of a moderate degree of extracellular dehydration, our study reported a discernible decrease in spleen size and mass, which aligns with findings from parallel research on the thyroid gland, albeit with nuanced differences in organ response [4]. The thyroid study, while also noting a reduction in organ weight, paradoxically observed an increase in the size of the thyroid follicles. This highlights an organ-specific adaptation to a moderate degree of extracellular dehydration, with the spleen uniformly shrinking in size, which may reflect its role in the body's response to fluid balance and blood filtration.

Vascular responses to a moderate degree of extracellular dehydration also appear to be a shared phenomenon across different organ systems. Similar to the adenohypophysial-thyroid axis study, our observations indicate a systemic vascular response to a moderate degree of extracellular dehydration in the spleen, marked by increased blood viscosity and signs of ischemia.

At the cellular level, mild dehydration induced a range of changes within the splenic tissue, including cytolysis, apoptosis, and necrosis, indicative of the organ's sensitivity to even slight alterations in hydration status. In contrast, the thyroid gland displayed more subtle cellular adaptations, such as modifications in tyrocyte morphology. This disparity underscores the differential cellular resilience and vulnerability of the spleen and thyroid to extracellular dehydration, likely reflecting their unique physiological roles and structural attributes. While both studies indicate that dehydration leads to significant morphological and functional changes in different organs, the specific changes observed reflect the distinct roles and structures of the spleen and the adenohypophysial-thyroid system [4].

Similar to the above-described changes in blood vessels of the hemomicrocirculatory channel were described in the spleen under the action of an acute stress reaction, which was reproduced by the method of fixing rats with an atraumatic clamp by the neck crease for six hours. At the microscopic level, signs of hemomicrocirculation disorders were revealed, manifested by the phenomenon of blood stasis, leukostasis, sludge syndrome with the formation of microthrombi. In addition, perivascular edema of the central arteries, an increase in the relative area of the periarterial zone of lymphoid nodules, and a decrease in the germinal center were observed [23]. This indicates that the state of dehydration is also stressful for the animal body.

Similar changes in the structure of the spleen parenchyma were detected under process of the reactive oxygen species caused by the action of silver, copper, and gold nanoparticles. The oxidative stress cause changes in cell membrane integrity, vacuolization of cells with indistinct white pulp, altered architecture and stagnation of red pulp, and multinucleated spleen cell formation. [24].

Observed changes in the structure and functionalities of the spleen at varying dehydration levels underscore

the imperative of maintaining optimal hydration to preserve immune defense capabilities.

Additionally, the results provide insight into the spleen's adaptability to various environmental challenges and stressors. The implications of this study extend to the understanding of the spleen's resilience and functional plasticity, suggesting that the adaptive responses of the spleen could serve as a potential biomarker for the systemic impact of dehydration and could inform therapeutic strategies to mitigate such stress-related damages in lymphoid organs.

CONCLUSIONS

The spleen and structural changes of all components of the parenchyma of the organ is dependent on the term of experimental extracellular dehydration. The first marks of cell of both the white and red pulp of the spleen damage are observed after 30 days of experiment. The number and volume of destructive changes in the tissues of the organ increases on the 60th day of the experiment. The end of the experiment is marked by significant changes in both mass and morphological parameters. Dehydration processes cause changes in the structure of macrophages and the density of the lymphocyte population. Also, the data obtained suggest that the mechanism of dehydration similar the mechanism of oxidative stress. The obtained data obtained give an idea of the adaptability of the spleen to external factors, which in the future will make it possible to develop appropriate therapeutic strategies of treatment.

PROSPECTS FOR FUTURE RESEARCH

The prospects for further development are associated with the study of organometric and morphometric changes in the structural components of the spleen in rats during readaptation after exposure to severe extracellular dehydration.

AUTHOR CONTRIBUTIONS

All authors substantively contributed to the drafting of the initial and revised versions of this paper. They take full responsibility for the integrity of all aspects of the work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

STUDY LIMITATIONS

The application of findings from adult male rat models to other species, particularly humans, is constrained by interspecies variations in physiology and hydration regulation. Caution must be exercised when extrapolating these results to human clinical contexts, necessitating further comparative research.

This investigation's scope, focusing on 30, 60, and 90-day periods of mild to severe dehydration, may not fully represent the broader spectrum of dehydration impact. The study's fixed timelines do not account for the dynamic nature of hydration states in real-world scenarios, nor do they fully explore the potential for organ recovery post-dehydration.

These statements succinctly address the concerns regarding the generalizability of the animal model to human populations and the limitations of the study design in reflecting the variable nature of dehydration.

REFERENCES

1. Mietlicki-Baase EG, Santollo J, Daniels D. Fluid intake, what's dopamine got to do with it? Physiology & Behavior. 2021;236:113418. https://doi.org/10.1016/j.physbeh.2021.113418.
2. Bailey TW, do Nascimento NC, Dos Santos AP, Sivasankar MP, Cox A. Comparative proteomic changes in rabbit vocal folds undergoing systemic dehydration and systemic rehydration. J Proteomics. 2023;270:104734. https://doi.org/10.1016/j.jprot.2022.104734.
3. Perry RJ, Rabin-Court A, Song JD, Cardone RL, Wang Y, Kibbey RG, et al. Dehydration and insulinopenia are necessary and sufficient for euglycemic ketoacidosis in SGLT2 inhibitor-treated rats. Nat Commun. 2019;10:548. https://doi.org/10.1038/s41467-019-08466-w.
4. Hryntsova NB, Khomenko IV, Romanyuk AM, Bumeister VI, Kravtsova IA. Morphological and morphometric rearrangements of the rat adenohypophysial-thyroid system under the experimental extracellular dehydration. World of

Medicine and Biology. 2019;2(68):174-9. https://doi.org/10.26724/2079-8334-2019-2-68-174-179
5. Santollo J, Myers KE, Rainer IL, Edwards AA. Gonadal hormones in female rats protect against dehydration-induced memory impairments in the novel object recognition paradigm. *Hormones and Behavior. 2019;114:*104547. https://doi.org/10.1016/j.yhbeh.2019.06.011
6. Santollo J, Edwards AA. How predictive is body weight on fluid intake in rats? It depends on sex. Physiology & Behavior. 2021;229:113262. https://doi.org/10.1016/j.physbeh.2020.113262.
7. Feng P, Wu J, Ren Y, Zhang L, Cao J, Yang L. Early pregnancy regulates the expression of prolactin and its receptor in the thymus, the liver, the spleen and lymph nodes in sheep. Domestic Animal Endocrinology. 2022;81:106731.
8. Chauhan A, Mamun AA, Spiegel G, Harris N, Zhu L, McCullough LD. Splenectomy protects aged mice from injury after experimental stroke. Neurobiology of Aging. 2018;61:102-11. https://doi.org/10.1016/j.neurobiolaging.2017.09.022.
9. Seifert HA, Offner H. The splenic response to stroke: from rodents to stroke subjects. J Neuroinflammation. 2018;15:195. https://doi.org/10.1186/s12974-018-1239-9.
10. Mehrvarz S, Shahabi S, Mohammadi Mofrad R, Sheikhbahaei E, Moslehi M. An experimental rat model of hilar splenic vessel ligation versus splenectomy for spleen trauma. Int J Burns Trauma. 2018;8(5):117-25.
11. Zhang J, Zhao Y, Sun N, Song M, Chen Y, Li L, [et al]. Lycopene Alleviates Chronic Stress-Induced Spleen Apoptosis and Immunosuppression via Inhibiting the Notch Signaling Pathway in Rats. J. Agric. Food Chem.2022;70,9:2889-97. https://doi.org/10.1021/acs.jafc.1c07550.
12. Prykhodko O, Sikora V. Structural changes of the thymus parenchyma under the condition of general dehydration. Journal of Morphological Sciences. 2023; 40:230–256.
13. Kovchun VY, Smiianov VA, Kovchun AV, Kachkovska VV, Sikora VZ. Ultramorphometric characteristics of acini and microvasculature of the pancreas in the presence of moderate dehydration. Wiad Lek. 2022;75(4 pt 2):998-1001. https://doi.org/10.36740/WLek202204214. PMID: 35633332.
14. Gillard BT, Amor N, Iraizoz FA, Pauža AG, Campbell C, Greenwood MP, Alagaili AN, Murphy D. Mobilisation of jerboa kidney gene networks during dehydration and opportunistic rehydration. iScience. 2023 Aug 9;26(9):107574. https://doi.org/10.1016/j.isci.2023.107574. PMID: 37664605; PMCID: PMC10470305.
15. Rybolovlev YR, Rybolovlev RS. Dosage of substances for mammals based on the constant of biological activity. Doklady AN SSSR. 1979;247(6):1513–1516.
16. Pogoryelov MV, Bumeyster VI, Tkach HF, Bolotna IV, Bonchev SD. Suchasni uyavlennya pro vodno-solyovyi obmin (oglyad literatury ta metody vlasnykh doslidzhen). Visnyk problem biolohyi i medytsyny. 2009; 2:8-14. [in Ukrainian].
17. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. 2020. Available via https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf.
18. Asim M, Alkadi MM, Asim H, Ghaffar A. Dehydration and volume depletion: How to handle the misconceptions. *World J Nephrol* 2019; 8(1): 23-32 [PMID: 30705869 https://doi.org/10.5527/wjn.v8.i1.23]
19. Mosendz T. Histo-ultrastruktura nervovo-miazovykh zakinchen ta hemomikrotsyrkuliatornoho rusla skeletnykh miaziv v normi i v umovakh eksperymentalnoi dehidratatsii [Histo-ultrastructure of neuromuscular endings and hemomicrocirculatory bed of skeletal muscles in normal conditions and in conditions of experimental dehydration]. Naukovyi visnyk Uzhhorodskoho universytetu: Seriia: Biolohiia. 2011;30:128-32.
20. Oleson S, Cox A, Liu Z, Sivasankar MP, Lu K-H. In Vivo Magnetic Resonance Imaging of the Rat Vocal Folds After Systemic Dehydration and Rehydration. Journal of Speech, Language, and Hearing Research. 2020;63(1):135-42. https://doi.org/10.1044/2019_JSLHR-19-00062.
21. Uno T, Hasegawa T, Horiuchi M. Combined stimuli of cold, hypoxia, and dehydration status on body temperature in rats: a pilot study with practical implications for humans. BMC Res Notes. 2020;13:530. https://doi.org/10.1186/s13104-020-05375-w.
22. Fetissov SO, Meguid MM. Food intake and meal pattern in response to hyperosmotic-induced dehydration in obese and lean Zucker rats. Nutrition: X. 2020;6:100011. https://doi.org/10.1016/j.nutx.2020.100011.
23. Makarenko OL, Koptev MM, Fylenko BM, Vynnyk NI, Kokovska OV, Makarenko AL [ta in.]. Vliyanie na selezenku ostrogo immobilizatsionnogo stressa, vizvannogo fiksatsiei kris za sheinuyu skladku [The effect on the spleen of acute immobilization stress caused by fixation of rats by the cervical fold]. Ukrainskyi zhurnal medytsyny, biolohii ta sportu. 2021;6,3(31):108-13.
24. Sharif S, Fatima R, Naz S. Toxic Effects of Metallic Nanoparticles on Rat's Spleen; a Review.Polish Journal of Environmental Studies. 2023;32(2):991-7. https://doi.org/10.15244/pjoes/156883.

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