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STATE OF CELL MORPHOFUNCTIONAL SYSTEMS UNDER ACTION OF SEAFARING LABOUR FACTORS: EXPERIMENTAL STUDY

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OBJECTIVE. Carry out morphological analysis of the tissues in the parenchymal organs after single x-ray exposure and in different intensity of decompressive venous gas embolism at the light-optical level.

MATERIALS AND METHODS. The study was carried out on experimental models: mice (acute radiation sickness, simulated by single x-ray exposure with the lethal absorbed dose of 7,8 Gy) and guinea pigs (acute decompression sickness (DS) and recurrent decompressive venous gas embolism of different intensity). Organ fragments of the experimental animals were recorded at different periods after removing from the experiment and processed using transmission electron microscopy to make and analyze semifine sections. There was comparative morphological analysis of semifine section of the liver, renal cortex and respiratory lung field.

RESULTS. There have been revealed reactive changes in various cell differons of the main parenchymal organs: lungs, liver and kidney, most sensitive to such adverse effects as radiation exposure and different levels of decompressive venous gas embolism, specific to professional activity of the crew of surface vessels and submarines. Reactive tissue changes at the cellular level are the initial link in the formation of pathological processes. The analysis of semifine organ sections of mice and guinea pigs has revealed non-specific reactive changes: vacuolization of parenchyma cell cytoplasm, erythrocyte stasis in microvasculature vessels and the formation of specific lesions – gas bubbles in acute DS. Reactive changes, observed in severe exposure, are also detected in cells and at lower loads of the studied factors. Their nature remains, but intensity of morphological manifestations reduces.

DISCUSSION. Studies of morphological changes in structural and functional units of the organ in acute conditions and accumulation of “residual changes” in cells, causing a chronic disease, are necessary for the evidence-based correction of seafarers’ working environment in sub-extreme and extreme conditions of service.

CONCLUSION. Morphological studies with the use of light-optical microscopy reveal initial reactive changes in tissues and cells of experimental animals, subjected to adverse factors, characteristic to professional activity of marine specialists. This contributes to identify factors of seafaring labour, having the most adverse effect on the body, and allows to improve the prevention system, creating conditions for increasing professional longevity of fleet specialists.

KEYWORDS: marine medicine, decompressive venous gas embolism, radiation, nephron, lung, hepatocytes, light-optical microscopy, seafarers’ professional longevity

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СОСТОЯНИЕ МОРФОФУНКЦИОНАЛЬНЫХ СИСТЕМ КЛЕТКИ ПРИ ДЕЙСТВИИ ФАКТОРОВ МОРСКОГО ТРУДА: ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ

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ЦЕЛЬ. Провести морфологический анализ тканей паренхиматозных органов после однократного рентгеновского облучения и при разной интенсивности декомпрессионной венозной газовой эмболии на светооптическом уровне.

МАТЕРИАЛЫ И МЕТОДЫ. Исследование выполнено на экспериментальных моделях: мышах (острая лучевая болезнь, моделированная однократным рентгеновским облучением с летальной поглощенной дозой 7,8 Грей) и морских свинок (острая декомпрессионная болезнь (ДБ) и повторяющаяся декомпрессионная венозная газовая эмболия различной интенсивности). Фрагменты органов экспериментальных животных фиксировали на разных сроках после выведения из эксперимента и обрабатывали по рутинной методике трансмиссионной электронной микроскопии для изготовления и анализа полутонких срезов. Проводили сравнительный морфологический анализ полутонких срезов печени, коркового вещества почки и респираторного отдела легкого.

РЕЗУЛЬТАТЫ. Выявлены реактивные изменения различных клеточных дифферонов основных паренхиматозных органов: легких, печени и почек, наиболее чувствительных к таким неблагоприятным воздействиям, как радиационное облучение и различные уровни декомпрессионной венозной газовой эмболии, характерные для профессиональной деятельности личного состава надводных кораблей и подводных лодок. Именно реактивные изменения тканей на клеточном уровне являются начальным звеном формирования патологических процессов. Анализ полутонких срезов органов мышей и морских свинок выявил неспецифические реактивные изменения: вакуолизацию цитоплазмы клеток паренхимы, стаз эритроцитов в сосудах микроциркуляторного русла и формирование специфических образований – газовых пузырьков при острой ДБ. Реактивные изменения, наблюдаемые при тяжелой степени воздействия, также обнаружены в клетках и при меньших нагрузках исследуемых факторов. Характер их сохраняется, но при этом снижается интенсивность морфологических проявлений.

ОБСУЖДЕНИЕ. Исследования морфологических изменений структурно-функциональных единиц органа при острых состояниях и при накоплении клетками «остаточных изменений», ведущих к хроническому течению заболевания, необходимы для научно-обоснованной коррекции условий труда моряков, находящихся в субэкстремальных и экстремальных условиях несения службы.

ЗАКЛЮЧЕНИЕ. Морфологические исследования с использованием светооптической микроскопии выявляют начальные реактивные изменения в тканях и клетках экспериментальных животных, подвергшихся неблагоприятным факторам, характерным для профессиональной деятельности морских специалистов. Это способствует выявлению факторов морского труда, оказывающих наибольшее неблагоприятное действие на организм, и позволяет совершенствовать систему профилактики, создавая условия для увеличения профессионального долголетия специалистов флота.

КЛЮЧЕВЫЕ СЛОВА: морская медицина, декомпрессионная венозная газовая эмболия, радиация, нефрон, легкое, гепатоциты, светооптическая микроскопия, профессиональное долголетие моряков

Introduction. Professional activity of fleet specialists is accompanied by a significant strain of physical and mental power. In the process of performing functional duties, sailors, especially in long autonomous voyages, are affected by a large number of unfavorable factors, different in modality, intensity and duration of action [1, 2]. These include increased pressure of gas and water environments and radioactive exposure. It should be noted that in addition to emergency situations, radiation injuries caused by low-dose and chronic exposure are also discussed in the scientific community [3–5].

During the period of national fleet formation, a large number of bench and field studies [6] have been carried out, allowing to normalize adverse factors and to achieve in most cases accident-free navigation, preserving not only

seafarers' health, but also an acceptable level of professional performance [7]. In response to the action of a complex of unusual stimuli, adaptive reactions develop in the seafarers' body, allowing it to adapt to the specific features of the activity. Professional adaptation is a continuous process of dynamic equilibrium in the system "subject of labor – professional environment". The basis of this process is a long and deep restructuring of the labor subject in response to stable changes in the professional environment. The reserve capabilities of the body spent on this process are replenished in the period of post-voyage rest and in the intervoyage period. The relevance of this issue led to the fact that a system of physiological support of long voyages was developed in the Navy under the leadership of I.A. Sapov in the 1970s [8].

However, the problem of preserving the professional longevity of seafarers remains poorly researched and its importance is increasing nowadays due to the limited human resources against the background of a rather high share of seafarers' discharge from service due to health problems¹ [9].

To date, conducting tests involving humans is associated with methodological difficulties. A possible promising solution to this problem is the determination of initial morphological changes in tissue cells of experimental animals under the action of adverse factors of maritime labor of different intensity, first isolated, and at the next stage - in the complex as well.

Thanks to modern histological methods of research, it was possible to identify and characterize many processes of cell vital activity under normal conditions and reactive changes in morphofunctional cell systems under the effect of damaging factors [10]. All morphofunctional cell systems are subject to reactive changes: covering and receptor systems (plasmolemma), internal metabolic environment (nucleus, organelles, inclusions) and locomotor systems (microfilaments, cilia, etc.). Reactive changes can be reversible, such as localized destruction of the inner membrane (cristae) of mitochondria, and irreversible, such as total destruction of mitochondrial cristae. The degree of severity of these changes is different, it depends on the time and strength of exposure and is individual in nature².

Reactive tissue changes at the cellular level are the initial link in the formation of pathological processes. Identification of morphological changes in structural and functional units of an organ in acute conditions and in accumulation of residual changes by cells causing chronic course of the disease are necessary for correction of labor conditions of persons working in sub-extreme and extreme conditions, which undoubtedly include seamen.

Objective. To carry out morphological analysis of parenchymatous organ tissues after single X-ray irradiation and at a different intensity of

decompression venous gas embolism at the light-optical level.

Materials and methods. Using the RUM-17 X-ray therapy unit, acute radiation sickness was modeled by exposure of male mice ($n = 18$) to X-rays in the dorsal-chest direction. Material was taken on the 9th and 30th day after exposure to a lethal absorbed dose of 7.8 Gy.

Acute decompression sickness (ADS) was modeled in a barocamera by 4-min non-stop decompression after saturating the guinea pigs' body with air nitrogen at a pressure of $P = 0.96$ MPa for 25 min (Group 1; $n = 4$). Animals with low intensity decompression venous gas embolism (DVGE) constituted group 2 ($n = 3$) and were decapitated 30-40 min after the end of decompression. DVGE was determined using an ultrasound unit operating on the Doppler principle. Animals were exposed to air pressure $P = 0.30$ MPa with isopression for 120 min and non-stop decompression for 2 min. Animals of group 3 ($n = 3$) were subjected to a series of consecutive exposures to increased air pressure with an interval of 2-3 days, decapitation was performed 30-40 min after the end of the fifteenth "dive". The pressure in the barocamera was used from 0.26 to 0.38 MPa with an exposure time of 120 min. Each next "dive" was performed at a pressure 0.02 MPa higher than the previous one. The intensity of DVGE never exceeded 0 points. Thus, the professional activity of divers was simulated. Guinea pigs of group 4 ($n = 3$) participated in the experiments together with animals of group 3, but were decapitated 30 h after the fourteenth "dive". Thus, the morphological picture obtained characterized the aftereffects of previous exposures to tissues and cells.

For morphological analysis fragments of organs of experimental animals were fixed in 2.5% glutaric aldehyde solution on 1% phosphate buffer with postfixation in 4% osmium tetroxide solution on 10% paraformaldehyde. After dehydration, the material was cast in epoxy resin mixture (Epon-Araldite) according to routine procedure for transmission electron microscopy. Semi-thin sections of 1 μm thickness were made on Ultramicrotome PT-PC device (RMC Boeckeler, USA) and stained with 1% toluidine/methylene blue solution. Morphological analysis was performed using a Scope A1 light microscope with an Axio-cam ERc 5s camera.

¹Military Field Therapy. National Guidelines, 2nd edition, revised and supplemented by E. V. Kryukov, Moscow: GEOTAR-Media; 2023. 736 p.

²Mirgorodskaya O. E. Reactive Changes in Tissue Cells under Damaging Effects / edited by prof. I. A. Odintsova. St. Petersburg: Military Medical Academy. 2022. 29 p.

All manipulations were guided by the rules of humane treatment of animals used for scientific purposes³.

Results. Reactive changes in the cytoplasm of hepatocytes, the leading cellular differon of the organ, were detected in the liver parenchyma in acute DS. The character of vacuolization of hepatocytes in the lobule is uneven: in hepatocytes adjacent to the central vein vacuolization is moderate, and at the periphery of the lobule almost the whole cytoplasm of cells is vacuolized (Fig. 1). Part of the liver sinusoidal capillaries is filled with erythrocytes tightly adhering to each other. At the periphery of the lobule, gas bubbles and vacuolized macrophages with phagocytic activity are seen in the lumen of sinusoid capillaries.

In guinea pigs of groups 3 and 4, who underwent a series of consecutive “dives”, the character of morphologic reactive changes remained, but was less severe. A large accumulation of lipid droplets, which is a sign of fatty degeneration of the liver, was also observed on the periphery of the lobules. At the same time stellate liver macrophages (Kupffer cells) were vacuolized to a greater extent than hepatocytes. Mitochondria of hepatocytes of the peripheral zone of the classical lobule were larger in diameter and more numerous than in the center, so the reactive changes were more pronounced. In some hepatocytes, the appearance of a single large vacuole without a membrane, many times larger than the diameter of mitochondria, was noted.

In the kidneys of experimental animals, the main reactive changes were detected in the structures of the cortical substance, represented by cortical nephrons consisting of renal tubules, distal and proximal convoluted tubules. In the guinea pigs of group 1 that died of DS, the capillaries of the nephron tubule are densely filled with erythrocytes and gas bubbles. The erythrocytes trapped by gas in the capillaries take on a multifaceted shape. The renal corpuscles of the nephrons are deformed. The space between the sheets of the capsule is not visible. Cells of

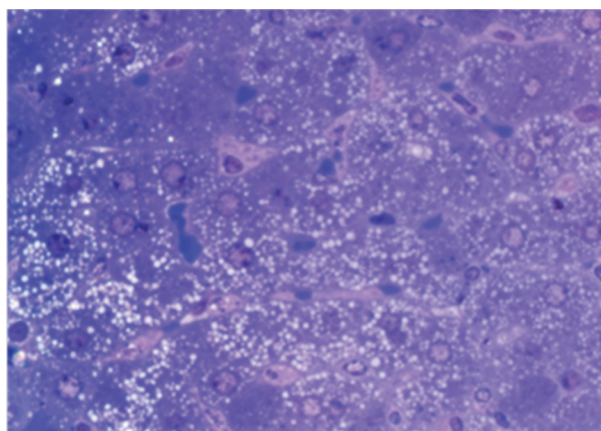


Fig. 1. Guinea pig liver in acute decompression sickness. Intense vacuolization of the cytoplasm of hepatocytes located along the periphery of the lobule is expressed. “Vacuoles” of a larger diameter, localized near the core, presumably “gas bubbles”. A semi-thin slice.

The color is toluidine blue. The scale: 20 μ m.

Рис. 1. Печень морской свинки при острой декомпрессионной болезни. Выражена интенсивная вакуолизации цитоплазмы гепатоцитов, расположенных по периферии дольки. Вакуоли более крупного диаметра, локализованные около ядра, предположительно, – газовые пузырьки. Полутонкий срез.

Окраска толуидиновым синим. Масштабный отрезок – 20 мкм.

convoluted tubules are abundantly vacuolized (Fig. 2). Gas bubbles are also seen in the lumen of the tubules. As in the case of liver hepatocytes, destruction of mitochondrial cristae and larger diameter inert gas bubbles in the cell are observed.

Autopsy of experimental animals with acute DS revealed bloody foam discharge from the lungs and other parenchymatous organs. In the respiratory section of the lungs at the light-optical level, the exit of blood formations into the lumen of alveoli was noted: in some of them, blood formations lay along the periphery, displaced by gas bubbles (Fig. 3). Areas of reduced airiness, compaction of lung parenchyma were detected. Alveolar macrophages localized in the lumen of alveoli were vacuolized. Their outgrowths are directed towards the direction of formed blood elements, which have exited into the lumen.

During a single X-ray irradiation of mice liver with a lethal dose of 7.8 Gy, we observed pronounced vacuolization in the cytoplasm of hepatocytes and an increase in their area, which causes partial narrowing of the lumen of sinusoidal capillaries.

³Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the Protection of Animals Used for Scientific Purposes. St. Petersburg: Rus-LASA "Association of Specialists in Working with Laboratory Animals", working group on translations and publication of thematic literature, 2012. 48 p.

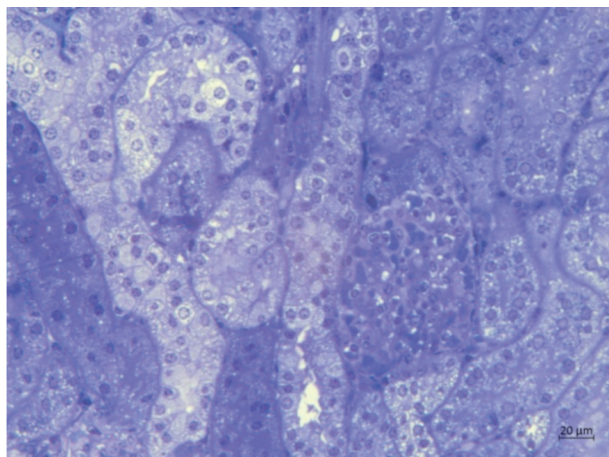


Fig. 2. Guinea pig kidney in acute decompression sickness. Fine-dispersed vacuolization and larger vacuolization in the structures of nephrons. Capillary stasis of the microcirculatory bed of the renal corpuscle. A semi-thin slice. The color is toluidine blue. The scale: 20 μm .

Рис. 2. Почка морской свинки при острой декомпрессионной болезни. Мелкодисперсная вакуолизация и более крупная вакуолизация в структурах нефронов. Стаз капилляров микроциркуляторного русла почечного тельца. Полутоновый срез. Окраска толуидиновым синим. Масштабный отрезок – 20 мкм.

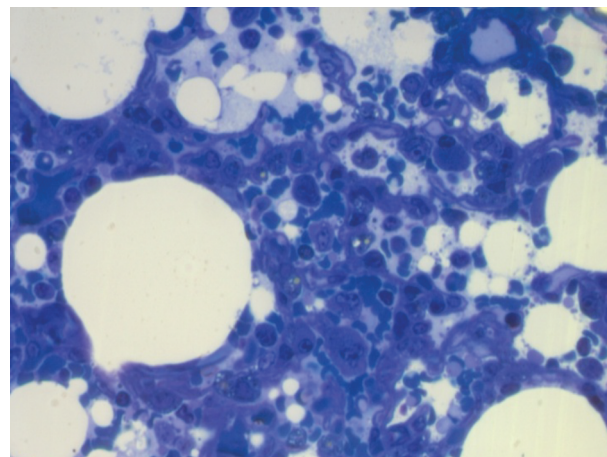


Fig. 3. The respiratory department of the guinea pig lung in acute decompression sickness. A semi-thin slice. The color is toluidine blue. The scale: 50 μm .

Рис. 3. Респираторный отдел легкого морской свинки при острой декомпрессионной болезни. Выход форменных элементов крови в просвет альвеол. Наличие пузырьков газа, деформирующих строму органа. Полутоновый срез. Окраска толуидиновым синим. Масштабный отрезок – 50 мкм.

At the same time, the reactively changed cells were evenly distributed in the liver lobule (Fig. 4).

In the kidney, erythrocyte stasis was observed in the capillary network of renal tubules of cortical nephrons at a lethal dose of X-ray irradiation. On the 9th day after irradiation in some renal tubules the lumen between the outer and inner sheets of the capsule is narrowed or absent, and the lumen of the capillaries of the tubules is dilated. Some cells of proximal tubules contain vacuolized cytoplasm, but the nucleus retains its native structure with predominance of euchromatin (Fig. 5). At later terms (30th day) after irradiation the character of reactive changes is more pronounced: practically in all renal tubules of nephrons the lumen between capsule sheets is absent, the lumen of capillaries of the tubular network is deformed, the lumen of proximal tubules is dilated, probably due to the loss of brush border height on the apical surface of cells [11]. Under the studied extreme factors on the kidney (decompression gassing and irradiation) no ruptures of plasmalemma cells were observed. Formed blood elements are located in the lumen of vessels, they are absent in the cavity between the sheets of the renal celiac capsule.

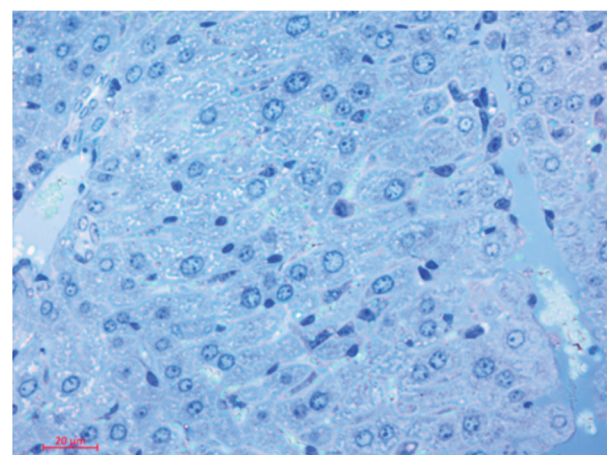


Fig. 4. Mouse liver at a lethal radiation dose of 7.8 Gy on the 9th day. A semi-thin slice. The color is methylene blue. The scale: 20 μm .

Рис. 4. Печень мыши при летальной дозе облучения 7,8 Гр на 9-е сутки. Гепатоциты с вакуолизированной цитоплазмой расположены в дольке равномерно. Полутоновый срез. Окраска метиленовым синим. Масштабный отрезок – 20 мкм.

In the lungs of experimental animals at autopsy after exposure to a single X-ray irradiation of 7.8 Gy on the 9th and 30th days, extensive foci of hemorrhages were observed. At the light-optical level, local compaction of exudate-soaked areas

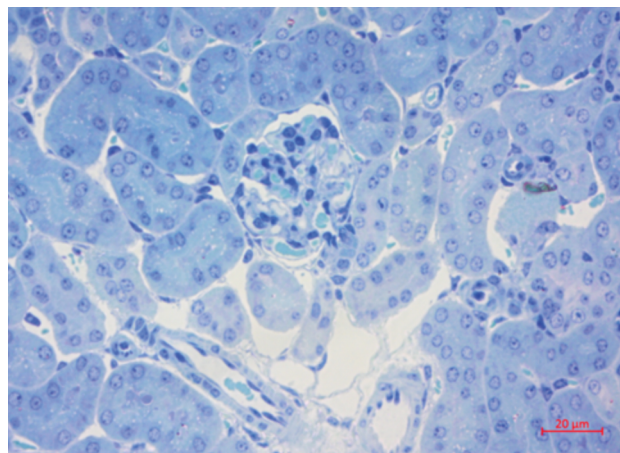


Fig. 5. Mouse kidney at a lethal radiation dose of 7.8 Gy on the 9th day. A semi-thin slice. The color is methylene blue. The scale: 20 μm .

Рис. 5. Почка мыши при летальной дозе облучения 7,8 Гр на 9-е сутки. Неравномерно расширенные просветы капиллярной сети почечного тельца. Мелкодисперсная вакуолизация нефроцитов канальцев. Полутонкий срез. Окраска метиленовым синим. Масштабный отрезок – 20 мкм.

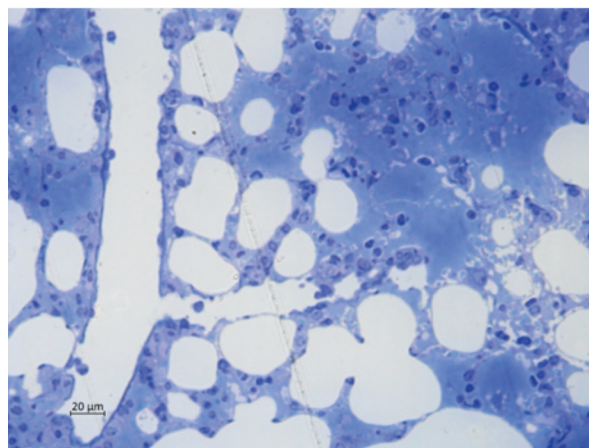


Fig. 6. The respiratory department of the mouse lung at a lethal radiation dose of 7.8 Gy on the 30th day. A semi-thin slice. The color is toluidine blue. The scale: 20 μm .

Рис. 6. Респираторный отдел легкого мыши при летальной дозе облучения 7,8 Гр на 30-е сутки. Часть альвеол заполнена экссудатом. Участки аэрогематического гистона отечны. Полутонкий срез. Окраска толуидиновым синим. Масштабный отрезок – 20 мкм.

of respiratory parenchyma near bronchioles and small vessels was revealed. The number of active macrophages increases in such areas. All lumen of bronchioles are filled with mucous secretion (Fig. 6). Areas of the aerochemical barrier (histone) are thickened, probably, as a result of edema.

Discussion. The uneven character of hepatocyte vacuolization and localization of gas bubbles in acute DS are probably associated with the peculiarities of blood supply to the liver lobules: its peripheral sections receive blood saturated with indifferent gas (in this case, nitrogen) brought from internal organs, which, taking into account the slow intra-organ blood flow, contributes to gas formation and hepatocyte damage in the form of intense vacuolization. Even moderate exposure to increased pressure, which does not lead to acute forms of DS, causes reactive changes in cells, which can subsequently develop into irreversible ones. Under different experimental effects, the character of vacuolization spreading can be different. It is known that the cytoplasm of hepatocytes contains more glycogen in the periphery of lobules, and lipids in the center; therefore, it is believed that many severe disorders develop in the central part of the lobule. However, as our previous studies at the ultrastructure level show, the formation of vacuoles can occur due to the

destruction of mitochondrial inner membranes (cristae) and be of a fine-dispersed character [12, 13]. The appearance of a single large “vacuole” without a membrane in some hepatocytes is interpreted by us as the formation of a gas bubble inside the cell [13]. Focal destruction of mitochondrial cristae in some cells – total, can cause disruption of lipid metabolism of hepatocytes and, as a consequence, excessive accumulation of lipid droplets in the cytoplasm of cells and blood plasma in experimental animals at extreme exposure. The detected signs of fatty liver dystrophy at systematic “diving” testify to the beginning of the formation of the so-called general pathological process, which in its various combinations and in different degrees of severity is observed in all types of pathology, regardless of the originality of their etiology, pathogenesis and clinical and anatomical picture^{4, 5}. Morphological changes in the respiratory part of the lungs, revealed at the

⁴Sarkisov D. S. Essays on the History of General Pathology / USSR Academy of Medical Sciences. Moscow: Medicine. 1988. 336 p.

⁵Professional pathology of Navy specialists. Pathophysiology: a guide for students and doctors of the Military Medical Academy and Military Medical Institutes / Ed. V. Yu. Shanin. SPb: ELBI-SPb. 2005. 639 p.

light-optical level, testify to the violation of permeability of the aerogemetic histone structures and are characteristic of pulmonary edema, resulting from massive embolization by gas bubbles of the vessels of the small circle of circulation [11].

Previously, at the ultrastructural level, we showed the content in the vacuoles of alveolar macrophages, membrane-like structures – unutilized lamellar bodies, which synthesize type II alveolocytes. Vacuolization of the cytoplasm of type II alveolocytes is caused by swelling and rupture of lamellar bodies, which are precursors of surfactant, as well as by destruction of the inner membrane of mitochondria and expansion of the cisterns of the endoplasmic network [12].

When studying the effect of extreme factors on the kidneys (decompression gassing and irradiation), it was found that the structures of the hematonephridial histone retain their integrity. This explains the absence of pronounced clinical manifestations of kidney damage.

Conclusion. Cells are the basic structural and functional unit of various tissues that form organs. The ability of cell structures to react, to respond with morphological changes, adapting to changing conditions of external and internal environment, is the basis of fundamental diagnostic signs of the developing pathological process. The search for objective ways to assess cell viability and reactive state of tissues is relevant in the development of a system of prevention of the main pathological conditions characteristic of specialists working in sub-extreme and extreme conditions. As an example of such prophylaxis in divers it is possible to give development of methods of increasing body resistance to decompression gas formation⁶.

It is logical that more pronounced morphological reactive tissue changes were observed under

extreme conditions: acute DS and lethal dose of X-ray irradiation, which allows us to describe and analyze the nature of morphological picture of each exposure most fully.

The study of reactive changes in the structure of tissues and cells on different terms after exposure to lethal doses of radiation and repeated exposure, permissible according to today's ideas, decompression venous gassing, allow us to assume the accumulative nature of morphological changes and characterize the initial stages of the pathogenesis of the chronic form of decompression disease [15]. The presented results of the experimental work indicate the prospect of morphological studies to identify the initial reactive changes in the body of experimental animals exposed to adverse factors characteristic of the professional activity of marine specialists. In this case we consider it reasonable to use the technique of light optical microscopy as more accessible for large-scale studies and at the same time possessing the necessary morphological informativeness. After identification of tissues and cells with the most pronounced disorders at the next stage of the study to determine the underlying pathogenetic mechanisms it is advisable to use histochemistry methods.

In the future it is necessary to study the effect on the body of animals of pre-critical doses acting on the personnel of surface ships and submarines for a long time, and then to model their complex effect. Thus, it will be possible to identify at the fundamental level the factors of marine labor and their combinations having the most adverse effect on the body. And this, in turn, will allow to improve the system of prevention and create conditions for increasing the professional longevity of fleet specialists.

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