Mikhail A. Kotskiy[1], Evgeny U. Bonitenko[1,2], Anton A. Tonshin[1], Polina V. Rodchenkova[1], Margarita P. Muravskaya[1], Julia V. Tkachuk[1], Alexander A. Cannibalotsky[3], Arman L. Kochoyan[4]

Liquid respiratory desaturation is a new method of preventing decompression sickness

[1]Izmerov Research Institute of Occupational Health, 31, Budyonnogo, Moscow, 105275;
[2]Sevastopol State University, 33, Universitetskaya St., Sevastopol, 299053;
[3]Research Institute of Ambulance named after N.V. Sklifosovsky Department of Health of the city of Moscow, 3, Bolshaya Sukharevskaya Sq., Moscow, 129090;
[4]Russian Center of Forensic Medical Examination, 12/13, Polikarpov St., Moscow, 125284

Introduction. According to the literature data, the method of liquid ventilation for the prevention of decompression sickness (DCS) was proposed only with the condition of its initiation before compression, that excluded the physical basis of the disease — an excess of metabolically indifferent gas in the tissues. However, as the analysis shows, in most cases, the evacuation of the crew of an emergency submarine is aggravated by being in gases' increased pressure environment. So the casualty tissues become obviously saturated with indifferent gas.

Experimental confirmation of the possibility of rapid tissues' desaturation of nitrogen during respiration by denitrogenizated and oxygenated in a normal conditions respiratory fluid (hereinafter — the method of liquid respiratory desaturation) was obtained on the biological model of DCS of Syrian hamsters.

The study aim is an experimental substantiation of the possibility to use liquid respiratory desaturation as a method of preventing the development of decompression disorders.

Materials and methods. Scientists have performed a study on 24 mature male Syrian hamsters weighing 165–185g, aged four months, using an experimental laboratory hyperbaric stand for temporary maintenance of small laboratory animals under high pressure of a gas or liquid medium with the possibility of switching from one medium to another in isobaric conditions. The research methodology is based on the assessment of the clinical presentation of decompression disorders and the results of ultrasound examination of gas formations in the heart, large veins and liver after the fast non-stop decompression, in the background of preliminary saturation of the animal's body with indifferent gas (nitrogen) by staying in the air under the pressure 0.6 MPa (60 MWC) for six hours. The effect on experimental groups animals deferens from the control group by the period of immersion and spontaneous breathing in the respiratory fluid (20, 30 and 40 minutes) before decompression.

Results. The authors analyzed the clinical picture of acute decompression disorders. The degree of gas formation in small laboratory animals was assessed by researchers using ultrasound using a semi-quantitative method. Spontaneous breathing with the prepared liquid, lasting 30 minutes or more, made it possible to remove excess nitrogen from the body of animals of experimental groups, providing etiopathogenetic prevention of DCS before decompression. The article presents the data of morphological studies.

Conclusion. *Liquid respiratory desaturation is a method of preventing decompression disorders based on the removal of metabolically indifferent gases from the body during liquid respiration, in the presence of a stress gradient from tissues into the respiratory fluid. The method allows the metabolically indifferent gases' rapid desaturation from the body by liquid ventilation before/or during decompression, thereby creating conditions of ultra-fast decompression modes without the risk of decompression disorders.*

Keywords: *liquid breathing; respiratory fluids; perfluorodecalin; perfluorohexane; hyperbaric; decompression disorders; liquid respiratory desaturation; DCS*

Ethics. Studies involving laboratory animals were conducted in compliance with the following regulations: Helsinki Declaration of 2000 "On humane treatment of animals", Order of the Ministry of Health of the USSR No. 755 of 12.08.1977 "Rules for carrying out work using experimental animals", Order of the Ministry of Health and Social Development of Russia No. 199n of 01.04.2016 "On approval of the rules of laboratory practice". The protocol of the study was approved by the Ethics Committee of the Izmerov Research Institute of Occupational Health.

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For correspondence: *Mikhail A. Kotsky,* Chief Researcher of the Target Search Laboratory of Translational Medicine, Izmerov Research Institute of Occupational Health. E-mail: 79031227522@yandex.ru

Contribution:
Kotsky M.A. — the concept and design of the study, data collection and processing, writing the text, editing;
Bonitenko E.Yu. — the concept and design of the study, writing the text;
Tonshin A.A. — the concept and design of the study, editing;
Rodchenkova P.V. — data collection and processing, conducting ultrasound research;
Muravskaya M.P. — data collection and processing;
Tkachuk Yu.V. — data collection and processing;
Kanibolotsky A.A. — conducting pathomorphological studies;
Kochoyan A.L. — conducting pathomorphological studies.

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Introduction. For the first time, in 1962, J. Kylstra and co-authors have suggested the idea of using liquid breathing (LB) to rescue people from underwater objects [1]. The authors describe cases of spontaneous LB of mice arriving in immersion under pressure up to 16.0 MPa in sterofundin saturated with oxygen under pressure. In 1968, J. Kilstra and co-authors decompressed mice independently inhaling perfluorocarbon respiratory fluid (PFC RF) from a depth of 1660 m in three seconds [2]. According to subsequent studies, mice immersed in perfluorocarbon respiratory fluid could tolerate decompression up to 25 MPa without harmful consequences (which corresponds to a depth of immersion in water at 2500 m). At the same time, decompression at a rate of 10 MPa/s did not lead to death and the development of decompression disorders [3–6].

The studies results of large animals' liquid respiration in a hyperbaric environment are also found in the scientific literature [7, 8]. In experiments on dogs whose lungs were

ventilated with PFC RF for 2 hours at an average pressure of 10 MPa, the researchers noted that the pH and partial pressure of carbon dioxide in arterial blood were maintained close to normal values [8].

This work confirmed the technical possibility of large laboratory animals (dogs) breathing PFC RF at depths of 500–700 meters of water, and the fact that the subsequent non-stop rapid decompression did not entail the development of decompression sickness (DCS) in animals. From the point of view of emergency wet evacuation from great depths, the use of liquid breathing provides advantages unattainable for gas breathing, namely: protection of the lungs and chest from extreme deformations at high compression and decompression rates, as well as prevention of decompression disorders at any rate of ascent from currently inaccessible depths [9].

It is necessary to pay attention to the fact that in order to prevent the development of decompression sickness (DCS), scientists previously proposed using the method of liquid breathing (LB) with the condition of its initiation before the start of compression to eliminate the physical basis of the disease — an excess of metabolically indifferent gas (in particular nitrogen) in tissues. However, the analysis shows that in most cases, the evacuation of the crew of a submarine in distress is aggravated by the presence of a period of stay in increased gases' pressure environment, the body of the evacuee is obviously saturated with indifferent gas.

We carried out mathematical modeling of the process of liquid respiration in hyperbaria and conducted computational experiments that confirmed the possibility of rapid desaturation of an organism that had previously been under increased gases' pressure conditions from nitrogen by breathing liquid denitrogenised and oxygenated under normal conditions. The next step was to confirm this hypothesis on the biological model of decompression sickness (DCS) in Syrian hamsters. Taking into account the fact that the research was planned to be carried out on small laboratory animals, the task of methods of lifetime visualization of gas formation in the body became actual.

Currently, the ultrasound method based on the Doppler effect the scientists widely used in diving medicine to study the mechanisms and features of intravascular gas formation in decompression sickness, as well as to monitor current regimes and develop new safety criteria for decompression [10]. However, in the available literature we have not found information about its use on small biological objects. In this regard, one of the goals of our study was to develop criteria for quantifying the degree of gas formation in small laboratory animals.

The study aim is experimental assessment of the possibility of using liquid respiratory desaturation as a method of preventing the development of decompression disorders.

Materials and methods. We have carried out the study on mature males of Syrian hamsters weighing 165–185 g, aged four months, obtained from Krol-Info LLC. Before the study, the animals were quarantined for 14 days. During quarantine and during the study, the animals were kept in accordance with the requirements of GOST 33215-2014[1] and GOST 33215-2014[2] on a standard water and food ration in a separate room.

The research methodology was based on the assessment of the clinical picture of decompression disorders and the results of ultrasound studies of gas formation in the chambers of the heart, large veins and liver after the fastest non-stop decompression of animals, against the background of preliminary saturation with indifferent gas (nitrogen) by staying in the air under high pressure.

The mode of exposure the experimental groups' animals differed from the control by the presence of a time-limited exposure to spontaneous breathing in immersion in respiratory fluid before decompression.

The scientists carried out research using an experimental laboratory hyperbaric stand designed for temporary maintenance of small laboratory animals under high pressure of a gas or liquid medium with the possibility of switching from one medium to another under isobaric conditions. The description of the stand, as well as the methods of the study are presented in the work of M.A. Kotsky and co-authors (2021) [9].

We used a mixture of perfluorodecalin (PFD) and perfluorohexane (PFH) in a volume ratio of 40:60 as a solution. The choice of such a composition, based on the results of preliminary studies, was found to be optimal in terms of the ratio of the duration of independent waiting to survival.

The authors have carried out oxygenation of the respiratory fluid (RF) and its denitrogenisation by bubbling with oxygen using a Nidek Mark 5 nuvo Lite concentrator (USA) for an hour at a flow rate of three l/min. The oxygen concentration in respiratory fluid was determined using the oxygen meter Aktakom ATT-3010 (Russia) with the function of a thermometer and was ~95.0 vol.% of the maximum possible saturation.

The choice of the mode of stay in a compressed air environment (6.0 hours under a pressure of 60.0 m of water) the scientists have determined the need to minimize the risk of oxygen poisoning with pronounced nitrogen saturation of rapidly, medium and slowly saturated tissues. We increased the pressure in the animal holding chamber by adding compressed air suitable for breathing to an excess pressure of 0.6 MPa for two minutes with a twofold deceleration of speed (up to 0.2 MPa/min), with a pressure set in the range from 0 to 0.2 MPa. The animals were kept in a hyperbaric environment for 360 minutes. Ventilation of the chamber was provided by constant replacement of the gas volume of the chamber due to automatic air supply when gas leaks through the pickling safety valve at a speed of about 1.0 l/min. To reduce the risk of lung barotrauma, decompression of control group animals was carried out without stopping for 30 seconds with a twofold deceleration of speed during the transition from an increased pressure of 0.2 MPa to normal conditions. Decompression of experimental animals when immersed in respiratory fluid was carried out at a constant rate for 15 seconds.

Researchers distributed 24 Syrian hamsters, males weighing 175±10 grams, into four groups. The characteristics of the experimental conditions for each of the groups are presented in ***Table*** 1.

[1] GOST 33215-2014 Guidelines for the maintenance and care of laboratory animals. Rules of equipment of premises and organization of procedures.

[2] GOST 33216-2014 Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory rodents and rabbits.

Table 1

Animals' groups distribution by the testing conditions

Group number	Compression time to the maximum pressure of 0.6 MPa, min	Duration, min.		Respiratory fluid temperature, °C	Decompression time, sec.
		exposure in compressed air at a pressure of 0.6 MPa	independent liquid breathing in immersion		
1	2	360	0	—	30*
2			20	24.0*	15
3			30		
4			40		

Note: * — decompression of the control group was carried out non-stop with a 2-fold speed deceleration during the transition from 0.2 MPa to normal conditions

During the experiments, the authors have recorded and evaluated following indicators:

- rectal temperature (°C) before and after the experiment;
- duration of the latent period before the manifestation of decompression sickness (min);
- the degree of gas formation by ultrasound diagnostics;
- severity of clinical manifestations of decompression disorders;
- the number of dead to the total number of animals in the group (%);
- the average time of death of animals from decompression sickness and other causes (min);
- results of pathomorphological examination (macro- and microscopic).

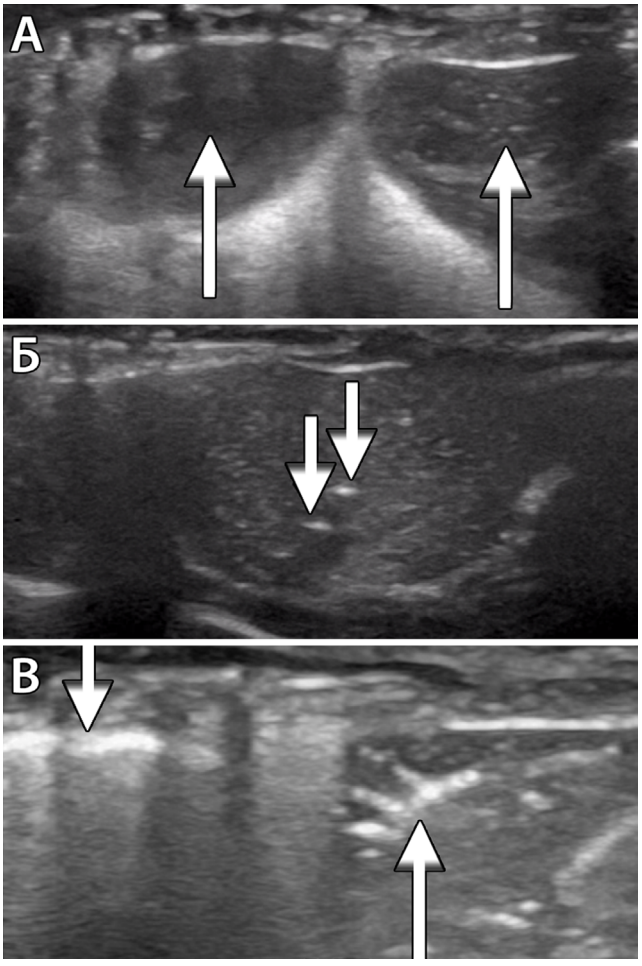

Fig. 1. Assessment of the gas formation's degree by ultrasonic method (A — the left arrow indicates the chambers of the heart, the right arrow indicates the intrahepatic structures are normal, no gas bubbles; B — the arrows indicate single gas bubbles in large liver veins; C — gas emboli in the chambers of the heart (left arrow), multiple gas bubbles in intrahepatic structures (right arrow))

To assess the severity of DCS, we have developed a semi-quantitative method of ultrasound assessment of the degree of gas formation in the cardiovascular system and in the intrahepatic structures of small laboratory animals. The study was conducted using the GE Healthcare LOGIQ A6 ultrasound scanning device (USA) as standard.

The developed indicators and evaluation criteria are presented below in ***Figure 1***:

" – " — there are no gas bubbles in the chambers of the heart, large veins (inferior vena cava and portal) and intrahepatic structures;

" + " — single gas bubbles in large veins (inferior vena cava and portal) of the liver and/or chambers of the heart;

" ++ " — the chambers of the heart and large veins (lower hollow and portal) are filled with gas, there are multiple gas bubbles in the intrahepatic structures.

Pathomorphological examination includes autopsy, macro- and microscopic (histological) examination of internal organs. The studied tissues were fixed in a 10% solution of buffered formalin for 24 hours, after which they underwent standard treatment in isopropyl alcohol and paraffin for the manufacture of histological preparations.

For microscopic examination, we stained the sections with hematoxylin and eosin. Morphological and micromorphometric studies were carried out using a Leica DM LS light-optical microscope (Germany) equipped with an ocular micrometer. The micrography was carried out using a Leica DC320 digital camera.

The Ethics Committee of the Izmerov Research Institute of Occupational Health have approved the Protocol of the study.

The scientists carried out statistical processing of the data obtained by methods of variational statistics using the Microsoft Excel program. Descriptive statistics were used to analyze the data: average values (M) and standard errors of the average value (m) were calculated. We checked the data for the normality of the distribution using the Shapiro–Wilk criterion.

The authors have analyzed intergroup differences by parametric methods using the Student's criterion. The differences were considered significant at $p<0.05$. We used the

nonparametric Mann–Whitney method for data belonging to a distribution other than normal.

Results. In the course of research, it was found that in the control group, clinical manifestations of severe DCS developed almost immediately after decompression. After extraction from the chamber, all animals showed a slight decrease in body temperature from 37.5±0.3 to 37.0±0.4°C. The clinical picture of decompression disorders developed rapidly after a short latent period of 4.3±2.1 min. ***(Table 2)***. At first, scratching and pronounced psychomotor agitation were recorded, which subsequently, against the background of rapidly progressing phenomena of acute respiratory and cardiovascular insufficiency, was quickly replaced by adynamia, single convulsive twitching and death. The number of adverse outcomes in the group was — 100% with an average time of development of fatal outcomes — 5.1±1.4 minutes ***(Table 3)***.

In the group two, the body temperature of the animals after removal from the stand was 32.0±0.1°C. Clinical manifestations of severe DCS developed after a short latent period, the duration of which was on average 2.88 times longer than in the control and was 12.4±2.0 minutes. In contrast to the control group, during the latent period, ataxia phenomena prevailed in animals. The beginning of the formation of gas bubbles in the cavities of the heart, large veins and liver was observed after 10.9±1.2 minutes, in contrast to the control group, in which they were recorded after 3.5±1.0 minutes after decompression is completed. However, the degree of gas formation in both groups was the same and was estimated as the maximum in " ++ " ***(Table 3)***.

Clinical manifestations of severe DCS in this group, as in the control group, are associated with an increase in the phenomena of acute respiratory and cardiovascular insufficiency with the background of severe ataxia.

These disorders were manifested by a rapid change of tachypnea to bradypnea, the appearance of pathological types of breathing and the subsequent cardiac arrest. The mortality rate in this group, as well as in the control group, was 100%, but the death of animals was observed later. Thus, the average time of death was 2.67 times longer than in the control and was 13.7±2.2 minutes.

In turn, in group three and group four 4, the body temperature after extraction was 28.9±0.3 and 27.1±0.3°C, respectively. We didn't observe clinical signs of decompression disorders in animals of these groups.

The animals' state after decompression and removal of respiratory fluid from the respiratory tract did not differ significantly from the animals to which the researchers performed respiratory fluid without compression in the framework of preliminary experiments. It should be noted that in animals of group four, gas formation in the chambers of the heart, large veins and intrahepatic structures was not registered during ultrasound examination. At the same time, we recorded one case of registration of single glass bubbles in the hepatic veins of an animal of group three, which regressed within an hour.

However, at the end of the first or beginning of the second day, the condition of the animals gradually worsened. The appearance of tachypnea was noted and dyspnea gradually increased, which was of a mixed nature. Against the background of progressive respiratory failure, the animals assumed a forced position. Later the phenomena of acute respiratory failure were joined by disorders of the cardiovascular system, which in turn led to the death of animals.

Unfavorable outcomes in groups three and four were 100% and, as a rule, were recorded at the end of the first — beginning of the second day after decompression. The average time of death of animals was 1578±258 and 1316±182 minutes accordingly. However, in one case, the death of an animal of the third group was registered after 4500 min. (at the beginning of four days).

Death causes' verification in the groups was carried out by conducting 24 pathoanatomical studies of dead animals (six in the control group and eighteen in the experimental groups) ***(Table 4)***.

Table 2

Timing of appearance and severity of decompression disorders

Group number	Ultrasound data		DCS	
	Bubble appearance time, min	The degree of gas formation	Hidden period, min	Severity of manifestations*
1	3.5±1.0	++	4.3±2.1	+
2	10.9±1.2	++	12.4±2.0	+
3	11.2	+	–	–
4	—	–	–	–

Note: there are no signs of decompression sickness — " – "; single gas bubbles in large liver veins — " + "; pronounced signs of decompression sickness — " ++ ".

Table 3

Causes and timing of laboratory animals' death

Group number	The number of dead to the total number of animals in the group	Average time of death, min		
		from DCS	not from DCS	
			early	late
1	6/6 (100)	5.1±1.4	—	—
2	6/6 (100)	13.7±2.2	—	—
3	6/6 (100)	—	1,578±258	4,500
4	6/6 (100)	—	1,716±402	—

Table 4

Typical changes observed in the pathoanatomical examination of control and experimental groups' dead animals

Indicators	Animal groups			
	one	two	three	four
Average time of death, min.	**5.1±1.4**	**13.7±2.2**	**1,578±258**	**1,316±182**
Macroscopic signs				
bloody discharge from the mouth and nasal passages	No	No	Yes	Yes
gas emboli in large veins and chambers of the heart	Yes	Yes	No	No
foaming after crossing large vessels of the thoracic and abdominal cavities	Yes	Yes	No	No
lungs drown in water	No	Yes	Yes	Yes
Microscopic signs				
Lungs	bronchi and alveoli of normal structure without signs of emphysema; fullness of the capillaries of the interalveolar septa	bronchi and alveoli are empty with signs of mild emphysema; fullness of the capillaries of the interalveolar septa	subpleural and alveolar hemorrhages from fresh erythrocytes; alveolar edema; fullness of the capillaries of the interalveolar septa; in part of the alveoli, leukocyte infiltration	
The brain	edema, mainly perivascular; capillary fullness, focal hemorrhages		fullness of the vessels of the soft meninges; perivascular edema	
Liver	the usual structure		pronounced fullness of the central veins and vessels of the triads	

As can be seen from the presented data, in the control and group No. two, the morphological picture corresponded to a severe degree of decompression sickness and was macroscopically manifested by the presence of gas emboli in large veins and in the chambers of the heart detected by ultrasound examination, as well as foaming at the intersection of large vessels of the thoracic and abdominal cavities.

While histological examination revealed cerebral edema (mainly perivascular) with multiple focal hemorrhages in the absence of changes from other organs.

Macroscopic examination of all animals that died in group three and group four drew attention to the presence of bloody discharge from the mouth and nasal passages, the absence of gas emboli in large veins and chambers of the heart. During histological examination of animals that died at the end of the first and beginning of the second day, we recorded the following changes in the lungs: subpleural and alveolar hemorrhages from fresh erythrocytes; edema of the alveoli; fullness of the capillaries of the interalveolar septa ***(Fig. 2A)***; leukocyte infiltration in part of the alveoli ***(Fig. 2B)***.

In the brain, there was a fullness of the vessels of the soft meninges and perivascular edema. Histological changes were not detected in other tissues and organs.

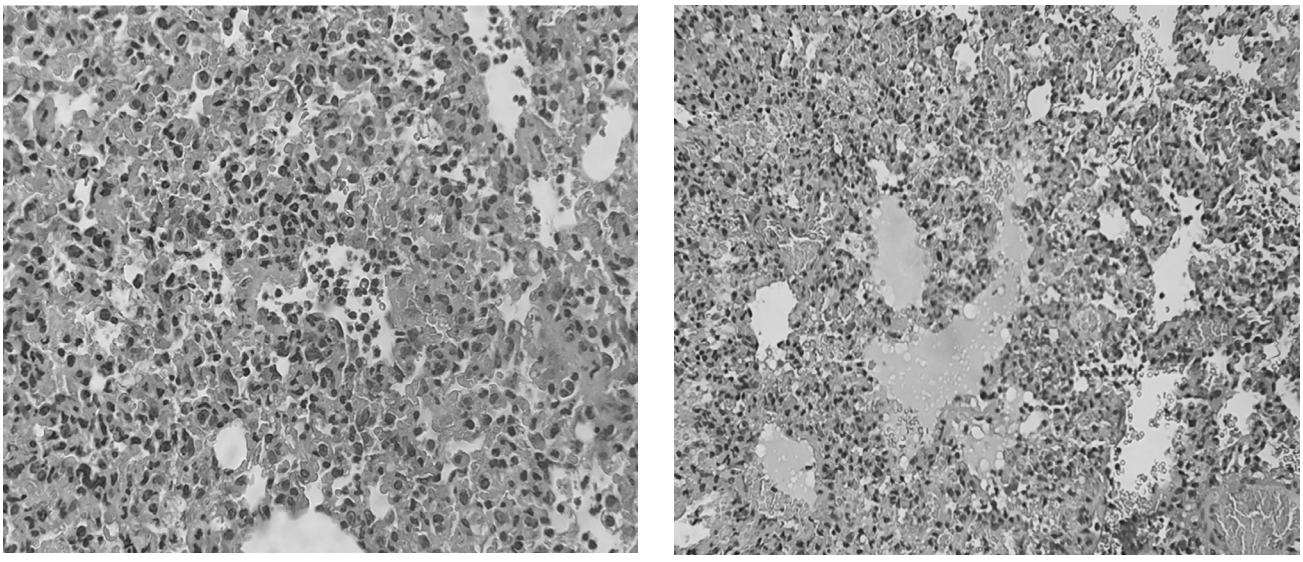

Fig. 2. Lung tissue of the 3rd group Syrian hamster dead in 31 hours after decompression (hematoxylin and eosin: A — × 400; B — × 100)

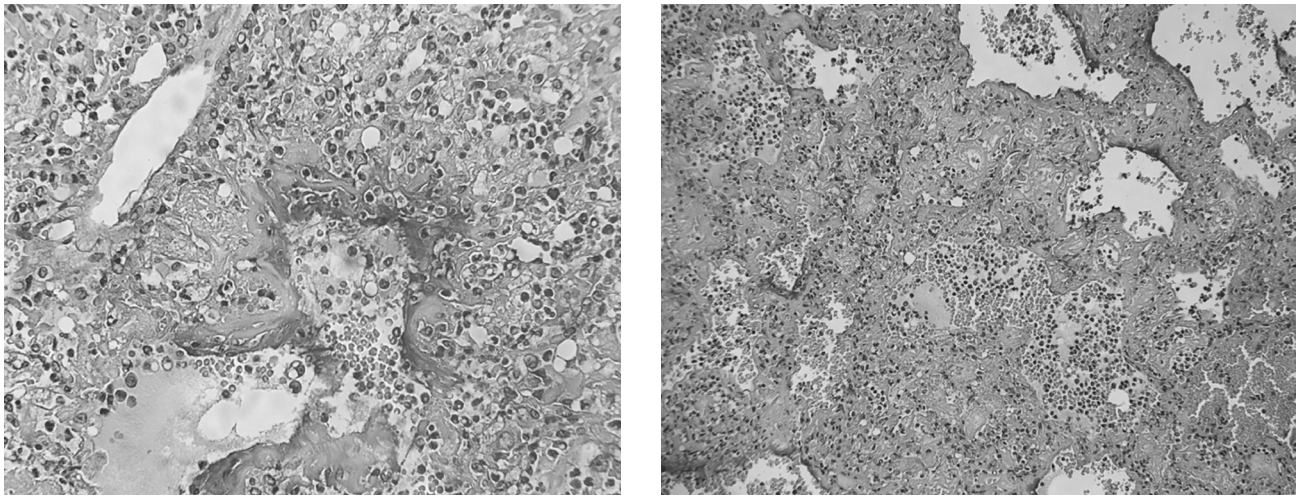

Fig. 3. Lung tissue of the 4th group Syrian hamster dead in 75 hours after decompression (hematoxylin and eosin: A — × 400; B — × 100)

In turn, in an animal from the third group that died at the beginning of the fourth day, during histological examination, changes were recorded in the lungs that were different from those observed in other animals that died at the end of the first or beginning of the second day. This animal had pronounced alveolar edema, loss of fibrin into the lumen of the alveoli, areas of fibrinoid necrosis of the hyaline membranes of the alveoli ***(Fig. 3A)***, as well as focal lymphohistiocytic infiltration, the presence of a large number of macrophages and pronounced vascular fullness ***(Fig. 3B)***.

Discussion. Based on the results obtained, it can be said that death in the control group is directly related to the development of severe DCS in animals. rapid and non-stop decompression.

In turn, an increased duration of the latent period before decompression disorders' manifestation and the time of death in the second group, as well as a decrease in intravascular and tissue gas formation may indicate insufficient duration of liquid breathing to remove nitrogen from the body accumulated during the stay of animals in conditions of increased pressure of the gas environment, and as a consequence — insufficient prevention of development DCS. In the third and fourth groups, we did not observe decompression disorders and pronounced formation of free gas, which indicated that liquid respiration lasting 30 minutes or more allowed removing excess nitrogen from the body and thereby preventing the development of DCS. It is noteworthy that we also did not observe barotraumatic disorders in animals of the control groups, despite rapid and non-stop decompression.

The data obtained suggest that a new method of prevention of decompression disorders was demonstrated in an animal experiment, called by the authors "liquid respiratory desaturation (LRD)". LRD is a method of prevention of decompression disorders based on the excretion of metabolically indifferent gases from the body during liquid breathing, in the presence of dissolved gas concentration gradient from tissues to respiratory fluid. The method makes it possible to saturate the body from metabolic indifferent gases during liquid respiration before and/or during decompression. Based on the conducted research, a patent for invention No. RU 2738015 "A method for the prevention of decompression sickness" was prepared [11].

When analyzing the causes of deaths in group three and group four, taking into account the clinical picture, as well as the data of the pathoanatomic study, it can be said that the immediate cause of death of animals at the end of the first — beginning of the second day was acute respiratory failure due to the development of the alveolar phase of pulmonary edema. In turn, in an animal from the third group, who died at the beginning of the fourth day, respiratory failure was associated with the development of acute respiratory distress syndrome, as evidenced by the corresponding histological picture.

Apparently, the development of both pulmonary edema and acute respiratory distress syndrome was due to the peculiarities of the physico-chemical properties of one of the components used in the respiratory fluid — perfluorohexane.

Perfluorohexane, as shown by studies conducted at the Golikov Scientific and Clinical Toxicological Center of the Federal Medical and Biological Agency, has a pronounced pulmonotoxic effect both in isolated and combined use with other perfluorocarbon compounds in the composition of the respiratory fluid. The use of PFD and PFH in the composition of respiratory fluid in a ratio of 40:60 is due to the improved rheological characteristics of respiratory fluid, which allow animals to breathe independently for a sufficiently long time in immersion in PFH under hyperbaric conditions.

Thus, not only the development of liquid lung ventilation devices with a liquid ventilation module, but also the development of a respiratory fluid that meets the requirements of efficiency and safety, will play an important role in the introduction of the method of liquid respiratory desaturation into practice.

Conclusions:

1. Liquid respiratory desaturation is a method of preventing decompression disorders based on the removal of metabolically indifferent gases (in particular nitrogen) from the body during liquid respiration, in the presence of dissolved gas concentration gradient from tissues into the respiratory fluid. The method makes it possible to saturate the body from metabolically indifferent gases during liquid respiration before and/or during decompression, thereby creating conditions for the use of ultra-short decompression modes without the risk of decompression disorders.

2. Removal from the body of indifferent gases (in particular nitrogen), dissolved in tissues during the stay in conditions of high pressure of the gas (air) environment, depends on the duration of the liquid breathing. At the same time, it is necessary to ensure the

presence of a stress gradient of the gases removed from the tissues in the respiratory fluid.

As part of the study, this problem was solved by hourly bubbling of the respiratory fluid with oxygen under atmospheric pressure, as well as by the absence of direct contact between the respiratory fluid and compressed air in the stand during the experiment.

3. The developed criteria for assessing the degree of gas formation using an ultrasound method based on the Doppler effect can be used to study the causes of decompression disorders in small laboratory animals.

4. The causes of death after breathing a liquid against the background of a preliminary stay in a high-pressure gas (air) environment may be:

- *insufficient removal of indifferent gases (in particular nitrogen) from the body, which leads to death due to DCS;*
- *pulmonotoxic effect of perfluorohexane, which may manifest itself in the development of pulmonary edema (at the end of the first — beginning of the second day), or respiratory distress syndrome (at the beginning of the fourth day).*

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