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Liquid respiratory desaturation. The first experience of application on large biological objects

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Introduction. This article is a continuation of the publication of the experimental studies results of prevention of acute decompression sickness (aDCS) in laboratory animals by the method of liquid respiratory desaturation (LRDS). LRDS is a method of preventing decompression disorders by the excretion of metabolically indifferent gases (in particular nitrogen) from the body during spontaneous respiration with liquid or artificial liquid lung ventilation (ALLV), by dissolved gas concentration gradient "from tissues to respiratory fluid". The method allows to provide rapid desaturation of the body from metabolically indifferent gases in the process of liquid respiration before and/or during decompression, thereby creating conditions for application of ultra-fast decompression profiles without the risk of decompression disorders.

The aim of the study was to confirm the possibility of LRDS being a method of DCS prevention on large laboratory animals (minipigs).

Materials and methods. The studies were performed on Wiessenau minipigs (*n*=8) — male and female, aged 4–5.5 months and weighing 7.1–11.2 kg. Animals of the control (*n*=4) and experimental (*n*=4) groups were subjected by compression by keeping in an air environment under high pressure (absolute 0.5 MPa for 60 minutes) to saturate tissues with indifferent gas (nitrogen). After compression experimental group was exposed to 30-minute ALLV with a liquid (perfluorodecalin) saturated with an oxygen under normobaric conditions. Air environment pressure during procedure of artificial lung ventilation was kept at 0.5 MPa. Decompression of the control and experimental groups was carried out in non-stop manner for 80 and 40 seconds respectively. The investigation of severity of clinical manifestations of DCS (changes in hemodynamic and respiratory parameters) including ultrasonic examination of gas bubbles in the heart and large vessels of the liver and as well as survival of animals in groups and pathoanatomical changes was performed.

Results of the study. Clinical manifestations, ultrasonic scanning of the heart and venous vessels of the liver, as well as morphological examination data indicated the development of severe acute post-decompression disorders (PDD) in animals of the control group, which caused deaths in 100% of cases. Meanwhile all animals in experimental group survived and their state was stable. According to ultrasonic examination, the presence of small number of gas bubbles in the right-side heart chambers and liver's venous vessels being noted, but they disappeared after several hours. Deviations of the respiration's parameters function from background values (shortness of breath of a mixed type with the participation of auxiliary muscles, etc.) observed from the 2nd to the 4th day after ALLV, as well as compensatory reactions from the cardiovascular system (heart rate variability and instability of hemodynamic parameters) were caused by ALLV.

Conclusions. *Compressed air exposure 0.5 MPa for 60 minutes followed by 80 seconds of non-stop decompression allows to provoke the severe acute PDD minipigs model, manifested by pronounced intravascular gas formation, the development of acute respiratory and cardiovascular insufficiency causing the development of adverse outcomes.*

The ultrasound method of visual assessment of intravascular gas formation severity, adapted for minipigs, together with the dynamics of changes in the indicators of respiratory and cardiovascular systems makes possible to assess not only state of animal under anesthesia, but also the effectiveness of PDD prevention measures.

The usage of ALLV with PFC liquid completely saturated with 100% oxygen under normobaric conditions makes possible partial removal of indifferent gas (nitrogen) dissolved in tissues of experimental animals during exposition in a compressed air environment before decompression and thereby carry out the prevention of DCS by LRDS method. It makes possible to implement successfully ultra-fast decompression profiles, incompatible with life in the control group.

The state of animals from experimental group after LRDS is characterized by lung impairment caused by ALLV in hyperbaric conditions accompanied by temporary changes of external respiration function and compensatory reactions of cardiovascular system, observed during first 4 days.

Ethics. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The Clinical Study Protocol was approved by local Ethics Committee of The Federal State Budgetary Scientific Institution «Izmerov Research Institute of Occupational Health».

Keywords: *liquid ventilation; artificial lung liquid ventilation; perfluorodecaline; decompression profile; postdecompression disorders; liquid respiratory desaturation*

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Introduction. Sometimes professional activity requires presence of workers in conditions with significant changes of the ambient pressure of the aqueous and/or gaseous environment. That entails the risk of specific diseases and injuries. For instance, decompression sickness (DCS) T70.3 by ICD-10 — an occupational disease of personal, working in altered gases and aqueous pressure conditions. It manifested by a complex of pathological reactions associated with the free gas bubbles` formation in the blood and other tissues supersaturated by indifferent gases caused by inadequate decompression [1, 2]. Divers, caisson workers, pilots and other specialists faces this disease [3].

Metabolically indifferent gases (oxygen-diluent gases, nitrogen and/or helium), being an essential component of respiratory gases mixtures and dissolved under pressure in tissues after inadequate decompression becomes gas bubbles in tissues and gas emboli. The only possible way to prevent their appearance in the body provides by the technology of usage liquids as a respiratory substrate. A liquid that practically does not change its volume by pressure shifts can provide gases exchange without risk of oxygen poisoning in the absence of diluent gases, that is, without the risk of aDCS development. The principal possibility of this was shown by usage of Sterofundin oxygenated at a pressure of 0.8 MPa [4].

Gollan F. and Clark L.C. [5] demonstrated similar results using perfluorocarbons (PFCs). Mice breathing PFCs survived after compression, exposure at 33.5 atm pressure and subsequent 5-second decompression. Meanwhile the gas-breathing animals died after they were removed from the hyperbaric chamber. In a later study, Lynch P.R. et al. [6] demonstrated the effect of compression, exposure (7 atm for 1 hour) and decompression (18 m/min) on physiological parameters, formation of vascular gas bubbles and survival of hamsters after respiration with gases or oxygenated PFCs liquid. After decompression, the survival rate in the group of animals undergoing spontaneous gas respiration was 9/15 for 15 minutes after extraction from the hyperbaric chamber. A pathoanatomical examination showed the presence of a large number of gas bubbles in these animals` right heart ventricle. While no deaths were observed in the group of animals breathing with PFCs liquid. 30 minutes after decompression, no gas bubbles were found in these animals` right heart parts. The experimentally confirmed possibility to eliminate DCS risk in liquid-breathing animal models placed in a high-pressure environment became the basis for the development of a new study direction — the mammals` tolerance of high hydrostatic pressures.

Early studies of total volumetric compression influence on isolated organs and tissues were indicate the central nervous system to be the most sensitive [7]. Kylstra J.A. et al. [8] in experiments on immersed in oxygenated PFCs and breathing spontaneously mice demonstrated that hydrostatic pressure 5–8 MPa causes a two-phase reaction: first in the form of increased locomotor activity, and with a further pressure increase — clonic-tonic seizures, areflexia and apnea. Thus, the liquid breathing (LB) technology made it possible to determine the extremely high hydrostatic pressure that is tolerable for mammals, although today this information has purely theoretical significance.

In this publication authors proposes to consider a fundamentally different approach of liquid ventilation technology usage — accelerated removal of indifferent gases from a pre-saturated organism after high pressure residence. This is realized by the possibility of breathing liquid to provide the main O_2 and CO_2 gases exchange in complete absence of diluent gases. That allows to eliminate indifferent gases from body tissues into environment during LB before and/or during decompression creating conditions for the usage of ultra-fast decompression profiles. This application is relevant in cases when the risk of aDCS is a setback for emergency escape, for example, for crew of disabled submarine or aquanauts from a hyperbaric chamber after long-term diving [9].

This article is a continuation of the publication of the experimental studies results of prevention of acute decompression sickness (aDCS) in laboratory animals by the method of liquid respiratory desaturation (LRDS). LRDS is a method of preventing decompression disorders by the excretion of metabolically indifferent gases (in particular nitrogen) from the body during spontaneous respiration with liquid or artificial liquid lung ventilation (ALLV), by dissolved gas concentration gradient "from tissues to respiratory fluid". The method allows to provide rapid desaturation of the body from metabolically indifferent gases in the process of liquid respiration before and/or during decompression, thereby creating conditions for application of ultra-fast decompression profiles without the risk of decompression disorders [10, 11].

The article presents the results of experimental modeling of aDCS on large laboratory animals (minipigs), criteria for assessing the severity of acute decompression disorders, including ultrasound imaging, as well as the results of prevention of intravascular gas formation by the LRDS method.

The aim of the study was to confirm the possibility of LRDS being a method of aDCS prevention on large laboratory animals (minipigs).

Materials and methods. The studies were performed on Wiessenau minipigs (*n*=8) — male and female, aged 4–5.5 months and weighing 7.1–11.2 kg. During 30 days of the quarantine, a daily inspection was carried out. The behavior and general condition were assessed. During quarantine and during the study, the animals were kept in accordance with the requirements of GOST 33215-20141 on a standard water and food ration in individual cells in a separate vivarium`s room. Healthy animals selected by the results of clinical examinations were included in the study. 8 minipigs were selected by body weight and randomized into two groups — control (*n*=4) and experimental (*n*=4). Animals of both groups were kept in an air environment under high pressure in order to saturate tissues by indifferent gas (nitrogen). The mode of exposure of experimental group was differed from the control one by additional incubation time under high pressure conditions, when artificial liquid lung ventilation (ALLV) was carried out supplied by PFCs liquid, that was preliminary saturated by oxygen under normobaric conditions. The characteristics of the research conditions for groups are presented in ***Table 1***.

The studies were performed using an experimental laboratory hyperbaric test bed (LHTB), which is an automated reservoir for modeling high pressure conditions of a gas and/or liquid environment and large laboratory animals to stay in. The scheme of the LHTB with a laboratory animal located in is shown in ***Figure 1***.

Each animal after intravenous premedication (atropine 0.02 mg/kg, tavegil 0.025 mg/kg, analgin 7 mg/kg; anesthetic support — zoletil-100 — 10.0 mg/kg and meditin 0.1% — 0.03 mg/kg; intubation by a tube 5–6.5 ID) was placed in the LHTB according to ***Figure 1***. To obtain controlled ventilation

Table 1

Characteristics of experimental conditions

Animal group	Compression time up to 0.5 MPa (absolute), min	Duration, min		Decompression time, sec
		Exposure at 0.5 MPa compressed air	ALLV at 0.5 MPa	
Control	2	60	0	80
Experimental			30	40

characteristics, as well as to place the necessary equipment, animals was positioned vertically. A soft-walled tank with oxygenated respiratory liquid (RL) was placed on the bottom of the LHTB. In order to minimize the gravitational effect of RL on the lungs during the ventilation process, the LHTB was filled with fresh water by ⅔. The water temperature was maintained at 32.0–33.0°C for stabilizing the temperature regime during experiments.

During the exposure the anesthetized animals in the LHTB breathed spontaneously. The remote switch provided the contact of intubation tube with gas phase inside LHTB. The pressure increase was achieved by compressed air from the gas tank (2), pressure control and pressure set rate were carried out using a pressure gauge and a stopwatch. To obtain a detailed case of post-decompression disorders, we kept the minipigs in compressed air environment 0.5 MPa (absolute) for 60 minutes. In order to prevent lung barotrauma, compression was carried out during the first minute to 0.1 MPa, after that the gas supply was accelerated and reached the target value by the end of the second minute. During the 60-minute exposure under the pressure, in order to remove carbon dioxide and stabilize the oxygen concentration in gas phase inside of LHTB, ventilation was carried out by constant supply of compressed air with rate about 40 l/min and discharge by partially opening the valve (6).

To carry out ALLV for experimental group animals an open respiratory circuit device to provide liquid ventilation for biological objects up to 20 kg body weight in a normal and hyperbaric conditions was designed ***(Fig. 2)***. ALLV device consists of 400 mm height sealed container 130 mm diameter with submersible micropumps located inside: providing inspiration — N1, N2 and exhalation — N3. A controlled valve (P1) is installed at the outlet of the pump N3. Micropumps are connected to a biological object (BO) by a T-fitting. An elastic latex shell — pressure compensator (KD) is installed to compensate the pressure inside the container. An external filter (F) is installed at the inlet of the RL intake. Taps Kr1 and Kr2 are designed for pouring dielectric liquid into the ventilator. A hermetic connector is installed in the lower part of the container to communicate with the control and power system.

Perfluorodecalin produced by HaloPolymer (TU 95.1233-92) was used as a RL. It was oxygenated by bubbling at atmospheric pressure using a Mark 5 nuvo Lite oxygen concentrator (Nidek Medical Products Inc., USA,) up to 85–95 vol.%. The degree of oxygen saturation of RL was determined using the Aktakom ATT-3010 (Aktakom LLC, Russia). The temperature of the liquid before and after the experiment was measured using a laboratory thermometer TL-4 (Thermopribor, Russia).

The experimental group animals ALLV started after intravenous administration of a pre-prepared dose of rocuronium bromide 6 mg/kg. After myorelaxant administration the electromagnetic valve (remote switch) was switched to the ALLV device`s circuit. ALLV device

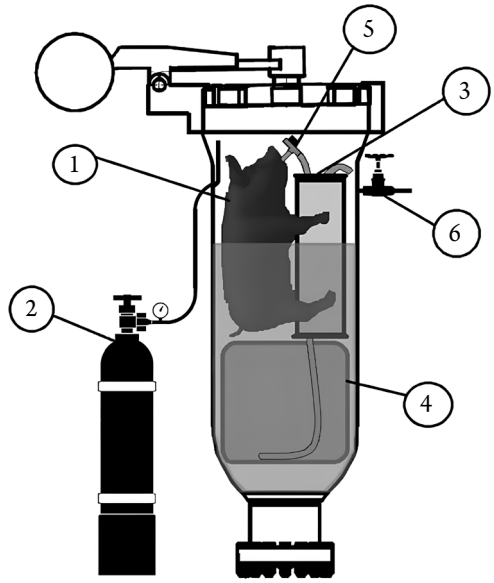


Fig. 1. The experimental laboratory hyperbaric test bed. (1 — biological object fixed in a hyperbaric test bed, partially flooded with water; 2 — a source of compressed air with a pressure control system; 3 — artificial lung liquid ventilation device; 4 — soft tank — a source of respiratory liquid; 5 — remote switch for switching from autonomous gas breathing to artificial lung liquid ventilation; 6 — valve for ventilation of the test bed's volume and decompression)

performed liquid ventilation for 30 minutes in volume control mode, respiratory rate 4 breaths/min, respiratory volume 20–22 ml/kg, inhalation : exhalation ratio 1 : 3. Each new portion of RL "for inspiration" came from a soft-walled tank. The "exhalation" was carried out forcibly by ALLV device. After evacuation from the lungs RL was poured into the internal space of the LGS.

Decompression in the control was performed non-stop in 80 seconds with a tendency to slow down from 0.2 MPa to atmospheric pressure in order to prevent lung barotrauma. Decompression of the experimental group was performed at a constant and maximum possible speed — in 40 seconds, because the effect of respiration substrate expansion during "the ascent" was absent.

After taking out from the LHTB the animals of the experimental group were transferred to CMV in the pressure control mode with F — 20–25 breath/min; Vt — 12–15 ml/kg; MV — 240–400 ml/kg/min, FiO_2 — 0.21. This mode maintained the acid-base state and the gas composition of arterial blood at normal values (pH — 7.45–7.55; partial pressure of carbon dioxide (p_aCO_2) — 35–42 mmHg; oxygen partial pressure (p_aO_2) — 100–110 mmHg; BE — 2–5 mmol/l). One hour after the animals were switched to spontaneous breathing. The experimental group animals got

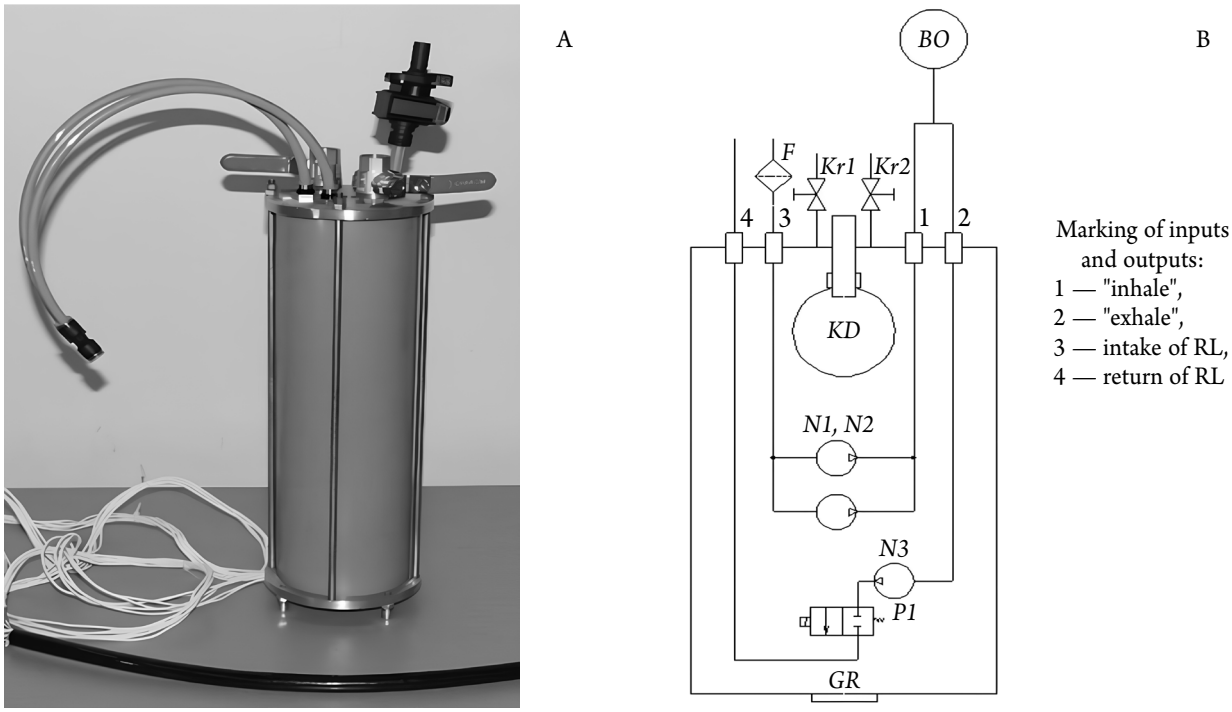


Fig. 2. Appearance (A) and hydraulic circuit (B) of the artificial lung liquid ventilation device

pharmacological correction: forced diuresis (crystalloid infusion — 12 ml/kg, mannitol — 150 mg/kg), inflammation reduce (prednisolone — 6 mg/kg; clemastine — 0.1 mg/kg) and antibacterial (ceftriaxone — 40 mg/kg) therapy. Cardiotonic (cordiamine — 25 mg/kg), painkillers (analgin — 20 mg/kg), nootropic (mexidol 2.5% — 5 mg/kg) drugs and anticoagulants (heparin — 200 ME/kg) were administrated.

CMV for the experimental group animals was performed by Zisline MV200 device (Triton-Electronics, Russia). Acid-base state and arterial blood gases and electrolytes monitoring was carried out by GEM Premier 3500 analyzer (Instrumentation Laboratory, USA).

Main physiological parameters were detected by a portable monitor MPR6-03 (Triton-Electronics, Russia) and by veterinary electrocardiograph Poly-Spectrum-8EX (Neurosoft, Russia). These parameters were: arterial hemoglobin oxygen saturation (SpO_2); frequency heart rate (HR) with electrocardiogram (ECG) registration; respiratory rate (F); tidal volume (Vt); minute respiratory volume (MV); rectal temperature (T_{rect}); noninvasive systolic (AD_{sist}) and diastolic (AD_{diast}) blood pressure. Detection of gas bubbles (GB) in the bloodstream usually managed by portable Doppler ultrasound indicators is widely spread in the practice of diving medicine. The fact of the possible presence of "silent" bubbles in the bloodstream, which do not cause complaints and do not lead to the development of DCS, is widely known [2]. However, in order to more clearly identify the preventive effect of experimental exposure, we used an ultrasound scanning device LOGIQ F6 series (GE Healthcare, USA), which allows to directly visualize gas structures in situ with reference to anatomical localization. The presence of gas structures was carried out in the heart chambers and the liver large vessels.

Clinical examination and assessment of GB`s presence by ultrasound were carried out with drug-induced sleeping animals. In order to characterize the severity of the animal's condition and distinguish asymptomatic intravascular gas formation, which does not pose a threat to life and health, from DCS (gas embolism), post-decompression disorders assessing criteria were formulated ***(Table 2)***. Evaluation of the GB`s registration results in the heart chambers was carried out by the extended Eftedal-Brubakk scales [12] and it is indicated in points: 0 — no GB in the field of vision; I — rare GB; II — one GB for every 4 cardiac cycles; III — one GB in each cardiac cycle; IVa — one GB per cm^2; IVb — at least three GB per cm^2; IVc — many GB distinguishable between by itself; V is a foamy state, GB is incalculable.

The presence of multiple GB in the left-side heart chambers (in the arterial stream) with the absence of atrial septal defects was chosen as an absolute criterion for severe DCS. The total blood count was evaluated by using an automatic hematology analyzer URI T-2900 VET PLUS (UNIT Medical Electronic Co., China). Biochemical blood parameters were counted by an automatic biochemical analyzer FUJI DRI-CHEM 4000i (FUJIFILM Co., Japan). The pathomorphological examination included autopsy, macro- and microscopic (histological) of internal organs` examination. The examined tissues were fixed in a 10% solution of buffered formalin for 24 hours, after that they had been underwent standard treatment in isopropyl alcohol and paraffin for the manufacture of histological preparations. For microscopic examination, the sections were stained by hematoxylin and eosin. Morphological and micromorphometric examinations were carried out using a Leica DM LS light-optical microscope (Leica Mikrosystems CMS GmbH, Germany) equipped with an eyepiece micrometer. Microphotography was carried out by Leica DC320 digital camera (Leica Microsystems CMS GmbH, Germany).

Statistical data processing was carried out 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 mean *(m)* were calculated. The data were checked for the normality of the

Table 2

Diagnostic signs of minipigs' acute post-decompression disorders

№	Diagnostic signs	The absence of acute PDD	Severity of acute PDD		
			Mild	Moderate	Severe
1	2	3	4	5	6
Clinical signs					
1.	Respiratory disorders	absent	absent	expressed	after decompression tachypnea is replaced by bradypnea or a pathological respiration type
2.	Violation of cardiac activity according to ECG data	absent	absent	absent	myocardial ischemia, conduction and rhythm disorders
3.	Neurological disorders, pain, scratching, etc.	absent	possible	highly possible	highly possible in case of survival
Ultrasound signs					
4.	Gas bubbles in the arterial stream and the left-side heart chambers	absent (0)	absent (0)	isolated, recorded during separate cardiac cycles (I–III)	numerous (IVa–IVc)
5.	Gas bubbles in the right-side heart chambers	isolated or numerous (I–III)	numerous, recorded during each cardiac cycles (III–IVc)	numerous, heart chamebers are not dilatated; blood flow is preserved (IVc)	numerous, heart chamebers are dilatated, the tricuspid valve flaps do not close, There is no blood flow (V)
6.	Gas bubbles in the liver vessels	absent or isolated	isolated or numerous (<3 per sm^2)	numerous (>3 per sm^2); blood flow is preserved	numerous, merge into emboli; the portal vein is completely gas filled, blood flow through the vessels is impaired

distribution using the Shapiro–Wilk criterion. The intergroup differences were analyzed by parametric methods using the Student's criterion. The differences were considered significant at $p<0.05$. The nonparametric Mann–Whitney method was used for data belonging to a distribution other than normal.

Results and discussion. After taking out from the LHTB the control group's animals breathed spontaneously in state of drug-induced sleep. The body temperature was 32.5±0.8°C (background temperature 38.4±0.5°C). In 12–20 minutes, respiratory rate was increased by 1.8 times and reached 65±6 breath/min (background — 36±3), tidal volume ~ 4.8 ml/kg (background — 10.1±2.4), MV ~ 398 ml/min/kg (background — 360.3±11.4). Heart rate was 112±12 beats/min (background — 78±10), heart rhythm was sinus by ECG data, blood pressure was not reliably determined by noninvasive method, SpO_2 — 53±2% (background — 97±2%). After that there was an acute decompensation of the respiratory and cardiovascular systems function. It was manifested by Cheyne–Stokes type breathing accompanied by loss of the ECG and pulse oximeter signals. Ventricular fibrillation, which quickly turned into asystole, was registered. All animals from control group were died in 37.5±8.4 minutes after decompression.

By the ultrasound examination of the control group, it was found that after decompression, GB appeared in:

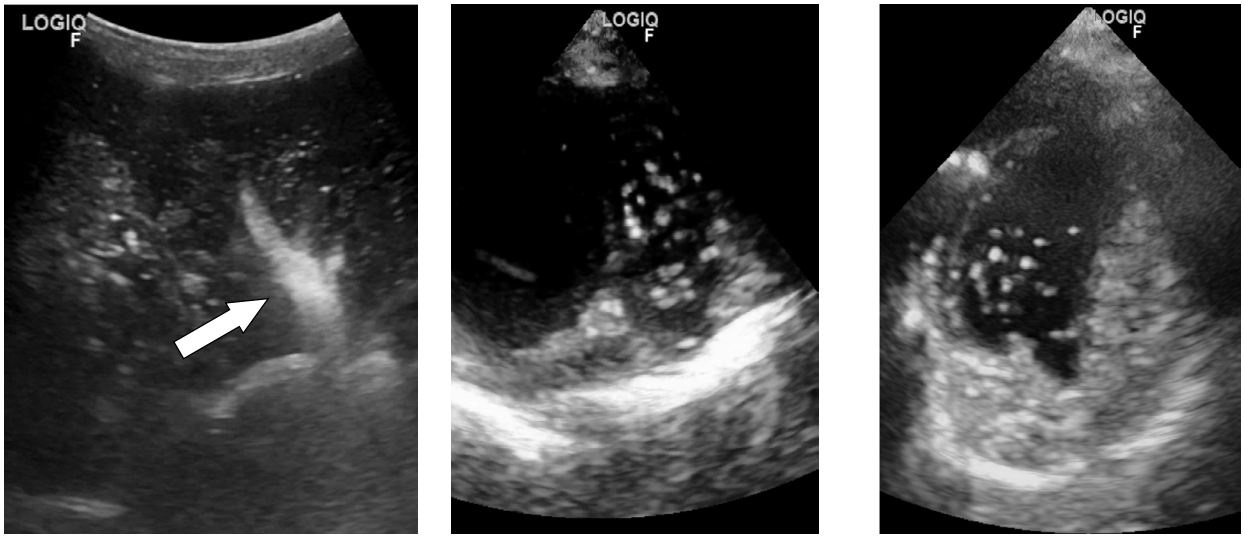
- the liver vessels in 5–7 min, isolated in beginning then numerous uncountable, the portal vein was gas filled, the blood flow through the vessels was disrupted ***(Fig. 3A)***;
- in the right-side heart chambers in 7–10 min isolated in beginning then numerous uncountable ***(Fig. 3B)***, up to 20–27 min heart chambers were dilatated, the tricuspid valve flaps did not close, no blood flow;
- in the left-side heart chambers in 10–20 min isolated in beginning then numerous ***(Fig. 3C)***, up to 25–30 min the blood flow stopped, the blood became foamy, gas accumulations prevented ultrasound examination.

According to the pathomorphological examination, all the control group animals were found to:

- the presence of foamy blood in the heart chambers and large vessels ***(Fig. 4)***;
- overfilled lungs blood vessels, capillaries and inter-alveolar septa, subpleural, peribronchial and alveolar hemorrhages, pronounced emphysema with subpleural cavities ***(Fig. 5A)***;
- erythrocyte stasis in capillaries and overfilled myocardial and epicardial vessels, liver, spleen and kidneys;
- overfilled membranes and substance of the brain vessels, perivascular edema of both the trunk and the cortical region of the large hemispheres.

The clinical manifestation (changes in hemodynamic and respiratory parameters), two-dimensional ultrasound data, as well as the results of morphological examination indicated the development of severe DCS in the control group animals, which caused the unfavorable outcome.

The experimental group examination was also carried out with drug sedation after taking out from the LHTB. The body temperature was 31.9±0.4°C (background 38.4±0.5°C). The skin was cold, and the visible mucous membranes normal color. All animals had bradycardia (heart rate — 52±4 beats/min), blood pressure, and SpO_2 were not determined.



A B C

Fig. 3. Ultrasonic signs of severe postdecompression disorders in animals of the control group (A — gas in the portal vein (marked with an arrow); B — multiple gas bubbles in the right-side heart chambers; C — multiple gas bubbles in the left-side heart chambers, the right-side chambers are filled with foam)

By the ultrasound examination of the experimental group, it was found that after decompression, GB appeared in:

- the liver vessels in 5 min; isolated in beginning then numerous (up to 1 per cm^2), blood flow through the vessels was preserved;
- in the right-side heart chambers in 5–7 min isolated isolated in beginning then numerous (up to 3 per cm^2), blood flow was preserved, the dimensions of the heart chambers were not changed, the tricuspid valve flaps closed completely;
- in the left-side heart chambers there were no GB.

One hour after decompression, the external respiration and systemic hemodynamics parameters were restored by temperature homeostasis normalization and by the ongoing therapy. The number of GB in the right-side heart chambers and portal vein progressively decreased, and 6 hours later they were not detected. In the experimental group, no adverse outcomes were recorded during the entire follow-up period (7 days). Severe decompression profile allowed us to fully demonstrate the differences between the groups. So, in the control, the chosen regime results in aDCS with a 100% fatal outcome, while the experimental group animals stage was stable, and the ultrasound examine results were interpreted as the asymptomatic presence of an insignificant amount of GP in the venous bed, since there were no behavioral disorders that could indirectly indicate the symptoms of DCS (neurological disorders, pain, scratching, etc.) were not registered by us [13].

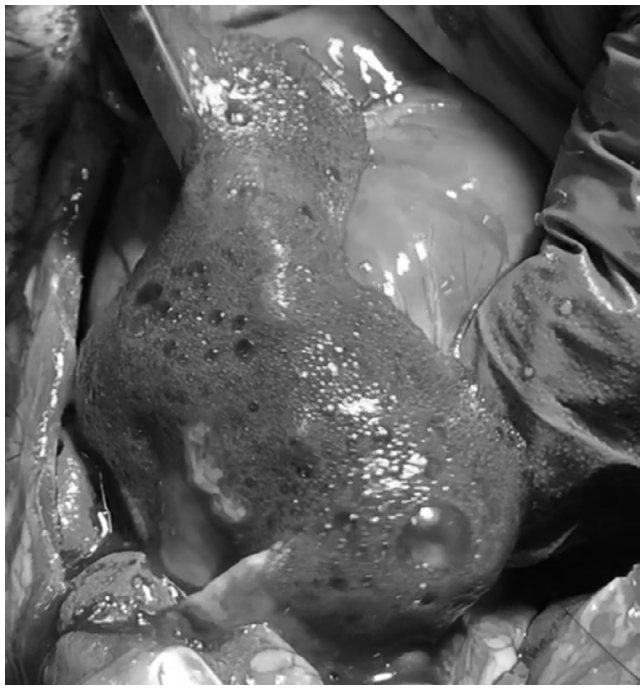


Fig. 4. Foamy contents of the control animals' right-side heart chambers

After decompression, the experimental group health status on day 1 was assessed as moderate severity. A significant decrease in appetite to 36±12% of background values was manifested by, tachycardia (136±29 beats/min; $p<0.05$), arterial hypertension (142.6±16.8/96.1±16.4 mmHg.; $p<0.05$), dyspnea of the inspiratory-expiratory type (respiratory rate — 78.4±15.0 breath/min; $p<0.05$; tidal volume — 7.2±1.2 ml/kg; MV — 565±47 ml/kg/min $p<0.05$) ***(Table 3)***. During the next 2 days, the animals health status improved progressively, food intake was restored, hemodynamic disorders and the severity of respiratory disorders decreased. On the fifth day all studied parameters did not differ from the background values.

All changes described above was caused by lung damage arisen in the process of ALLV in hyperbaric conditions, as well as compensatory reactions of the cardiovascular system — heart rate variability and instability of hemodynamic parameters ***(Table 3)***. At the same time no changes were detected on the ECG. During the entire observation period by the chest X-ray results — the lungs were without visible focal changes, the pulmonary pattern was enhanced in the basal and posterobasal sections mainly due to the vascular component, the roots behind the median shadow, the sinuses were free.

The cumulative cause of the hematological and biochemical blood parameters dynamics ***(Table 4)*** may be

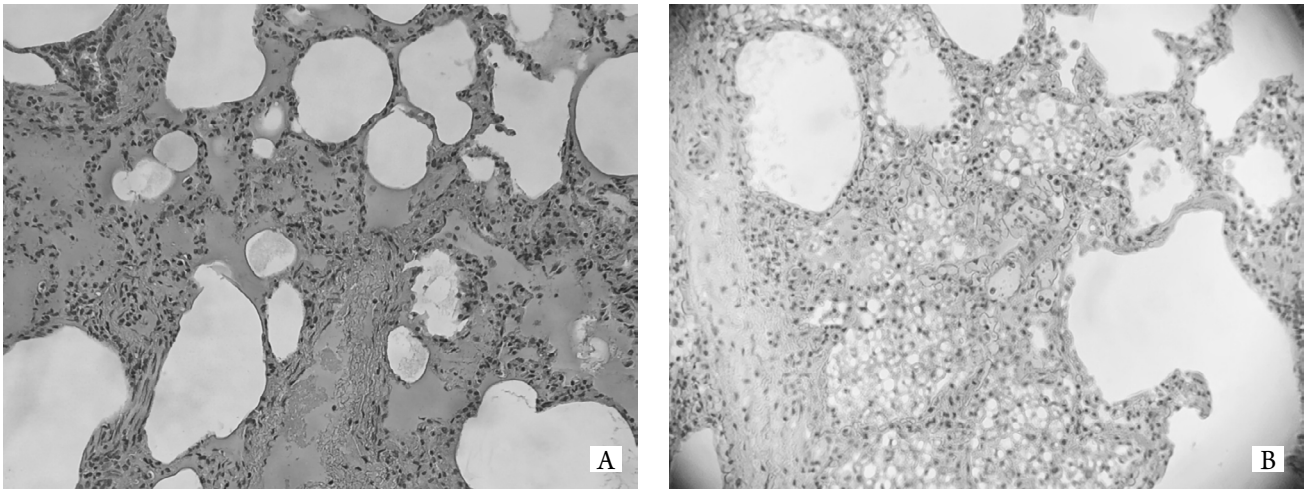


Fig. 5. Microslides of the lung tissues in control (A) and experimental (B) groups (hematoxylin and eosin, magnification ×400)

a complex of overlapping body reactions caused by damage of lung tissue during maladaptive liquid ventilation in an unnatural position of the body (when the weight of RL creates an additional traumatic effect), as well as the recovery process. Thus, an increase in the number of leukocytes (p<0.05) may be due not only to the damaging effect of forced liquid ventilation, but also to the reaction of the immune system, primarily neutrophils and macrophages, to the presence of insoluble PFCs in the lung tissue [14]. In turn, an increase in serum creatine kinase (CK) and lactate dehydrogenase (LDH) activity, detected on day 3 (p<0.05) after ALLV with a tendency to a subsequent decrease by

Table 3

The clinical parameters' changes of the experimental group *(M±m)* after liquid respiratory desaturation

Parameters	Background values	Days after ALLV						
		1	2	3	4	5	6	7
Health status (condition)	good	fair	fair	fair	good	good	good	good
Physical activity	common	decreased sharply	decreased sharply	decreased sharply	decreased	decreased	common	common
Meal intake, % of the norm	100	36±12	53±14	78±16	89±10	100	100	100
T_{rect}, °C	38.7±0.2	39.3±0.3	39.7±0.2	39.3±0.1	39.1±0.2	38.8±0.2	39.0±0.2	38.6±0.3
Body weight, % of initial	100.0	unkn	unkn	73.3±0.9	unkn	86.5±1.1	unkn	93.5±0.9
The cardiovascular system								
Heart rate, beats/min	91.6±7.6	136.3±19.5*	unkn	120.0±10.8*	unkn	113.6±8.0	unkn	108.1±9.2
AD_{sist}, mmHg	110.0±13.2	142.6±16.8*	unkn	138.3±10.3	unkn	113.0±13.2	unkn	105.0±9.2
AD_{diast}, mmHg	63.5±15.4	96.1±16.4	unkn	100.8±23.2	unkn	75.3±9.6	unkn	78.2±9.6
The respiratory system								
Respiratory rate, breath/min	35.0±7.4	78.4±15.0	unkn	59.1±12.1*	unkn	43.5±18.2	unkn	40.2±11.7
Tidal volume, ml/kg	9.2±0.5	7.2±1.2	unkn	7.3±1.1	unkn	7.9±0.8	unkn	7.8±0.6
Minute volume ml/kg/min	322±24	565±47	unkn	432±43*	unkn	344±29	unkn	313±21
Auxiliary muscles articipation in breathing	–	+++	++	++	+	+	–	–
Breathing profile								
Forced inhalation	–	++	++	++	+	–	–	–
Forced exhalation	–	+	+	+	–	–	–	–
Wheezing	–	dry	dry	dry	–	–	–	–

Note: the sign is missing — «–»; the sign is expressed — «+++», not significantly expressed — «++», not expressed — «+».
* — statistically significant (p<0,05) differences from background values.

Table 4

Hematological and biochemical blood parameters *(M±m)* of the experimental group after liquid respiratory desaturation

Parameters	Days after ALLV		
	0 (Background) (*n*=4)	**3 (*n*=4)**	**7 (*n*=4)**
Leukocytes, 10^9/l	14.9±0.4	20.7±0.8*	12.1±2.6
Erythrocytes, 10^{12}/l	7.5±1.2	8.6±0.3	8.9±0.5
Hemoglobin, g/l	160±18	159±2	162±5
Hematocrit, %	42±5	40±2	43±1
Platelets, 10^9/l	411±77	258±13*	289±17*
AST, U/l	48±11	64±3	62±7
AlAT, U/l	64±18	86±13	76±14
LD, U/l	678±134	971±85	866±33
CPK, U/l	342±138	1947±211*	1177±146*
CK-MB, U/l	278±3	235±9*	290±11
Total protein, g/l	71.0±6.1	85.2±5.1	73.3±6.1
Albumin, г/л	44±3	46±4	46±2
Blood urea nitrogen, mmol/l	3.90±0.31	4.55±0.42	4.43±0.51
Creatinine, umol/l	50±3	55±5	49±5
Total bilirubin, umol/l	5.30±1.10	7.29±0.89	5.21±0.31

Note: AST — Aspartate aminotransferase; AlAT — Alanine aminotransferase. * — statistically significant (p<0,05) differences from background values.

day 7 (p<0.05), may be caused by the active participation of auxiliary muscles in the act of respiration with pronounced inspiratory-expiratory tachypnea.

By the results of pathomorphological investigation of the experimental group euthanized on the 7th day — moderate alveolar edema, focal clusters of foamy macrophages, emphysema and thickening of the interalveolar septa were observed on the part of the lungs in some cases ***(Fig. 5B)***. No pathological changes of other organs were found. The results of the pathoanatomical investigation confirms the previously declared opinion that lung damage in the process of ALLV is the leading one, while changes of other organs and systems are just compensatory reactions.

Conclusions:

1. Compressed air exposure 0.5 MPa for 60 minutes followed by 80 seconds of non-stop decompression allows to provoke the severe acute PDD minipigs model, manifested by pronounced intravascular gas formation, the development of acute respiratory and cardiovascular insufficiency causing the development of adverse outcomes.

2. The ultrasound method of visual assessment of intravascular gas formation severity, adapted for minipigs, together with the dynamics of changes in the indicators of respiratory and cardiovascular systems makes possible to assess not only state of animal under anesthesia, but also the effectiveness of PDD prevention measures.

3. The usage of ALLV with PFC liquid completely saturated with 100% oxygen under normobaric conditions makes possible partial removal of indifferent gas (nitrogen) dissolved in tissues of experimental animals during exposition in a compressed air environment before decompression and thereby carry out the prevention of DCS by LRDS method. It makes possible to implement successfully ultra-fast decompression profiles, incompatible with life in the control group.

4. The state of animals from experimental group after LRDS is characterized by lung impairment caused by ALLV in hyperbaric conditions accompanied by temporary changes of external respiration function and compensatory reactions of cardiovascular system, observed during first 4 days.

References

1. On approval of the list of occupational diseases. https://clck.ru/39WVSt (Order of the Ministry of Health and Social Development of the Russian Federation dated 04/27/2012 No. 417N (Revision dated 04/27/2012)). Access mode: free.
2. Smolin V.V., Sokolov G.M., Pavlov B.N. *Diving descents up to 60 meters and their medical support. Ed. 4th, revised and expanded.* M.: Firma «Slovo»; 2013.
3. *Physiology of scuba diving and emergency rescue:* Textbook, ed. prof. I.A. Sapova. L.: Voen-med. akad. im. S.M. Kirova, 1986. – S. 8.
4. Kylstra J.A., Tissing M.O., Van der Maen A. Of mice as fish. *Trans. Am. Soc. Artif. Intern. Organs.* 1962; 8: 378–83. https://doi.org/10.1097/00002480-196204000-00077
5. Gollan F., Clark L.C. Prevention of bends by breathing an organic liquid. *Trans. Assoc. Am. Physicians.* 1967; 29: 102–109.
6. Lynch P.R., Wilson J.S., Shaffer T.H., Cohen N. Decompression incidence in air- and liquid-breathing hamsters. *Undersea Biomed Res.* 1983; 10(1): 1–10.
7. Kriss A.E. *Life processes and hydrostatic pressure.* M.: «Nauka»; 1973.
8. Kylstra J.A., Nantz R., Crowe J., Wagner W., Saltzman H.A. Hydraulic compression of mice to 166 atmospheres. *Science.* 1967; 158: 793–794.
9. Kotskij M.A., Bonitenko E.Yu., Ton'shin A.A., Rodchenkova P.V. Opportunity of liquid ventilation method implementation in advanced technologies of accidental submarine crews rescue. *Med. truda i prom. ekol.* 2022; 62(9): 566–578. https://doi.org/10.31089/1026-9428-2022-62-9-566-578
10. Kotskij M.A., Bonitenko E.Yu., Makarov A.F., Kanibolockij A.A., Kochoyan A.L., Litvinov N.A. About possibility of using liquid respiration to prevent the development of decompression

disorders. *Med. truda i prom. ekol.* 2022; 62(2): 91–100. https://doi.org/10.31089/1026-9428-2022-62-2-91-100
11. Kotskij M.A., Bonitenko E.Yu., Ton'shin A.A., Rodchenkova P.V., Muravskaya M.P., Tkachuk Yu.V., Kanibolockij A.A., Kochoyan A.L. Liquid respiratory desaturation is a new method of preventing decompression sickness. *Med. truda i prom. ekol.* 2023; 63(1): 4–17.
12. Møllerløkken A., Blogg S.L., Doolette D.J., Nishi R.Y., Pollock N.W. Consensus guidelines for the use of ultrasound for diving research caisson. *Diving Hyperb. Med.* 2016; 46(1): 26–32.
13. Zverev D.P., Bychkov S.A., Myasnikov A.A., Yarkov A.M., Haustov A.B., Klenkov I.R., Fokin S.G. Possibilities of ultrasound methods in the diagnosis of decompression sickness. *Morskaya meditsina.* 2021; 7(4): 75–83. https://doi.org/10.22328/2413-5747-2021-7-4-75-83
14. Carpenter J.W. Carpenter S. Exotic animal formulary. *Elsevier Science.* 2022. ISBN: 9780323833929. ISBN-10: 0323833926.

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