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COMBINED IMPACT OF GAMMA AND LASER RADIATION ON PERIPHERAL BLOOD OF RATS *IN VIVO*

G. A. Zalesskaya,[a*] L. E. Batay,[a] I. V. Koshlan,[b] V. M. Nasek,[c] R. D. Zilberman,[c] T. I. Milevich,[d] R. D. Govorun,[b] N. A. Koshlan,[b] and P. Blaga[b,e]

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The impact of γ radiation of ^{137}Cs (doses of 1 and 3 Gy), low-intensity laser radiation (λ = 670 nm, 5.3 or 10.6 J/cm^2) as well as the influence of consecutive laser and γ radiation on peripheral blood and blood cells (erythrocytes, leukocytes, lymphocytes, granulocytes) were studied by analyzing the number of blood cells, blood absorption spectra, and activity of antioxidant defense enzymes. Two series of experiments were performed on four groups of rats. The rats of the control group (group 1) were not exposed to γ or laser radiation. In the experimental groups, single irradiation of the whole body of rats with γ radiation (group 2), three- or four-day over-vein irradiation of blood in the tail vein by low-intensity laser radiation (group 3), and successive three- or four-day irradiation of blood by laser and then a single irradiation of the whole body with γ radiation (group 4) were performed. It was shown that changes of the blood cell content in the experimental groups are accompanied by changes in the spectral characteristics of the blood and the activity of antioxidant defense enzymes. The radioprotective effect of low-intensity laser radiation is manifested as an increase in the average number of leukocytes and lymphocytes in the group as compared with the postradiation, as well as an increase in the activity of antioxidant protection enzymes. The possibility of using low-intensity optical radiation for correction of hematological disorders caused by ionizing radiation is discussed.

Keywords: *low-intensity laser radiation, blood absorption spectra, ionizing radiation, blood, blood cells, superoxide dismutase, catalase.*

Introduction. Radiation damage to human and animal skin has been studied for several decades in connection with the widespread use of ionizing radiation (IR) in industry and in medicine (1). The action of IR on blood and its components *in vitro* is used widely in medical practise to sterilize blood and prolong its storage [2]. In [1, 3] it was shown that the strength of the effect of IR on the living cells of blood depends on a number of factors: the type of radiation, the type of cell, the dose, the strength of the radiation, the time elapsed from the moment of exposure, and others. Because of the change of components in the blood and destruction of its cells observed during the action of IR on blood *in vitro* and *in vivo* it is necessary to study its radiation stability not only to determine safe procedures for the use of IR in medicine and technology but also for the development of new effective radioprotectors. Experts in various scientific fields are actively engaged in the search for new methods and agents for radiation protection. The ability of low-intensity optical radiation (LIOR) to have a radioprotective effect was discussed in [4–8], where both positive and negative results are described. Analysis of published data shows that study of the radioprotective action of LIOR is at present in the initial stage, and the mechanics of its protective action have not been substantiated [4–8].

The aim of the present work was to undertake model investigations on the combined influence of low-intensity laser radiation (LILR) and IR on blood and on the cells of the peripheral blood of rats (erythrocytes, leukocytes, lymphocytes, granulocytes) based on comprehensive analysis of the hematological parameters, the spectral characteristics of the blood,

*To whom correspondence should be addressed.

[a]B. I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, 68 Nezavisimost′ Ave., Minsk, 220072, Belarus; email: zalesskaya@imaph.bas-net.by; [b]Joint Institute for Nuclear Research, Dubna, Russia; [c]Institute of Bioorganic Chemistry, Minsk, Belarus; [d]Institute of Radiobiology, Gomel, Belarus; [e]Czech Technical University, Prague, Czech Republic. Translated from Zhurnal Prikladnoi Spektroskopii, Vol. 84, No. 5, pp. 737–745, September–October, 2017. Original article submitted April 4, 2017.

and changes in the activity of enzymes of antioxidant protection (superoxide dismutase (SOD) and catalase (Cat)). Study of the mechanics of the action of small doses of IR on the living organism numbers among the fundamental tasks of molecular physics.

Materials and Methods. The model investigations were carried out in two series of experiments on four groups of rats of the Wistar line 200–250 g in weight. The effects of γ radiation from ^{137}Cs (1 and 3 Gr), LILR (670 nm, 5.3 or 10.6 J/cm^2), and the successive action of LILR and γ radiation of peripheral blood and its components (erythrocytes, hemoglobin, leukocytes, lymphocytes, granulocytes) were compared. The rats of the control group (group 1) were not subjected to γ radiation or LILR. The following procedures were used in the experimental groups: a single exposure of the whole body of the rats to γ radiation produced during the decay of ^{137}Cs (the Igur apparatus of the Institute of Radiobiology, National Academy of Sciences of Belarus, dose 3 Gr, exposure rate 0.67 Gr/min) (group 2); three- or four-day supravenous irradiation of the blood (SVIB) in the caudal (tail) vein (group 3); successive three- or four-day LILR and then single exposure of the whole body to γ radiation (group 4); combined irradiation with γ radiation and then single SVIB (16 J/cm^2). The two series of experiments differed in that the rats were subjected in the first series to three-day (10.6 J/cm^2) and in the second series to four-day SVIB for blood in the caudal vein (5.3 J/cm^2, emitter Lyuzar therapeutic instrument, Minsk). In the rats of group 3 successive three-day action of laser radiation of the blood in the first series of experiments and four-day action in the second was followed after 24 h by whole-body exposure to γ radiation. In both series of experiments the blood samples were taken from the jugular vein of the live rats on the fourth day after γ irradiation and on the second day after laser irradiation. During use of the experimental animals (rats) the rules set out in the Helsinki declaration on the humane treatment of animals were observed.

For the model investigations of the radioprotective processes 216 blood samples from the rats taken before and after the action of the physical factors were studied *in vivo*. The hematological indices were determined on a Humacount hematology analyzer (Germany). The blood samples were stabilized with ethylenediamine tetraacetate (EDTA). The activity of SOD in the whole blood was determined by the reaction of superoxide-dependent oxidation of quercetin in an alkaline medium, which was conducted by means of a set of SOD:100/2 reagents (from NTPK "Analiz X") [9]. The activity of Cat in the blood serum was determined from the ability of H_2O_2 to form stable colored complexes with ammonium molybdate [10].

Irradiation of the blood by a noninvasive supravenous technique (SVIB) was used to study the radioprotective action of LILR as the most effective method of influencing the organism. The proportions of radiation falling onto the surface of the skin reaching the blood in the caudal vein were estimated in order to select the principal parameters of the action (the wavelengths λ and the strength of optical radiation suitable for SVIB). For this purpose under the conditions of multiple scattering we calculated the attenuation (I/I_0) of the radiation passing through layers of skin tissue with thicknesses of 1.5 and 2 mm with allowance for the absorption coefficients μ_a, scattering coefficients μ_s, and the anisotropy factors g of the irradiated tissues.

Figure 1 shows the dependence of I/I_0 on λ, calculated as $I(z) = I_0 \exp(-\mu_{ef} z)$, where I_0 is the intensity of the incident radiation, z is the distance from the irradiated surface, μ_{ef} is the effective attenuation coefficient of the radiation in the layer of blood, $\mu_s' = \mu_s(1 - g)$ is the reduced (or transport) scattering coefficient. The optical characteristics of the cutaneous tissues of rats obtained in [11] were used. From the spectrum for $I(z)/I_0$ on λ we estimated the transmission of the cutaneous tissues at various wavelengths and the fraction of the radiation reaching the blood in the caudal vein. From analysis of the results of calculation in the spectral region most effective for SVIB we selected the wavelength λ = 670 nm at which for SVIB about 40% if the radiation falling on the surface reaches the blood in the caudal vein.

The absorption spectra of the samples of blood from the rats taken before and after treatment with LILR and γ radiation were recorded in the region of 300–1200 nm on a Cary spectrometer (Cary, USA). The changes in the absorption spectra in the regions sensitive to oxygenation of the blood, which include the Soret band and longwave absorption in the region of 650–1000 nm, were used to evaluate the effect of physical factors on the degree of saturation of the hemoglobin (Hb) of the venous blood with oxygen (S_tO_2). The S_tO_2 values were determined from the ratio of the optical densities at λ = 60 nm and at the isobestic point λ = 805 nm. The results of the measurements were processed by variation statistics with determination of the reliability against the Student t test.

Results and Discussion. *Photoradiobiological changes in the concentration of Hb, amounts of erythrocytes (RBC), Hematocrit (Hct) in peripheral blood of rats.* It is known that even small doses of IR can not only affect the hematosis processes but can also lead to destruction of mature erythrocytes. Both processes are dose-dependent and are capable of causing anemia, which appears only a few days after exposure and then remains for several weeks.

The possibility of development of anemia initiated by γ radiation was assessed from the amount of RBC (C_{RBC}), the hematocrit (Hct), and the concentration of Hb (C_{Hb}) in the peripheral blood of the rats, the values of which averaged among

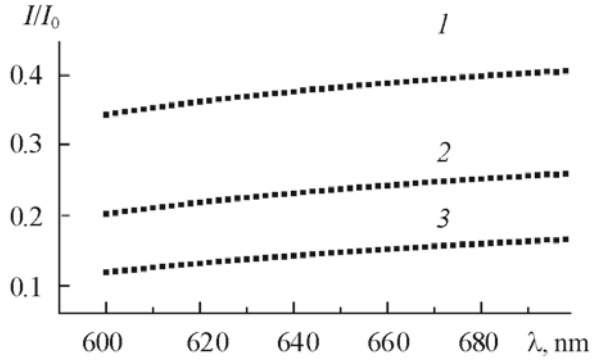

Fig. 1. The transmission spectra of the cutaneous tissues of rats for layers with thicknesses of 1 (1), 1.5 (2), and 2 mm (3).

TABLE 1. Relative Changes in the Average Amount of RBC, Concentration of Hb, and Hct for Various Versions of Laser and γ Radiation

Series No.	γ Radiation (3 Gr)/control				Combined radiation/γ radiation (3 Gr)			
	Ratio of indices	Blood indices			Ratio of indices	Blood indices		
		RBC	Hb	Hct		RBC	Hb	Hct
I	γ/C	0.82	0.82	0.91	(l + γ)/γ	1.03	1.01	1.01
II	γ/C	0.94	0.93	0.97	(l + γ)/γ	0.99	1.00	1.00
Ia*	γ/C	0.81	0.84	0.90	(γ + l)/γ	1.13	1.07	1.02

Note: C = control, γ = after γ irradiation with dose 3 Gr, (l + γ) = with successive action of laser and γ radiation; Ia* = ratio of indies for γ-(3 Gr) + laser irradiation (5 min) to γ irradiation.

the groups and measured on the fourth day after irradiation with a dose of 3 Gr are compared in Table 1 for the two series of experiments. According to the presented data, for γ irradiation with a dose of 3 Gr in the first series of experiments the amount of RBC on the fourth day after irradiation had decreased in comparison with the initial amount and amounted to 82% of the original value, the Hct had decreased to 91%, while the concentration of Hb had decreased to 82% of the original value. At the same time on the fourth day after γ irradiation with a dose of 1 Gr all the values (C_{RBC}, Hct, and C_{Hb}) had decreased to 94% of the original values. In the second series of experiments the changes in C_{RBC}, Hct, and C_{Hb} were smaller. We note that in the two series of experiments after the action of γ radiation with a dose of 3 Gr the C_{RBC}, Hct, and C_{Hb} values only approximated to the lower limit of permissible values, while not falling to the values characteristic of anemia. The greatest changes were in the amount of RBC in the series of experiments I and Ia, decreasing in the first series from 8.22 ± 0.26 to $6.74 \pm 0.30 \cdot 10^9$ L^{-1}. The results confirm the data on the higher radio stability of akaryocyte RBC cells compared with other blood cells [3].

The small decrease in the concentration of Hb and the decrease of Hct and the amount of RBC that appear on the fourth day after γ irradiation may be due both to destruction of mature erythrocytes and to interruption of the hematosis process. However, the decrease in the concentration of Hb with decrease in the amount of RBC, like the retention here of the average content of Hb in the RBC (MCH = 16.6 ± 0.3 pg before and 17.3 ± 0.3 pg after γ irradiation) and the small changes in the average concentration of Hb in the RBC (MCHC = 323 ± 2 g/L before and 298 ± 4 g/L after γ irradiation) reflect an insignificant contribution from hemolysis of the RBC due to radiation-induced destruction of their membranes.

Preliminary SVIB did not have a negative effect on the amount of RBC or on the concentration of Hb and also did not substantially change the results of the action of γ radiation with dose 3 Gr on C_{RBC}, Hct, and C_{Hb} in either series I or series II experiments. It should be noted that increase of the duration of laser action to 5 min (16 J/cm^2), used in the series of experiments Ia after γ irradiation, did not impair the results obtained after preliminary SVIB. Since the final result of the action depends on the ratio of post radiation loss of cells and their cytothesis the presented results show that on the fourth day

TABLE 2. Relative Changes in the of Average Amount of WBC, PLT, LYM, and GRA for Various Versions of Laser and γ Irradiation

Experiment series	Relationship types	Blood cells					
		WBC	LYM	LYM, %	PLT	GRA	GRA%
I	C/γ	4.50	7.90	2.34	0.96	1.02	0.25
II	C/γ	4.07	4.95	1.66	0.80	1.09	0.37
I	(l + γ)/γ	1.12	1.80	1.48	0.94	1.04	0.70
II	(l + γ)/γ	1.22	1.33	1.31	1.06	1.07	0.74
Ia*	(γ + l)/γ	1.30	2.20	1.47	1.08	0.81	0.80

Note. The notation for the irradiation is the same as in Table 1.

the action of SVIB only showed a tendency toward acceleration of the reparation processes. According to [1, 2], restoration of the population of RBC can take several weeks. At doses lower than the specified values their cytothesis as a rule takes place over periods significantly longer than our investigation (four days).

Photoradiobiological changes in the amount of leukocytes (WBC), thrombocytes (PLT), lymphocytes (LYM), and granulocytes (GRA) in peripheral blood of rats. Together with the death of blood cells the number of known negative effects of γ radiation include suppression of hematosis. According to the results obtained in the two series of experiments the WBCs were very sensitive to the action of γ radiation (Table 2). In series I on the fourth day after whole body irradiation with a dose of 3 Gr their amount was reduced by 4.5 times and on the second day by 4 times, but with a dose of 1 Gr it was reduced by only 2.6 times, i.e., the loss of WBC was dose-dependent. Estimates of the number of LYM and GRA entering into the WBC pool show that the amounts in series I and II change differently under γ irradiation with a dose of 3 Gr. The average amount of LYM decreased most strongly, indicating that they have the highest sensitivity to γ irradiation among the blood cells. With a dose of 3 Gr the strong decrease in the average amount of LYM in the two series of experiments [to $(0.82 \pm 0.09)\cdot10^9$ in I and $(1.15 \pm 0.16)\cdot10^9$ L^{-1}] and also of WBC [to $(2.43 \pm 0.21)\cdot10^9$ in I and $(1.88 \pm 0.19)\cdot10^9$ l^{-1} in II] is significantly lower than the permissible values and bears witness to the hematological toxicity of the employed moderate dose of γ radiation. The action of γ radiation also leads to different changes in the relative amount of cells entering into the WBC pool (Table 2), thereby demonstrating the different radiosensitivity in the cells of the subpopulation. The average amount of GRA remains practically unchanged in series I [$(1.44 \pm 0.27)\cdot10^9$ before and $(1.41 \pm 0.25)\cdot10^9$ L^{-1} after γ irradiation], although their relative content in the cell pool decreases. The weak dependence of the amount of GRA and also PTL on moderate doses of γ radiation was also noted in [2].

The preliminary three-day *in vivo* action of laser radiation on the venous blood of rats in series I did not lead to appreciable growth of the amount of WBC compared with the amount obtained after γ irradiation with a dose of 3 Gr, but the amount of LYM did increase (by 1.8 times); the amount of PLA and GRA did not change, but their relative content in the population decreased by 0.7 times. Combined irradiation with γ radiation (3 Gr) followed by single laser irradiation (16 J/cm^2) in this series led to a larger increase in the amount of WBC (1.3 times) and LYM (2.2 times).

In the experiments of series II preliminary four-day SVIB increased the average amount of WBC by 1.22 times, the average amount of LYM by 1.33 times, and their relative content in the population by 1.31 times. The average amount of PLT did not change. As in series I, the amount of GRA changed little, but their relative content in the population decreased by 0.74 times. During analysis of the results of series II it is necessary to take account of the fact that four-day preliminary SVIB reduced the amount of WBC by 1.12 times and of LYM by 1.27 times compared with the control group, which was undoubtedly reflected in the results of the combined treatment (laser + γ radiation).

The results show that under the influence of LILR there was a positive tendency for increase both in the amounts of WBC and LYM reduced by the action of a 3-Gr dose and in the relative amount of LYM in the peripheral blood. Preliminary SVIB did not have a significant effect on the amount of PLT. According to [3, 12], increase of the time interval between the action of the γ radiation and taking the blood samples could change the recorded results toward the better side compared with the use of the four-day regime.

As far as the differences in the radiosensitivity of the investigated blood cells are concerned, according to collective theories, it is determined by a series of factors [1]. The more sensitive the blood cells are to ionizing radiation, the more

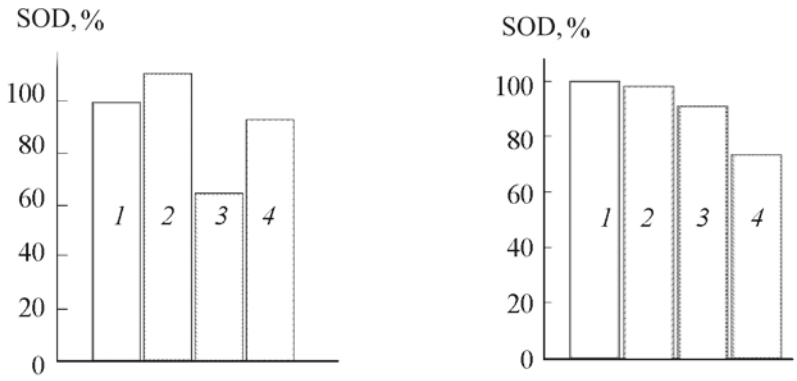

Fig. 2. Average activity of SOD among the groups: a): 1) control; 2) preliminary three-day laser + 1 γ radiation 3 Gr; 3) γ radiation 3 Gr; 4) γ radiation + 1 followed by laser radiation for 5 min. b): 1) control; 2) four-day laser radiation; 3) preliminary four-day laser + 1 γ radiation 3 Gr; 4) γ radiation 3 Gr.

quickly they multiply, the more prolonged the mitosis phase in them, and the less differentiated they are. The results of the investigations show that leukopenia and lymphopenia developed in the rats under a dose of 3 Gr, which may be due both to destruction of cells and to interruption of the hematosis process. The discovered high radiosensitivity of the WBC corresponds to the theories that have accumulated and to existing data [1, 8]. The WBCs are highly dividing and poorly differentiated cells and as a rule exhibit high radiosensitivity. The LYM cells are a known exception in that they do not divide but have high sensitivity to ionizing radiation. The radiosensitivity of the WBC and LYM cells is affected, among other factors, by the high radiosensitivity of the hemopoietic cells of the bone marrow responsible for their reproduction.

Effect of laser and γ radiation on the activity of antioxidant protection enzymes. In the two series of measurements the activity of the key antioxidant protection enzymes SOD and Cat decreased under the influence of γ radiation in comparison with the control group and depended on the dose of γ radiation. Thus, on the fourth day after γ radiation the activity of SOD averaged for the group with a dose of 1 Gr amounted to 90% of the activity obtained in the control group, while the analogous value for Cat was 85%. At the same time with a dose of 3 Gr the average activity of SOD decreased to 64% in series I and 73% of the control value in series II, while the activity of Cat decreased to 44% in I and 62% of the control value in II; this indicates a decrease in the reserves of antioxidant protection of the organism under the action of γ radiation. SOD is the most important element of antioxidant protection that accelerates the formation of hydrogen peroxide (H_2O_2) from the superoxide radical O_2^- by four orders of magnitude. Cat, which enters the second echelon of antioxidant protection, splits H_2O_2 into H_2O and O_2, which prevent the formation of the $^*OH$ radicals that have highly destructive action.

The preliminary three-day SVIB (10.6 J/cm^2) in series I led to a reliable increase of 1.7 times in the activity of SOD reduced by γ radiation and 1.9 times for Cat, and the activity of SOD exceeded the initial value (Fig. 2a). In series II with preliminary four-day SVIB (5.3 J/cm^2) the activity of SOD increased by 1.24 and that of Cat by 1.17 times. Thus, preliminary three- and four-day SVIB creates favorable conditions for retarding the oxidation processes initiated by γ radiation.

Photoactivation of the antioxidant protection enzymes, which leads to an increase in the group average activity of SOD and Cat compared with the values reduced by the effect of the 3-Gr dose, must be regarded as one of the important mechanisms for the radioprotective action of LIOR. Photoactivation of the antioxidant system of the blood cells, initiated by the preceding SVIB, arises from intensification of the antioxidant protection system of the organism that counteracts the development of oxidative stress due to the γ radiation. The obtained results on the photoactivation of SOD and Cat confirm the existing data on the possibility of using LIOR to improve the antioxidant protection of the organism.

Photoradiobiological changes in the absorption spectra of venous blood. Analysis of the absorption spectra of samples of venous blood before and after all three types of action (γ, laser, and laser + γ) in the regions sensitive to oxygenation of the blood provides information about the change in the degree of saturation of the Hb with oxygen (S_VO_2) needed to determine the mechanisms of activation of antioxidant protection. In [13–15] it was shown that the action of LIOR with the wavelengths absorbed by blood leads to changes in the content of oxy- and deoxyhemoglobin in the blood, and these appear as changes of the spectra in the regions sensitive to oxygenation of the blood. The spectra presented in Fig. 3 are normalized with respect to optical density at the isobestic points on account of differences in the intensities due to changes in the concentration of Hb

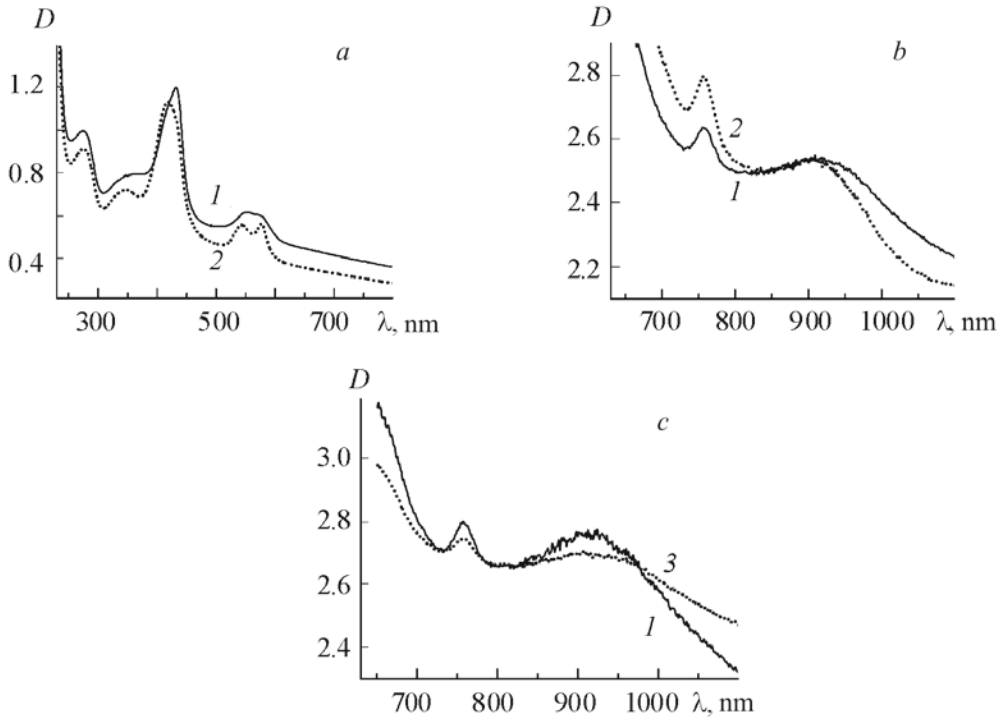

Fig. 3. Absorption spectra of samples of venous blood of rats: a) before irradiation, S_VO_2 = 50% (1); after the laser + γ irradiation that increased the oxygenation of the venous blood, S_VO_2 = 67% (2); b) before irradiation, S_VO_2 = 60% (1) and after the laser + γ irradiation that reduced the oxygenation of the venous blood, S_VO_2 = 50% (2); c) before irradiation, S_VO_2 = 54% (1) and after γ irradiation, S_VO_2 = 67% (3).

and S_VO_2 under the influence of physical factors. Under the influence of LILR in the samples of blood studied in series I and II the low values of $S_VO_2 \approx 50\%$ were increased to 70%; analogous results were obtained for the combined laser + γ treatment. In the samples in which the oxygenation of the blood increased both after laser and after laser + γ treatment the activity of the SOD was higher than in the samples with reduced oxygenation. However, in certain samples of blood after laser + γ treatment the oxygenation of the blood remained low (Fig. 3b). An interesting experimental fact to which it is necessary to pay attention is the high oxygenation of the blood of individual rats not only after SVIB and combined (laser + γ) irradiation but also after single γ irradiation. For instance Fig. 3c shows the absorption spectrum of the blood of a rat with high oxygenation of the blood after γ irradiation.

The results show that SVIB improves the oxygenation of the blood of the rats both during laser irradiation and during combined laser and γ irradiation. Approach of S_VO_2 to the normal values for venous blood normalizes the oxygen-dependent processes, improves the transport of O_2, and improves its utilization in the cells.

Primary molecular mechanisms of photoradiobiological action. The mechanisms of radiation damage to the molecules of biotissues (proteins, lipids, nucleic acids) are being actively investigated. A feature of the action of IR on bio subjects is the multistage character and the combination of direct and indirect action [1]. At the primary or physical stage the energy of IR absorbed by the molecules of the biotissues leads to rupture of the molecular bonds and the appearance of radicals ($RC^{\bullet}$, $RO^{\bullet}$, $RS^{\bullet}$, $RN^{\bullet}$, etc.) and also ionized and excited atoms and molecules in the irradiated substance the cells of which do not have structures that undergo preferential absorption of IR energy. It is known that the formation of free radicals, active forms of oxygen (AFO), and low-molecular endogenous prooxidants triggers a complex chain of reactions that give rise to oxidative stress. As a result the direct damage to the biomolecules by the primary IR energy is intensified by the defects due to the development of physicochemical processes. Active forms of oxygen are extremely effective at intensifying radiation damage to macromolecules. During radiolysis of water their amount increases and comprises some 80–90% of the mass of the cell. Increase of the O_2 content of the surrounding medium and in the cells leads to the formation of an additional amount of AFOs, which intensify the radiation damage to the living organism. The dependence of the severity of radiation

damage on the oxygen content of the medium, established at the beginning of the twentieth century, was called the oxygen effect. Recent investigations have confirmed the effect of the O_2 concentration in the surrounding medium on growth of the cells, their radiosensitivity, and their survival [16].

In recent years negative effects due to the action of IR on the biostructures have been attributed more and more to the development of oxidative stress, during which interaction of the observed biological effects with the formation of AFO must occur [12]. When LILR is used as radioprotector its effect on the oxygenation of the blood, its cells, and antioxidant protection enzymes is determined by the molecular mechanism of the action of LIOR [11]. By influencing the oxygen-dependant processes and entry of the oxygen into the cells LIOR changes the amount of AFO not only during SVIB but also during combined action of laser + γ irradiation. It should be noted that at the optimum physiologically permissible doses AFOs act as signal molecules for the oxidative stress that appears and participate in various intra- and intercellular regulation processes counteracting the destructive development of free radical reactions in the organism [17].

The prooxidant action of LILR on blood can reduce its radioprotective effect. During the combined action of laser and γ radiation it is therefore desirable that the selected LILR regimes favor change in the O_2 content of the blood to values that secure normal functioning of the organism. Photoinduced increase of oxygenation of the blood should not negatively affect the radioproctive effect of LILR. The absence of an effect or a negative but not radioprotective effect of laser + ionizing radiation, observed in [8, 18], may be caused by increase of the amount of AFO above the physiologically permissible norm. In view of the fact that the largest increase in the oxygen content of the blood of animals occurs during the irradiation procedure [15] γ irradiation in the experiments was only conducted 24 h after the last SVIB procedure.

Conclusions. It was shown that whole body γ irradiation of rats with a 3 Gr dose induces leukopenia and lymphopenia. The decrease in the average concentration of Hb and the amount of RBC circulating in the peripheral blood is less clearly defined. The C_{RBC}, Hct, and C_{Hb} values only approximate to the lower limit of the permissible values without falling to the values characteristic of anemia. The average amount of PLT remains unchanged. Among the positive changes that indicate radioprotective activity for LIOR it is necessary to include the increase of the group average number of LIM initiated by them in comparison with the post radiation value: the increase in the activity of antioxidant protection enzymes (SOD and Cat) in comparison with the value obtained after γ irradiation. The important role of antioxidant enzymes in normalization of postradiation damage is confirmed by the increase of the activity of SOD and Cat, reduced by γ irradiation, that accompanies the increase in the number of WBC and LIM. It was shown that SVIB improves the oxygenation of the rats' blood both during laser irradiation and during the combined action of laser and γ radiation. Approximation of S_VO_2 to the normal values for venous blood normalizes the oxygen-dependent processes and improves the transport of O_2 and its consumption in the cells.

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