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REGULATORY EFFECT OF LOW-INTENSITY OPTICAL RADIATION ON OXYGENATION OF BLOOD IRRADIATED *IN VIVO* AND METABOLIC PROCESSES

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For three series of blood samples, we have studied the effect of therapeutic doses of low-intensity optical radiation (LOR) on oxygenation parameters of blood irradiated in vivo, and also on the levels of some metabolites: lactate, glucose, cholesterol. The quality of blood oxygenation was assessed using three parameters: the partial pressure of oxygen p_VO_2, the oxygen saturation of hemoglobin S_VO_2, and the oxygen level in arterial and venous blood, varying under the influence of low-intensity optical radiation due to photodissociation of hemoglobin/ligand complexes. We have established that during photohemotherapy (PHT), including extracorporeal, supravascular, and intravenous blood irradiation, positive changes occur in the oxygenation parameters and the metabolite levels, while after the courses of PHT have been completed, the individual changes in such parameters in individual patients were both positive and negative. The regulatory effect of PHT was apparent in the tendency toward a decrease in high initial values and an increase in low initial values both for the oxygenation parameters and for the metabolites; but at the doses recommended for use, PHT had a regulatory but still not a normalizing effect.

Keywords: *photohemotherapy, blood oxygenation parameters, lactate, glucose, cholesterol.*

Introduction. Application of low-intensity optical radiation (LOR) for medical purposes has considerably outrun experimental and theoretical studies of its effect. Positive outcomes from application of LOR in treatment of various diseases have been mainly noted in the scientific literature. Generally phototherapy conditions, radiation sources, and wavelengths are selected empirically. In this case, differences in the photophysical processes initiated in biological tissues by absorption and scattering of radiation at different wavelengths as well as the individual sensitivity of patients to LOR procedures have not been considered. It is attempted to justify such an approach by the lack of accepted ideas about the primary photoacceptors (molecules absorbing radiation) and photoprocesses initiated by LOR in biological tissues, despite the undoubted progress in understanding these questions that has been achieved to date [1–4]. At the same time, by now a fair amount is known about the mechanisms for realization of the therapeutic effects of phototherapy, which allows us to analyze the reasons for the contradictory nature of the data on the biological activity of LOR.

The aim of this work was to use as a basis the theoretically and experimentally substantiated mechanism of action for photohemotherapy (PHT) to analyze the details of its effect on metabolic processes occurring in different patients, comparing the identified patterns for photomodification of blood and radiation-initiated changes in the levels of some metabolites under the influence of LOR at different wavelengths absorbed by blood.

Materials and Methods. We studied changes in the blood oxygenation characteristics and metabolite concentrations, directly during individual procedures and also 20–30 min after completion of the course of treatment, in three series of blood samples from patients with cardiovascular diseases. The first series of samples was obtained for extracorporeal UV blood irradiation (UBI) in patients (n = 30) using light from a mercury lamp (λ = 254 nm, power density at the surface of the cuvet 1.5 mW/cm^2); the course of treatment consisted of five daily procedures. The samples in the second series were blood from patients (n = 20) who had received intravenous blood irradiation (intravenous LBI) procedures daily for seven days in the ulnar vein using radiation from a semiconductor laser (λ = 670 nm, 2 mW at the output of the optical fiber, t = 20 min). The samples in the third series were obtained during supravascular blood irradiation (SLBI) in the patients (n

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= 26) using emission from a semiconductor laser (λ = 670 nm, power 20 mW, t = 20 min). As shown by our estimates, with such an exposure method, about 70% of the radiation incident on the skin surface reaches the blood in the vein. For each course of PHT, for two or three patients we determined the time variation of the oxygenation parameters: the oxygen saturation of hemoglobin (Hb), the partial pressures of blood gases, the individual Hb fractions, and also levels of some metabolites before and after each procedure in the course of treatment. The results obtained for each of the three PHT methods were compared with the results for the comparison groups (n = 15) of patients with cardiovascular disease who had not received the PHT procedures. Blood samples drawn for analysis were stabilized by heparin. Blood irradiation was carried out according to the standard technique approved by the Ministry of Health of Belarus with the informed consent of the patients.

We used optical oximetry results, complete blood counts, and chemistry panels to test for some metabolites before and after PHT. The absorption spectra in the 200–1200 nm region were recorded on a Cary 500 spectrometer (Varian, USA). Using the spectrophotometric unit of the ABL-800 analyzer (Radiometer, Denmark), which determines the optical transmission of a blood sample at 128 wavelengths in the 478–678 nm range in a single measurement, we measured the oxyhemoglobin fraction ($F_V(HbO_2)$), the oxygen saturation of venous blood (S_VO_2), and the lactate, glucose, and bilirubin levels in venous blood. The concentration of products of lipid metabolism was determined on an FP-901 chemistry analyzer (Labsystems, Finland). The oxygen saturation of arterial blood (S_AO_2) was evaluated from pulse oximetry data (YuOM-300, YuTAS, Ukraine). OMNIC software was used to process the spectra. The results were treated statistically with estimation of the significance of the differences using the Student's t-test. The correlation between the quantitatively normally distributed parameters was determined using the Pearson correlation test.

Results and Discussion. *Effect of photohemotherapy on oxygenation processes.* Results of our previous research [5–8] showed that intravenous LBI (λ = 632.8, 670 nm), UBI (λ = 254 nm), and SLBI (λ = 670, 800 nm) lead to similar changes in absorption by blood and erythrocytes in regions of the spectrum that are sensitive to blood oxygenation: the Soret band, the doublet at 540 nm and 570 nm, the 650–950-nm region. Radiation at the wavelengths used is absorbed by blood. Analysis of the spectral signs of photochemical reactions initiated by therapeutic doses of optical radiation allowed us to formulate ideas about the primary photoacceptors and the mechanisms of action of optical radiation on blood. Hemoglobin (Hb), having absorption coefficients at these wavelengths that significantly exceed the absorption coefficients for other molecular blood components, is the primary photoacceptor for low-intensity optical radiation. The observed spectral changes are due to changes in the oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb) fractions during photodissociation of HbO_2 directly in the erythrocytes. For neither PHT method at the doses used did we observe hemolysis of erythrocytes, the number of which (monitored during the entire course of treatment) remained within the normal range. Thus when blood is exposed to UV radiation, which is considered to be the most photochemically active, the normal erythrocyte count is preserved: $\langle C_{RBC}\rangle = (4.79 \pm 0.5)\cdot 10^{12}$ cells/L before UBI and $\langle C_{RBC}\rangle = (4.73 \pm 0.35)\cdot 10^{12}$ cells/L after the course of treatment is completed.

Photodissociation of Hb/ligand complexes leads to changes in the blood oxygenation characteristics: $F_V(HbO_2)$, S_VO_2, partial pressure p_VO_2, and consequently the oxygen level in venous blood $Ct_VO_2 = [Hb]\cdot 1.39(S_VO_2/100)$. Comparison of the oxygen characteristics in blood samples drawn before irradiation, during the PHT procedures, and after the treatment is completed at fixed times relative to the radiation exposure allowed us to establish the details of the regulatory effect of PHT in different stages of irradiation.

The regulatory effect of PHT on the oxygenation parameters is quantitatively different in different stages of the course of treatment. For all three methods, the values of p_VO_2 and S_VO_2 obtained during the PHT procedures and 20–30 minutes after completion are different. During the course of PHT treatment, we saw oscillations in the blood oxygenation characteristics. Fig. 1 shows the fluctuations in the oxygen level in venous blood Ct_VO_2 during courses of SLBI and UBI treatment, which are similar to the oscillations obtained for p_VO_2 and S_VO_2 [2, 4]. The values of p_VO_2, S_VO_2, and Ct_VO_2 increased during the procedures, and decreased after the treatment was completed. In the studied blood samples from patients with cardiovascular disease, the initial values were 15 torr < p_VO_2 < 30 torr, 15% < S_VO_2 < 35% below the normal values for venous blood ($p_VO_2 \approx 40$ torr and $S_VO_2 \approx$ 65–70%). Therefore the tendency toward normalization of p_VO_2 to 40 torr, S_VO_2 to 70–80%, and Ct_VO_2 to 13–15 vol.% during the procedures indicates a regulatory increase in the lowered initial values, responsible for the positive therapeutic effect of PHT. However, positive changes in these values during the procedure changed to a decrease down to the initial values or close to them by the beginning of the next procedure. Sometimes the p_VO_2 and S_VO_2 values by the middle of the course of treatment became even lower than the initial values, increasing the O_2 deficiency in venous blood. The noted changes in the oxygenation characteristics during the procedures were similar for laser radiation sources and a mercury lamp [2, 3].

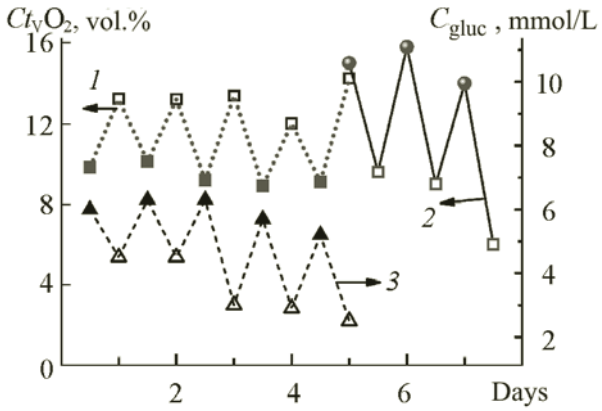

Fig. 1. Oxygen level oscillations in venous blood Ct_VO_2 for intravenous laser blood irradiation (intravenous LBI) (1) (before the procedure (■), during the procedure (□)) and UV blood irradiation (UBI) (2) (before the procedure (●), during the procedure (○)) and the glucose concentration C_{gluc} (3) (before the procedure (▲), during the procedure (Δ)).

Of special interest for assessment of the therapeutic effect are regulatory changes in both group-averaged and individual oxygenation parameters after completion of the courses of PHT. With regard to the arithmetic averages over the entire group of values, for the test patients the average values of $\langle p_VO_2 \rangle$ did not change after completion of the courses of UBI treatment, remaining below the normal values for venous blood (23.3 torr and 24 torr before and after UBI). However, after the course of SLIB treatment, the values of $\langle p_VO_2 \rangle$ increased from 19.8 torr to 25.8 torr. The arithmetic group averages for the oxygen saturation of hemoglobin $\langle S_VO_2 \rangle$ decreased from 41.6% down to 34.5% after a course of UBI treatment, increased from 31.5% to 50% after a course of SLBI treatment, and decreased from 52% to 40% after a course of intravenous LBI treatment. As follows from the presented data, for the doses recommended for use, the average values of the oxygenation parameters obtained by the end of all the courses of phototherapy did not reach the optimal values for venous blood.

The regulatory effect of PHT on each of the oxygenation parameters by the end of the course of treatment was studied over two subgroups of an ordered series, divided up according to the initial value of the analyte parameter using the median method. One group included patients with initial values of the parameter under consideration that were higher than the medium, while the other group included patients with initial values of the parameter that were below the median. We note that over these two subgroups, the average values of the oxygenation parameters remained below the normal values by the end of the course of treatment, changing in different ways under the influence of both SLBI and UBI. The regulatory effect of SLBI was apparent in the smaller increase in the average values $\langle S_VO_2 \rangle$, $\langle p_VO_2 \rangle$ in the subgroups with the higher initial values, while the regulatory effect of UBI was apparent even in a decrease in $\langle S_VO_2 \rangle$, $\langle p_VO_2 \rangle$ in the subgroups with the higher initial values (Fig. 2). For both methods, by the end of the courses of PHT treatment, the average values of the oxygenation parameters in the two subgroups came closer together but did not coincide. As follows from Fig. 2, straight lines passing through the initial and final values intersect at values which might be achieved for a longer duration of the courses of PHT treatment. By the end of the courses of treatment, the oxygenation parameters were below the optimal values, i.e., at the recommended doses, PHT had a regulatory but still not normalizing effect on the oxygenation parameters.

Analysis of the individual changes ΔS_VO_2, Δp_VO_2, and ΔCt_VO_2 shows that after completion of the courses of intravenous LBI, UBI, or SLBI, the effect of PHT on these parameters is selective and is different for different patients. Due to the individual sensitivity of the patients to blood irradiation, the changes Δp_VO_2 and Δp_VCO_2 in venous blood of individual patients were quite different, by the end of the course of treatment demonstrating both an increase in p_VO_2 and a decrease compared with the initial values [2–4]. The values of Δp_VO_2 increased linearly while the values of Δp_VCO_2 decreased as ΔS_VO_2 increased. The changes in p_VCO_2 under the influence of PHT are opposite to those obtained for p_VO_2. Thus by the end of the courses of PHT treatment, identical radiation doses in different patients initiated changes in S_VO_2, p_VO_2, and Ct_VO_2 not only different in magnitude but also in different directions, which depend on the photo-induced changes in the oxygen saturation of venous blood ΔS_VO_2 and the initial value of S_VO_2 [2–4].

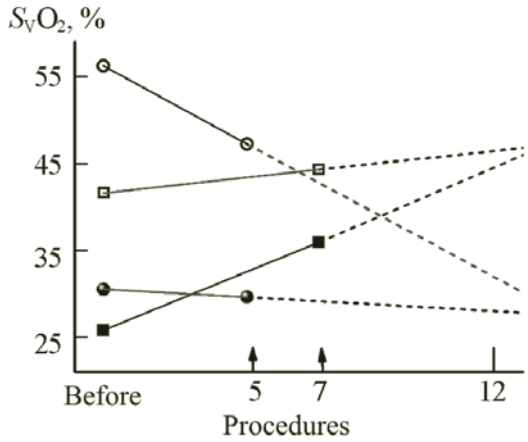

Fig. 2. Regulatory effect on oxygen saturation of hemoglobin from five UBI procedures for patients in the subgroup with initial S_VO_2 values below the median (●) and above the median (○), and seven SLBI procedures for patients in the subgroup with initial S_VO_2 values below the median (■) and above the median (□).

The changes obtained in the oxygen saturation of hemoglobin (ΔS_VO_2) allow us to estimate the effect of PHT on the O_2 demand by the tissues. The rate of metabolic processes in the body is determined by the amount of oxygen that can be absorbed by the tissues from the capillary bed, which can be estimated using the O_2 utilization coefficient: $K_{util} = (Ct_AO_2 - Ct_VO_2)/Ct_AO_2$, where the difference ($Ct_AO_2 - Ct_VO_2$) determines the concentration of the extractable oxygen. Pulse oximetry measurements show that changes in the oxygen level in arterial blood Ct_AO_2 under the influence of PHT are not large, since S_AO_2 increases at the end of the course of treatment by not more than 1%–2%. For individual patients, the course of PHT treatment has a regulatory effect on K_{util}, causing changes in K_{util} of different magnitudes that can even be in different directions, similar to what happens for the methods used in [3]. If the doses used correspond to the optimal doses for the given patient and lead to an increase in S_VO_2, then the course of PHT treatment makes it possible to reduce consumption of venous oxygen stores typical of patients with cardiovascular disease, and the O_2 level in venous blood (Ct_VO_2) increases. Oxygen utilization by tissues is normalized with positive regulatory changes in oxygen exchange. Individual pathologically elevated utilization coefficients are reduced down to 40%, approaching normal values ($22\% < K_{util} < 32\%$). For optimal doses, courses of PHT treatment balance the correspondence between tissue oxygen demand and oxygen delivery that is disturbed in patients with cardiovascular disease. Improvement in the oxygenation parameters was established in 24% of the test patients after completion of the course of UBI treatment and in 56% of the test patients after the course of SLBI treatment. The results given above show that courses of UBI, SLBI, and intravenous LBI have a pronounced positive effect on oxygen exchange only in some patients with cardiovascular disease, for whom a tendency toward an increase in O_2 level and a decrease in CO_2 level in venous blood is apparent by the end of a course of PHT treatment.

Photo-induced changes in metabolites during photohemotherapy. Before now, sufficient attention was not focused on studying the effect of photo-induced changes in oxygenation parameters on metabolic processes. Quantitative analysis of the metabolite levels before and after PHT shows that photo-induced changes in oxygenation parameters and metabolites are correlated. During courses of PHT treatment, for the metabolites, as for the oxygenation parameters, we observe periodic oscillations in the concentrations, and the changes in them occur even in the first PHT procedure (Fig. 1). The regulatory effect of PHT is apparent in the fact that the elevated initial concentrations are reduced during the irradiation procedure, but increase up to the initial values by the beginning of the next session. As for the oxygenation parameters, for the metabolites we observe a tendency toward normalization of the metabolite levels during the PHT procedures.

The regulatory effect of UBI and SLBI was studied from the change in cholesterol, glucose, and lactate levels at the end of the course of treatment. We compared changes in the average concentrations in two subgroups of an ordered series, divided up according to the median of the initial values so that the first subgroup included concentrations higher than the median and the second subgroup included concentrations lower than the median. The regulatory effect of SLBI and UBI is demonstrated in Fig. 3, using the example of changes in the average cholesterol (Chol) concentration $\langle C_{Chol} \rangle$ in

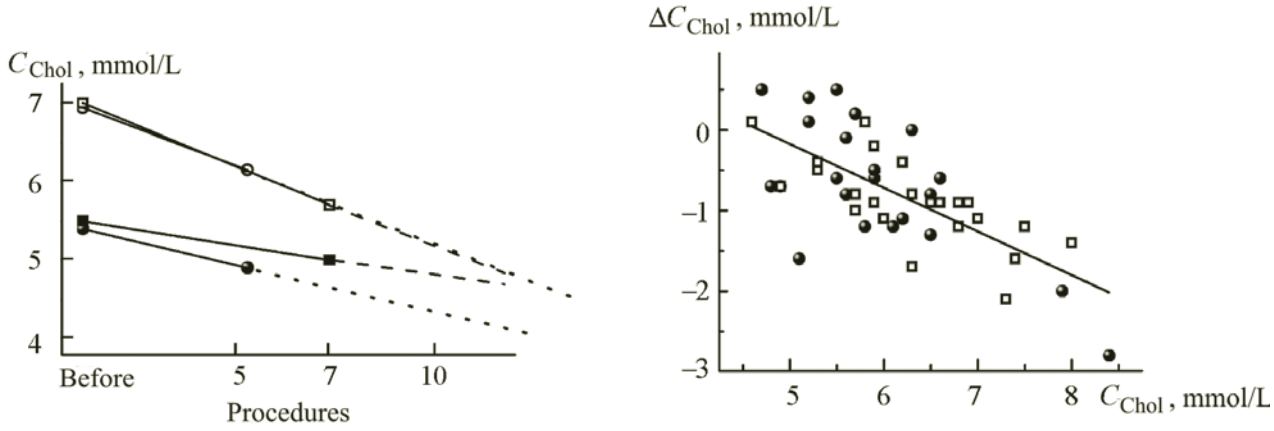

Fig. 3. Regulatory effect on cholesterol level C_{Chol} from five UBI procedures for patients in the subgroup with initial cholesterol level below the median (●) and above the median (○), and seven SLBI procedures for patients in the subgroup with initial cholesterol values below the median (■) and above the median (□).

Fig. 4. Individual changes in cholesterol level ΔC_{Chol} vs. initial concentration C_{Chol} in blood of individual patients after completion of courses of UBI (●) and SLBI (□) (Pearson linear correlation coefficient $r = -0.69$, $p < 0.0001$). A decrease in concentration under the influence of PHT is shown as a negative result.

TABLE 1. Regulatory Effect of Seven Supravascular Blood Irradiation (SLBI) and Five UV Blood Irradiation (UBI) Procedures on Metabolite Levels

Metabolite	SLBI		UBI	
	I	II	I	II
Glucose	8.6% increase	4.2% decrease	8.6% increase	8.3% decrease
Cholesterol	8.3% decrease	18.5% decrease	9.2% decrease	11.3% decrease
Lactate	18% increase	4% increase	33.8% increase	28.5% decrease

Note. I is the subgroup with low initial values, II is the subgroup with high initial values.

the two subgroups in which the initial average cholesterol concentrations were above the optimal values for patients with cardiovascular disease. The regulatory effect of PHT is apparent in the stronger reduction in cholesterol concentration in the subgroup with the higher values. For glucose and lactate, the regulatory changes in the concentrations are presented in Table 1. We should point out that in the subgroups with initial levels lower than the median, the average glucose and lactate concentrations, which were lower than the upper values in the normal range (5.5 mmol/L for glucose, 1.6 mmol/L for lactate), increase under the influence of PHT, but they decrease in the subgroups with average level above the normal range.

The regulatory nature of the effect of PHT on individual metabolite level is responsible for the dependence of the observed changes in their concentration on the initial level. Our results show that the changes can be both positive and negative. Under the influence of PHT, the concentrations decrease for elevated initial values and increase for reduced initial values, as was demonstrated for cholesterol (Fig. 4). The effect of photo-induced changes in the oxygenation characteristics on the metabolite levels is apparent in the dependence of changes in their concentration on ΔS_VO_2. The dependence of ΔC_{lac} on ΔS_VO_2 given in Fig. 5 shows that the strongest changes in the initial metabolite concentrations occur in a limited ΔS_VO_2 range. The results of our study allow us to conclude that therapeutic doses of PHT have a systemic effect on the body, which is apparent in the correlated changes in the oxygenation parameters and the various metabolic characteristics even during the first procedure and in each of the subsequent procedures.

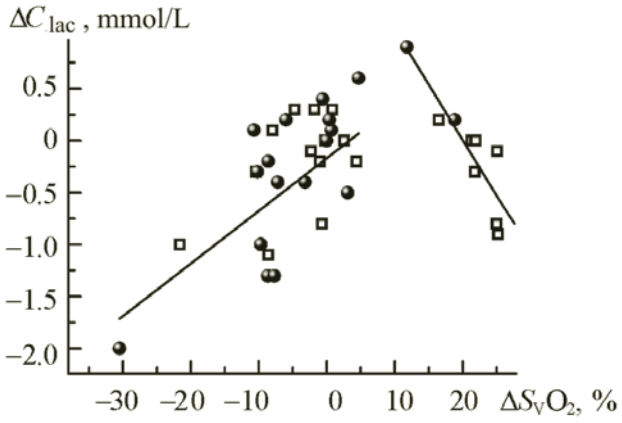

Fig. 5. Changes in lactate concentration (ΔC_{lac}) vs. changes in the oxygen saturation of Hb initiated by UBI (●) and SLBI (□). A decrease in concentration C_{lac} is shown as a positive result.

With regard to literature data, in recent years results confirming the regulatory effect of phototherapy have been obtained in [9, 10] at the end of courses of intravenous LBI treatment, and also in [11] for exposure to broadband emission from a halogen lamp (a Bioptron device). Both elevation and lowering of the studied characteristics under the influence of intravenous LBI have been observed for immunomodulating [11], anti-inflammatory and wound-healing [12] activity. The regulatory effect of intravenous LBI was analyzed in [9] on the degree of aggregation of erythrocytes and platelets and in [10] on the overall concentration of nitrates and nitrites. It has been repeatedly noted [13, 14] that for the laser exposure parameters recommended for phototherapy, both positive and negative changes in clinical parameters have been observed. However, none of the indicated papers were able to identify the nature of the regulatory effect in different directions for therapeutic doses of LOR.

The mechanism of action for phototherapy we proposed in [1–4] lets us explain the possibility of an effect from LOR in different directions on processes occurring in the bodies of the patients. Activation of the body is represented as the following stages: absorption of LOR by hemoglobin in the erythrocytes; reversible photodissociation of Hb/ligand complexes; change in oxygenation parameters on detachment of O_2, affecting oxygen saturation of tissues; activation of intracellular processes to form active oxygen species (AOS), playing the role of physiologically active compounds. The ability of the same AOSs to participate both in cell and tissue damage and in intracellular and intercellular regulation processes counteracting destructive development of free-radical reactions in the body explains why there are optimal doses for PHT, below which we observe positive clinical effects and above which we observe negative clinical effects due to depletion of the anti-oxidant defense system. The negative treatment outcomes obtained for individual patients when using different types of phototherapy may be connected with depletion of the anti-oxidant defense system due to overdosing or for an unsatisfactory initial state of the body, not allowing for normalization of the balance between intracellular AOS generation and AOS inhibition by anti-oxidant systems of the body.

Conclusions. We have shown that therapeutic doses of photohemotherapy have a systemic effect on the body, which is apparent in the correlated changes in the oxygenation and various metabolic characteristics even during the first procedure and then in each of the subsequent procedures. The most pronounced regulatory effect, leading to positive changes in the oxygen characteristics and metabolite levels, occurs during the procedures. At the end of the courses of photohemotherapy, the regulatory effect is apparent in the dependence of the metabolite concentrations on the initial concentration and photo-induced changes in the oxygen saturation of hemoglobin (Hb). Under the influence of photohemotherapy, the concentrations are reduced for elevated initial values and raised for lowered values. At the end of the courses of treatment, the regulatory nature of low-intensity optical radiation (LOR) is apparent in stimulation of only those processes which were impaired for the patients compared with healthy patients.

The results obtained confirm the mechanism proposed in [1–4] for the therapeutic effect of low-intensity optical radiation. Photomodification of blood due to absorption of optical radiation by the blood is the major mechanism of action on the body for therapeutic doses of optical radiation having a systemic effect. Phototherapy affects oxygen exchange in the body, changes both the tissue oxygen demand and oxygen delivery to the cells, promoting normalization of intracellular

processes for production of active oxygen species which participate in many intramolecular and intermolecular processes in the body. Correction of the balance between production of active oxygen species and AOS inhibition by components of the anti-oxidant defense system is considered as the process making it possible to stimulate the positive therapeutic effects of phototherapy.

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