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PROCESSES OF TRANSFER IN RHEOLOGICAL MEDIA

DIRECT RHEOLOGICAL, CYTOMETRIC, AND CARDIOLOGICAL EFFECT OF INTRAVENOUS LASER IRRADIATION OF BLOOD

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As a result of the first session of intravenous laser irradiation of blood, it has been established that changes in the blood cellular elements, according to the data of cytometry, and in the blood and plasma viscosity are analogous to the changes occurring when a blood specimen is being incubated at a temperature of 48°C for 1 h or during its storage for 18 days at 12°C in a refrigerator: average dimensions of leukocytes decrease, thrombocytes decline in number, the deformability of erythrocytes is impaired, and the viscosity of the blood increases. Analogous changes in the cytometric and rheological indices, as well as a small hemolysis of erythrocytes, were observed on irradiation of a blood specimen in vitro. The suggestion has been made that the therapeutic effect of intravenous laser irradiation of blood is due to the generation of young cellular elements and their ejection into the blood channel instead of those damaged by the laser radiation.

Introduction. The physiotherapeutic method of intravenous laser irradiation of blood (ILIB) is applied in the cases of myocardial ischemia, hypertension, stenocardia, anginas, maxillary sinusitis, acne, and nonspecific diseases of lungs; ILIB also helps in relieving stress and raising the working capacity of sportsmen [1–4]. A course of ILIB improves the deformability of erythrocytes and reduces the quantity of membrane-attached hemoglobin, thus favoring better oxygenation of tissues [5]. As a result of laser therapy of spinal injury the regeneration of the nervous tissue is improved, but with suppression of the activation of leukocytes [6]. But there is abundant experimental material pointing to the injurious effect of laser radiation on blood cells both *in vitro* and *in vivo*. Information is available in the literature on the rise in the activity of antioxidant mechanisms as a result of applying a course of ILIB [1, 2]. This fact, as well as the positive outcome of applying antioxidant therapy in the period of ILIB-induced exacerbation of disease symptoms, allow an assumption that the mechanism underlying the injury of blood cells as a result of an ILIB session is oxidative in character. As a result of laser irradiation, the singlet oxygen formed does damage to the membranes of the cellular elements of blood, altering their permeability [7], which in turn disturbs the electrolyte balance of the cells. Laser radiation alters the structure of erythrocytes and leads to accumulation of methemoglobin in them [8]. Laser radiation affects both the lipids and proteins of the erythrocyte membranes [9], the activity of the membrane acetylcholinesterase [10], and Na^+–K^+–ATPase [11]. There is a definite inconsistency in the above-mentioned facts: despite the direct damaging effect of laser radiation on blood cells, there is a positive therapeutic effect of a course of ILIB.

Since laser therapy is applied in a wide range of pathologies, the mechanism of its effect must relate to the most general principles of self-regulation in an organism. With the possibility of investigating the cellular elements of blood by means of modern automatic blood analyzers (ABA), the deformability of erythrocytes by the method of filtration, as well as the viscosity of blood and plasma, we carried out investigation of the direct effect of laser irradiation of blood *in vivo* (ILIB session) and *in vitro* on the macro- and microrheology of blood and compared the results

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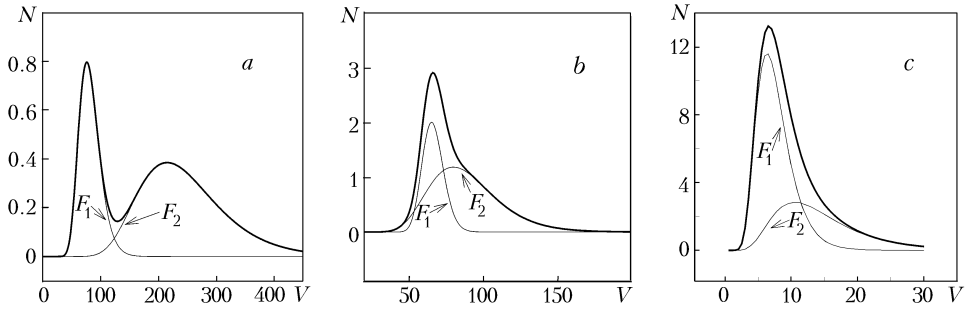

Fig. 1. Distribution of leukocytes (a), erythrocytes (b), and thrombocytes (c) over the volume. *V*, fl; *N*, %.

with the influence exerted on the blood cells by heating and storage in a refrigerator. In processing the experimental data, we employed our own rheological model (developed in our laboratory) of blood and mathematical expansion of the histograms of the cellular elements of blood into Gauss' lognormal functions.

Material and Methods. A course of ILIB (from three to seven sessions using a "Mulat" device with a wavelength of 633 nm, radiation power of 1.1 mW, 20 min for the first session and 30 min for all subsequent ones) was carried out to raise the working capacity. Taking part in the experiment were ten young sportsmen of both sexes of age 17 to 34. The blood was irradiated *in vivo* with the aid of a light guide introduced through a syringe needle into the elbow vein. Blood for cytometry was drawn into ethylenediamine tetraacetate (EDTA) from a finger before and after ILIB in such a way that no more than half an hour elapses after the procedure. For rheological measurements 10 ml of blood was taken from the elbow vein into a test-tube containing 120 μl of 7.5% EDTA solution. Irradiation of blood *in vitro* was made in a test-tube for 20 min with constant stirring by a light guide. Cytometric investigations were made on a Micros-60 OT ABA (France). The histograms of the distributions of leukocytes, erythrocytes, and thrombocytes over the volume of the cells (Fig. 1) were converted into a digital form, normalized (the area under the entire curve S = 100%), and by the least squares method were adjusted in the form of the sum of n lognormal Gauss' functions with the aid of the mathematical program SigmaPlotWindows according to the formulas

$$F(V) = \sum_{i=1}^{n} F_i, \quad F_i = k_{i1} \exp\left(-k_{i2} \left(\ln(V) - \ln(V_i)\right)^2\right). \tag{1}$$

The anisotropies of elementary distributions were calculated from the formulas

$$A_i = \frac{V_i}{k_{i1}}\left[\exp\left(\sqrt{\frac{\ln(2)}{k_{i2}}}\right) - \exp\left(-\sqrt{\frac{\ln(2)}{k_{i2}}}\right)\right], \quad i = 1, ..., n. \tag{2}$$

For erythrocytes and thrombocytes, n = 2; for leukocytes, from two to four elementary distributions manifest themselves individually (in a maximum variant two for lymphocytes and two for granulocytes, Fig. 1a). We restricted ourselves to two lognormal distributions: F_1 describes the distribution of lymphocytes over the volume and F_2, the distribution of granulocytes (see Table 1).

Measurements of the curve for the flow of a suspension of washed off erythrocytes in an isotonic solution of NaCl as well as of blood plasma was made on a Brookfield DV-II+PRO CP viscosimeter at room temperature. The viscosity of the whole blood was measured on a VIR-78 coaxial-cylindrical viscosimeter (Russia) at 30°C, with the range of shear rates being from 2 to 90 sec^{-1}. The curves of blood flow were processed with the aid of the rheological model of [12] modified in the process of our further work:

$$\ln(\eta/\eta_{pl}) = A \cdot \text{HCT}^B, \quad A = a_0 \exp\left(a_1 \exp\left(-\frac{\gamma}{\gamma_1}\right) + a_2 \exp\left(-\frac{\gamma}{\gamma_2}\right)\right). \tag{3}$$

TABLE 1. Changes in the Concentrations and Predicted Parameters of the Distributions of Cellular Elements of Blood over the Volume in the Process of Storage and Heating

Concentrations of cellular elements of blood and parameters (1) and (2)	Storage of blood in a refrigerator at 12°C, days		Heating of blood at 48°C	
	2	18	Original blood	After 1 h of heating
		Erythrocytes		
RBC, 10^{12}/l	3.56	3.61	4.62	4.49
V_1, fl	69.5±0.1	76.4±0.3	69.3±0.1	71.4±0.2
V_2, fl	82.1±0.6	108±1	82.8±1.1	88.7±3.8
V_2/V_1	1.18	1.42	1.20	1.24
S_1, %	27.47	79.0	33.1	55.8
S_2, %	72.53	21.0	66.9	44.2
S_2/S_1	2.6	0.27	2.0	0.8
A_1, fl	12.6	12.8	8.6	9.8
A_2, fl	36.3	45.9	35.8	66.6
		Leukocytes		
WBC, 10^9/l	5.1	4.9	5.5	17.3
V_1, fl	69.8±0.3	61.0±0.4	57.4±0.1	49.3±0.6
V_2, fl	207.3±0.6	128.5±1.0	194.0±1	128.0±4
V_2/V_1	2.97	2.11	3.4	2.6
S_1, %	26.7	36.8	29.7	35.4
S_2, %	73.3	63.2	70.3	64.6
S_2/S_1	2.75	1.72	2.4	1.8
A_1, fl	53.3	38.0	37.0	44.9
A_2, fl	123.25	87.0	312.3	282.3
		Thrombocytes		
PLT, 10^9/l	338	135	356	149
V_1, fl	5.9±0.2	5.1±0.2	5.7±0.3	5.0±0.1
V_2, fl	8.7±10.8	8.3±1.4	8.2±1.1	6.5±0.3
V_2/V_1	1.48	1.61	1.45	1.30
S_1, %	76.0	58.5	38.0	25.0
S_2, %	24.0	41.5	62.0	75.0
S_2/S_1	0.32	0.71	1.63	3.01
A_1, fl	0.23	0.18	0.38	0.21
A_2, fl	3.01	1.00	0.70	0.99

Using Eq. (3), it is possible to compare the values of the relative viscosity of blood and of erythrocyte suspensions, if the value of HCT undergoes a change as a result of any effect. The values of the standard relative viscosity $(\eta/\eta_m)_{st}$ were calculated at HCT = 0.4.

The deformability of erythrocytes was measured on an IDA-4 device (Russia) by the method of filtration under gravity through filters with pores of an average size of 5 μm. The calculation of data was made using the formula of [13]:

$$T_{er} = \frac{128\,(T_s - T_b)\,L\eta_{20}d}{T_b\pi D^4 \Delta p \mathrm{RBC}}. \quad (4)$$

The whole blood was stored in a refrigerator at 12°C; in regular time intervals, after careful mixing 1 ml was taken by a syringe through a rubber cork for cytometric and rheological investigations.

The heating of the whole blood during fixed intervals of time at 48°C was made in a water thermostat; thereafter the blood was cooled to room temperature, and cytometric investigations of specimens on the ABA were made.

The state of the cardiovascular system of the sportsmen were estimated with the aid of an Omega-S automated cardiograph. The main characteristics were the heart rate (HR), the index of vegetative equilibrium (IVE), the

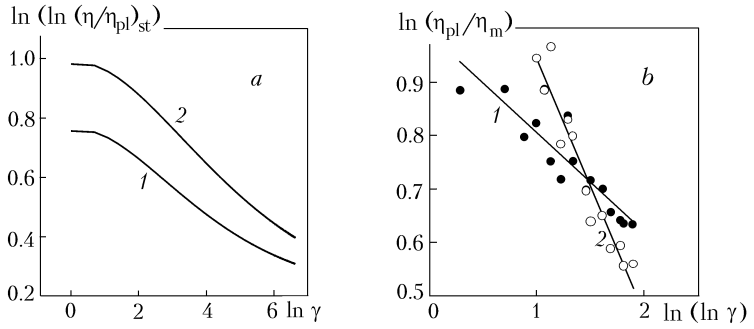

Fig. 2. Relative standard (at HCT = 0.4) viscosity of blood (a) and relative viscosity of plasma (b) before (1) and after (2) of the first session of ILIB. Points, experiment. γ, sec^{-1}.

index of tension (IT), the index of adequacy of the regulation processes (IARP), the vegetative index of rhythm (VIR), and the general assessment given in marks.

Results and Discussion. The heating of blood at temperatures close to the temperature of denaturation of cytoskeletal protein of the spectrine erythrocytes (50°C) impairs their deformability. At a low temperature of blood storage, depletion of adenosine triphosphate acid (ATP) occurs, together with poisoning of the cells by the products of the metabolism proper, first of all by lactate, lowering of pH, and inhibition of Na^+–K^+–ATPase. Passive streams of ions and water rush through the membrane and as a result the erythrocytes swell up, their deformability is impaired, and the viscosity of the blood increases. Experiments on heating of whole blood at 48°C (the temperature close to the temperature of spectrine denaturation) *in vitro* have shown that with an increase in the time of heating up to 1 h the maxima of elementary distributions of leukocytes shift to the side of smaller cell volumes. Simultaneously the concentration of white cells (WBC) increased up to 10 min of heating, after which it began to decrease. According to ABA data, storage causes analogous changes in white blood, but without a substantial change in the concentration of leukocytes. It is known from the literature that heating influences the size of thrombocytes [14], allowing an assumption that they decompose into smaller parts, just as in the case of leukocytes. The concentration of thrombocytes (PLT) decreased with heating. During storage of blood the PLT value also decreased. As a result of heating, the maximum of the second (F_2) lognormal distribution of the population of erythrocytes (V_2) shifted to the side of higher values. The relative content of the second population of cells S_2 decreased, whereas the anisotropy (A_2) increased. Analogous changes in the erythrocytes were observed during storage of blood in a refrigerator. Earlier we showed that heating at temperatures close to the temperature of spectrine denaturation leads to an increase in the blood viscosity due to the swelling and impairment in the deformability of erythrocytes [15]. The deformability of erythrocytes as a result of both storage for 18 days and heating of blood was so impaired that the motion of a specimen through a filter in the IDA-4 device was not observed at all. Thus, the impairment of the deformability of erythrocytes, the increase in the relative viscosity of blood at high shear rates, the decrease in the average size of granulocytes, and the decrease in the quantity of thrombocytes occurred in correlation with the effects that damage the blood: heating and storage in a refrigerator. The most sensitive and most easily measured (20 μl of blood from a finger into EDTA, measurement on an ABA) index of these changes is the state of granulocytes, the average volume V_2 of which decreased substantially (see Table 1).

According to the literature data, the influence of laser irradiation on the cellular elements of blood *in vitro* can be characterized as a damaging one of oxidizing character. In our experiments, when blood was irradiated *in vitro*, the standard relative viscosity of blood increased by 6.1 ± 0.2% in the entire range of shear rates, the plasma viscosity did not change, and a slight hemolysis of erythrocytes was observed. After the first session of ILIB the standard relative viscosity of blood was increased (Fig. 2a). The viscosity of plasma also changed: at low shear rates it increased and at high ones it decreased as compared to the initial one (Fig. 2b). The deformability of erythrocytes after the first session of ILIB was impaired noticeably (T_{er} = 0.47 ± 0.06 sec before the ILIB and T_{er} = 0.93 ± 0.10 sec after the ILIB). The action of the first and subsequent ILIBs on leukocytes sometimes resulted in an increase in V_2 and sometimes in its decrease. The values of PLT usually decreased ($P < 0.05$). After the second and subsequent sessions of ILIB the PLT increased, the deformability of erythrocytes improved, and the relative viscosity of plasma and blood de-

creased at high shear rates (90 sec^{-1}). Thus, we observed adaptation of the organism to the damaging effect of laser irradiation and a rapid (within half an hour) release of young thrombocytes into the blood channel from the blood depot. Judging by the viscosimetry data, the same occurs with erythrocytes, although no reliable information was obtained on the increase in the average volume of cells.

The data of cardiology correlated with the data of the cytometry of leukocytes. As a cytometric test we have taken the change in the average volume of granulocytes V_2 as a result of the ILIB session and as the cardiological one — the change in the HR, IVE, IT, IARP, and VIR, and the assessment of the state of the cardiovascular system in marks. The decrease in the average volume of granulocytes was accompanied by the impairment of the cardiological indices of a patient.

We have described the normal progress of the ILIB course, the final result of which was the improvement of the state of the health and the working capacity of sportsmen. However, not all of ILIB courses had such an outcome. In some cases, not only after the first session of ILIB but also after the second one could the impaired state of the pool of granulocytes be observed, that is, a decrease in the average volume of cells V_2 and also a decrease in PLT. The general assessment of the cardiovascular system decreased, and the pulse, IVE, and IT ($P < 0.05$) increased, which is an indication of the impairment of the working capacity. These processes were accompanied by the subjective impairment of the general condition of sportsmen, sometimes to such an extent that a patient refused to continue the therapy. There was also a third variant of the progress of the ILIB course. A sportsman was initially in a very good form, judging from the data of cardiography, as well as from the fact that already after the first session of ILIB, according to the data of cytography, the average volume of granulocytes V_2 and PLT increased. In such cases there was no correlation between the cardiological and cytometric indices. A course of ILIB neither improved the health state of the sportsman nor the state of his cardiovascular system, nor the qualitative composition of blood.

By the mechanism of action many forms of physiotherapy can be compared to setting-up exercises, training, bloodletting, or vaccination. The very session of therapy exerts a damaging effect on the organism or its elements, in our case the cellular elements of blood, in response to which the organism mobilizes its defenses, and all the factors of the immune system accumulate in a somewhat larger volume than needed to compensate the damaging effect. In the case of immune deficit even a small quantity of weakened microbes (inoculation) may turn to be an excessive loading with which the organism cannot cope, and the disease against which the inoculation was made develops in the organism. The same may occur as a result of the hardening-type of ILIB physiotherapy. Just as inoculation, it is not applied to an ailing organism. Characteristic for the hardening-type therapy are the period of aggravation of symptoms after the first sessions and then improvement of the immune, cardiological, and rheological indices. But if the therapy was applied when it must not be done, only impairment of the state was observed.

Laser irradiation of blood impairs all three forms of cellular elements: leukocytes, thrombocytes, and erythrocytes. The changes that occur in the blood cells directly as a result of the effect of laser irradiation are phenomenologically similar to the changes brought about by an elevated temperature or storage of preserved blood. As a result of the action of a course of ILIB, the organism has to replenish the store of blood cells, which leads to the strengthening of immunity and improvement of the deformability of erythrocytes (the decrease in the amount of hemoglobin bound with the membrane is attributed to the increase in the fraction of young erythrocytes in the population [5]); however, it additionally stresses the blood-forming system, as any loss of blood. This brings a short-term positive effect but depletes the store of protective defenses of an organism from a remote perspective.

CONCLUSIONS

1. ILIB is a hardening-type physiotherapy, therefore it must not be used for a weakened organism in the period of disease aggravation.

2. The average volume of granulocytes is a sensitive test for estimating the efficiency of the physiotherapy, along with cardiological indices and the state of the health of a patient. A normal course of ILIB physiotherapy is accompanied by a decrease in the index V_2 after the first session and by its increase after subsequent sessions. If after a number of sessions the value of V_2 is decreased regularly, the course should be discontinued. If an increase in V_2 is observed already after the first session of ILIB, the sportsman is in good form and is not in need of a stimulating therapy.

NOTATION

A_i, anisotropy of the ith distribution function F_i, fl; a_0, a_1, a_2, parameters that correlate with the effective radius of hydrodynamic resistance (ERHR) of an ultimately deformed erythrocyte, average ERHR of aggregates of erythrocytes and ERHR of a undeformed erythrocyte, respectively; B, correction for the near-membrane layer of the medium that increases the effective value of the hematocrit index; d, the extent of dilution of the original blood in a solution of isotropic NaCl, $d = 40$; D, diameter of a filter, $D = 5 \cdot 10^{-6}$ m; HCT, hematocrit index, volumetric concentration of erythrocytes; k_{i1}, k_{i2}, parameters corresponding to the ith distribution function F_i; L, filter thickness, $L = 10^{-8}$ m; n, number of lognormal distribution functions into which an experimental histogram of the cellular element of blood is expanded; N, quantity of cells, %; PLT, concentration of thrombocytes in the original blood, 10^9/liter; RBC, concentration of erythrocytes in the original blood, 10^{12}/liter; S_i, normalized area under the curve of ith distribution, %; T_{er}, time of the passage of average erythrocyte through a pore, sec; T_b, time of the passage of the isotonic solution of NaCl through a filter, sec; T_s, time of the passage of a specimen through the filter, sec; V_i, volume of cells that corresponds to the maximum of ith distribution, fl; WBC, concentration of leukocytes in the original blood, 10^9/liter; γ, shear rate, sec^{-1}; γ_1, γ_2, characteristic shear rates of disaggregation and deformation of erythrocytes, respectively, sec^{-1}; Δp, pressure difference in the working node, $\Delta p = 588$ Pa; η, viscosity, mPa·sec; η_m, viscosity of the medium, mPa·sec; η_{pl}, viscosity of plasma, mPa·sec; η_{20}, viscosity of isotonic solution of NaCl at 20°C, $\eta_{20} = 10^{-3}$, Pa·sec. Subscripts: m, medium; pl, plasma; st, standard; s, suspension; b, buffer; er, erythrocyte.

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