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INTERACTION OF LOW-INTENSITY LASER LIGHT WITH HUMAN BLOOD

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The processes of light attenuation in biological media are considered in details. The techniques of defining attenuation coefficient, plotting scattering indicatrix, calculating erythrocytes, measuring blood temperature under the action of laser radiation are described. The coefficient values depending on the radiation wave length and concentration of scattering centre have been obtained in the experiments. The angular radiation dispersion is plotted, the temperature dynamics is found out. Influence of erythrocytes on laser light attenuation when passing through blood is described. The graphs of attenuation coefficient dependence on the quantity of red cells per 1 mkl of blood for the wavelength of 632 and 890 nm are plotted. Plotting indicatrix and temperature measurement is performed for the wavelength of 632 nm.

At present laser is used as a surgical tool, diagnostic and therapeutic means. Low-efficient laser radiation as a form of physiotherapy has found wide application in clinical practice for treatment of different diseases. In our opinion, there are two unsolved problems in laser therapy. The first one is the absence of clear concept on mechanism of therapeutic action for every concrete disease; the second one is that the range of radiation parameters in laser therapy has not defined yet. To choose the parameters one needs to answer the question on how blood absorbs, weakens, scatter laser radiation. Knowing the weakening value one can determine the depth of laser radiation penetration.

The purpose of the given work is to study experimentally the interaction of laser light with the wavelength of 632 and 890 nm and human blood.

The effects and results on investigation of biological tissue optics with various structures considered before show the necessity of advanced study on optical characteristics. Many scientific laboratories are engaged in research on laser radiation influence on blood. A number of questions relating to changes in blood structure, cell deformation etc. are investigated. Only a few of experts are involved in studying changes of laser radiation physical parameters at penetration through the blood layer. Such research is carried out in M.V. Lomonosov's Moscow University at the chair of general physics and wave processes of physical department [1], in Saratov State University [2], in Altay State University [3].

When passing light in blood dispersion and absorption play an important role. Light dispersion by blood is connected with its structure consisting of a large number of dispersing centres random distributed in volume. The process of dispersion causes changes in spatial distribution of light intensity. In this connection, one of the main characteristics in studying light dispersion is an indicatrix determining light intensity as a function of scattering angle. Measurement of scattering indicatrix consists in exposure of tray with blood to the light beam and recording scattered radiation intensity at different angles. In close-packed particle system, which is blood, scattering indicatrix can somewhat differ from corresponding value for an isolated particle [4].

To study the regularities of radiation penetration through close-packed media it is appropriate to choose blood. On the one hand, radiation propagation through blood is of great interest for clinical medicine as a means for disease diagnostics. On the other hand, the selected methods of calculation in multiple scattering can be applied to the other close-packed media similar in optical properties.

Under the influence of laser radiation on biological substrates there appear thermal, kinetic, ultrasound, electrochemical, photochemical and other effects. Most of lasers used nowadays operate in the red and infrared spectrum ranges, the radiant energy absorbed in atoms and molecules reinforcing their oscillatory and rotational motions, i. e. it is converted into thermal energy. Warming-up tissues was one of the main physiotherapeutic means. The first field of coherent radiation application was physical therapy. In warming-up chemical and biochemical reactions accelerate, that defines physiological effect. The advantages of warming-up by means of coherent waves are explained by the fact that these waves penetrate deep into organism and heat is released to a large extent directly in tissues at some distance from the surface [5]. In this case superfluous warming-up surface tissue decreases, which is inevitable if sources of heat affect the body surface. It has been stated that thermal effect of radiation from optical quantum generators differs from conventional thermal lesions by immediate increase in temperature of tissue along the beam keeping adjacent tissue intact.

The first and most understandable mechanism from the physical point of view is dissipation of absorbed energy into heat. There is an opinion and numerous experimental data that at energy levels used in cold laser therapy warming-up tissue does not exceed 0,1 °C, which is negligibly small [6]. It is true if we speak on average tissue temperature in the region of radiation, though the distinction between the norm and general disease state is in the range of 0,3...0,4 °C.

Describing heat transfer of biological tissue one should pay attention to two aspects. As physical investigations show, endothermal chemical reactions depend on not only average energy input, but also on rate and frequency of energy input. Besides, when measuring temperature fields in biological tissues it is necessary to pay attention not only to possible presence of gradients on small geometric scale, but also to the fact that under the condition of metastable solutions thermal energy is immediately spent for chemical reaction. The temperature gradient exists only before the beginning of chemical reaction absorbing heat. On the basis of temperature measurement in tumors under the action of laser radiation using thermocouple sensors it was found that after radiation the temperature descends slowly [7]. It should be pointed out that under the action of laser radiation on biological objects the following factors influence the temperature raise: density of radiation energy, reflection of laser radiation, degree of tissue hydration, its secondary environment, electrical conductivity.

Choice of blood as an object of investigation is explained by the fact that blood is a component of nearly all biological tissue. Taking into consideration the fact that attenuation is a result of scattering and absorption it is necessary to define the input value of every process. The main centres of scattering and absorption in blood are erythrocytes, since their number is so large in comparison with that of other corpuscles. That is why for us it is, first of all, interesting to make experiment on determination of dependence of attenuation factor on the number of erythrocytes. Experiments on determination of attenuation factor were carried out at the devices designed by us with helium-neon (λ=0,632 mkm, P=12 mW) and semiconductor (λ=0,89 mkm, P=25 mW) lasers. The choice of the given types of lasers is explained by their wide application in medicine. Optical quantum generator (helium-neon laser with the radiation wavelength 632 nm, power density 200 mW/sm^2) as a source of radiation was chosen in terms of the following reasons:

1. Small angular divergence of laser beam allows for doing without complex optical system, which is necessary in using other sources as light.
2. Owing to high specific power density of laser light one can restrict oneself by relatively small volume of blood in the investigation.
3. Monochromaticity of the source simplifies sufficiently theoretical calculations of indicatrix in small angles necessary for comparison of experimental results with the theory.

Attenuation factor was measured in the following way. At the output helium-neon laser generates nearly parallel beam, which falling on the tray with blood, scatters. After this radiation is collected by the lens and focused on photoelectric multiplier. In this case scattered radiation is repelled by the shield that permits for consideration of complete divergence angle 1°. Readings were taken from millivoltmeter. Radiation of semiconductor laser coming through the tray and a system of two lenses, focused on photodiode, before which there is the shield (complete divergence angle is 3,8°). In Fig. 1, 2 the scheme of experimental equipment for determination of attenuation factor is shown.

To calculate attenuation factor the Bouguer law was used considering transmittance factor of quartz tray with transparent windows in which there was blood

$$\Phi(d,\lambda) = \Phi_0(\lambda)\tau(\lambda)\exp\{-\alpha(\lambda)d\},$$

where $\Phi(d,\lambda)$ is radiation passing through blood layer; $\Phi_0(\lambda)$ is the falling radiation; $\alpha(\lambda)$ is attenuation factor; d is the thickness of absorbing layer; $\tau(\lambda)=(1-\rho_1)^2\cdot(1-\rho_2)^2$ is the transmittance factor of the tray, ρ_1, ρ_2 are the reflection coefficient at the glass – air and glass – blood boundary, respectively. The reflection coefficients are calculated by the formula $\rho = \left(\frac{1-n}{1+n}\right)^2$, where n is the refraction index. For quartz glass the refraction index is n=1,54 for λ=0,63 mkm, n=1,53 for λ=0,91mkm. For blood the refraction index is defined by the Abbe refractometer, and it is equal to n=1,34. The refraction index of blood was changed at different periods of time, before and after radiation. When introduced the Bouguer law it is considered that light scattering is negligibly small.

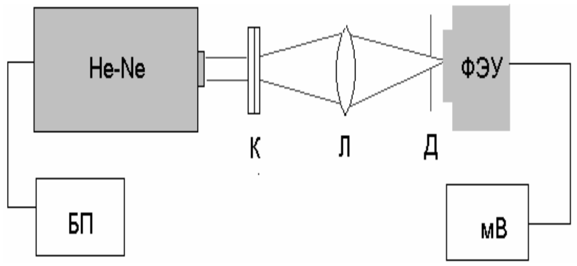

Fig. 1. *The scheme of experimental equipment with He-Ne laser for determination of attenuation factor (He-Ne – helium-neon laser, БП – power supply, мВ – millivolmeter)*

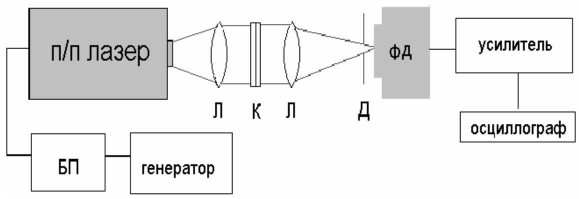

Fig. 2. *The scheme of experimental equipment with semiconductor laser for determination of attenuation factor (п/п лазер – semiconductor laser, БП – power supply, ФД – photodiode)*

The number of erythrocytes was changed by dilution of blood with its plasma. Calculation of erythrocytes was performed using the Goryaev's grid. It consists of 225 large squares, 25 of which are divided into smaller ones, 16 squares per each. Erythrocytes are calculated in 5 large squares divided into small ones [8]. Their number in 1 mkl of blood is calculated by the formula:

$$x = \frac{a \cdot 4000 \cdot b}{c},$$

where x is the number of erythrocytes per 1 mkl of blood; a is the number of erythrocytes counted in a definite number of small squares; c is the number of small squares in which erythrocytes are counted; b is the degree of blood dilution; 1/4000 is the volume of small square (multiplying by 4000, we reduce it to the volume of 1 mkl blood).

Spread in obtained values of attenuation coefficient for the samples of undiluted blood under study at the same layer thickness d=1,08 mm and λ=0,63 mkm amounted 8,74...8,97 mm^{-1}, for λ=0,89 mkm – 7,92...8,25 mm^{-1}, which is explained by different sizes of erythrocytes. The nimber of erythrocytes varied in the range from to per 1 mkl of blood. For the given dilution of blood the attenuation factor changed from 8,97 to 7,91 mm^{-1} for helium-neon laser and from 8,15 to 6,58 mm^{-1} for semiconductor lasers. Thus, one can say that having changed the concentration by nearly 25 times, the attenuation factor and, consequently, the depth of laser radiation penetration changed negligibly, i. e. therapeutic action on blood as a system with close-packed particles is the same at unchanged parameters of laser radiation. In Fig. 3 the graph of dependence of attenuation factor on the number of erythrocytes for helium-neon and semiconductor lasers is presented.

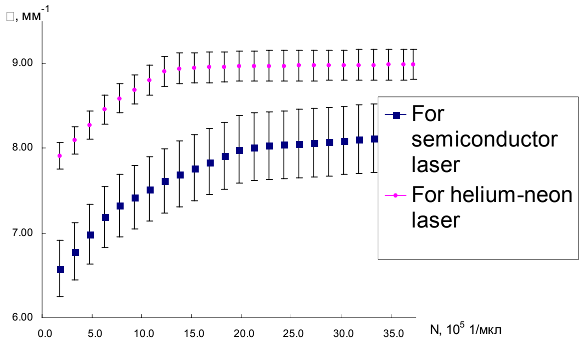

Fig. 3. *Graph of dependence of attenuation factor on erythrocyte concentration in one microlitre of blood*

Erythrocyte concentration in 1 mkl of blood is described by the magnitude called hematocrit H. Hematocrit shows the relation of erythrocytes and blood plasma, but not general number of erythrocytes. In normal blood H=0,4. Hematocrit H is connected with erythrocyte concentration N and volume of one erythrocyte V_e by the relation $N=H/V_e$. At hematocrit H<0,2 scattering coefficient is calculated by the formula $N\sigma_S=H\sigma_S/V_e$. At H>0,2 particles become close-packed, but medium becomes nearly homogeneous. For venous blood at significant closeness of packing all blood can be considered as a homogeneous medium consisting of haemoglobin, in which plasma particles are interspersed surrounding red corpuscles. In this case it is not particles that mainly scatter, but intervals between them [9].

Experiments on determination of scattering indicatrix are carried out in the following way. Laser beam passes through the tray with blood and gets to photoelectric multiplier (PEM-68), before which there is the system of three shields (angular field of vision is 1°). The given system skips the beams in small space angle. The signal from PEM goes to registering device (digital voltmeter of direct current Щ1516). Shifting the shield together with PEM perpendicular to the on-axis beam one can register light scattered at different angles. The intensity is measured in every 5°. The scheme of experimental equipment for determination of light scattering angular dependence is presented in Fig. 4. Dependencies of laser radiation intensity on scattering angle for different blood concentrations are shown in Fig. 5.

Analysis of the results obtained shows that when decreasing the blood concentration by 25 times the values of scattered radiation intensity increases. Angular dependence of diluted blood is situated higher angular dependence of laser light when passing through whole blood. It is in good agreement with the fact that the main scattering centres in blood are erythrocytes. Whole blood is considered to be a close-packed medium, with the increase of which the scattering factor decreases, and, hence, scattering light intensity. In the range within which the blood packing factor achieves its maximum value, the scattering factor tends to zero. With decrease in the number of erythrocyte contribution of laser radiation intensity forward sufficiently increases, but scattering in the opposite direction decreases. In general, qualitative view of scattering indicatrix does not change.

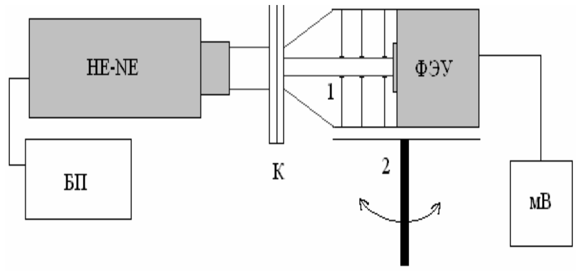

Fig. 4. *The scheme of experimental device for studying angular distribution: 1) Set of shields. 2) Turning table. He-Ne – helium-neon laser. БП – laser power supply. K – tray. ФЭУ – photoelectric multiplier. мВ – millivoltmeter*

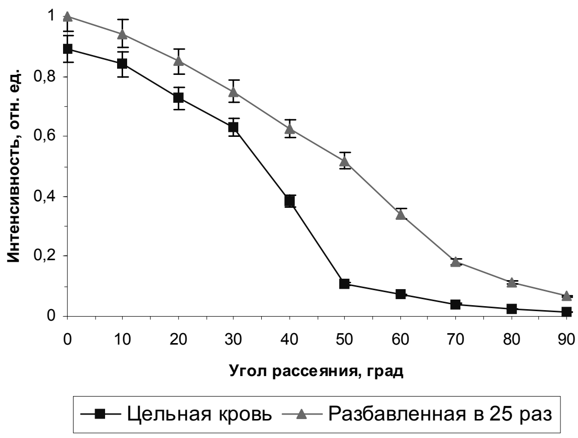

Fig. 5. *Dependence of scattering radiation intensity on divergence angle*

When introducing the Bouguer law scattering is assumed to be negligibly small. In other words, attenuation of laser radiation depends only on absorbing capacity of an object – venous blood. However, in the given region of spectrum scattering prevails over absorption. This fact explains the absence of any changes in attenuation factor depending on erythrocyte concentration, while scattering explicitly depends on their number.

We have made the experiments on measuring blood temperature under radiation of helium-neon laser. Experiments were carried out using platinum-platinum-rhodium thermocouple [10]. In Fig. 6 the scheme of experimental installation is presented. Laser radiation passing through the blood layer K (5 mm), transfers some quantity of heat to it. By means of thermocouple T, connected with millivoltmeter of DC Щ1518 the change of signal was registered. From calibration graph of platinum-platinum-rhodium thermocouple it is evident that 8 mkV change of the signal corresponds to 1° C temperature change. In the course of the experiment changes in temperature were registered at the exposure time of laser radiation to 30 sec. At the time exceeding 30 sec, changes in temperature were not fixed.

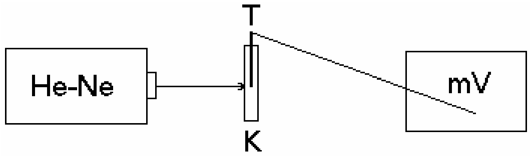

Fig. 6. *The scheme of experimental equipment for determination of warming-up blood layer*

The temperature distribution along the beam axis and perpendicular to it was also obtained. Analysing the results it became clear that at irradiation of blood the temperature of lower layers becomes higher than that of the upper layers. Such a temperature distribution is connected with the blood structure. Blood is a suspension of blood corpuscles in liquid part – plasma. Behaviour of erythrocytes in time is characterised by sedimentation rate, therefore, a particle received some quantity of heat precipitates and, in this way, heats the lower layers. The experiment showed that temperature gradient along the beam amounted 0,4°C, in other directions – 0,1 °C. The error in determination of temperature amounted 0,05 °C, which is explained by the choice of thermocouple and measuring device.

Main results and conclusions

1. A set of experimental devices for determination of attenuation factors, temperature of warming-up blood, construction of radiation scattering indicatrix in the process of interaction of cold laser radiation with human blood has been created and corresponding methods have been developed.
2. The technique of measuring dynamics of blood layer temperature in the region of action cold helium-neon laser is developed. Experimental results which are in excellent agreement with theoretical, calculated on the basis of the heat-balance equation of are obtained. It resides in 0,1...0,5 °C temperature increase, which is essential for solution of problems on laser therapy.
3. It is stated that when changing the concentration of erythrocytes 25 times therapeutic action on human blood is unchangeable, since attenuation coefficient for laser light increases 1,1 times, but then it is nearly unchangeable for concentration from $1,25\cdot10^6$ to $3,7\cdot10^6$ 1/mkl.

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