

Modification of biological objects in water media by CO₂-laser radiation

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Abstract. The modification of biological objects (polysaccharides and cells) by CO₂-laser radiation in water added drop by drop into the interaction region is studied theoretically and experimentally. Calculations are performed by using the models describing gas-dynamic and heterogeneous processes caused by absorption of laser radiation by water drops. It is found experimentally that the laser modification of polysaccharides leads to the formation of low-molecular derivatives with immunostimulating properties. A dose of the product of laser activation of the yeast culture *Saccharomyces cerevisiae* prevented the development of a toxic emphysema in mice and protected them against lethal grippe and also prevented a decrease of survival rate, increased the average life, and prevented the development of metabolic and immune disorders in mice exposed to sublethal gamma-radiation doses.

Keywords: laser application, interaction of radiation with biological objects, polysaccharide, yeast culture, immunity.

1. Introduction

It is known that the chemical or physical modification of natural polysaccharides affects their immunostimulating and adaptogene properties. The low-molecular fractions of polysaccharides can be obtained in the enzymic or acid hydrolysis of alginates and pectins, upon their radiochemical degradation induced by highly intense ionising radiation or ultrasonic modification. The low-molecular fractions have a high biological activity, increase the content of bifidobacteria in an enteric microflora, penetrate through the mucous barriers of a gastrointestinal tract inside the organism and interact with receptors (lectins) on the surface of immunocompetent cells [1, 2]. They are also efficient for the therapy of chronic infections, ulcer, and immunodeficiency.

All this stimulated our studies on the modification of

polysaccharides by laser radiation for obtaining low-molecular derivatives involved in the regulation of adhesion and glycolysis of cells, activation of some enzymes and immunocompetent cells. We have performed experimental and theoretical investigations in this direction for several years. We use a CO₂ laser emitting at a wavelength of 10.6 μm as a radiation source because the absorption coefficient of water and aqueous solutions of biological objects at this wavelength is four orders of magnitude higher than at 1.06 μm. In addition, the 10.6-μm CO₂ lasers are most economical and widespread.

2. Experimental conditions

The experimental setup uses a 220-W cw CO₂ laser. The laser beam of diameter $d_{\text{las}} = 1.6$ mm was directed perpendicular to the free-fall trajectory of drops of the aqueous solution of polysaccharides. The drop diameter d_d was 2.6 mm with an accuracy of $\pm 12\%$, their velocity v_d in the region of interaction with the laser beam was 0.485 m s^{-1} , and the interaction time was 8.7 ms. The drops were collected in a heat-insulating collector. We measured in experiments the horizontal displacement of a drop from the interaction site, the water temperature in a collector, and the mass of evaporated water. Also, the process of interaction of a drop with the laser beam was recorded with a video camera.

We determined in experiments the radiation power providing the maximum yield of the product (low-molecular polysaccharide fractions). The mass of fractions was measured with a liquid mass-spectrometer by a standard method. The change in the molecular composition of the solution of the Iceland moss shown in Table 1 demonstrates the substitution of high-molecular fractions (with the molecular weight from 80000 to 400000) by low-molecular fractions (from 20000 to 60000). One can see that at the radiation power density on a drop $q = 0.9 \text{ kW cm}^{-2}$, the concentration of low-molecular fractions of polysaccharides increases by 40 %.

Table 1.

Radiation power density on a drop/kW cm ⁻²	Molecular composition of a biological material (%)	
	Low-molecular fractions	High-molecular fractions
0	24.94	75.05
0.2	24.95	75.04
0.6	30.92	68.06
0.9	34.74	64.49

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Received 25 August 2005; revision received 13 July 2005
Kvantovaya Elektronika 35 (9) 867–872 (2005)
Translated by M.N. Sapozhnikov

Our experiments revealed the existence of the laser radiation threshold below which no depolymerisation of polysaccharides was observed, while above the threshold the yield of low-molecular fractions increased with power. The existence of the upper radiation power threshold above which depolymerisation terminated proved to be unexpected.

We proposed the working hypothesis of the hydrodynamic modification of biological objects [3], which is based on the estimate of the fractions of a laser-beam energy redistributed among different channels including the absorption of energy by a vapour-gas cloud formed upon water evaporation, reflection of light from a surface, absorption in a volume, and the kinetic acceleration of a drop.

The calculation by Fresnel formulas showed that the reflection of radiation from the surface can be neglected. The measurements of the radiation power propagated through a cloud of evaporated water showed that the signal was not attenuated within the measurement error (better than 5%). These two factors suggest that the laser-beam energy is absorbed in the drop volume, more precisely, in its thin near-surface layer because, according to the Lambert–Bouguer law, the attenuation of the radiation power is $K = \exp(-\alpha L_{\text{abs}})$, where L_{abs} is the absorbing-layer depth (in centimetres); $\alpha = 4\pi\chi/\lambda$ is the Bouguer linear absorption coefficient; and χ is the absorption index ($\chi_{\text{H}_2\text{O}} = 0.091$). For $K = 0.001$, the absorption depth of the CO₂-laser radiation is $L_{\text{abs}} = 64 \mu\text{m}$, $\sim 90\%$ of energy being absorbed in a layer of depth $\sim 20 \mu\text{m}$.

The absorbed energy is spent to heat (E_{h}) and evaporate (E_{evap}) a drop:

$$E_{\text{in}} = E_{\text{h}} + E_{\text{evap}}. \quad (1)$$

By measuring directly the water temperature in a heat-insulation collector in the incident radiation power range $Q_{\text{in}} = 50 - 216 \text{ W}$, we found that

$$E_{\text{h}} = 2.1 \times 10^{-3} Q_{\text{in}}. \quad (2)$$

The experimental increase ΔT in the water temperature averaged over the volume was $5.5 \times 10^{-2} Q_{\text{in}}$, where $\Delta T = T_{\text{d}} - T_0$ ($T_0 = 288.5 \text{ K}$). For $Q_{\text{in}} = 50 \text{ W}$, the value of ΔT is very small (2.75 K), which suggests that the heating of a drop cannot cause the depolymerisation of polysaccharides.

The fraction of evaporated water is also small. Indeed, by assuming that evaporation occurs at the temperature 100°C , we represent the laser energy spent for evaporation in the form

$$E_{\text{evap}} = m[c_p(373 \text{ K} - T_0) + r], \quad (3)$$

where r is the heat of evaporation; m is the mass of evaporated water; and c_p is the heat capacity. The value of m calculated by the maximum power of the beam, taking (1)–(3) into account, was less than 5%, in accordance with the experimental measurements of the volume of a drop before and after its irradiation.

Therefore, a small fraction of evaporated water, a small penetration depth of radiation into the drop volume, and a small increase in the drop temperature suggest that the destruction of polysaccharides is not caused by the electro-

magnetic action of the laser beam. Therefore, the only reason of destruction is the hydrodynamic action caused by the absorption of the laser-beam energy in a thin near-surface layer of a drop.

Note here that despite the relatively long irradiation time ($\sim 10^{-2} \text{ s}$) of a drop intersecting the laser beam, the generation of the compression and rarefaction waves in the drop is caused by a nonstationary heating of the drop surface. The following mechanism takes place: the heating rate in the radiation front propagating in the drop from bottom to top is rather high, providing the conditions for a brief nonequilibrium overheating of water, which is characterised by a high pressure whose ‘discharging’ exerts a pulsed action on the drop volume.

Below, we present successively the models and results of calculations: first we use the one-dimensional hydrodynamic model to analyse the depolymerisation of large molecules and then we employ the models of external flowing around and heterogeneous processes on the drop surface to explain the possible reason for the existence of the limited laser radiation power range within which depolymerisation occurs [4].

3. Hydrodynamic processes inside a drop that cause the depolymerisation of the low-molecular fractions of polysaccharides

We used the one-dimensional hydrodynamic model to estimate the parameters of an acoustic pulse caused by the volume expansion of water upon heating of the near-surface layer of a drop by a laser beam. Because this criterion is determined by scaling quantities, the one-dimensional approximation prove to be sufficient for the description of the propagation of perturbations inside the drop along the normal to its surface. The complete evolution of these perturbations in a three-dimensional drop is more complicated since it is determined by the decay, interference, and reflection of perturbations from a free surface, and because the latter is mobile, it is necessary already to analyse a two-liquid system for the consistent description of the evolution of perturbations. We assume that such a complication of the model of the internal hydrodynamic of the drop is premature; it should be preceded by a deeper understanding of the microhydrodynamic mechanism of transformation of macromolecules.

The model includes the equation for calculating temperature, written in the coordinate system moving at the velocity of the evaporating surface [5]:

$$\rho c \frac{\partial T}{\partial t} - \rho c v_s(T_s) \frac{\partial T}{\partial y} = \lambda \frac{\partial^2 T}{\partial y^2} + \alpha I \exp(-\alpha y). \quad (4)$$

The boundary condition on the surface has the form

$$\lambda \frac{\partial T}{\partial y} = \rho \varkappa v_s(T_s), \quad (5)$$

where t is the time; y is the coordinate directed along the normal to the surface; λ is the heat conduction; c is the heat capacity; \varkappa is the specific heat of evaporation of water; ρ is the density; T_s is the surface temperature; I is the laser radiation power density; and v_s is the surface-motion velocity. The value of v_s depends both on the surface temperature and the condensation coefficient, and is

determined in this model by expressions presented in [5]. In the next model, v_s is calculated self-consistently.

We determined from Eqn (4) the heating rate of the near-surface layer of the drop, on which the generation of a pressure pulse and, therefore, the propagation of the compression and rarefaction waves depend. The latter, along with other hydrodynamic parameters, are described by the momentum and continuity equations

$$\frac{\partial \rho v}{\partial t} + \frac{\partial(\rho v^2 + p)}{\partial y} = \mu \frac{\partial^2 v}{\partial y^2}, \quad (6)$$

$$\frac{\partial \rho}{\partial t} + \frac{\partial \rho v}{\partial y} = 0, \quad (7)$$

where v is the velocity of the medium; p is the pressure; and μ is the viscosity. The relation between the water density and pressure was described by the Tate equation. Despite the fact that the initial reason of the inner motion of water in a drop is the surface heating, we use Eqns (6) and (7) for an isothermal liquid in a fixed coordinate system. This is justified because the propagation velocity of the compression wave in the drop is much greater than the propagation velocity of heat from the drop surface. The boundary conditions on a free surface for Eqns (6) and (7) were specified by the expressions $\partial v / \partial y = 0$ and $\partial p / \partial y = 0$.

The boundary conditions were specified by assuming that the nonequilibrium heating of a layer of thickness $L = 20 \mu\text{m}$ (absorbing 90% of energy) is determined by the parameter $\delta T = \tau(dT/dt)$, where τ is the propagation time of sound in the absorbing layer of water (for $v_{\text{sound}} = 1500 \text{ m s}^{-1}$, the time $\tau = L/v_{\text{sound}} = 1.33 \times 10^{-8} \text{ s}$); dT/dt is the average heating rate of the layer. For the radiation power $Q_1 = 50 \text{ W}$, it follows from (4) that $dT/dt = 10^5 \text{ K s}^{-1}$, i.e., $\delta T \sim 1.33 \times 10^{-3} \text{ K}$. Because the coefficient of thermal expansion at the temperature 20°C is $\xi = 2 \times 10^{-4} \text{ K}^{-1}$, the pressure ‘spike’ δP in the surface layer at the initial instant for $Q_1 = 50 \text{ W}$ was $\sim 1.3 \times 10^5 \text{ Pa}$. This value was assumed the excess pressure in the near-surface layer.

We estimated the conditions of the beginning of transformation of macromolecules from the deformation rate $\partial v / \partial y$ of water determined from expressions (6) and (7). According to [6], a change in the macromolecular form is small until the absolute value of $\partial v / \partial y$ is an order of magnitude smaller than

$$\left| \frac{\partial v}{\partial y} \right|_{\text{cr}} = \frac{kT}{\mu \langle h^2 \rangle^{3/2}}, \quad (8)$$

where k is the Boltzmann constant and $\langle h^2 \rangle^{1/2}$ is the root-mean-square distance between the free ends of a macromolecule.

Figure 1 shows the deformation rate calculated at different instants. One can see that a pulse propagates in water at the velocity of sound (1500 m s^{-1}), while its amplitude decreases as the pulse moves away from the drop surface. For the amylopectin molecule, $\langle h^2 \rangle^{1/2} = 0.214 \mu\text{m}$ [7], corresponding to the critical deformation rate $(\partial v / \partial y)_{\text{cr}} = 500 \text{ s}^{-1}$, which is of the order of magnitude of the deformation rate of water obtained at the radiation power $Q_1 = 50 \text{ W}$. For $Q_2 = 200 \text{ W}$ and $t = 0.5 \mu\text{s}$, the maximum deformation rate is $\partial v / \partial y = 6825 \text{ s}^{-1}$, which is already an order of magnitude higher than $(\partial v / \partial y)_{\text{cr}}$. Therefore, at the threshold deformation rate of water

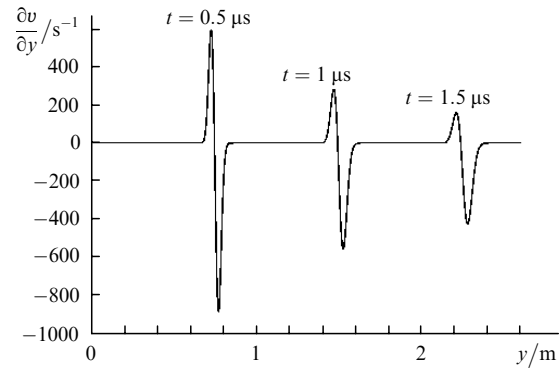


Figure 1. Deformation rate of water at different instants for $L = 20 \mu\text{m}$ and $\delta P = 1.3 \times 10^5 \text{ Pa}$.

achieved in the experiment, the form of the amylopectin molecule could change, which explains the modification process.

In this way we have determined the possible hydrodynamic mechanism of destruction of large polysaccharide molecules. However, the above-described mathematical model requires the development because the evaporation rate v_s was calculated by using expressions containing the condensation coefficient γ whose value is uncertain (according to [8], $\gamma = 0.04$, while according to [9], $\gamma = 1.0$).

The surface temperature strongly depends on γ . For example, we calculated the following temperatures at the instant of termination of irradiation of the central region of a drop: for $Q_1 = 50 \text{ W}$ and $\gamma = 0.04$, the surface temperature is $T_s = 605 \text{ K}$ and the maximum temperature inside the absorbing layer is $T_{\text{max}} = 632 \text{ K}$; for $Q_1 = 50 \text{ W}$ and $\gamma = 1$, $T_s = 389 \text{ K}$ and $T_{\text{max}} = 437 \text{ K}$; for $Q_2 = 200 \text{ W}$ and $\gamma = 0.04$, $T_s = 705 \text{ K}$ and $T_{\text{max}} = 790 \text{ K}$; and for $Q_2 = 200 \text{ W}$ and $\gamma = 1$, $T_s = 418 \text{ K}$ and $T_{\text{max}} = 475 \text{ K}$. It follows from these data that at the fixed power, depending on the condensation coefficient, either the evaporation regime is realised or the explosive boiling up of water, which begins at $T = 600 \text{ K}$. We determined the value of γ by solving the problem of external flowing around a drop.

4. External flowing around a drop upon interaction with a laser beam

The flowing around model describes the motion of air with water vapour, heterogeneous evaporation and condensation processes on the surface, and the heating of the near-surface layer of a liquid. The motion of the gas mixture was calculated by using the nonstationary system of Navier–Stokes equations and mass and thermal transfer equations:

$$\rho \frac{\partial \mathbf{v}}{\partial t} + \rho(\mathbf{v} \nabla) \mathbf{v} = \rho \mathbf{g} - \nabla p + \nabla \mu(\nabla \mathbf{v}), \quad (9)$$

$$\frac{\partial \rho}{\partial t} + \nabla(\rho \mathbf{v}) = 0, \quad (10)$$

$$c_p \rho \frac{\partial T}{\partial t} + c_p \rho \mathbf{v}(\nabla T) = \nabla \lambda(\nabla T), \quad (11)$$

$$\frac{\partial c_i}{\partial t} + \mathbf{v}(\nabla c_i) = \nabla D_i(\nabla c_i), \quad (12)$$

$$p = \rho RT \sum_{i=1}^{N_i} \frac{c_i}{m_i}, \quad (13)$$

where \mathbf{v} is the velocity vector; R is the universal gas constant; N_i is the number of components of the gas mixture; m_i and c_i are the molecular weight and the mass fraction of the i th component of the gas mixture; and D_i is the diffusion coefficient of the i th component. The viscosity, diffusion, and heat conductivity coefficients were calculated by using the formulas of Wilky and Mason–Saksena [10]. The mass of the water vapour flow at the drop–gas interface was calculated from the Hertz–Knudsen expression

$$R_{\text{H}_2\text{O}} = \frac{\gamma m_{\text{H}_2\text{O}} (p_{\text{H}_2\text{O}}^* - p_{\text{H}_2\text{O}})}{(2\pi m_{\text{H}_2\text{O}} RT_s)^{1/2}}, \quad (14)$$

where $p_{\text{H}_2\text{O}}^*$ is the saturation pressure of water at the surface temperature; $p_{\text{H}_2\text{O}}$ is the partial pressure of water near the surface; and $m_{\text{H}_2\text{O}}$ is the molecular weight of water.

The temperature distribution in the near-surface layer of a drop was calculated along the normal to its surface by using Eqn (4); however, in this model, unlike the above-described model, the velocity of movement of the liquid surface during evaporation was calculated by the expression

$$v_s = \frac{R_{\text{H}_2\text{O}}}{\rho^*}, \quad (15)$$

where ρ^* is the water density.

The condensation coefficient was determined by calculating the reactive force acting on the drop in the beam direction,

$$F_x = \int_S v_{xg}^2 \rho ds, \quad (16)$$

where v_{xg} is the horizontal component of the gas velocity. The horizontal displacement of the drop from the site of interaction with the laser beam was determined from the calculated value of F_x , and the value of v_{xg} was found by comparing the calculated value of γ with its experimental value. The absolute value of the gas velocity vector on the drop surface was determined from the expression

$$v_g = v_s \frac{\rho^*}{\rho}. \quad (17)$$

Figure 2 shows the dependences of the horizontal displacement of the drop on the laser-beam power. One

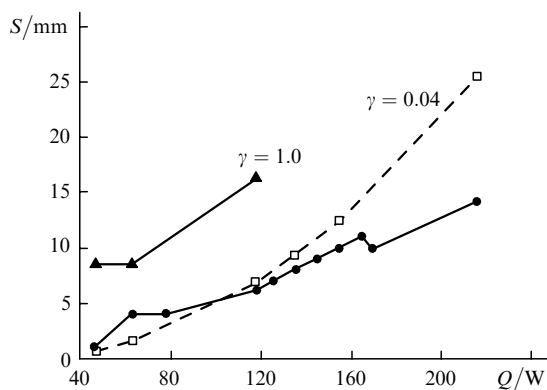


Figure 2. Dependences of the horizontal displacement S of a drop on the laser beam power Q for different values of γ (black triangles and circles are experiment).

can see that for $\gamma = 1.0$, there exist a considerable discrepancy between the calculated and experimental data. The best agreement is observed for $\gamma = 0.04$, in accordance with [8]. This value of γ was used in calculations using the external flowing around model.

Note that, by determining γ , we simulated simultaneously the evaporation of water for the conditions of optoacoustic experiments [11]. However, the amplitudes of the recoil pressure in calculations for $\gamma = 0.04$ and 1.0 were within the experimental error.

Figure 3 presents the distributions of the molar fraction of water molecules near the drop, obtained by using Eqns (9)–(14) at instants $t = 4.35$, 7.35, and 11.75 ms (the interaction time of radiation with the drop is 8.7×10^{-3} s). Near the surface the supersaturated vapour was formed. The yield of water vapour from the drop and the cloud shape agree with the photographs of the process.

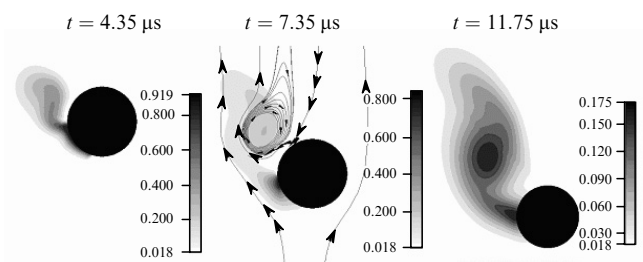


Figure 3. Distributions of the molar fraction of water molecules at different instants.

The analysis of the results of calculations obtained with the found value of γ suggests the possible reason for the existence of the upper limit of the radiation power, above which the depolymerisation of polysaccharides ceases: as the laser beam power is increased, the temperature of the surface layer of the drop at the beginning of irradiation achieves the level at which the explosive ('instant') boiling up of water starts. After the boiling up of a part of the drop surface (during the fall of the latter), the boiling front, appearing at temperature 373 K, moves upward over the drop behind the irradiation region. In the surface boiling regime, the quasi-equilibrium regime of energy absorption is realised in which the nonequilibrium overheating of the surface layer is absent. (This overheating is characterised by the anomalously high local pressure increase giving rise to the compression and rarefaction waves, which, as shown above, cause the deformation of amylopectin molecules.)

5. Results of the study of biological activity of products of laser modification of biological objects

It is known that galacto-containing low-molecular derivatives of polysaccharides accelerate the healing of stomach ulcer and prevent the tumour metastasize [1,2]. The different methods of synthesis of these derivatives are listed in the beginning of the paper [12–15]. We studied the modification of water solutions of polysaccharides from the Iceland moss *Cetraria Islandica* and from the sea grass *Zostera* [16, 17] in the form of falling drops by using laser radiation to obtain low-molecular derivatives of these solutions having the immunostimulating and antiulcer

activity. It is found that low-molecular fractions (less than 20 kDa) enhanced the functional activity of the cellular immunity T-unit and were efficient in the therapy of chronic persisting infections (herpes, hepatitis B) and ulcers.

We also studied the modification of yeast cells by laser radiation and found that the CO₂-laser radiation induced the metabolic activation of the yeast culture *Saccharomyces cerevisiae* and the secretion of thermal-shock proteins BTSh-104 and BTSh-70 into the cultural medium. We studied the adaptogen properties of the product of laser activation of the 10 % yeast culture *Saccharomyces cerevisiae*. The degree of its activation (an increase in the biomass) was measured by the method of microcapillometry. The content of metabolites (CO₂ and ethanol) was measured by the chromatography method. Laser activation caused the increase in the yeast biomass by a factor of 3–4 and enhanced their functional activity related to the increase in the production of metabolites, CO₂ and ethanol, by a factor of 1.8–3.4. After laser activation of the yeast culture, the content of BTSh-70 and BTSh-104 in it increased by a factor of 5–7.

The model of the immunodeficiency state caused by gamma irradiation (5.5 Gy) of white mice (570 species) was used to determine the biological activity of the product of laser activation of the yeast culture. Its radioprotective activity was estimated by detecting the variation dynamics of the mass and average life of test mice in the postirradiation period and measuring the Calusiner coefficient characterising the death rate of irradiated mice. The survival rate was measured within 24 hours after the beginning of the experiment. The dynamics of immune and metabolic variations in the organism of irradiated mice was measured.

The prophylactic dose of the product of laser activation of the yeast culture (PLAYC) 7 days before irradiation resulted in a considerable increase in the survival rate, up to 93 % (compared to 53 % in the control group) and prevented the development of hypotrophy (body mass deficiency) upon exposure to ionising radiation. The effect was most pronounced in the group of mice receiving the pasteurised PLAYC. The ionising radiation suppressed the aerobic mechanisms providing the structural-functional integrity of leucocytes. The PLAYC dose prevented the development of these changes and stimulated the anaerobic processes of the energy supply to provide the structural-functional integrity of leucocytes beginning with the third week after irradiation.

In the control group, the progressing functional deficiency of the cell immunity T-unit was detected beginning with the third week of the experiment. The PLAYC dose prevented its development and stimulated the secretion of cytokines activating the functional activity of the immunity T-unit beginning with the third week of observations (the maximum effect was observed during the fifth–sixth week). The ionising radiation activated the formation of peroxide compounds in leucocytes with the maximum effect during the first and second weeks of the experiment. The PLAYC dose prevented the development of peroxide processes in leucocytes of mice. We found that the PLAYC protected cells from the damage by lysosomal enzymes, and its prophylactic doses considerably increased the phagocytic activity of neutrophils of the peripheral blood.

We also performed experimental studies using the model of hemorrhagic emphysema caused by inhalation infection by the gripe virus A/Aichi/2/68 (H3N2) pathogenic for

mice. The 70-mg kg⁻¹ remantadine dose injected by the prophylactic scheme 24 and 1 hour before infection was used as a reference compound. The virus was injected through a nose under weak ether narcosis in doses of 3 and 30 LD₅₀. Animals (each of the groups contained 10 mice) were observed for 14 days. The death rate (the ratio of the number of mice perished for 14 days to the total number of infected mice in the group) and the protection index (the ratio of the difference of the number of deaths in the control and test groups to the number of deaths in the control group) were calculated from the data obtained. We found that the death rate and the protection index of the PLAYC for the sum of two virus doses were: for injecting the PLAYC for 14 days 41 % and 36 %; for 7 days 45 % and 29 %; for injecting the pasteurised nonactivated product of the yeast culture 63 % and 0%; and of remantadine 14 % and 59%. The PLAYC dose increased the survival rate and prevented the development of the lung tissue damage. The results of our study demonstrate the dose-dependent protective activity of the PLAYC *Saccharomyces cerevisiae* for the lethal gripe infection of test animals and the outlook for further investigations of the efficiency of this compound for prophylactic and complex medical treatment of emphysema upon infection and acute toxic inhalation injuries of human beings.

6. Conclusions

We have shown experimentally the efficiency of the laser method of depolymerisation of polysaccharides and metabolic activation of the yeast culture *Saccharomyces cerevisiae* by using a CO₂ laser for irradiating their water solutions supplied to the interaction region drop by drop. The mathematical models proposed in the paper explain the mechanism of depolarisation of molecules, which begins after the achievement of a laser-radiation threshold at which the deformation waves appear in water. The termination of depolymerisation at high radiation power is explained by the beginning of explosive burning of the surface layer, decreasing the nonequilibrium overheating of water.

We have shown that galacto-containing low-molecular derivatives of polysaccharides obtained from pectins of the sea grass *Zostera* and the Iceland moss by laser modification of their water solutions are promising for therapy of chronic persisting infections (herpes, hepatitis) and stomach and duodenum ulcers. It is shown that the objective product obtained by the laser activation of the yeast culture *Saccharomyces cerevisiae* is promising for the preventive treatment of immunodeficiency states of human beings subjected to the adverse exposure to ionising radiations, for the prophylactic and complex medical treatment of toxic emphysema, for protecting the organism from injury caused by various biological pathogens, for example, the gripe virus, and from the action of adverse exotoxic and endotoxic factors.

Acknowledgements. The authors thank P.G. Lyashed'ko, A.V. Zemlyanoi, E.N. Mikhal'tsova, and A.I. Gubanov for their creative participation in the experimental study of the laser-activation products and the processing of the results, and also N.F. Morozov, V.A. Levin, and D.A. Indeitsev for useful discussions of the theoretical models describing the interaction of laser radiation with a liquid jet. This work

was supported by the Russian Foundation for Basic Research (Grant No. 04-01-00661).

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