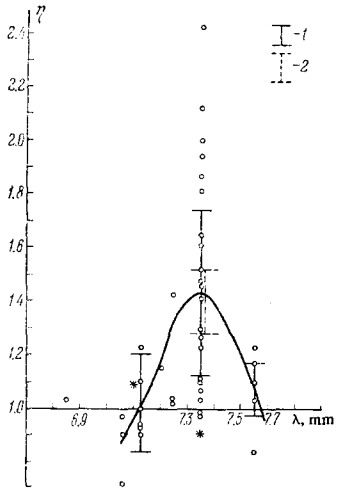


FIG. 1. Coefficient η of increase in Hb stability to acid splitting as a function of irradiation wavelength (according to [3]). $\eta = x_{contr}/x_{irrad}$, where the percentage splitting $X = 1 - C_{hydr}/C_{init}$ and C is the albumin concentration in the initial state (C_{init}) and the concentration of the remaining unsplit albumin after hydrolysis (C_{hydr}). The points indicate the scatter of the data from the various experiments; 1—rms scatter; 2—scatter after reduction of experimental data within the framework of a model taking account of the effect of microwaves on the kinetics of the oxy-met transition; asterisks indicate values of the coefficient η that we obtained for lyophylically dried Hb preparations.

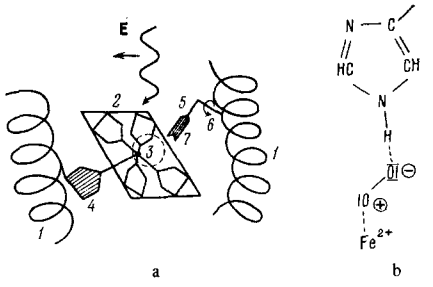


FIG. 2. Schematic representation of the structure of the active center of one of the four chains of the hemoglobin molecule (a) and the O-O bridge between the iron of the heme and the hydrogen of the distal histidine (b). 1—Spiral segments of the protein part of the micromolecule; 2—heme; 3—iron ion at center of heme; 4—proximal histidine F8 linking heme to protein part of molecule; 5—distal histidine E7, which is set in rotational rocking motion about the "axis" 6 by the microwaves; 7—conditional boundaries of region in which distal histidine interacts with iron ion.

V. I. Gaĭduk, Yu. I. Khurgin, and V. A. Kudryashova. Outlook for Study of the Mechanisms of the Nonthermal Effects of Millimeter- and Submillimeter-band Electromagnetic Radiation on Biologically Active Compounds

1. We irradiated aqueous solutions of hemoglobin (Hb) in the millimeter band. Not only was the complete molecular structure of this protein known[1], but Komov, Chistyakova, and Manĭolov had previously obtained an effect in which microwave exposure produced irreversible nonthermal changes in dried Hb preparations (see[2]). We had observed[3] a change in the chemical properties of 5% aqueous solutions of native Hb obtained from human erythrocytes. The material was irradiated at wavelengths around 7.35 mm for 5 hours at a radiation intensity on the order of 1 mW/cm^2 and temperatures of 37–40°C. On completion of the radiation exposures, the percentage acid hydrolysis of the Hb (splitting off of heme) was lower than that in the control (Fig. 1), i.e., exposure to microwaves resulted in increased strength of the bond between the heme and the protein. This can be interpreted as an increase in the stability of the Hb to the transition (during irradiation) from the active oxy form to the inactive met form, since the rate of acid hydrolysis of oxy-Hb is much lower than that for met-Hb[4]. Consideration[3] of the kinetics of this process makes it possible to improve the reliability of the experimental data.

2. According to the hypothesis of[5], a single molecular group in the active center can be regarded as responsible for the changes that take place; this is the distal histidine E7, which is essential to the Hb function[1] (Fig. 2). It can describe pivoting motions with frequencies on the order of a few cm^{-1}. The energy of the histidine oscillation and its average position with respect to the iron atom may be changed under the influence of the microwave field. Under certain conditions, the oxygen molecule in Hb forms[6] a bridge between the iron atom and the hydrogen of the E7 histidine, over which an electron is removed from the Fe atom and the Hb converted to the met form. Excitation of the histidine E7 oscillations can be inhibited by the formation of this bridge, thus preventing the inactivation of the Hb. However, the effect of the microwaves on the histidine E7 itself is not the only possibility; in principle, it can also be transferred to the histidine as a result of excitation of vibrations of the macromolecule as a single entity. This mechanism is based on the protein-machine hypothesis[7]. It is not now possible to choose between the two hypotheses or to synthesize them, and it has by no means been proven that changes in the disposition of the histidine E7 are responsible for the observed effects. Moreover, $kT/h\nu \approx 150$ under the conditions of our experiments, i.e., there are weak effects on the chemical behavior of the molecular systems (see, for example,[8]); it must be remembered that the walls of the "cavity" within which the E7 histidine is situated can fluctuate. The frequency and amplitude of these fluctuations (more precisely, their spectral distribution) are determined not only by temperature, but also by the physicomechanical properties of the macromolecule, which somehow transforms the energy of random collisions of molecules of the aqueous medium against the surface of the Hb. We may hope that microwave irradiation will assist us in finding the degrees of freedom through which most of the energy is "transferred," and that the importance of the nonlinearity[9,10] of the dipoles (which results from the energy dependence of their oscillation frequency) interacting with the microwave field will be established.

3. In principle, it would be interesting to use microwaves for resonance control of biological processes, e.g., of the addition of oxygen to Hb, in order to regulate enzyme reactions and to study the properties of the biopolymers themselves, e.g., their catalytic activity. The use of physicochemical methods to indicate microwave exposure is also promising. Selectivity of biopolymer absorption in aqueous media has not yet been observed in the microwave band. The presence of smooth dispersion and the influence of the type of solvent on absorption indicate that the interaction of the microwaves with matter is predominantly of relaxation nature as the wavelength is reduced all the way down to $\lambda \approx 0.1$ mm. In the submillimeter band, the inertia of the molecules in their oscillations about their temporary equilibrium positions begins to make itself felt[11]. Nevertheless, study of microwave absorption yields much information

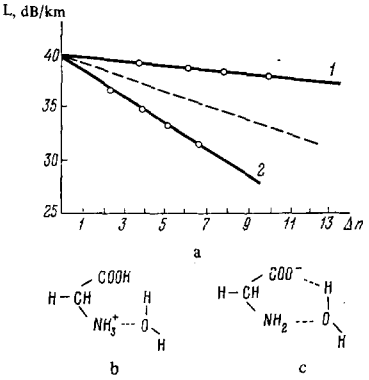
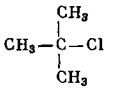


FIG. 3. Curves of absorption of aqueous glycine solution in acidic (1) and alkaline (2) media as a function of water content in the solution (a) and a schematic interpretation of the data shown (b, c). a) The dashed line indicates the decrease in absorption of pure water on a corresponding decrease in the amount of it ($\Delta n \approx 55.5 - n_1$, where n_1 is the water content (mole/liter) in the solution and Δn is the decrease of solution water content due to solution of the amino acid); b) cationic form (acidic solution of amino acid): the water molecule is not strongly bound and has freedom of displacement with respect to the hydrogen bond; c) anionic form (alkaline solution of amino acid); several water molecules are strongly bound and have lost freedom of displacement; for simplicity, the diagram shows only one strongly bound water molecule.

even now. For example, in the related compounds



(tert-butyl chloride; $\mu \approx 2.14$ D) and $CH_3-CH_2-CH_2-CH_2-Cl$ (n-butyl chloride; $\mu \approx 2.08$ D), whose molecular dipole moments μ differ (in the gaseous phase) by only 3%, solutions of these compounds in nonpolar solvents give absorption values differing by a factor of about two, like the pure liquids—the explanation for which may be found in the difference between the potentials of the intermolecular interactions that results from the shape differences between the molecules and their polarization ellipsoids. Data on the influence of the interaction of amino acids with solvent (water) molecules as it affects absorption are highly interesting. Absorption decreases with decreasing amount of water in the solution, by approximately three times as much in an alkaline medium, in which the amino acids are in the cationic form, than in an acidic medium (Fig. 3). This is explained by strong bonding of about 2 water molecules by the amino acid in alkaline media, which is qualitatively consistent with the conclusions obtained in[12] by IR spectroscopy. Several strongly bound water molecules (and not only one, as in[12]) can be registered in the microwave band. These results indicate the possibility of developing a microwave method for measuring the degree of hydration of biopolymers in solution at different temperatures (NMR and microcalorimetric measurements yield the amount of free water when frozen solutions are melted). The proposed method would yield information on the interaction of amino acids and other biologically active compounds with water and could be used to construct a theory of resonant microwave effects on biopolymers. In addition, direct determination of the hydration number would be helpful to understanding of the nature of the reactivity of organic compounds in the liquid phase and certain specific properties of the macromolecules that are determined by their interaction with the aqueous medium.

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D. S. Chernavskiĭ. I should like to make a few remarks on two possible mechanisms of the effect of microwaves on hemoglobin.

One of them relates to the direct action of the microwaves on the histidine, and the other to an effect on the molecule as a whole and the excitation of elastic oscillations of the entire structure of the protein. It seems to me that these mechanisms should be regarded not as alternatives, but instead as complementing one another, i.e., as two aspects of the same mechanism. To clarify, it might be appropriate to recall the hypothesis of the role of elastic deformations in enzymatic catalysis. According to this hypothesis, the energy needed to lower the activation barrier is stored in the polypeptide in the form of elastic deformations and released (or converted to another form) at the instant of the enzymatic event. It is important to stress that the deformations must be elastic, since otherwise the stored energy is dissipated. To resort to a simile: the enzyme-protein works like a machine: it contains a stressed region ("spring"), and at the proper moment the energy is transferred through a system of levers into the region of the active center, where the substrate molecule is located. As a result, a certain bond in the substrate molecule is ruptured and the desired reaction takes place.

Estimates have indicated[1] that the dimensions of the spring should be in the tens of angstrom units, i.e., of the same order of magnitude as the entire polypeptide globule. According to the estimates of[2], a system of these dimensions has a natural vibration frequency $\omega = 10^{11}$ Hz, i.e., of the same order of magnitude as the resonance frequency in the spectrum of the microwave effect. It appears that the elastic vibrations themselves