

of the problems whose solution may aid in understanding of other general biological processes associated with manifestation of the ultimate effects of irradiation.

R. I. Kiselev and N. P. Zalyubovskaya. Effects of Millimeter-band Electromagnetic Waves in the Cell and Certain Structural Elements of the Cell

Study of the mechanism by which electromagnetic waves in the millimeter band act on biological objects acquires substantial importance for the use of these waves in biology and medicine. During recent years, we have studied the influence of the millimeter band on isolated human and animal cells. Such cells offered a convenient model that enabled the experimenter to obtain individual cells in a monolayer form in which they were readily accessible to microwave exposure and subsequent study of its effects. In addition, structural elements of cells, viruses, and microorganisms were irradiated with microwaves. The basic criteria for evaluation of millimeter-wave effects were the morphological and biochemical indicators, survival rates, and changes in the antigenic, culturing, and virulence properties of the irradiated objects.

These studies indicated that millimeter-wave irradiation of isolated cells resulted in damage to the cell membrane, degeneration of protoplasm, and an increase in the sizes of the cells (control $5904 \pm 183 \mu^3$, irradiated at 6.5 mm $6985 \pm 185 \mu^3$; $p < 0.01$) and the nuclei (control $492 \pm 62 \mu^3$, irradiated at 6.5 mm $590 \pm 43 \mu^3$; $p < 0.01$).

The total nucleic acids and albumin contents of cells irradiated at 6.50 mm showed an increase. While the control had RNA $74.9 \pm 5.1 \mu\text{g}$, DNA $96.8 \pm 9.4 \mu\text{g}$, and albumins $109.8 \pm 6.7 \text{mg}$, the figures after irradiation were RNA $97.3 \pm 3.6 \mu\text{g}$, DNA $137.7 \pm 6.2 \mu\text{g}$, albumins $130 \pm 8.6 \text{mg}$; $p < 0.01$. It is possible that, by affecting cell metabolism, microwaves influence synthetic processes.

We noted a decrease in the number of viable cells after irradiation at the various wavelengths. In the range 5.90–7.50 mm, the 6.50-mm wavelength showed more conspicuous biological activity (Figs. 1 and 2).

After irradiation of red blood cells (erythrocytes) at 6.50 mm, we noted significant changes in hemolytic stability, an indication that these cells are sensitive to such radiation and that functional and structural changes occur under exposure to it.

Changes in the nucleic acid and albumin contents took place after irradiation in nuclei and mitochondria that were separated from liver cells. The extremely weak luminescence of the irradiated cell elements was down considerably in comparison with the control, and there was a sharp decrease in the rate of buildup of chemoluminescence intensity on heating (Fig. 3).

Millimeter-wave irradiation of various viruses (adenoviruses, measles virus, vesicular stomatitis virus, and others) resulted in a quantitative reduction of the virus particles (on irradiation of the whole virus) by a factor of 2–3. The lowered infectious activity of irradiated adenoviruses and measles virus was manifested in a delay of the cytopathogenic effect on a tissue culture.

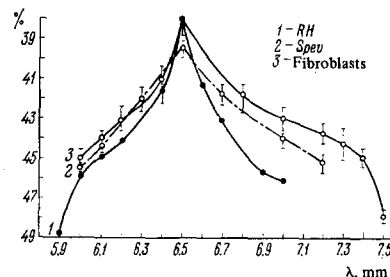


FIG. 1. Nature of millimeter-band microwave effect on various types of tissue cultures.

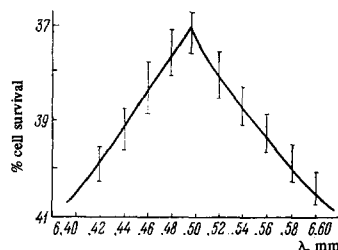


FIG. 2

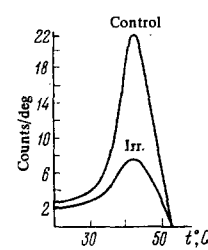


FIG. 3

FIG. 2. Influence of millimeter-band microwave irradiation on survival rate of tissue culture.

FIG. 3. Growth rate of chemiluminescence intensity of cell nuclei after millimeter-wave irradiation.

A decrease in infectious activity was observed after irradiation of virus DNA preparations (isolated from adenoviruses) as compared to unirradiated specimens. While the cytopathogenic effect was observed on the 10th day in tissue cultures that had been treated with unirradiated DNA and was morphologically similar to the manifestations of adenovirus cell infection, the infectious activity in tissue cultures treated with DNA that had been irradiated at 6.50 mm appeared between days 15 and 16 and corresponded morphologically to a manifestation of the whole virus. It appears that millimeter-band microwave irradiation of the virus DNA resulted in this case in a partial loss of infectious activity, although transforming activity was not lost, as manifested in the later appearance of the cytopathogenic effect.

We judged the influence of microwave irradiation on the cellular genome from the increase in latent-phage and colicin productive activity after irradiation of lyso-genic and colicinogenic microbe strains. After irradiation of the latter at 6.50 mm, the colicin titer increased to 320 conventional units. Increased production of phage particles as compared with the control was observed in the lysogenic microbe strains after millimeter-band microwave irradiation. Thus, while the number of phage particles was 1471 ± 152.0 in the control, it was 2934 ± 64.0 after irradiation at 5.9 mm, 4042 ± 152.0 after irradiation at 6.1 mm, 5725 ± 129.2 after irradiation at 6.50 mm, and 1296 ± 60.4 after irradiation at 7.5 mm; $p < 0.01$.

Thus, these studies indicated that millimeter-band electromagnetic waves affect both cells and cell structures.

The data obtained may serve as a basis for the use of millimeter-band electromagnetic waves in experiments toward controlled modification of viruses and microbes.

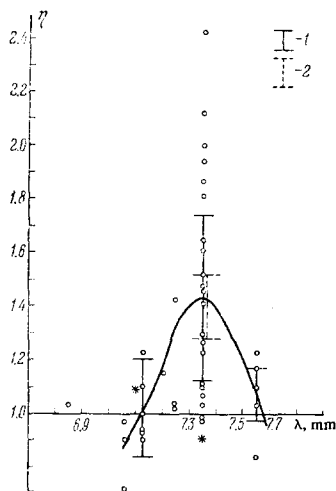


FIG. 1. Coefficient η of increase in Hb stability to acid splitting as a function of irradiation wavelength (according to [3]). $\eta = x_{\text{contr}}/x_{\text{irrad}}$, where the percentage splitting $X = 1 - C_{\text{hydr}}/C_{\text{init}}$ and C is the albumin concentration in the initial state (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 lyophilically dried Hb preparations.

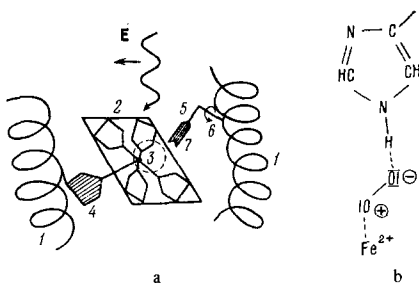


FIG. 2. Schematic representation of the structure of the active center of one of the four chains of the hemoglobin molecule (a) and the O-O bridge between the iron of the heme and the hydrogen of the distal histidine (b). 1—Spiral segments of the protein part of the micromolecule; 2—heme; 3—iron ion 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.

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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 Maniǎlov 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/cm² 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 physicochemical 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