

Several series of experiments were set up. In one series, we studied the effects of different wavelengths (7.2 and 7.6 mm) on the *Staphylococcus*, an aerobic microbe. In the second series, we investigated the protein metabolism of the individual amino acids in the various microbes after irradiation at the same wavelength (7.2 mm). The resulting data were processed statistically. As an example, we present data (see the figure) on the effects of various wavelengths on the *Staphylococcus*. As the figure shows, the following amino acids belong to the first group after irradiation at 7.2 mm: methionine, valine, alanine, threonine, and proline (five amino acids); on irradiation at 7.6 mm, methionine, valine, alanine, proline, tryptophan, glutamic acid, and ascorbic acid (seven amino acids). In the second group after irradiation at 7.2 mm: glutamic acid 19%, aspartic acid 72%, arginine 19%, tryptophan 13%, serine 16%, tyrosine 3.2%. After irradiation at 7.6 mm: arginine 14%, serine 12%, tyrosine 45%, glycine 16%, histidine 14%, lysine 14%, threonine 50%. In group 3 after 7.2-mm irradiation: leucine 1.2%, glycine 41%, lysine 30%, phenylalanine 20.5%, histidine 25%. After irradiation at 7.6 mm: leucine 7%, phenylalanine 15%. Consequently, there are qualitative and quantitative differences in the effects of the different wavelengths (7.2 and 7.6 mm).

In analysis of the factual material, our attention is drawn to two groups of amino acids: those with acidic properties (glutamic and aspartic acids) and those with alkaline properties (histidine, lysine, and arginine). While the number of "acidic" amino acids in the nutrient medium increases after growth of the microbes irradiated at 7.2 mm, no differences in their contents in the medium are detected after irradiation at 7.6 mm.

As for the "alkaline" amino acids, the amounts are smaller (group 3) during growth of bacteria irradiated at 7.2 mm and larger in the case of 7.6 mm. Changes in the contents of "acidic" and "alkaline" amino acids are also observed after irradiation of aerobes, anaerobes, and fungi at 7.2 mm. The metabolism of other amino acids is subject to substantial variations, both qualitative and quantitative. How can these facts be explained? We believe that electromagnetic radiation in the millimeter band has a definite influence on the protein metabolism of bacteria. This is manifested either in the form of activation or inactivation of proteolytic enzymes or in a change in the activity of enzymes participating in the metabolism of the individual amino acids.

<sup>1</sup> V. E. Manoilov, et al., Sb. Trudov LkhFI, No. 21, 1, 78 (1967).

<sup>2</sup> N. D. Devyatkov, Elektronnaya Tekhnika SVCh, Part 4, Sov. Radio, 1970, p. 190.

<sup>3</sup> V. F. Kondrat'eva and E. N. Chistyakova, in <sup>[1]</sup>, pp. 1 and 83.

### N. P. Zalyubovskaya. Reactions of Living Organisms to Exposure to Millimeter-band Electromagnetic Waves

We have been investigating the effects of millimeter-band electromagnetic waves on intact organisms, isolated cells, and cellular structures since 1966. To establish the biological effects of millimeter-band radiation, we studied the reactions of organisms in various stages of evolutionary development (viruses, microbes, insects, birds, and mammals).

Exposure of microorganisms (*Staphylococcus*, *Streptococcus*, *B. coli*, typhoid bacillus) to millimeter waves lowered their survival rates by 60% and more, affected the morphological, culturing, and biochemical properties, increased their sensitivity to antibiotics, and modified their antigenic properties. The infective activity of irradiated viruses was lowered.

The biological effects of the millimeter waves depended on wavelength and exposure time. The bactericidal action of millimeter waves was most pronounced at a wavelength of 6.5 mm. These studies permitted the conclusion that millimeter-band electromagnetic waves influence the viability of microorganisms.

In the experiments in which insects (*Drosophila*) were irradiated, we studied the influence of millimeter waves on the survival rates of the irradiated individuals, their ability to reproduce, and the influence of such irradiation on their offspring in the first and second generations.

Irradiation (for 15–60 min) of adult male and female *Drosophila* individuals was not lethal; they showed no externally evident changes, and breeding of such insects generally produced normal offspring. However, the offspring were fewer in number, and the fertility of the insects depended on the wavelength of the radiation to which they had been exposed (Fig. 1) and on the exposure time (Fig. 2).

Prolonged exposure to millimeter waves (for 3, 4, and 5 hours) resulted in significant changes in the first and second generations of *Drosophila*. Male individuals obtained from irradiated parents in the second generation were characterized by lower than normal viability; many perished 3–6 days after crossing. In most cases, female individuals laid no eggs.

Mutants seldom appeared in the first generation; most of them were observed in the second generation after prolonged exposure to radiation at 6.5 mm.

Thus, genetic changes were observed after exposure to millimeter waves in the insect experiments, and were manifested in lowered fertility and viability of the offspring. The observed changes apparently took place in reproductive cells, since the offspring inherited them. Individual genes obviously exhibited definite sensitivity to millimeter waves; this was indicated by multiple occurrences of the mutations in the offspring of the irradiated *Drosophila*.

With the object of studying the influence of millimeter-band microwaves on the formation, growth, and development of living organisms at a more advanced

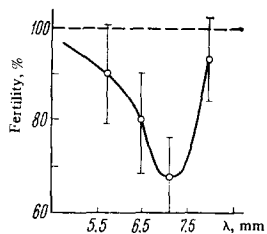


FIG. 1. Effects of various millimeter-band wavelengths on fertility of *Drosophila* in the second generation.

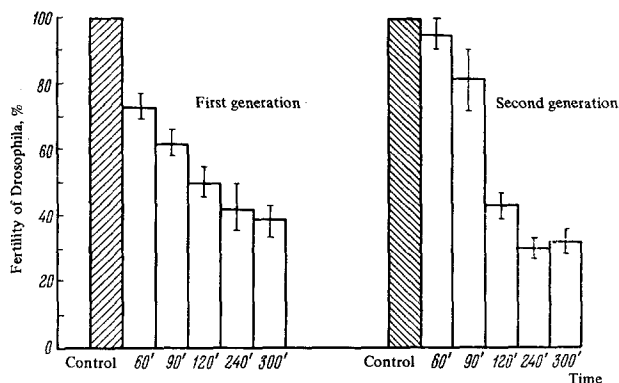


FIG. 2. Variation of fertility in first and second generations of *Drosophila* after irradiation for various exposure times at a wavelength of 6.5 mm.

stage in evolutionary development, we irradiated chick embryos and then followed them through their embryonal and post-embryonal states.

Beginning at the 7-th day, chick embryos were irradiated with millimeter waves five times for 30 minutes at a time. None of them perished after irradiation, and no loss of weight of the embryos as compared with the control was observed during the incubation period, but the incubation period was lengthened by 2–3 days.

The chicks that developed from the exposed embryos were found to be somewhat retarded in their development, especially when the irradiation had been at 6.5 mm. These chicks were unable to stand on their feet and began to peck at food later than the others; all of the irradiated chicks feathered out poorly.

The chicks that developed from the irradiated embryos were observed for 50 days. Up to the seventh day, their weights differed little from those of the controls, but they began to lag behind in this respect beginning at the 10th to 12th day. This decrease in weight continued to the last (50th) day (Fig. 3).

Thus, the results indicate a distinct effect of millimeter waves on the vital activity of chicks developed from irradiated embryos. The degree of the effect on post-embryonal development depended on wavelength.

Processes unfolding at all levels of organization of the particular organism take place in shaping the response reactions to millimeter-wave exposure in multicellular organisms.

In the experiments on mammals, we were interested in the response reactions associated with functional changes in the organism due to disturbances of the various complex functional systems. The tests showed that irradiation of the experimental animals (white rats and mice) over 40–50 days for 10–15 minutes per day was not lethal. However, these animals were sluggish and

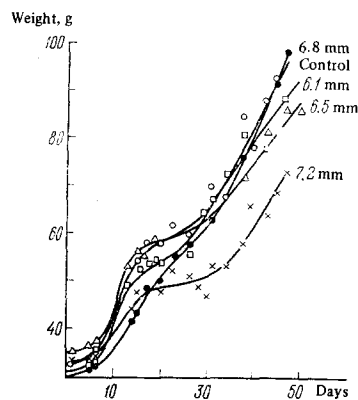


FIG. 3. Weight variations of chicks developed from embryos irradiated with millimeter waves.

their fur dishevelled; they refused food and drink for some time. The hair on shaved skin areas (under local irradiation) failed to grow back over the entire course of the exposures. Biopsies of irradiated skin areas showed atrophy of the Malpighian layer, sclerosis of the derma, and signs of accumulation of fat with penetration of fatty vacuoles into the derma, i.e., abnormalities in the outer layers of the skin and the underlying muscular layer were observed after irradiation.

The sensitivity of the organism's humoral system to millimeter irradiation was inferred from hematological indicators. The blood coagulation rate was higher after irradiation ( $68.2 \pm 1.5$  sec in the control,  $35.0 \pm 1.3$  sec after irradiation at 6.5 mm;  $p < 0.01$ ), and hemoglobin content decreased to 11.0 g-% as compared with 16.0 g-% in the unirradiated animals. Blood-serum albumin was lower by 30% and more in the irradiated animals as compared with the intact ones.

Decreases in total nucleic acids and albumins were observed in the blood-forming organs (liver and spleen) of the irradiated animals. Thus, while the total nucleic acid content in the control was  $312 \pm 8.24$   $\mu\text{g}$ , with  $94.8 \pm 3.3$   $\mu\text{g}$  of RNA,  $217.3 \pm 7.2$   $\mu\text{g}$  of DNA, and  $51.76 \pm 1.1$  mg of albumins, the respective concentrations decreased after irradiation at 6.5 mm: total nucleic acids to  $250.0 \pm 6.4$   $\mu\text{g}$ , RNA  $109.0 \pm 6.0$   $\mu\text{g}$ , DNA  $140.0 \pm 7.6$   $\mu\text{g}$ , and albumins  $38.0 \pm 2.2$  mg;  $p < 0.05$ . The irradiated animals showed lower resistance to infection. Their antibody (agglutinin) and blood lysozyme levels were half those of the control. Irradiation of immunized animals had no effect on specific resistance; these animals remained stable against infection.

Thus, the studies showed that simple and highly organized animals are sensitive to electromagnetic waves in the millimeter band. This suggests that the action of millimeter waves is a general biological one and is not limited by phylogenetic differences between organisms. The effects of millimeter waves on the living organism were manifested in functional and systemic changes, although certain distinctive features of the reaction were noted in specific organisms. The biological effect of the millimeter waves depended on wavelength and exposure time. In the 5–8-mm band, 6.5-mm microwaves were characterized by higher biological effectiveness.

The biological effects of the millimeter waves were manifested in changes in many vital processes. The very diversity of these effects makes their investigation one

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.

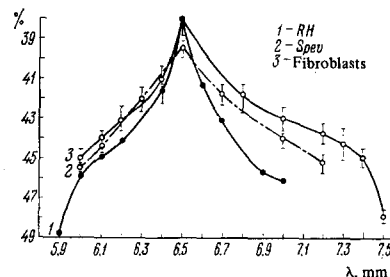


FIG. 1. Nature of millimeter-band microwave effect on various types of tissue cultures.

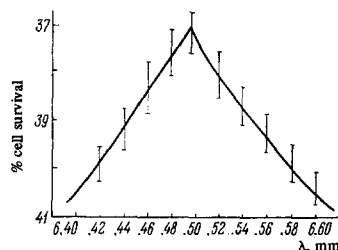


FIG. 2

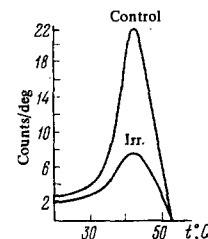


FIG. 3

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  $1471 \pm 152.0$  in the control, it was  $2934 \pm 64.0$  after irradiation at 5.9 mm,  $4042 \pm 152.0$  after irradiation at 6.1 mm,  $5725 \pm 129.2$  after irradiation at 6.50 mm, and  $1296 \pm 60.4$  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.