

certain properties of bacteria. Three strains of *Cl. sporogenes*, two of *Cl. histolyticum*—anaerobic spore bacteria with conspicuous proteolytic properties—and three strains of *Bact. prodigiosum*—an aerobic bacterium that differs substantially in a number of properties from the other two—were chosen as objects.

Each strain was irradiated 20 times at a wavelength of 7.20 mm for three hours at a time. Morphology, sporulation (in the anaerobes), the nature of growth on culture media, saccharolytic, proteolytic, and antigenic properties, and, in the case of *hystolyticum*, also pathogenicity, were studied after irradiation.

After irradiation, *sporogenes* and *histolyticum* shrank to half the size of the controls, and seldom appeared in pairs and chains. A strong and consistent decrease in spore-forming ability was observed in both anaerobes. This was especially pronounced in the case of sporogenesis: unirradiated cultures grown on Kitt-Tarozzi medium had 50–54 cells with spores per hundred after twenty-four hours, while irradiated cells showed only 5–20 spore cells per hundred. Two cultures lost their ability to form spores altogether. It was not recovered after the cultures had been stored for a year and subcultured 20 times.

There were changes in growth on dense nutrient media. Rounder colonies with only slightly convoluted margins and smoother surfaces were encountered among the *sporogenes* colonies. Beginning at the 8th to 10th exposure, *prodigiosum* cultures began to grow in the form of pale pink colonies that did not redden in the light. Suspensions prepared from irradiated cultures that had grown for twenty-four hours were colorless or slightly pinkish, while their controls were deep pink or bright red.

The antigenic properties of the bacteria were affected. Irradiated *sporogenes* and *histolyticum* cultures began to agglutinate at titers $\frac{1}{2}$ to $\frac{1}{4}$ those of the controls. Antigens from the irradiated cultures had a positive precipitation reaction in agar gel in dilutions 1–3 smaller than the control dilutions. The intensity of the reaction was also weaker. The control cultures formed three lines of precipitation in the reaction between undiluted and 1:2 diluted sera and antigens, but there were seldom two lines, and usually only one, in irradiated cultures with the same antigen concentrations.

There was no change in the saccharolytic activity of the bacteria, but proteolytic activity declined. The irradiated bacteria began to peptonize milk 2–6 days later than the controls. Irradiated *sporogenes* cultures were slower to decompose the fragments of meat in the Kitt-Tarozzi medium. The pieces normally vanish completely within 20–30 days, but 35–52 days were required when irradiated bacteria were cultured.

The decrease in the ability to peptonize milk and decompose meat indicates a change in protein metabolism.

To investigate the virulence of *histolyticum*, rabbits and white mice were inoculated with cultures that had been growing for four days. The cultures were administered intramuscularly, undiluted and in successive twofold dilutions up to 1:32, in 0.5-ml doses to the mice and in 1-ml doses to the rabbits. Eight irradiated and 3 unirradiated cultures were used in the experiment. The animals were observed for six days.

The animals inoculated with the unirradiated cultures perished during the first four days up to a maximum dilution of 1:8.

Local effects were observed in the rabbits given the 1:16 dilution, and also in one of the animals given the 1:32 dilution.

Of the eight rabbits inoculated with the irradiated cultures, one perished at the 1:8 dilution, and the rest after administration of undiluted or 1:2 diluted cultures. Three rabbits showed local effects from the 1:4 dilution, and one even from 1:8.

We also studied the influence of microwaves on the survivability of the bacteria. It had been established originally that the 7.2-mm wavelength was most injurious. We investigated the effects of wavelengths $\lambda = 7.1, 7.15, 7.16, 7.17, 7.18, 7.19,$ and 7.20 mm to define a narrower wavelength band between 7.1 and 7.2 mm.

In all experiments, the number of microorganisms was smaller after exposure than in the controls; the strongest effect was observed from the 7.15-mm wavelength.

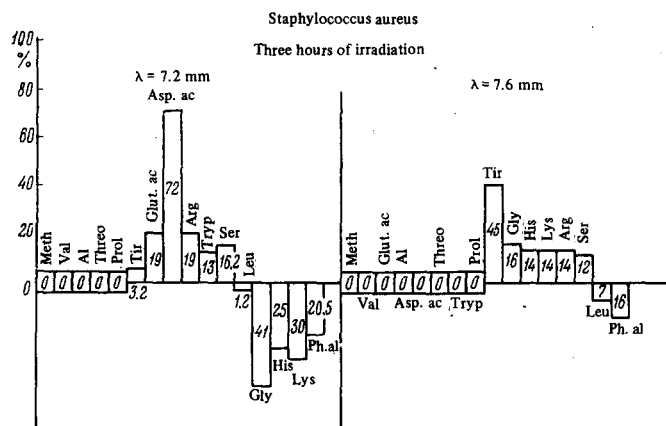
Thus, millimeter waves have a substantial lethal effect on bacteria. Survival time was found to depend on wavelength. Sporogenesis, antigenic and proteolytic properties, and virulence are affected by microwave exposure.

S. E. Manóilov, E. N. Chistyakova, V. F. Kondrat'eva, and M. A. Strelkova. Effects of Millimeter-band Electromagnetic Waves on Certain Aspects of Protein Metabolism in Bacteria

It had been shown in earlier studies that this type of radiation has a very strong influence on the functions both of biologically active substances—hemoglobin, the cytochromes, etc.^[1,2] and on microorganisms^[3]. In the present study, we report material from an investigation of the effects of millimeter radio waves on certain aspects of the protein metabolism of anaerobic and aerobic bacteria and on fungi, whose protein metabolisms exhibit qualitative differences.

The following species were the objects of investigation: *Cl. sporogenes*, *Cl. histolyticum* (anaerobes), *B. prodigiosum*, *Staphylococcus aureus* (aerobes), *Act. norsie*, *Pen. nigricans* (fungi). The microbes were irradiated at wavelengths of 7.2 and 7.6 mm by a backward-wave-tube source at an average power flux density of 4–5 mW/cm² in the radiation incident on the object. Equal numbers of irradiated and unirradiated microorganisms were introduced into the nutrient medium, and the free amino acid contents were determined by Baudet's method in this medium after one day of growth.

All data were calculated as percentages of the amino acid contents in the nutrient medium after unirradiated microorganisms had been cultivated on it. The amino acid contents for all of the bacteria studied in the nutrient medium can be classified into three groups: in the first group, there is no difference between the quantities of amino acids in the nutrient medium between the irradiated and unirradiated bacteria; in the second group, the quantity of amino acids in the medium was larger when irradiated microbes were cultured than in the case of unirradiated microbes; in the third group, the amounts of amino acids in the medium were smaller for irradiated than unirradiated microorganisms.



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.

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

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

³ V. F. Kondrat'eva and E. N. Chistyakova, in [1], 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