lacunas[1], in which the numbers of individual colicin-synthesizing bacteria are counted. The effect was evaluated with the aid of the so-called induction coefficient, which is determined by the ratio of the lacuna-formation frequencies in the experiment and the control:

$$K_i = \frac{L_e K_c}{K_e L_c},$$

where L_e is the number of cells forming colicin in the experiment, K_e is the total number of colicinogenic cells in the experiment, L_c is the number of cells forming colicin in the control, and K_c is the total number of colicinogenic cells in the control.

It was found that the number of colicin-synthesizing cells increased sharply on irradiation of the colicinogenic strain with millimeter waves of certain wavelengths. Thus, the number of cells that synthesized colicin increased by an average of 300% on irradiation at wavelengths of 5.8, 6.5, and 7.1 mm. At the same time, neighboring wavelengths, 6.15 and 6.57 mm, showed no such effect. The results obtained were reproduced with high regularity.

Thus, it was observed that millimeter waves of certain wavelengths are capable of inducing the synthesis of colicin by colicinogenic bacteria. This indicates that these waves may be able to influence the regulation of functional activity in certain (in this case of extrachromosomic) genetic elements of bacterial cells.

The behavior of the induction coefficient of the bacterial colicin synthesis as a function of wavelength was investigated in greater detail in the range 6.50—6.59 mm, because it was precisely in this range that an "active" wavelength (6.50 mm) and an "inactive" one (6.57 mm) had been detected.

Investigation of 11 points with the aid of a specially adapted wavemeter capable of measuring wavelengths with a resolution of 0.01% produced the curve in Fig. 1. It follows from the figure that colicin synthesis is a resonant function of wavelength. Note must also be taken of the high sensitivity of the biological system to variation of wavelength. The statistical significance ($P \leq 0.001$) of the differences between the comparison indicators in the control and experimental systems was demonstrated on statistical reduction of the results of repeated experiments (from 15 to 25 at each point). The effect was directly dependent on irradiation time. Irradiation for 30 minutes at t = 20°C had no influence on colicin synthesis; the numbers of cells synthesizing colicin increased by a factor of 1.5—2 after irradiation for one hour and reached a maximum after 2 hours (Fig. 2). At 37°C, colicin synthesis was induced by as little as 30 minutes of irradiation. This would apparently be associated with the higher functional activity of all systems of the cell under these conditions.

We then studied the influence of the power flux density of the radiation on induction of the colicin synthesis. Variation of the power flux density through a factor of 100, from 0.01 to 1.00 mW/cm^2, had no influence on the induction coefficient, and only a further reduction of the power to 0.01 mW/cm^2 resulted in a sharp decrease in the biological effect (Fig. 3).

Thus, the magnitude of the biological effect is affected differently by variations of exposure time, power density, and temperature. While exposure time has a very strong influence, variation of the power of the radiation over a broad range leaves the magnitude of

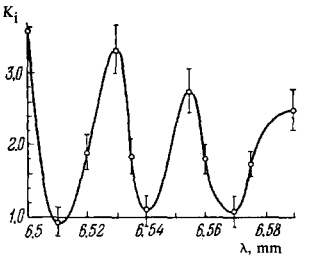

FIG. 1. Induction coefficient K_i of colicin synthesis as a function of wavelength.

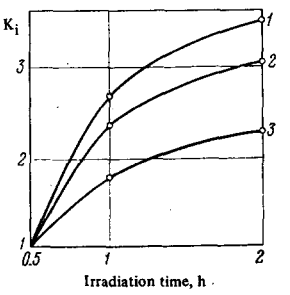

FIG. 2

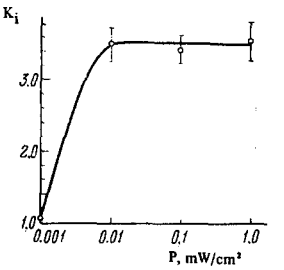

FIG. 3

FIG. 2. Induction coefficient of colicin synthesis as a function of irradiation time. λ(mm) = 6.5 (1), 5.8 (2), and 7.1 (3).

FIG. 3. Induction coefficient of colicin synthesis as a function of power flux density.

the effect practically unchanged. That the effect does not depend on power is another weighty argument in favor of the nonthermal effects of millimeter waves, since all thermal effects depend primarily on flux intensity. Direct temperature measurements with a thermocouple indicated that the bacterial suspensions in the experimental and control systems had practically the same temperature during irradiation.

Up to the present time, the ability of various agents (both physical and chemical) to induce the colicin synthesis, which is lethal to the bacterial cell, has been linked basically to the ability of these agents to disintegrate DNA or to block its synthesis. The classical inductors of the colicin syntheses of other similar genetic systems (for example, that of temperate phage)—UV irradiation or mitomycin C[2]—also exhibit these properties. As we know, both agents rupture chemical bonds in the DNA molecule, with formation of pyrimidine-base dimers. From this point of view, millimeter-band radiation can be regarded as a fundamentally new agent that disturbs the functional regulatory mechanism of genetic elements in the cell, and extrachromosomic elements in particular, without causing direct damage to the DNA molecule.

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V. F. Kondrat'eva, E. N. Chistyakova, I. F. Shmakova, N. B. Ivanova, and A. A. Treskunov. Effects of Millimeter-band Radio Waves on Certain Properties of Bacteria

Over a number of years beginning in 1965, we investigated the influence of millimeter-band radio waves on

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 anerobes. 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. Manoĭlov, 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/cm^2 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.