

FIG. 1. Changes in number of bone-marrow cells  $(N/N_0)$  and skin temperature  $(\Delta t)$  of irradiated animal as functions of power flux density. 1–Number of bone-marrow cells (control); 2–exposure to x-radiation; 3–combined exposure to microwaves and x-rays; 4–change of skin surface temperature.



FIG. 2. Variation of number of bone-marrow cells with microwave irradiation time. 1–Control (unirradiated-animals); 2–x-irradiation; 3–microwave field and x-irradiation.



FIG. 3. Variation of number of bone-marrow cells with wavelength. 1-Control (unirradiated animals); 2-x-irradiation; 3-microwave field and x-irradiation.

the animals has no influence whatever on  $N/N_0$  up to a power flux density  $P = 9 \text{ mW/cm}^2$ . Thus, there is a certain threshold power flux density below which the microwave field has no effect. Then, as P is increased, the number of undamaged cells increases practically jumpwise to 0.85. A further increase in P is not accompanied by an increase in  $N/N_0$ . The same plot indicates the increase in the skin temperature of the irradiated animal as a function of P. Temperature does not change below  $P = 10 \text{ mW/cm}^2$ . We then observe a slow temperature increase during which the slope of the  $\Delta t$  (°C) line is  $2.5 \times 10^{-2} \text{ deg/mW} \cdot \text{cm}^{-2}$ . We see from comparison of curves 3 and 4 in Fig. 1 that the magnitude of the biological effect does not correlate with the variation of the animal's skin temperature.

Thus, the optimum power flux density, at which the microwaves can be observed to have a protective effect on bone marrow but do not cause heating of the skin, is around 10 mW/cm<sup>2</sup>. It was this circumstance that dictated selection of a power density of 10 mW/cm<sup>2</sup>.

When the microwave exposure times of the animals were varied, it was found that no microwave effect appears at all before t = 30 min (Fig. 2). As the irradiation time increases to 60 minutes, we observe an increase in the protective effect and  $N/N_0$  reaches 0.8. Further increase of the exposure is not accompanied by any appreciable increase in the number of cells that remain undamaged by x-rays. Thus, the optimum irradiation time was found to be 60 minutes.

We were most interested in investigating  $N/N_0$  as a function of microwave wavelength in experiments with combined exposure to microwaves and x-rays. The wavelength of the microwaves was varied from 6.6 to 7.7 mm. The results of these measurements appear in Fig. 3. It was found that the protective effect of preliminary microwave exposure of the animals is distinctly selective in nature. Thus, the undamaged-cell count rises from 0.5 to 0.85 at the wavelengths of 6.7 and 6.82 mm, in the range 7.09-7.16 mm, and at 7.26 and 7.7 mm, while there was no protective effect at all at the same microwave power density at the other wavelengths studied (6.6, 6.85, 7.0, 7.07, 7.17, 7.19, and 7.4 mm). This behavior of the  $N/N_0(\lambda)$  relationship suggests a resonant mechanism for the action of the microwave field.

 <sup>1</sup>L. A. Sevast'yanova, S. L. Potapov, V. G. Adamenko, and R. L. Vilenskaya, Biol. Nauki, No. 6, 46 (1969).
<sup>2</sup>L. A. Sevast'yanova, S. L. Potapov, V. G. Adamenko, and R. L. Vilenskaya, Changes in Hemopoiesis Under the Influence of Microwave and X-Radiation. Morphological and Hematological Aspects of the Biological Effects of Ionizing Radiation and Cytostatic Preparations, Fifth Conference of the Central Scientific Research Laboratory, Tomsk, 1970.

- <sup>3</sup>R. L. Vilenskaya, L. A. Sevast'yanova, and A. S. Faleev, A Study of the Absorption of Millimeter Waves in the Skin of Experimental Animals. Elektronnaya Tekhnika, Ser. 1 (Elektronika SVCh), No. 7, 97 (1971).
- <sup>4</sup>L. A Sevast'yanova, M. B. Golant, V.G. Adamenko, and
- R. L. Vilenskaya. Biol. Nauki, No. 6, 58 (1971).
- <sup>5</sup>R. L. Vilenskaya, É. A. Gel'vich, M. B. Golant, and

A. Z. Smolyanskaya, ibid., No. 7, 69 (1972).

A. Z. Smolyanskaya and R. L. Vilenskaya. Effects of Millimeter-band Electromagnetic Radiation on the Functional Activity of Certain Genetic Elements of Bacterial Cells

The effects of millimeter waves on intracellular systems responsible for lethal synthesis in bacteria, i.e., the synthesis of substances that result in the depth of the cell, were investigated. The colicinogenic factor of <u>Bacillus coli</u> was chosen as the test object. The colfactor is an extrachromosomic genetic element. The functional activity of this element is normally repressed. Suppression of the col-factor results in synthesis of a special proteic substance known as colicin; the cell then perishes. The colicin that it has produced has an antibacterial action with respect to other bacteria of the same or similar species.

We studied the influence of millimeter waves on colicin synthesis in the colicinogenic strain <u>E. coli</u> C600 (E<sub>1</sub>) and in the strain <u>E. coli</u> K12S, which is sensitive to the colicin of the former. The activity of the colicin synthesis was determined by the method of lacunas<sup>[1]</sup>, in which the numbers of individual colicinsynthesizing bacteria are counted. The effect was evaluated with the aid of the so-called induction coefficient, which is determined by the ratio of the lacunaformation 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, Ke 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  $(P \le 0.001)$  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  $t = 20^{\circ}C$  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  $0.01 \text{ mW/cm}^2$  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 Ki of colicin synthesis as a function of wavelength.

ĸ



FIG. 2. Induction coefficient of colicin synthesis as a function of irradiation time.  $\lambda(mm) = 6.5$  (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<sup>[2]</sup>-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.

<sup>1</sup>H. Ozeki, B. A. D. Stocker, and H. Margeri, Nature 184, 337 (1959).

<sup>2</sup>V. G. Likhoded, Mikrobiologiya, No. 7, 116 (1963); P. Amati, J. Mol. Biol. 8, 239 (1964); W. deWitt and D. Helinsky, ibid. 13, 692 (1965); P. Fredericq, J. Theor. Biol. 4, 159 (1963).

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

572 Sov. Phys. Usp., Vol. 16, No. 4, January-February 1974