INDUCTION OF COLICIN SYNTHESIS BY MILLIMETER RADIATION

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Irradiation of a colicinogenic strain of Escherichia coli C600 (E_1) by electromagnetic waves in the millimeter waveband and of intensity too low to produce heat can induce colicin synthesis. The induction effect is dependent on the wavelength and duration of irradiation and the temperature of the test object.

It has been shown [1, 3, 5] that irradiation by millimeter waves of intensity insufficient to produce heat can cause death of bacterial cells.

In the investigation described below the action of radiation in the millimeter waveband on the phenotypic expression of bacterial genes responsible for lethal synthesis was studied.

EXPERIMENTAL METHOD

The colicinogenic factor, an extrachromosomal genetic element of bacteria controlling the lethal synthesis of the protein substrate colicin, which has antibacterial action against bacteria of the same or related species, was chosen as the test object. Experiments were carried out with colicinogenic strain Escherichia coli C600 (E_1) and strain E. coli K12S, obtained from the Laboratory of Genetics and Selection of the I. I. Mechnikov Institute, and sensitive to the colicin produced by it.

The activity of colicin synthesis was determined by the gap method [15], in which individual bacteria synthezing colicin are counted. A 2-h culture of bacteria grown in Martin's broth, in a Teflon cell (transparent) for millimeter radiation, was irradiated.

A type OV-612 backward-wave tube with electronic tuning within the range from 5.7 to 8 mm and with a maximum power for continuous operation of about 1 000 mW, was used as the generator of electromagnetic waves [4]. Irradiation was given from a horn-type antenna at wavelength of 5.8, 6.15, 6.5, 6.57, and 7.1 mm. The power flux density was 1 mW/cm^2.

Optimal doses of culture and nutrient medium to allow reliable measurements of the transmitted power when the culture preserved its biological activity were chosen. The thickness of the layer of bacterial suspension in the Teflon cell was 0.6 mm. The effect was estimated by the values of the induction coefficient

$$K_i = \frac{N_{gi}/n_{ci}}{N_{gu}/n_{cu}},$$

where N_{gi} is the mean number of gaps in the irradiated culture; N_{gu} the mean number of gaps in the control culture in the same dilution; n_{ci} the mean number of irradiated cells; n_{cu} the mean number of cells in the control culture in the same dilutions.

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TABLE 1. Induction Coefficient of Colicin Synthesis During Irradiation with Millimeter Waves

Index	Wavelength (in mm)				
	5.8	6.15	6.5	6.57	7.1
Number of experiments	18	24	29	18	23
K_i (M ± m)	3.05±0.25	1.06±0.13	3.54±0.39	1.08±0.22	2.3±0.34
Index of statistical significance	6.8		6.0		3.74
Probability of error (in percent)	0.1		0.1		0.15

EXPERIMENTAL RESULTS

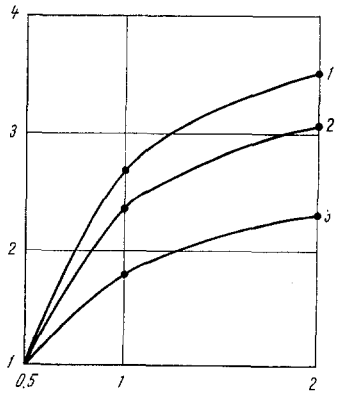

Fig. 1. Induction coefficient of colicin synthesis as a function of duration of irradiation. Abscissa, duration of irradiation (in h); ordinate, induction coefficient. 1) λ=6.5 mm; 2) λ=5.8 mm; 3) λ=7.1 mm.

Induction of gap formation by millimeter radiation was found to depend on the duration of the irradiation (Fig. 1). Irradiation during the first 30 min did not affect colicin synthesis. Irradiation for 1 h led to a sharp increase in the number of colicin-synthesizing cells at the corresponding wavelength. An increase in the duration of irradiation to 2 h led to a further increase in the induction coefficient. However, an increase in the duration of irradiation beyond this point did not yield definite results.

The induction activity of the millimeter radiation depended chiefly on the wavelength (Table 1). Waves of length 5.8, 6.5, and 7.1 mm induced colicin synthesis; at wavelengths of 6.15 and 6.57 mm no significant effect was observed. Statistical analysis of the results demonstrated their significance, which was also verified by control experiments without irradiation. In this case the values of the induction coefficient were constant and were close to one.

Millimeter radiation is known to raise the temperature in the irradiated object. Kohijama and Nomura [13] showed that colicin synthesis can be induced by a rise of temperature in a thermosensitive mutant of the colicinogenic strain containing colicin E_2. Although the intensities of irradiation used in the present experiments were deliberately chosen to be small, not exceeding 1 mW/cm^2, so that elevation of the temperature was unlikely, and although strain E. coli C600 (E_1) is not thermosensitive, because of these facts it was decided to monitor the temperature in irradiated and unirradiated systems. The temperature of the investigated system (the medium containing the suspension of bacteria) was measured by means of a thermocouple. No difference of temperature could be detected with an accuracy of reading of 0.1-0.2°C. It was accordingly concluded that the effect was nonthermal in character.

In order to strengthen the obtained effect, irradiation was carried out in an incubator at 37°C. In this case induction was observed after irradiation for 30 min also. However, the effect could not be substantially increased.

The experiments thus showed that irradiation by electromagnetic waves in the millimeter waveband can induce colicin synthesis in colicinogenic bacteria at certain wavelengths.

Hitherto the ability of substances to induce colicin synthesis, lethal to the colicinogenic cell, has been attributed mainly to their DNA-disintegrating properties or to their ability to block their DNA synthesis. These properties are possessed by UV radiation [17, 10, 12] and by certain chemicals which disturb DNA synthesis in the cell and damage the DNA molecule [2, 6, 8, 9, 11, 14]. The mechanism of induction by millimeter waves must differ qualitatively from that of induction induced by these agents acting on DNA, for the quantum energy of millimeter waves is extremely small (10^4 times smaller than the quantum energy of UV irradiation), and too small to rupture any chemical bonds or to damage the DNA molecule. From this point of view millimeter radiation can be regarded as, in principle, a new agent which, without directly damaging the DNA in the molecule, leads to a disturbance of the mechanism regulating the function of episomal genes in the cell.

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