Nonthermal Effects of Extremely High-Frequency Microwaves on Chromatin Conformation in Cells *in vitro*—Dependence on Physical, Physiological, and Genetic Factors

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***Abstract*—There is a substantial number of studies showing biological effects of microwaves of extremely high-frequency range [i.e., millimeter waves (MMWs)] at nonthermal intensities, but poor reproducibility was reported in few replication studies. One possible explanation could be the dependence of the MMW effects on some parameters, which were not controlled in replications. We studied MMW effects on chromatin conformation in *Escherichia coli* (*E. coli*) cells and rat thymocytes. Strong dependence of MMW effects on frequency and polarization was observed at nonthermal power densities. Several other factors were important, such as the genotype of a strain under study, growth stage of the bacterial cultures, and time between exposure to microwaves and recording of the effect. MMW effects were dependent on cell density during exposure. This finding suggested an interaction of microwaves with cell-to-cell communication. Such dependence on several genetic, physiological, and physical variables might be a reason why, in some studies, the authors failed to reproduce the original data of others.**

***Index Terms*—Biological applications of electromagnetic radiation, biomedical effects of electromagnetic radiation, genetics, polarization.**

I. Introduction

MICROWAVES in the frequency range of 30–300 GHz are often called millimeter waves (MMWs) because the wavelength in vacuum belongs to the interval of 1–10 mm. The biological effects of MMWs have been studied for over 20 years starting with investigations of Webb [1], Vilenskaya *et al.* [2], Devyatkov [3], and Gründler *et al.* [4]–[9]. Several reviews were devoted to the effects of MMWs [7], [10]–[14]. The most recent review summarized more than 100 MMW investigations in biology and medicine and indicated several problems in this field of research [14]. One of them is the question about so-called nonthermal effects.

Due to the efficient absorption of MMWs in water solutions and biological tissues, significant variations in specific absorption rate (SAR) is observed through an irradiated sample. Khizhnyak and Ziskin [15] found specific microoscillations of temperature in irradiated water solutions. Such phenomena were supposed to explain at least some bioeffects of MMWs. MMW irradiation of thin layers results in significant heating at power density (PD) above 1 mW/cm^2. MMW bioeffects at this and higher levels are usually attributed to induced heating. Nevertheless, the observed MMW effects were not always explained by heating, even at the thermal levels of exposure [16].

The well-known example for nonthermal effects of MMWs is the study of Gründler *et al.* [4]–[9]. For over ten years, this group consistently reported the resonance effects of MMWs on the growth of yeast cells. Different exposure systems and analytical facilities were used, leading to the same conclusions about resonance response of yeast cells to nonthermal MMWs. Sophisticated system for image processing recognition was used, which allowed a very precise analysis of the cell cycle in individual cells. The effects were observed at PD of 10^{-12} W/cm^2 and could not be explained by heating [9].

Despite of a variety of reported MMW bioeffects, only a few independent replications were performed [17]–[19]. The apparent conclusion of these replications is that the original data on MMW effects are poorly reproduced in independent experiments.

Significant effects of nonthermal MMWs on the chromatin conformational state (CCS) in *Escherichia coli* (*E. coli*) cells and thymocytes of rats have been observed by our group [20]–[30]. MMW effects on CCS were dependent on several physical, physiological, and genetic parameters. The data suggested that a number of variables should be controlled in original experiments and in replication studies. In this paper, we describe the dependence of MMW effects on all these parameters based mainly on the data obtained by our group and in comparison with the recently published data of others.

II. AVTD Technique

The main body of results analyzed in this paper was obtained with the method of anomalous viscosity time dependence (AVTD). This technique is based on the radial migration of high molecular weight DNA–protein complexes such as nucleoids in rotary viscometer [31]. The physical model of AVTD was developed by Kryuchkov *et al.* [32] based on the theory

Manuscript received November 10, 1999; revised May 3, 2000. This work was supported by the Swedish Council for Work Life Research and by the Swedish Radiation Protection Institute.

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Publisher Item Identifier S 0018-9480(00)09714-3.

of radial migration [33]–[35]. The changes in AVTD were observed in *E. coli* cells of several strains and rat thymocytes after exposure to microwaves *in vitro* [20]–[30]. The AVTD changes have been also observed upon treatment of cells with DNA/chromatin-specific chemicals such as ethidium bromide (EtBr) and etoposide VP-16 [28], [36], [37]. Several experimental observations have suggested that an increase in AVTD in response to MMWs is caused by relaxation of DNA domains and, consequently, decrease in AVTD is caused by chromatin condensation. Single-cell gel electrophoresis confirmed this suggestion [38].

III. Frequency and Power Dependencies of MMW Effects

Effects of low-intensity microwaves on repair of radiation-induced DNA breaks were studied by the AVTD method in *E. coli* K12 AB1157 [20]. Significant suppression of repair was found when X-irradiated cells were exposed to microwaves within frequency ranges of 51.62–51.84 and 41.25–41.50 GHz. In both ranges, the effect had a pronounced resonance character with resonance frequencies of 51.76 and 41.32 GHz, respectively [20], [23]. The effect of microwaves did not depend on the sequence of cell exposure to X-rays and MMWs. The MMW effect could not be explained by heating. First, statistically significant suppression of repair was observed at a very low PD of 1 μW/cm^2. Second, no suppression of repair was observed upon heating of cell suspension by 5 °C. Third, the PD averaged over the exposed surface did not depend on frequency within observed resonances.

It was established that the reduction of PD resulted in significant narrowing of the resonance response of *E. coli* cells to MMW exposure [23], [28]. Ups to 15 frequencies were investigated inside each resonance range and all frequency dependencies obtained fitted well to Gaussian distribution [28]. The experimental conditions allowed determination of the resonance frequency with an error of ± 1 MHz. Within this error, the resonance frequency of 51.755 GHz was stable with decreasing of PD from 3×10^{-3} to 10^{-19} W/cm^2. At the same time, the half-width of the resonance decreased from almost 100 to 3 MHz. The dependence of half-width of the 51.755-GHz resonance effect on PD had the steep decrease from 3×10^{-3} to 10^{-7} W/cm^2 followed by slow decreasing from 10^{-7} to 10^{-19} W/cm^2. The question then arose: what happened in the frequency range of 51.65–51.85 GHz upon narrowing of the 51.755-GHz resonance? The cell response to MMWs at a PD of 10^{-10} W/cm^2 was studied in this frequency range [28], [29]. Three additional resonances were detected: 51.675 ± 0.001, 51.805 ± 0.002, and 51.835 ± 0.005 GHz. The half-widths of all resonance including the main one, i.e., 51.755 ± 0.001 GHz, were about 10 MHz at the PD of 10^{-10} W/cm^2. Therefore, sharp narrowing of the 51.755-GHz resonance in the PD range from 3×10^{-3} to 10^{-7} W/cm^2 was followed by an emergence of new resonances. These data were interpreted as a splitting of the main resonance 51.755 GHz [28]. Dependence of the MMW effect on PD was investigated at one of these resonances, i.e., 51.675 GHz [29]. This dependence had the shape of a "window" in the PD range from 10^{-18} to 10^{-8} W/cm^2. It is important that no MMW effect was observed at subthermal and thermal PDs. This type of PD dependence clearly indicated nonthermal mechanism of the MMW effects observed. The frequency dependencies were studied around 51.675 GHz at different PDs and this resonance frequency was shown to be stable within the range of 10^{-18}–10^{-8} W/cm^2. Along with disappearance of the 51.675-GHz resonance response at a higher PD of 10^{-6}–10^{-3} W/cm^2, a new resonance effect arose at 51.688 ± 0.002 GHz [29]. This resonance frequency was also stable within the studied PD range. Taken together, these data strongly suggested a sharp rearrangement of resonance spectra, which was induced by MMWs of the subthermal PD range. The half-widths of three studied resonances showed rather different dependencies on the PD, changing from 2 to 3 MHz to 16 to 17 MHz (51.675 and 51.668 GHz) or from 2 to 3 MHz to 100 MHz (51.755 GHz) [28], [29].

Significant narrowing in resonance response was found when studying the growth rate in yeast cells [9] and chromatin conformation in thymocytes of rats [27]. In the study of Gründler *et al.*, the half-width decreased from 16 to 4 MHz as the PD was decreased within the range of 10^{-2}–10^{-12} W/cm^2 [9]. Based on these studies with different cell types, one may assume that narrowing of the resonance upon decease in the PD is one of the basic regularities in cell response to MMWs. On the other hand, different dependencies of a half-width on the PD may be expected for different resonance frequencies.

It was established that the dependence of the MMW effects on the PD had a linear section followed by a plateau [3]. This type of PD dependence was observed in [7], and [10]–[14]. The data obtained in experiments with *E coli* cells and rat thymocytes provided new evidence for this type of PD dependence and indicated that PD dependencies might have the shape of a "window" [22], [27]–[29]. The summary of the data on PD dependencies is given in the Table I. The position of the window varied between different resonance frequencies and depended on cell density during exposure of cells [29]. Nevertheless, window-like PD dependence was observed when studying MMW effect at different resonances. The most striking window was observed at the resonance frequency of 51.755 GHz [28]. When exposing the *E. coli* cells at the cell density of 4×10^8 cell/mL, the effect reached saturation at the PD of 10^{-18}–10^{-17} W/cm^2 and did not change up to PD of 10^{-3} W/cm^2. In these experiments, the direct measurements of PD below 10^{-7} W/cm^2 were not available and lowest PDs were obtained using calibrated attenuators. Osepchuk and Petersen [39] have suggested that MMW effects could be explained by the presence of temporal harmonics, but the body of our data did not support the hypothesis of Osepchuk and Petersen [40]. The background MMW radiation has been estimated as 10^{-21}–10^{-19} W/m^2/Hz [41]. Since the experimentally determined half-width of resonance was in the order of 1 MHz [28], background PD was estimated as 10^{-19}–10^{-17} W/cm^2 within the resonance. The MMW effects were observed at these PD in experiments with *E. coli* cells [24], [26], [28], [29]. The data suggested that the PD dependence of MMW effect might not have a threshold.

TABLE I
WINDOWS IN THE PD DEPENDENCIES OF MMW EFFECTS AS MEASURED WITH THE AVTD TECHNIQUE IN *E. COLI* AND RAT THYMOCYTES

Cells and cell density during exposure, cell/ml	Frequency, GHz	Polarisation	Window in the PD dependence, W/cm^2	Reference
Rat thymocytes, 5x10^6	41.62	linear	10^{-7}-10^{-4}	27
E. coli K12 AB1157, 4x10^7	41.32	circular	10^{-5}-2x10^{-4}	22
X-irradiated *E. coli* K12 AB1157, 4x10^7	41.32	linear	10^{-6}-2x10^{-4}	20
E. coli K12 AB1157, 4x10^7	51.675	linear	10^{-18}-10^{-8}	29
E. coli K12 AB1157, 4x10^8	51.675	linear	10^{-17}-10^{-3}	29
E. coli K12 AB1157, 4x10^7	51.668	linear	10^{-14}-3x10^{-3}	29
E. coli K12 AB1157, 4x10^8	51.668	linear	10^{-8}-10^{-2}	29
E. coli K12 AB1157, 4x10^7	51.755	linear	10^{-18}-10^{-3}	28
E. coli K12 AB1157, 4x10^8	51.755	linear	10^{-19}-10^{-3}	28
E. coli K12 AB1157, 4x10^7	51.674	linear	10^{-17}-10^{-5}	28

IV. DEPENDENCE OF MMW EFFECTS ON DURATION OF EXPOSURE AND TIME AFTER EXPOSURE

Usually, the duration of exposure was 5–10 min in experiments with *E. coli* cells and rat thymocytes at the PD of 10^{-5}–10^{-3} W/cm^2 [20], [21], [27]. In order to achieve the same effect at lower PD of 10^{-14}–10^{-17} W/cm^2, the time of exposure should be prolonged to 20–40 min. This time should be even longer, more than 1 h, at lowest PD of 10^{-19} W/cm^2 [26]. Therefore, the same MMW effect could be achieved by prolongation of exposure if the PD decreased.

The MMW effect on the CCS of *E. coli* cells depended on post-exposure time. Usually, this dependence had an initial phase of increase in the MMW effect. This phase was about 100 min [24], [26] and followed by the phase, which was close to a plateau. The plateau lasted around 100 min [26]. A trend to decrease in effect was observed at longer times up to 300 min [24]. Significant changes in AVTD were observed when rat thymocytes were lyzed in between 30–60 min after exposure to MMWs [27]. This effect nearly disappeared if the cells were incubated more than 80 min after exposure. The data suggested that there is a time window for observation of effect on the CCS, which may be dependent on cell types, cell density during exposure, duration, and PD of exposure.

V. POLARIZATION

The effects of circularly polarized (CP) MMWs were studied in *E. coli* cells at the frequencies from the two resonances identified with linearly polarized MMWs, i.e., 51.62–51.84 and 41.25–41.50 GHz. At the resonance frequency of 51.76 GHz, right-hand CP microwaves suppressed repair of X-rays induced damages as measured with AVTD [21], [23]. Left-hand CP MMWs had virtually no effect on repair, while the efficiency of linearly polarized MMWs was in between two circular polarizations. Inversion in effective circular polarization was observed at another resonance frequency, i.e., 41.32 GHz. Left-hand CP microwaves significantly suppressed repair, while right-hand polarization was almost ineffective. It is important that MMWs of the same CP affected or correspondingly did not affect cells at several tested frequencies within each resonance [22], [21], [23]. Therefore, the sign of effective CP was the attribute of the whole resonance.

In the beginning of experimentation, left- or right-hand spiral waveguides were used to produce CP MMWs [21]. The installation with spiral waveguides provided an ellipticity coefficient of 1.2 ± 0.1. In subsequent experiments, another installation with a better ellipticity coefficient, i.e., 1.05 ± 0.05 was used for exposure [23], [22], [29]. In this installation, CP MMWs was obtained by means of the quarter-wave mica plates. Simultaneous exposure of three different samples with linear, left- and right-hand CP MMWs was available. Stronger difference between effects of left- and right-hand CP MMWs on repair of X-rays-induced damages was observed using installation with the quarter-wave mica plates in comparison to the spiral waveguides [22]. Nevertheless, even with the ellipticity coefficient virtually equal to unity, a statistically significant, though relatively weak, effect of "ineffective" polarization was observed (Table II). This could indicate the presence of a small number of targets in corresponding (nondominant) conformation that are able to interact with MMWs at ineffective polarization. It was found that pre-irradiation of *E. coli* cells to X-rays inverted the sign of effective polarization [22], [23]. This inversion was observed for two resonances (Table II). It is important that neither resonance frequencies, nor half-widths of the resonance changed during inversion of effective CP. The effects of left-and right-hand MMWs become the same at 50 cGy [23]. At this dose, about one single stranded DNA break per haploid genome was induced and the dose was too low to damage significantly any cellular structure, except for DNA. It is known that a nucleoid in *E. coli* cells consists of the supercoiled DNA domains.

TABLE II
EFFECTS OF MICROWAVES AT 100 μW/cm^2 ON THE CHROMATIN CONFORMATIONAL STATE OF INTACT CELLS AND ON REPAIR OF DNA DAMAGES INDUCED BY X-RAYS (20 Gy) AS MEASURED WITH AVTD. ALL EFFECTS WERE NORMALIZED TO THE EFFECT OF THAT TYPE OF CIRCULAR POLARIZATION, WHICH PRODUCED A MAXIMUM EFFECT AT THE GIVEN RESONANCE FREQUENCY. AVERAGE FROM SIX INDEPENDENT EXPERIMENTS AND STANDARD ERROR IS GIVEN. THE DATA WERE ADAPTED FROM [22]

Treatment, polarisation	Resonance 41.32 GHz			Resonance 51.76 GHz		
	Right	Linear	Left	Right	Linear	Left
MMW	1.00±0.08	0.40±0.08	0.08±0.06	0.32±0.08	0.56±0.08	1.00±0.08
MMW and X-rays	0.23±0.12	0.52±0.06	1.00±0.07	1.00±0.06	0.56±0.07	0.28±0.06
X-rays and MMW	0.09±0.09	0.5±0.1	1.0±0.1	1.00±0.04	0.7±0.1	0.24±0.06

It is believed that the majority of DNA in living cells has a right-hand helicity (B-form), but a minor part, in order of 1%, may be in the form of a left-hand helix (Z-form). Radiation-induced DNA breaks result in relaxation of DNA domains. On the other hand, supercoiling is connected with transitions between right-hand B-form to left-hand Z-form in some DNA sequences. Therefore, the data suggested that difference in biological effects of polarized MMWs was connected with DNA helicity and supercoiling of DNA domains.

The supercoiling of DNA domains is changed during the cell cycle because of elementary genetic processes such as transcription, replication, and recombination. It can also be changed by means of DNA-specific intercalators such as ethidium bromide (EtBr). Changing the supercoiling, EtBr facilitates the transition of the left-hand DNA sequences (Z-form) to the right-hand B-form. Preincubation of *E. coli* AB1157 cells with EtBr (1 μg/mL) inverted the effective polarization and right-hand MMWs at the resonance frequency of 51.755 GHz became more effective than left-hand polarization [30]. EtBr changed the supercoiling of DNA domains starting at a concentration of 1 μg/mL, as measured with the AVTD in lysates of different cell types including *E. coli* [28], [37], [38]. The data provided evidence that DNA is a target of MMW effects.

In all experiments, the effect of linear polarized MMWs was in between the effects of two circular polarizations. Unexpectedly, the same circular polarization was more effective if the cells were exposed to MMWs both before and after X-irradiation (Table II). The combined exposure of cells to MMWs at different CP resulted in nonadditive effects [24]. This nonadditivity was explained in terms that each CP stimulated transitions of certain DNA sequences into a form of a corresponding helicity, but the subsequent exposure to MMWs at another polarization might affect this process. More studies are needed to elucidate the mechanism of combined effects of CP MMWs and to characterize the target responsible for dependence of the resonance MMW effects on polarization. Nevertheless, recent investigations of 11 resonances in *E. coli* cells and two resonances in Wistar rat thymocytes indicated that one of two circular polarizations was always more effective than another one [27], [29], [42]. These data are summarized in Table III.

Obviously, the difference in effects of right- and left-hand polarizations could not be explained by heating or by mechanism dealing with "hot-spots" due to unequal SAR distribution. The data about the difference in effects of differently polarized MMWs, inversion of effective circular polarization between resonances, and after irradiation of cells with X-rays, provided clear evidence for nonthermal mechanisms of MMW effects.

VI. MODULATION

There is an experimental evidence for the role of modulation for the microwave-induced effects both *in vitro* and *in vivo* [43]–[45]. Gapeev *et al.* [46] analyzed the role of modulation for the effects of MMWs. The authors studied the respiratory burst induced by calcium ionophore A23187 and phorbol ester PMA in the peritoneal neutrophils of mice. MMWs at the PD of 50 μW/cm^2 inhibited the respiratory burst. MMW effect depended on frequency and was maximal at the frequency of 41.95 GHz. The opposite effect, stimulation of the respiratory burst, was observed upon modulation of MMWs with the frequency of 1 Hz. Only this modulation out of four tested (0.1, 1, 16, and 50 Hz) resulted in stimulation of the respiratory burst.

VII. ELECTROMAGNETIC ENVIRONMENT

Litovitz *et al.* provided evidence that the extremely low-frequency (ELF) magnetic noise of 2 μT inhibited the effects of microwaves on ornithine decarboxylase in L929 cells [45]. Usually, all electric devices were shut down during Gründler *et al.*'s experiments with yeast cells in order to decrease the electromagnetic noise (personal communication). The static magnetic field was controlled in Gründler *et al.*'s experiments and in the replications of these experiments by Gos *et al.* [19]. Background electromagnetic fields might be important for effects of MMWs on the chromatin conformation. This suggestion followed from the observation that both static magnetic field and ELF magnetic fields at low intensities affected the CCS in cells [37], [47], [48]. The changes in static magnetic fields during exposure to MMWs affected response of *E. coli* to MMWs (unpublished results of Shcheglov *et al.*). Therefore, the static magnetic field was controlled and all electric devices were shut down during our experiments with MMWs.

TABLE III
EFFECTIVE POLARIZATION IN RESONANCE RESPONSE OF *E. COLI* CELLS AND WISTAR RAT THYMOCYTES TO NONTHERMAL MMWS AS MEASURED WITH AVTD TECHNIQUE

Cells	Resonance frequency, GHz	Effective circular polarisation
E. coli K12 N99(λ,λ*imm*^{434}*bio*^{10})	41.277±0.002	Right-handed
Wistar rat thymocytes	41.303±0.001	Right-handed
E. coli K12 N99(λ)	41.305±0.001	Right-handed
E. coli K12 AB1157	41.32±0.01	Right-handed
E. coli K12 N99	41.324±0.001	Right-handed
Wistar rat thymocytes	41.61±0.01	Left-handed
E. coli K12 AB1157	51.675±0.001	Left-handed
E. coli K12 N99(λ,λ*imm*^{434}*bio*^{10})	51.723±0.001	Left-handed
E. coli K12 N99(λ)	51.740±0.001	Left-handed
E. coli K12 AB1157	51.755±0.001	Left-handed
E. coli K12 N99	51.765±0.002	Left-handed
E. coli K12 AB1157	51.805±0.002	Right-handed
E. coli K12 AB1157	51.835±0.005	Left-handed

VIII. CELL-TO-CELL INTERACTION IN RESPONSE TO MMWS

Usually, the *E. coli* cells were exposed to MMWs at the cell density of 4×10^7 cell/mL. When the cell density of exposed cells was increased to 4×10^8 cell/mL, the resonance MMW effect grew substantially [26]. Experiments were performed with different PD levels and times of exposure to MMWs at 51.76 GHz. All AVTD measurements were performed at the same cell density of 4×10^7 cell/mL. When the results for the same values of PD and exposure times were compared, the effect of MMWs increased by a factor of 4.7±0.5 on average with increase in cell density by one order of magnitude. The data suggested a cooperative nature of cell response to MMWs, which is based on cell-to-cell interaction during exposure. This suggestion was confirmed by the observed partial synchronization of cells after exposure to MMWs [26]. Due to this synchronization, cell density of the exposed cells could be either higher or lower in comparison to control level in dependence on time after exposure.

A significant MMW effect on synchronization of *Saccharomyces carlsbergensis* yeast cells were observed by Golant *et al.* [49]. Exposure to MMWs at 30 μW/cm^2 and 46 GHz was performed at the cell density of about 10^5 cell/mL. MMWs induced synchronization as measured by cell density and bud formation. This synchronization lasted more than 20 cell cycles after exposure. The authors concluded that MMWs induced cell-to-cell interaction resulting in the synchronization observed.

In recent studies with *E. coli* cells, the cooperative effect was confirmed for the resonance frequency of 51.755 GHz and found at two other resonances of 51.675 and 51.688 GHz [28], [29]. The data suggested that, within different resonances, the response of cells to MMWs might depend on the cell density during exposure. The average intercellular distance was approximately 13 μm at the cell density of 4×10^8 cells/mL. Therefore, no direct physical contact seemed to be involved in the cell-to-cell interaction. Two mechanisms were suggested to account for the cooperative nature in the resonance response to MMWs [26]. In the chemical-diffusional mechanism, the cells, which have responded to MMWs, released chemical messengers. These messengers could reach other cells via diffusion, thus causing secondary reactions. In the electromagnetic mechanism, the affected cells might be a source of a secondary irradiation. The dependence of the effect on cell density was modeled based on both mechanisms, and the electromagnetic one provided better fit to experimental data [26].

Although the dependence of the MMW effect on PD showed considerable difference between two cell densities, 4×10^7 cells/mL and 4×10^8 cells/mL, the 51.755-GHz resonance frequency did not change with changes of cell density [28]. The half-width of the resonance did not depend on cell density either. Contrary to the 51.755-GHz resonance response, the half-width of the 51.675-GHz resonance depended on the cell density [29]. The data suggested that intracellular interaction affected a subcellular target for the effects of MMWs at 51.675 GHz.

The dependence of the resonance response on cell density was studied both at stationary and logarithmic phase of growth during exposure to MMWs in the range of 10^{-18} to 3×10^{-3} W/cm^2 [50]. Relatively weak response to MMWs was observed in exponentially growing cells. Partially synchronized stationary cells were more sensitive, especially at cell densities above 10^8 cell/mL. A significant shift in the resonance frequency was observed between logarithmic and stationary phase. The data suggested that the cooperative response of cells to MMWs might be different at different phases of growth. The data indicated also that response to MMWs might not be limited by the reaction of single cells, but the cooperative reaction of the exposed cell population might be involved. Even at the highest cell densities, the cells occupied a negligible part of the exposed volume and could not change the absorption of microwaves. The significant difference in cell response at the

different cell densities provided strong evidence for nonthermal mechanism of the MMW effects.

IX. Dependence of MMW Effects on Genotype

The effects of MMWs were studied in *E. coli* cells with different length of chromosomal DNA [25]. Bacterial chromosome was lengthened by inserting DNA of λ and $\lambda\mathrm{imm}^{434}\mathrm{bio}^{10}$ phages. Strain N99 of wild type, lysogenic strain N99(λ) and N99($\lambda, \lambda\mathrm{imm}^{434}\mathrm{bio}^{10}$) was used. Response of each strain was studied at 10–17 frequencies inside 41.24–41.37 and 51.69–51.795 GHz at 10^{-10} W/cm^2. Clear resonance response was observed for each strain in both frequency ranges (Table III). Significant shifts of both resonance frequencies were found between strains. The shifted resonances had the same amplitude and half-width as for N99 cells. Upon shifting, no changes in effective circular polarization were observed.

The shifts in resonance frequencies could not be explained by activity of additional genes inserted with the phage DNA. For example, cI and rex genes are active in lysogenic N99(λ) strain. Nevertheless, the $\lambda\mathrm{imm}^{434}\mathrm{bio}^{10}$ insertion did not contain immunity region and, therefore, the cI and rex genes. Moreover, this genome is identical to the genome of phage λ, but about 23% shorter because of bio^{10} deletion. Therefore, it was unlikely that shifts of resonances were caused by additional gene activity upon insertion of $\lambda\mathrm{imm}^{434}\mathrm{bio}^{10}$. On the other hand, the theoretical consideration based on mutual vibrations of separate domains regarding a whole nucleoid provided good correlation between experimental data and calculated shifts in resonances [25].

A detailed analysis of MMW effects in AB1157 cells at 10^{-10} W/cm^2 revealed the resonance frequency of 51.755 ± 0.001 GHz. This value was statistically significantly different from the corresponding resonant frequency of the N99 strain, 51.765 ± 0.002 [28]. It should be noted that both strains are considered as wild-type strains. Nevertheless, those strains were different in genotypes by several specific gene markers [20], [51]. The data suggested that strains of different origin, even being considered of wild type, may posses different resonance responses.

X. Dependence of MMW Effects on Physiological Variables

The importance of physiological parameters, which may include all conditions of cell culture growth such as aeration, the composition of the growth, and exposure media, has been previously discussed by Gründler *et al.* [7]. Recently, Lai and Singh described effects of microwaves on the rat brain cells as measured using a microgel electrophoresis assay [52]. These effects were significantly blocked by treatment of rats either with the spin-trap compound N–tert–butyl–α–phenylnitrone or with melatonin. These data indicated that radicals might be involved in effects of microwaves and provided further evidence for dependence these effects on physiological variables.

In our investigations, *E. coli* cells were exposed to CP or linearly polarized MMWs (100 μW/cm^2) at the resonance frequencies, i.e., 41.32 or 51.76 GHz [24], [26]. Both value and direction of the MMW effects strongly depended on the phase of culture growth. At the logarithmic phase of growth, MMWs at all polarizations resulted in decrease in the AVTD peaks and, contrarily, the AVTD peaks increased after MMW exposure at stationary phase of growth. Higher variability in effects was observed for the logarithmic phase and effects were more stable for the stationary phase. There was no effect at all if cells were exposed at the end of the logarithmic phase where the MMW effects changed their direction [26].

Another stage of particular interest was the beginning of the logarithmic stage, where the effect of MMWs was relatively weak. Nevertheless, only a decrease of AVTD peaks was observed in cell response at different stages of the logarithmic phase. The AVTD data were confirmed by electrophoretic analysis of proteins bound to DNA [24]. The main feature of the effect in the stationary phase was a decrease in the quantity of several DNA-bound proteins with molecular weights of 61, 59, 56, 26, and 15 kDa. The main trend was an increase in some proteins, 61, 56, 51, and 43 kDa, as AVTD peaks decreased after exposure at the logarithmic phase. Thus, the decrease in the level of proteins bound to DNA increased maximum viscosity and vice versa.

The *E. coli* cells were usually grown in Luria broth before exposure to microwaves. Prior to exposure, the cells were collected by centrifuging and suspended in an M9 buffer. In experiments under the same conditions of exposure to MMWs, we observed a dependence of the MMW effect on time between preparation of cell suspension and exposure (Shcheglov *et al.*, in preparation). The control levels of AVTD did not change. The unpublished data of Ushakov *et al.* indicated that the MMW effects correlated with the concentration of oxygen in cell suspension during exposure. This correlation might suggest that oxygen concentration should be indicated in order to improve reproducibility.

XI. Discussion

Our experimental data have revealed several regularities in the effects of the low-intensity microwaves on the chromatin conformation in cells *in vitro*: frequency dependencies of resonance type, dependence of the resonance effects on polarization, "window" dependence on PD, narrowing of the resonances, and rearrangement of frequency spectra of action with decrease in the PD. The MMW effects depended on the genotype of *E. coli* cells under study, the growth stage of the bacterial culture, the cell density, the static magnetic field during exposure, the time between microwave exposure, and recording of the effect. The experimental data provided strong support for nonthermal MMW effects, which have been discovered by Webb and Booth, Vilenskaya *et al.*, Devyatkov, and Gründler *et al.* [1]–[4]. The discussion of mechanisms and biological significance of these effects is beyond the scope of this paper. We would like just to stress that the MMW effects depend on a number of physical, physiological, and genetic parameters. Obviously, not taking into account the dependence of the MMW effects on all those parameters may lead to a negative conclusion regarding the reproducibility. In respect to reproducibility, especially important might be the observations that MMWs could inhibit or stimulate the same functions [14]. Under different conditions of exposure, MMWs either increased or decreased the growth rate

of yeast cells [4]–[9], the radiation-induced damages in mice [52], the respiratory burst in neutrophils of mice [46], and the condensation of nucleoids in *E coli* cells [24], [26]. Potentially bidirectional effects of MMWs should be taken into account to improve reproducibility.

Despite a considerable body of investigations with MMWs in biology, only a few studies were performed to replicate the original data on nonthermal MMW effects. The best-known attempt to replicate the results of Gründler *et al*. was the recent study of Gos *et al.* [19]. No MMW effect was observed in this well-described research. However, a deviation in routine protocol might account for poor reproducibility. For example, synchronized cells were used in studies of Gründler *et al.*Contrary to Gründler *et al.*'s original protocol, exponentially growing cells were used by Gos *et al.*If the MMW effects in yeast cells are dependent on stage of growth, cell density, and intercellular interactions, as described for *E. coli* cells [24], [26], [28], [29], no response might be expected within some stages of the logarithmic phase of growth. Gos *et al.*used a *S. cerevisiae* strain with the auxotrophy mutations for leucine and uracil. The wild type strain was used by Gründler *et al.*. We observed various responses to microwaves between *E. coli* cells with different genotypes, including wild-type strains of different origin. It might suggest another cause for deviations between data of Gründler *et al.* and Gos *et al.*

The number of possible variables in reproducibility of MMW effects seem to be far beyond the limits of usually controlled parameters in biological experiments. Nevertheless, successful application of MMWs in therapy of various diseases [14] provided intriguing perspective for further development of MMWs research in biology and medicine.

ACKNOWLEDGMENT

The authors thank Prof. M. Harms-Ringdahl, Stockholm University, Stockholm, Sweden, for reading the manuscript and fruitful comments.

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