

Resonance Effect of Low-Intensity Millimeter Waves on the Chromatin Conformational State of Rat Thymocytes

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The method of anomalous viscosity time dependencies (AVTD) was modified for the study of the changes in the chromatin conformational state (CCS) of rat thymocytes of the Wistar line. The response of the thymocytes of male rats to low-intensity millimeter waves (MMW) was examined. It was shown that MMW at power densities (PD) of $1 \mu\text{W}/\text{cm}^2$ produced a resonance effect on the CCS in the frequency range of 41.56–41.67 GHz. The resonance frequency of the cell response did not vary significantly among five examined rats and was determined to be 41.61 ± 0.01 GHz. A halfwidth of resonances was averaged to 40 MHz. The power dependence of the resonance effect was measured in the range of 10^{-11} – 10^{-4} W/cm². Statistically significant changes in CCS were registered, starting with 10^{-9} W/cm². Right- and left-handed circularly polarized MMW were shown to differ in efficiency at the resonance frequency.

The established regularities in the thymocyte response to low-intensity MMW was very similar to those which have been previously found for *E. coli* cells.

Introduction

The role of coherent electromagnetic fields of the millimeter range in regulation of the living systems was first grounded by Fröhlich (Fröhlich, 1968). This concept was developed later in the works of other researchers (Keilmann, 1986; Sitko *et al.*, 1988). Fröhlich regarded the high sensitivity of living cells to irradiation with non-thermal millimeter waves (MMW) at particular, so-called resonance, frequencies as a possible result of his theory.

Several reviews are devoted to the effect of low intensity (non-thermal) millimeter waves on living cells (Postow and Swicord, 1986; Gründler *et al.*, 1988; Belyaev, 1992). The most intensive studies were concentrated on the effect of MMW on *S. cerevisiae* and *E. coli* cells. MMW were shown to influence simultaneously: cell division, gene expression, macromolecular synthesis, and the genome conformational state of *E. coli* cells (Belyaev, 1992; Alipov *et al.*, 1993; Belyaev *et al.*, 1994). The established strong frequency dependences of the MMW effect on living cells permit one

to regard the cellular response as resonant. High quality of resonances, cell sensitivity to super low intensities of MMW, and dependence of the effect on MMW polarization were determined (Gründler, 1992; Belyaev *et al.*, 1992a, 1992b, 1993a, 1994). These results are in good agreement with the Fröhlich concept (Fröhlich, 1968) and provide the evidence for an quantum-mechanical approach in physical modelling of a MMW bioaction (Keilmann, 1986; Sitko *et al.*, 1988; Belyaev *et al.*, 1994).

At the same time there is a sufficient lack of information dealing with the MMW effect on the cells of higher eukaryots. We tried to partially fill this gap in the present work. For this purpose, the reaction to MMW of thymocytes from the Wistar rats was examined. First, it was necessary to figure out whether the conformation of chromatin in these cells was as sensitive to MMW as, for instance, the chromatin conformational state (CCS) of *E. coli* cells. The resonant response of rat thymocytes to low intensity MMW within the frequency range of 41.5–41.7 GHz has been established previously (Shtemler *et al.*, 1992). In this paper we investigated the resonance effect of MMW on the CCS of thymocytes by means of a modified technique of the anomalous viscosity time dependencies (AVTD). We attempted to establish the power density threshold of MMW

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effect at the resonance frequency. This threshold seems to be at least no more than 10^{-9} W/cm².

Second, to determine possible individual deviations of the resonant reaction, we studied the frequency dependence of the MMW effect on cells from different Wistar rats.

Third, the dependence of resonance effect on the MMW polarization was also examined.

The established regularities of the reaction of rat thymocytes to MMW turned out to be similar to regularities of MMW effect on *E. coli* cells, which have been described previously (Belyaev, 1992; Belyaev *et al.*, 1992a, 1993a, 1994).

Materials and Methods

Thymocytes were obtained from male Wistar rats weighing usually 160–200 g, by means of a standard method (Klaus, 1987). Immediately after dissection, the thymus was placed in cooled medium 199. The tissue was then cut into pieces and the suspension was strained through a nylon filter. Then the cells were twice washed in 10 ml of medium 199 and subsequently precipitated by centrifuging (10 min, $300 \times g$). The concentration of cells and the number of viable thymocytes were measured in a hemocytometric camera. The number of dead cells, measured by an uptake of trypan blue, did not exceed 3%.

A block diagram of the unit used for irradiation with linearly polarized MMW has been previously described (Belyaev *et al.*, 1992c). Frequency deviation did not exceed 1 MHz. Error in the measurement of the output power was $\pm 10\%$. The value of the voltage standing wave ratio in a waveguide did not exceed 1.4. Irradiation of cell suspension was carried out by means of a pyramidal horn, dimensions being 40×50 mm². A foam plastic gasket having 7 mm thickness was situated on top of the horn. Cell suspension was irradiated in nitrocellulose tubes of 14 mm in diameter (Beckman, 331370). Three or four tubes, placed vertically on the gasket by means of a foam plastic holder, were simultaneously irradiated. The tubes were arranged symmetrically around the axis of the horn. Each irradiated tube contained 0.1 ml of cell suspension in medium 199. Cells were exposed to circularly polarized MMW in the same way, as with linear polarized MMW, by means of a device described previously (Belyaev *et al.*, 1992b).

The average value of power density (PD) was calculated knowing the output power and horn dimensions. The surface distribution of the PD on the gasket surface was measured at the 60 mW output power by means of a dipole MMW probe (Belyaev *et al.*, 1992c). With the used irradiation frequencies, the local PD values differed no more than one order of magnitude on the gasket surface. Average PD, measured at the cell suspension surface of each tube, was equal to the calculated one. The specific absorption rate (SAR) in cell suspension was measured for each tube by a calorimetric method using a microthermocouple with an error of 0.1 °C. The SAR value was 5 mW/g for the 1 mW/cm² PD at 41.61 GHz.

During the MMW irradiation and subsequent incubation until lysis, the cells were in a geomagnetic field of 46 ± 7 μ T.

Thymocytes were lysed by adding to each tube 3.2 ml of lysing solution: 0.7% sarcosyl (Serva, F.R.G.), 0.5 M Na₂EDTA (Sigma, U.S.A.), 0.01 M Tris-HCl (Serva), pH 7.1. Afterwards tubes were kept in water bath at 46 °C for 10 min and finally placed in a thermostat (33 °C).

The AVTD was measured 60 min after the beginning of lysis by a rotary viscosimeter of the Zimm-Crothers type according to the previously described technique (Belyaev *et al.*, 1992c, 1993b). To this end, a tube with lysate was placed in a thermostatically controlled (33 °C) jacket of the viscosimeter. Then a cylindrical glass rotor 30 mm long and 8 mm in diameter was suspended on the meniscus of the lysate. Measurements were carried out at a shear rate of 1.7 s⁻¹ and shear stress of 0.002 N/m². Three or four independent AVTD measurements were made in each version of experiment. Based on this, the average maximum viscosities in sham control ($\bar{\eta}_{\max \text{ cont}}$) and after cell exposure ($\bar{\eta}_{\max \text{ eff}}$) were determined. The value of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\max \text{ eff}} / \bar{\eta}_{\max \text{ cont}}$ was used to estimate the effect of MMW on the CCS in all experiments. The difference between the exposed cell suspensions and sham control was determined using the Student's test on the basis of not less than three independent AVTD measurements.

Results

In the preliminary experiments, we determined the dependence of the maximum viscosity on the

temperature of heating the lysate of the intact cells (Fig. 1). Immediately after adding the lysing solution to the cells the lysates were placed in a water bath at the required temperature for 10 min. The AVTD was measured in the lysates 3–4 h after the end of heating. The maximum viscosity was observed at 46 °C. Heating at this temperature, which leads to partial denaturation of the proteins bound with DNA and to partial relaxation of nucleoids, was previously used in viscosimetric measurements (Chase and Shafer, 1979). In all the subsequent experiments, the lysates were heated to 46 °C at the beginning of lysis.

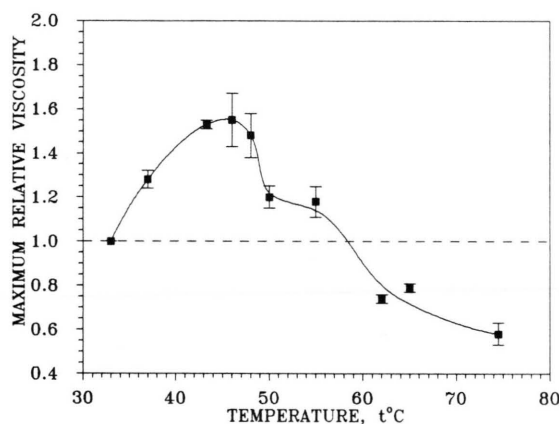


Fig. 1. Dependence of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\max t} / \bar{\eta}_{\max 33^\circ\text{C}}$ on the temperature during 10 min of heating the lysate in the beginning of lysis. All values are normalized to the maximum viscosity without heating. The presented values are obtained by averaging the five experiments with thymocytes of male Wistar rats weighing 150–200 g. In this and following figures the standard error is given.

The dependence of the AVTD maximum viscosity on the time after heating the lysate is presented in Fig. 2. Only small variations of AVTD peaks were observed in first 1–1.5 h of lysis. Then the maximum relative viscosity tended to increase considerably and nearly duplicated after 4 h from the beginning of lysis.

In more than ten preliminary experiments with the AVTD technique modified in this work, we reproduced the previously reported effect of MMW on the CCS within 41.5–41.7 GHz (Shtemler *et al.*, 1992; data are not shown).

The MMW effect strongly depended on the time of the cell incubation between irradiation and lysis

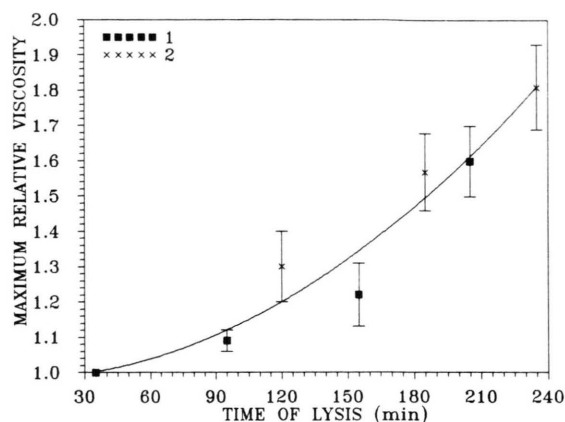


Fig. 2. Dependence of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\max t \text{ min}} / \bar{\eta}_{\max 30 \text{ min}}$ on the time of lysis. All values are normalized to the maximum viscosity obtained after 30 min lysis; 1. thymocytes of rat weighing 120 g; 2. thymocytes of rat weighing 250 g.

(Fig. 3). Increase of AVTD peaks was maximum when lysing the cells 30–60 min after irradiation. The effect nearly vanished after incubation in medium 199 for more than 80 min.

To determine the individual features of the thymocyte reaction we studied the frequency dependence of the MMW effect on the cells of five rats. Thymocytes were subjected to MMW at 1 $\mu\text{W}/\text{cm}^2$

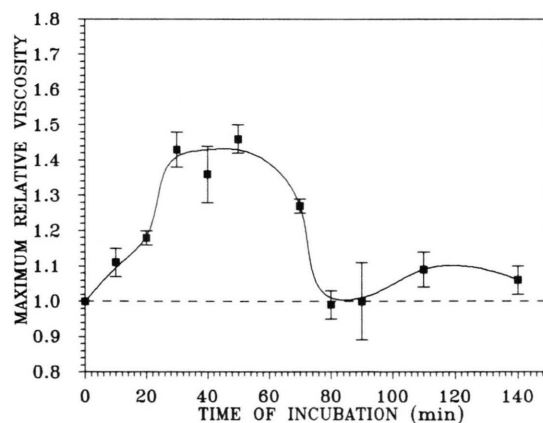


Fig. 3. Dependence of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\max \text{ MMW}} / \bar{\eta}_{\max \text{ cont}}$ on the time of incubation of irradiated cells in medium 199 before lysis. Cells were irradiated with MMW (41.62 GHz; 1 $\mu\text{W}/\text{cm}^2$; 10 min) in medium 199 at a concentration of 10^7 cell/ml. The presented values were found by averaging the data obtained from the experiments with the cells of 8 male Wistar rats (140–260 g).

at 4–7 different frequencies within the range of 41.56–41.67 GHz and then incubated in 199 medium 30 min before lysis. Cells of all examined rats displayed a statistically significant reaction to MMW at particular frequencies from the studied range. Three of the obtained frequency dependencies are given in Fig. 4. It is clear, that these dependences have a resonance nature. All results, dealing with the study on the frequency dependences for thymocytes of five rats, are summarized in Table I. The values of resonance frequencies $\langle f \rangle_{\text{res calc}}$ and halfwidths of resonances were calculated from an approximation of the experimental results using Gauss's distribution, as it has been previously described (Belyaev *et al.*, 1992d). The resonance frequency of cellular response did not vary among different rats within the error limits and was determined to be 41.61 ± 0.01 GHz in

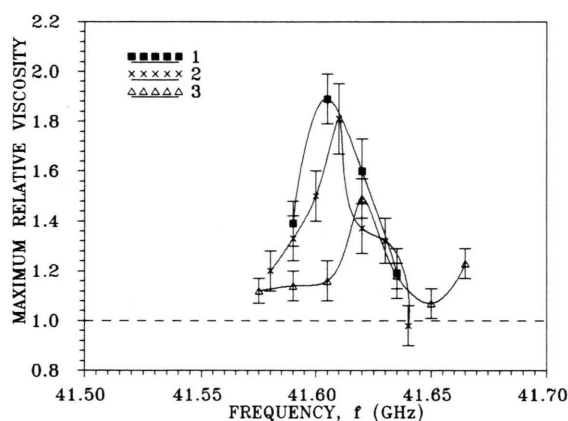


Fig. 4. Frequency dependence of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\text{max MMW}} / \bar{\eta}_{\text{max cont}}$. Thymocytes were irradiated by MMW at $1 \mu\text{W}/\text{cm}^2$ for 10 min: 1; 2; 3 – thymocytes of rats weighing 200; 220; 160 g.

average. Resonance halfwidth was averaged to 40 MHz.

The PD dependence of the effect was studied in the range of 10^{-11} – 10^{-4} W/cm^2 (Fig. 5). Statistically significant changes of AVTD peaks were observed starting with $\text{PD} = 10^{-9}$ W/cm^2 . The effect reached saturation at power density 10^{-7} W/cm^2 , which is less than thermal PD in at least three orders of magnitude. Detectable 0.1°C heating of cell suspension was observed at $0.2 \text{ mW}/\text{cm}^2$.

In a set of four experiments the thymocytes of male rats were irradiated for 10 min with left- and right-handed circularly polarized MMW at the frequency of 41.61 GHz and $\text{PD} = 1 \mu\text{W}/\text{cm}^2$. The cells were exposed at the 10^6 cell/ml concentration

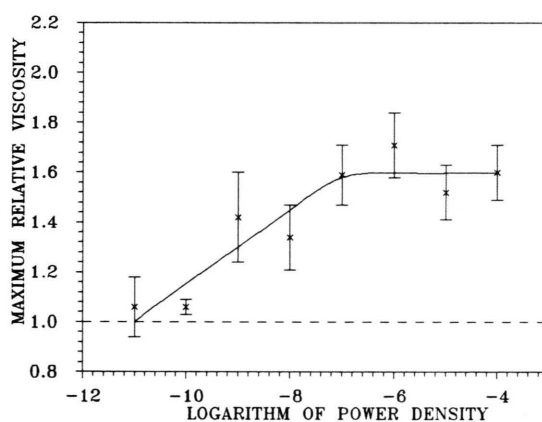


Fig. 5. Dependence of the maximum relative viscosity $\bar{\eta} = \bar{\eta}_{\text{max MMW}} / \bar{\eta}_{\text{max cont}}$ on a logarithm of power density. The power density is given in W/cm^2 . Cells at a concentration of 5×10^6 cell/ml were MMW irradiated for 10 min at a frequency of 41.62 GHz. The measurements were performed 60 min after the beginning of lysis. The averaged data were obtained from 8 independent experiments with thymocytes of male Wistar rats weighing 160–220 g.

Table I. Parameters of resonance response of rat male thymocytes on MMW at $1 \mu\text{W}/\text{cm}^2$ in frequency range of 41.56–41.67 GHz.

Weight of rat [g]	Cell concentration during exposure [cell/ml]	$f_{\text{res exp}}$ GHz	$\langle f \rangle_{\text{res calc}}$ GHz	Halfwidth of resonance [MHz]	$\bar{\eta}_{\text{max res}} \pm \text{Se}$	Significance level as compared with sham control
160	5×10^6	$41.620 \pm 0.015^*$	41.614	48	1.49 ± 0.08	$p < 0.003$
170	5×10^5	41.610 ± 0.010	41.617	23	1.26 ± 0.06	$p < 0.003$
200	5×10^5	41.605 ± 0.015	41.610	32	1.89 ± 0.12	$p < 0.0003$
210	5×10^5	41.620 ± 0.015	41.609	51	1.37 ± 0.10	$p < 0.014$
220	5×10^6	41.610 ± 0.010	41.607	34	1.81 ± 0.14	$p < 0.001$

* The step of changing the frequency in experiment is given.

and lysed after 30 min incubation in 199 medium. Only left-handed polarization was shown to have a statistically significant effect on the cells of rats 3 and 4 from Table II. Both circularly polarized MMW had a significant effect on the CCS of rat thymocytes in experiments 1 and 2 (Table II). However, left-handed circularly polarized MMW were more effective than right-handed in all experiments.

Discussion

A few of the latest reviews are devoted to the resonant effects of low-intensity MMW on the living cells (Postow and Swicord, 1986; Gründler *et al.*, 1988; Belyaev, 1992). It should be noted that the vast majority of well documented experiments were performed using procaryotic and yeast cells. The resonance reaction of the cells of higher eukaryots to MMW has been investigated to a lesser extent. At the same time, if MMW play fundamental role in the regulation of living systems, the cells of different biological species and types of differentiation could display resonance response to MMW (Belyaev, 1992).

It has been assumed that the processes of excitation in chromosomal DNA should play a significant role in the resonance response of cells to MMW (Belyaev *et al.*, 1992c). Possible physical mechanisms of generation of collective modes in DNA and resonant MMW interaction with living cells, based on the consideration of self-consistent intracellular excitations, have been proposed (Arinichev *et al.*, 1993; Belyaev *et al.*, 1993c). Changes in the conformational state of chromatin should be ranked among those essential changes which occurred in cells according to the proposed mechanisms. The CCS changes have been found while exposing the *E. coli* cells to low-intensity

MMW at resonance frequencies (Belyaev, 1992; Alipov *et al.*, 1993; Belyaev *et al.*, 1992a, 1992d, 1993a, 1993c, 1994). These changes were also observed in preliminary experiments with human leukocytes (Belyaev *et al.*, 1993d) and when exposing the thymocytes of rats (Shtemler *et al.*, 1992). In the last case a strong resonance dependence of MMW effect on frequency was found within 41.5–41.7 GHz.

In the present work we analyzed in more details the CCS changes during the response of thymocytes of different male Wistar rats to non-thermal MMW in the frequency range of 41.56–41.67 GHz. Modified conditions of thymocyte lysis and AVTD measurements were used. Nevertheless, we were able to reproduce the previously reported effect of weak MMW on the CCS of thymocytes (Shtemler *et al.*, 1992). The main result obtained here was that the thymocytes of five examined rats exhibited a statistically significant resonance response in the given frequency range. The established resonance reaction of thymocytes is a further experimental evidence for the hypothesis of the fundamental role of MMW in the regulation of living systems (Fröhlich, 1968). Furthermore, thymocytes of rats are already the third type of cells experimentally proved to change the CCS under the resonance effect of MMW.

The extended analysis of the functional changes during the resonance reaction to MMW of *E. coli* cells has revealed the complex nature of this reaction. Coincidentally with the CCS, such important indices as gene expression, rate of cell division as well as the rates of DNA and protein synthesis were affected by MMW (Belyaev, 1992; Alipov *et al.*, 1993). Experimental evidence have been obtained that chromosomal DNA is a target of resonance interaction of *E. coli* cells with MMW (Belyaev *et al.*, 1992d). The relation between the

Table II. Efficiency of right-handed polarized MMW in relation to left-handed polarized MMW at 41.61 GHz and 1 $\mu\text{W}/\text{cm}^2$ when influencing the CCS of thymocytes of different Wistar rats.

NN	Weight of rat	$\bar{\eta}_{\text{right}} \pm \text{SE}$	P_{right}^*	$\bar{\eta}_{\text{left}} \pm \text{SE}$	P_{left}^*	$\bar{\eta}_{\text{right}}/\bar{\eta}_{\text{left}} \pm \text{SE}$	Significance level
1	165	1.59 ± 0.13	$p < 0.002$	2.16 ± 0.24	$p < 0.005$	0.74 ± 0.10	$p < 0.02$
2	155	1.40 ± 0.10	$p < 0.005$	1.77 ± 0.15	$p < 0.002$	0.79 ± 0.09	$p < 0.01$
3	110	0.99 ± 0.04	$p < 0.8$	1.27 ± 0.08	$p < 0.003$	0.78 ± 0.06	$p < 0.03$
4	140	1.06 ± 0.27	$p < 0.8$	1.43 ± 0.16	$p < 0.045$	0.74 ± 0.21	$p < 0.30$

* Significance level as compared with control level.

resonance MMW frequencies and the genome length have been demonstrated (Belyaev *et al.*, 1993c). We suppose that the resonance frequencies of other cells, including thymocytes of rats, are determined by the structure of their chromosomal DNA. The coincidence of resonance frequencies of all rats examined in this work can reflect the fact that a given resonance frequency is determined by a specific peculiarity in the organization of the Wistar rat genome.

It should be noted, on the other hand, that reducing the error of the resonance frequency determination, which comprised 10–15 MHz in the present study, can reveal more delicate variations of the resonance frequency between the thymocytes of different rats, due to their genetic distinction. Deviations of resonance frequencies on this order of magnitude have been established, for example, upon insertion of prophage DNA into the chromosome DNA of *E. coli* cells (Belyaev *et al.*, 1993c).

The quality of the resonances described here is on the order of 10^3 . Approximately the same quality is characteristic for the MMW resonance effect on the CCS of *E. coli* cells at $1 \mu\text{W}/\text{cm}^2$ (Belyaev *et al.*, 1993a). The same values of halfwidths of resonances, about 100 MHz, have been established previously for response of rat thymocytes and *E. coli* cells on MMW at $0.2 \text{ mW}/\text{cm}^2$ (Shtemler *et al.*, 1992; Belyaev *et al.*, 1992c). Therefore, the qualities of resonance MMW effects on the CCS of different cells seems to be equal at the same PD values. Note, that strong PD dependence of quality of resonance response to MMW have been also found in experiments with yeast cells (Gründler, 1992). The possible explanation of the dependence of resonance halfwidth on PD will be discussed elsewhere (Belyaev *et al.*, in preparation).

Experimental evidence for the resonance effect of MMW at very low intensity is another important result of this work. The PD dependence has a section of logarithmic growth from 10^{-11} to $10^{-7} \text{ W}/\text{cm}^2$, and a plateau in the range of 10^{-7} –

$10^{-4} \text{ W}/\text{cm}^2$. The power dependence of the MMW effect on the CCS of rat thymocytes closely resembles that one established previously for *E. coli* cells (Belyaev *et al.*, 1994). It should be emphasized that the MMW effects at the PD involved can not be explained by trivial heating. Statistically significant changes in CCS were induced by MMW at $10^{-9} \text{ W}/\text{cm}^2$. These PD values are at least five orders of magnitude less than the values, that result in detectable (0.1°C) heating of the irradiated suspension. Note, that similar kind of power dependences for non-thermal MMW effects were previously described by other authors (Gründler, 1992).

The third result of the present work is that circularly polarized MMW at resonance frequency were experimentally shown to differ in efficiency. It have been verified, that at least in case of *E. coli* cells, the sign of effective circular polarization at a resonance frequency was determined by the helicity of DNA (Belyaev *et al.*, 1992d). Stage of cell growth was a critical parameter for manifesting of differently polarized MMW effects (Belyaev *et al.*, 1993e). Therefore, one can not exclude that observed variations in MMW effects on the thymocytes of different rats are due to the differences in their age.

Thus, the resonance reaction of rat thymocytes to low-intensity MMW closely parallels one of *E. coli* cells in physical regularities, studied in this work. We also emphasize that, as in the case of *E. coli* cells, the CCS changes of rat thymocytes are determined, at a molecular level, by the changes in the cooperative binding of a number of structural and functional proteins to chromosomal DNA (Belyaev *et al.*, 1993a; Radko *et al.*, 1992).

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