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Short communication

Effects of low-frequency magnetic fields on bacteria *Escherichia coli*

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Abstract

The effects of low-frequency magnetic fields (Bm = 2.7–10 mT, f = 50 Hz, time of exposure t = 0–12 min, laboratory temperature) on the viability and oxidoreductive activity of gram-negative bacteria *Escherichia coli* were investigated. The growth of these bacteria was negatively affected by such fields. We compared two experimental systems — solenoid [Sb. Lek. 99 (1998) 455] and a cylindrical spool — to find differences between nonhomogeneous and "more homogeneous" magnetic fields. We observed analogous effects in both experimental conditions. The growth curve of the exposed bacteria was lower than the control one. The ability of bacteria to form colonies decreased with increasing magnetic field intensity and with increasing time of exposure. The oxidoreductive activity was measured using reduction of a tetrazolium salt. The decrease in oxidoreductive activity with increasing time of exposure was observed, but the effect was due to a lower amount of bacteria surviving the exposure to the magnetic fields. The decrease in oxidoreductive activity and ability to form colonies were compared with the assumption that the effect of magnetic field is probably bactericidal.

Keywords: ELF magnetic fields; *Escherichia coli*

1. Introduction

One of the mostly discussed contemporary problem in the biophysics is whether low-frequency magnetic fields can affect living systems. A lot of papers concerning this topic have been published in the last 20 years, but the results are very controversial [2–5]. A big number of attempts to explain magnetic field effects on the molecular level have been given [6]. It was shown that magnetic fields can affect biological functions of organisms by changes of the concentration of hormones, by changes of the activity of enzymes or of the transport of ions by cell membranes, by changes in the synthesis or transcription of DNA, etc [2,7,8].

In this work, we continue in the investigation of the extremely low-frequency magnetic field effect on gram-negative bacteria *Escherichia coli*. We have found previously [1] that nonhomogeneous magnetic fields (B_m = 5–20 mT, f = 50 Hz, time of exposure t = 0–24 min) negatively affect the growth of our bacteria. The effect depends on the duration of exposure and on the magnitude of magnetic induction. The growth curve of the exposed bacteria is changed as well. There is a difference in the exposure of the bacteria in broth and on the agar plates. The bacteriophage BF23 is bound less to bacteria exposed and the magnetic field did not induce any production of bacteriophage from the lysogenic strain. We suppose that magnetic fields did not damage DNA of the bacteria exposed.

We repeated now these experiments using a more homogeneous magnetic field to find if the effects depend on the homogeneity of the fields. We decided to measure the dynamics of the growth and the oxidoreductive activity to find whether the magnetic fields effects are bactericidal (killing the bacteria) or bacteriostatic (blocking their growth during the exposure).

2. Experimental

The magnetic fields were generated by a cylindrical coil. This was charged by a regulated transformer. The maximal

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effective current was 1.9 A, the frequency 50 Hz. The temperature was kept on the value of the laboratory temperature (20–25 °C) and it was measured by the thermometer. The samples were placed on the nonconductive stand in the centre of the coil.

The bacteria *E. coli* (strain K12 Row, genotype 58–161 *metB*1*rpsL* 1^{+} F^{def} P. Fredericq), from the Department of Biology Medical Faculty of the Masaryk University Brno, were used. TY broth (8 g tryptone, 5 g yeast extract—HiMedia Lab., Bombay, 5 g NaCl—Lachema Brno/1 l of water) and basic nutrient agar (40 g/l—Imuna, Šarišské Michalany) were used for cultivation of the bacteria.

One percent (w.v.) 2,3,5-triphenyl tetrazolium chloride (TTCl, Lachema Brno) was used as indicator in the oxidoreductive activity experiments.

The number of colony forming units (CFU) was used to quantify our results. Fresh bacterial cultures were used throughout the experiments. Control cultures were kept in the same conditions as the exposed ones except the sole exposition to the magnetic fields.

In the experiments using varying exposure times or magnetic induction, appropriately diluted bacterial cultures were exposed to magnetic fields on agar plates in the phase of their logarithmic growth (4.5 h since inoculation). For studies of the dynamics of growth, broth cultures were exposed to the magnetic fields in the logarithmic growth phase at time intervals, samples thereof were transferred to agar plates for CFU counts. To measure the oxidoreductive activity, cultures (12 h after the inoculation) were exposed to magnetic fields in broth, then 0.4 ml of 1% TTCl were added to 4 ml samples of the culture and samples placed into a thermostat (37 °C, 1 h). Then, after the TTCl reaction was finished, the samples were spun down (15 min/4000/min) and the colony indicator in each sediment was diluted in 2 ml of acetone. After a complete reduction of the dye, its optical transparency was measured by a photometer (Spekol, Carl-Zeiss Jena) using $\lambda = 490$ nm.

For statistical analysis of the results, the *t*-test was used at the 0.95 level of significance.

3. Results and discussion

3.1. Dependence of CFU on the time of exposure

We exposed bacterial cells to the magnetic field and varied the duration of the exposure. We found that the number of CFU decreases with the time of exposure. The decrease is exponential. This result corresponds to the one for a nonhomogeneous magnetic field.

3.2. Dependence of CFU on the magnitude of magnetic induction

Bacteria were exposed to the magnetic field and the magnitude of the magnetic induction was changed in the interval 2.7–10 mT. We ascertained again an exponential decrease of the number of CFU in the exposed culture. The result was again the same as for nonhomogeneous magnetic fields.

3.3. The study of the growth dynamics

We tried to find out if the inhibitive effects of the magnetic field are bacteriostatic or bactericidal. We counted the number of CFU during exposure of the culture and compared it with the control (Fig. 1). After the analysis of the data, we ascertained that the slope of the dependence of CFU on the time of the exposure does not equal to zero, but it is as to the slope of the control curve. We suppose that cells in the magnetic field do not lose their ability to divide. The decrease of the CFU number is caused by death of some bacteria in the culture. The effect of magnetic fields probably is not bacteriostatic.

3.4. Measuring of the oxidoreductive activity in the cells exposed

We studied oxidoreductive activity of the bacterial cells using reduction of tetrazolium. Bacteria reduced tetrazolium on the phormasane and we quantified this activity by photometric measurement of the transparency of the solution of phormasane formed. We found an increase of the transparency depending on the time of the exposure; it means that the oxidoreductive activity of the bacteria decreases with the time of exposure (Fig. 2a). The next step was to measure the dependence of the transparency on the number of CFU in the sample. We compared two curves, the one of cells exposed to magnetic field and the other of control cells. By statistic analysis, we found that the slopes of both curves are similar (Fig. 2b). From this fact, we assume that magnetic field exerts no effect on the metabolism of the bacteria exposed. On the other hand, the

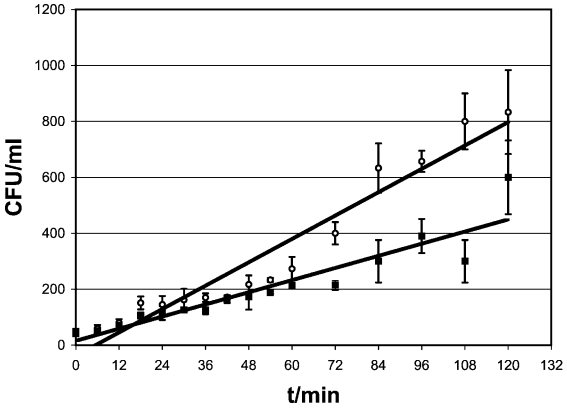

Fig. 1. The dependence of the colony forming units on the time of exposure. The magnetic fields was turn off in the $t = 60$ min. ○—Control culture; ■—culture exposed.

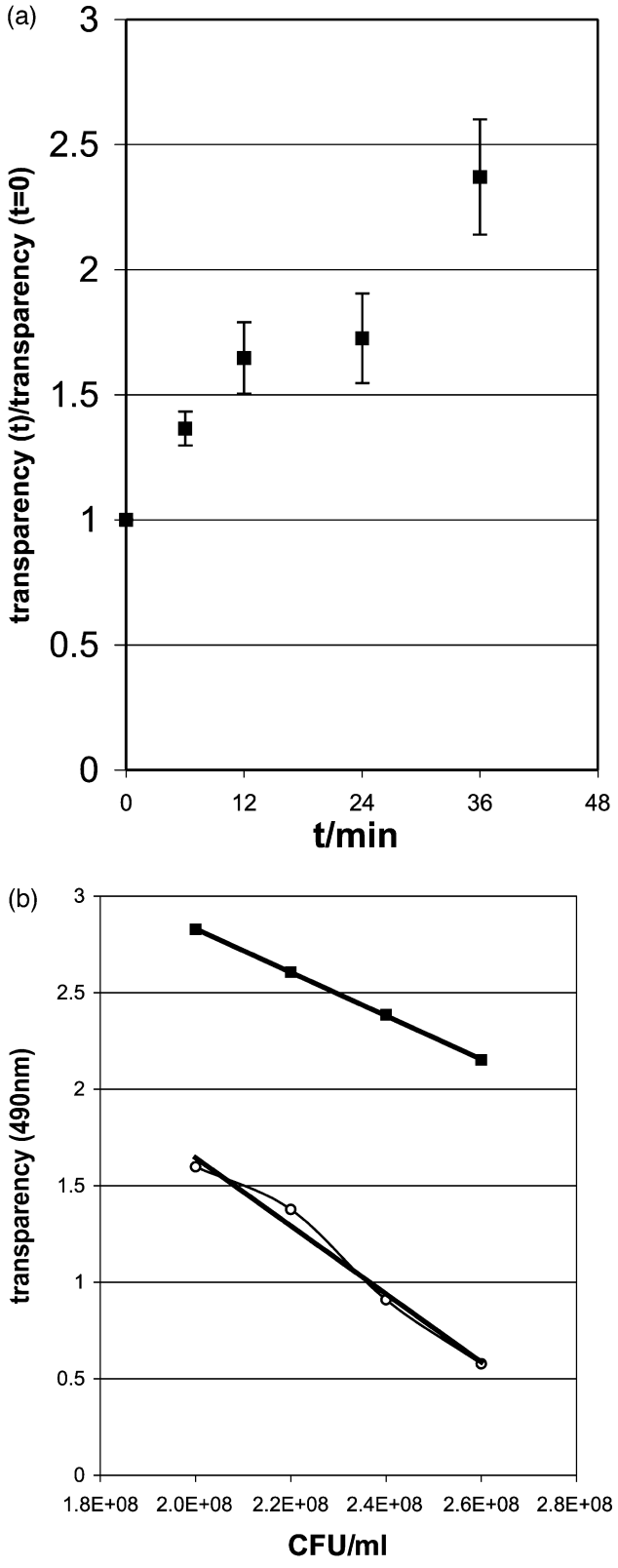

Fig. 2. Measuring of the oxidoreductive activity. (a) Dependence of the transparency, $\lambda = 490$ nm, of the samples after TTCl reaction on the time of the exposure (higher transparency means lower activity); (b) Dependence of the transparency of the samples on the number of CFU for unexposed (○—the slope = (− 1.7 ± 0.8) e − 8) and exposed (■—the slope = (− 1.1 ± 0.5) e − 8) cultures.

oxidoreductive activity is a good means for measuring the magnetic field effects. Because the magnetic field has no effect on the metabolism of bacteria and the metabolism of the culture decreases with the time of exposure as does the number of CFU, we concluded that magnetic field kills a small part of bacteria from the culture, the majority of them developing without provable disturbance.

4. Conclusion

We found that magnetic fields (50 Hz, 2.7–10 mT, 0–12 min) can affect the bacteria *E. coli*. We confirmed our previous results. The effect of the nonhomogeneous and homogeneous fields is approximately the same. By measuring the dynamics, we found that the effects of magnetic fields are not bacteriostatic; the number of bacteria increases during exposure of the growing culture to the magnetic fields but is less than in the control culture. Magnetic fields have no effect on the metabolism of the bacteria. From all the results, we assume that the magnetic fields kill a part of bacteria exposed.

The question of how the magnetic field can kill bacteria is not solved by our experiments. The main theories that try to explain the biological effects of electromagnetic fields are based on the possible effect of electromagnetic field on the permeability of the ionic channels in the membrane. This can affect the ion transport into the cells and it can result in biological changes of the organism. The other possible effect is the formation of free radicals due to magnetic field exposure. On the other hand, our previous experiments reported no changes in DNA structure due to magnetic field [1].

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