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Applications of Electromagnetic Phenomena in Electrical and Mechanical Systems
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MUTAGENIC AND PHYSIOLOGICAL EFFECTS ON THE BACTERIAL CELL BY EXPOSURE TO MAGNETIC FIELDS

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Abstract

Mutagenic and physiological effects of extremely low frequency (ELF) magnetic fields (60 Hz, 45 mT) were estimated using bacteriophage lambda. By exposure to ELF magnetic fields for about 8 hours, the bacteriophage lambda of lysogenic growth state in *E.coli*, shifted to lytic growth 2 times as often as control. The mitomycin C, mutagenic reagent, was shifted the bacteriophages from lysogenic to lytic growth 46-times as often as control. These results suggested that ELF magnetic fields (60 Hz, 45 mT) did not induce DNA damage in the bacterial cell as mitomycin C action. But ELF magnetic fields (60 Hz, 45 mT) might induce physiological damage in the bacterial cells.

1. INTRODUCTION

At present, there are many reports about the influence of an electromagnetic field on the living organisms. International health organization (WHO) and international cancer research organization (IARC) categorized ELF magnetic fields as "2B: It may have the possibility of cancer " in 2001. However, since the valuation is based on data deficient in an objective standard and molecular level data, it is not clear how magnetic fields affect the living organisms.

We have investigated influence of magnetic fields in biochemistry and molecular biology level, for example, on catalase activity, restriction enzyme activity, nucleic acid synthesis or DNA repair etc. *in vitro*. We could not detect remarkable effects on these enzymatic activities except one of catalase.

We also used the nematode *C. elegance* for the *in vivo* experiment, and have clarified the following things. The expression of heat shock protein was increased by exposure to ELF magnetic fields [1]. Recently we identified 20 genes potentially responsible to ELF magnetic fields by the differential display method [2].

In this study, we investigated whether the magnetic fields (ELF) induce DNA damage or physiological abnormality in the bacterial cell using lysogenic bacteriophage lambda. Furthermore, the availability of the magnetic fields for bio-control (regulation of gene expression etc.), another objective, is discussed in this paper.

2. MATERIALS AND METHODS

2.1 Magnetic field generator

ELF magnetic field generator used in this study was shown in Fig. 1. The one direction coil of orthogonal oriented was excited and generated the magnetic fields of 60 Hz, 45 mT. The experimental area (Φ= 52 mm, h = 75 mm) was maintained at about 32 ℃ using circulating water during exposure to magnetic fields.

2.2 Bacterial strain

The *Escherichia coli* W3110 lambda_857, which have lambda provirus (lysogenic growth state) and *E. coli* W3110 were from National Institute of Genetics (Japan). Cells of *E. coli* were grown in LB broth.

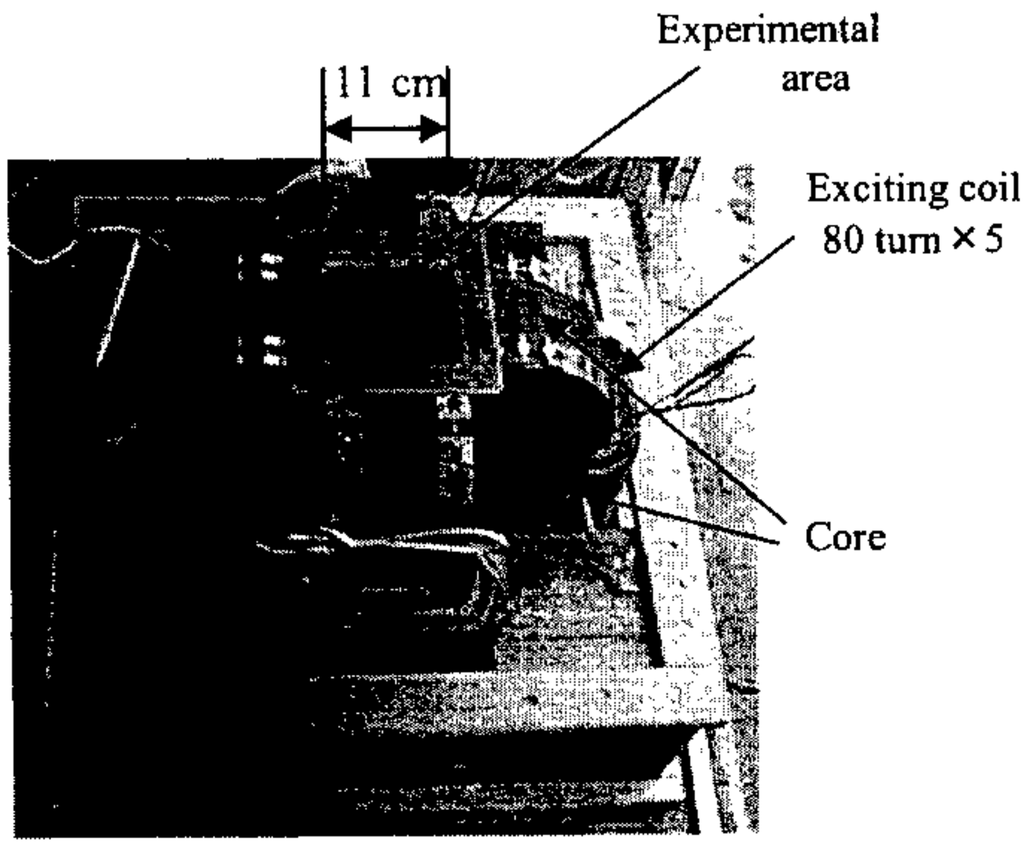


Fig. 1 Extremely low frequency magnetic field generator

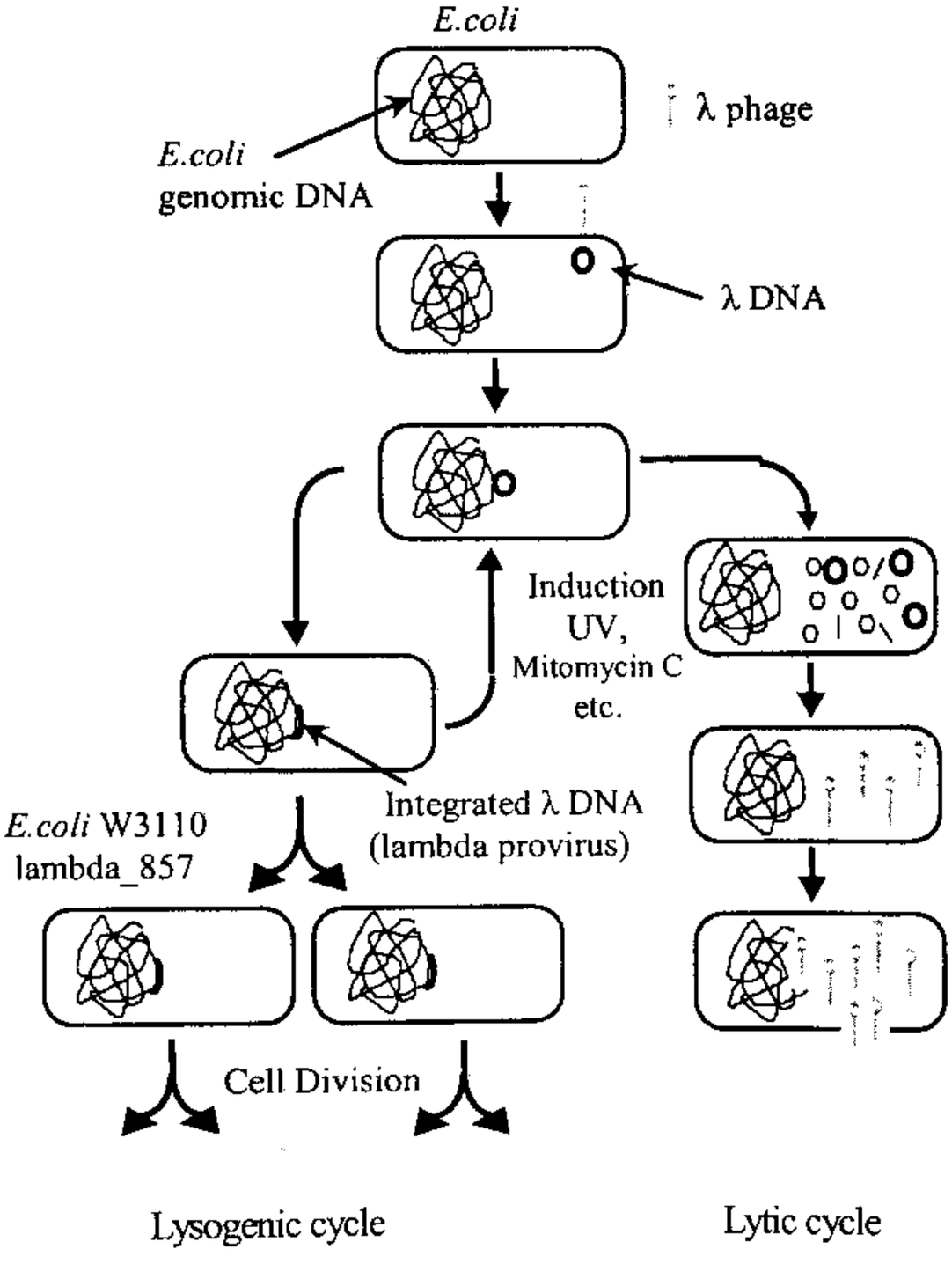


Fig. 2 Life cycle of λ phage

E.coli W3110 lambda_857 ($1\times10^{7\text{-}8}$ cfu/ml) 0.2 ml
(control, exposed)

↓

Centrifugation, 12,000 rpm for 3 min

↓

0.1ml of supernatant was added
0.2 ml of *E.coli* W3110

↓

Room temp. for 15 min

↓

Plating with 2.5 ml of Soft agar

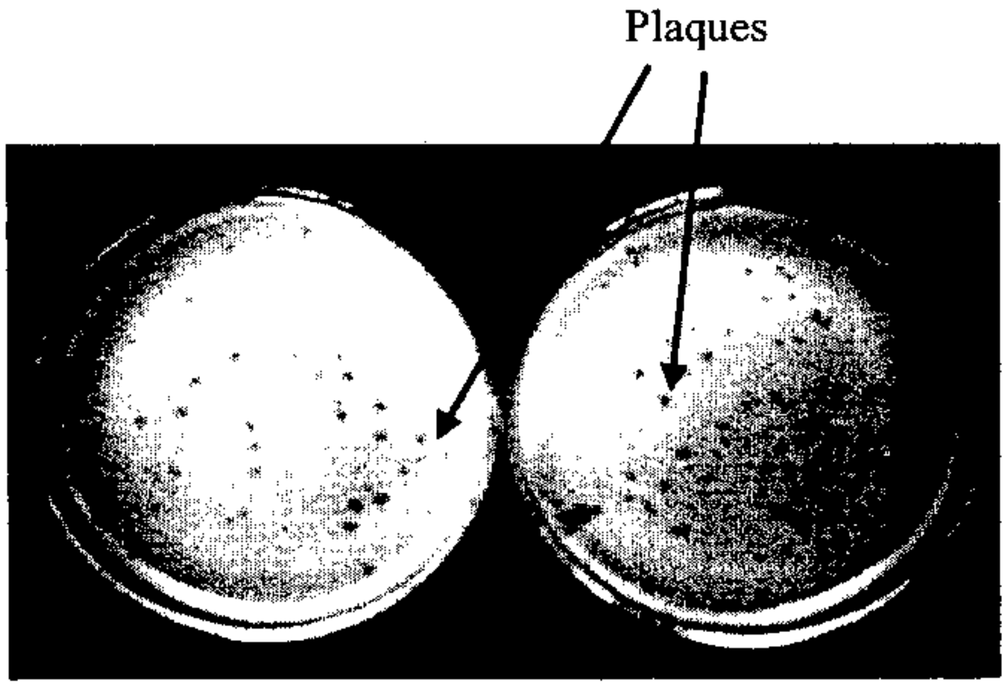
↓

Incubation for overnight at 38℃

↓

Count of the plaques

(a) Method



(b) Detection of the plaques

Fig. 3 Plaque Assays

2.3 Life cycle of bacteriophage lambda

The bacteriophage lambda (λ) can multiply by either a lytic or a lysogenic pathway in the *E. coli* bacterium (Fig. 2). When the bacteriophage is growing in the lysogenic state, damage to the cell (e.g. DNA damage by exposure to UV light or mitomycin C) causes the integrated viral DNA (provirus) to exit from the host chromosome and shift to lytic growth, regarded as bacteriophage induction [3], [4].

In this study, the *E. coli* W3110 lambda_857, which have lambda provirus, was exposed to ELF magnetic fields, and the number of lytic growth phage was measured by plaque assay [3] as magnetic field effect on the bacterial cell.

2.4 Plaque assay

Fig. 3 shows the plaque assay in this study. Cell suspension of *E.coli* W3110 lambda_857 ($1\times10^{7\text{-}8}$ cfu/ml) was incubated at 32 ℃ in a magnetic field or in a sham space as a control. Each cell suspension (0.2 ml) was sampled at each incubation time, centrifuged at 12000 rpm for 3 min. Then 0.1 ml of supernatant including bacteriophage was mixed with top agar and indicator *E.coli* and plated on LB agar plates. The plate was incubated at 39 ℃ for overnight and the numbers of plaques were counted. Each time of experiments were repeated at least several times. As positive control, cell suspension of *E.coli* W3110 lambda_857 added mitomycin C was prepared.

3. RESULTS

3.1 Effects of ELF magnetic fields on the induction of lambda phage

To test the DNA and physiological damage to the bacterial cell by ELF magnetic fields (60 Hz, 45 mT), cells of the *E. coli* W3110 lambda_857, which have lambda provirus, were incubated under ELF magnetic fields and the numbers of lytic growth phage were measured by plaque assay every 1 hour.

By exposure to magnetic fields for about 8 hours, the bacteriophages shifted to lytic growth 1.71 times as often as control (shown in Table 1). The value was average of 8 times experiments and shown statistically significant difference (n=8, P<0.012). On the other hand, the mitomycin C, mutagenic reagent, was shifted the bacteriophages from lysogenic to lytic growth 46-times as often as control.

These results suggest that ELF magnetic fields (60 Hz, 45 mT) do not induce DNA damage mitomycin C in the bacterial cell as mitomycin C. However ELF magnetic field (60 Hz, 45 mT) might induce physiological damage in the bacterial cells

3.2 Effects of ELF magnetic fields and mitomycin C on the induction of lambda phage

It is reported that the frequency of chromosomal aberration increases during exposure to magnetic field with mitomycin C or X-ray [5]. We, therefore, measured the shift rate of provirus to lytic growth by combined use of ELF magnetic fields and mitomycin C.

As shown in Table 2, the relative number of induced bacteriophage was increased 2.37 times by exposure ELF magnetic fields in combination with mitomycin C for 3 hours. That is, the lysogenic growth phage in *E.coli* W3110 exposed ELF magnetic fields and mitomycin C were shifted to lytic growth 2 times as soon as only mitomycin C.

The result suggested that ELF magnetic fields might enhance mitomycin C action synergistically.

Table 1 Effects of magnetic fields on induction of λ phage

*n=8, p<0.012

	Induced Phage Titer : pfu/ml (Relative Titer)		
	Control	MF (45 mT)	MMC (10 μg/ml)
Time (h)		Exposure	Treatment
0	122 (1.00)	122 (1.00)	124 (1.02)
1	80 (1.00)	70 (0.87)	55 (0.69)
2	77.5 (1.00)	127.5 (1.65)	65 (0.84)
3	217 (1.00)	269.5 (1.24)	9985 (46.01)
4	171 (1.00)	170 (0.99)	
5	141 (1.00)	140 (0.99)	
6	325.5 (1.00)	392.7 (1.21)	
7	373 (1.00)	515.5 (1.38) *	
8	1786.7 (1.00)	3060 (1.71) *	
12	1860 (1.00)	2450 (1.32)	

Table 2. Effects of magnetic fields and mitomycin C on induction of λ phage

*n= 6, p<0.0010

	Induced Phage Titer :pfu/ml (Relative Titer)	
	MMC	MMC (10 μg/ml) +
Time (h)	(10 μg/ml)	MF(45 mT) Exposure
0	295 (1.0)	310 (1.05)
2	153.1 (1.0)	181.3 (1.18)
2.5	420.9 (1.0)	281.3 (0.67)
3	421.2 (1.0)	996.4 (2.37) *
4	14404.2 (1.0)	25075 (1.79) *

4. CONCLUSION

In this study, the lysogenic growth lambda bacteriophages in *E.coli* W3110 were shifted to lytic growth 1.71 times as often as control by exposure to magnetic fields for about 8 hours. These results suggested that ELF magnetic fields (60 Hz, 45 mT) did not cause DNA damage strongly in the bacterial cell as mitomycin C. At this point, our result is agreed with other report that 50 Hz, 40 mT magnetic fields is not mutagenic in bacterial mutation assay [6]. However, ELF magnetic fields (60 Hz, 45 mT) might induce physiological damage in the bacterial cells.

By the combination experiments of ELF magnetic fields and mitomycin C, the shift rate of the lambda bacteriophage from lysogenic to lytic growth was 2 times faster than only mitomycin C. The results suggest that ELF magnetic fields might enhance the action of mitomycin C.

When the bacteriophages shift from lysogenic to lytic growth, the gene expression pattern is changed in the phage genome. Therefore, the magnetic fields might have possibilities to control the gene expressions.

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