# Investigation of Potential Genotoxic Effects of Low Frequency Electromagnetic Fields on *Escherichia coli**

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**Abstract**—Exposure of growing cells of *Escherichia coli* strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m$^{-1}$ did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 *umuC* or TK501 *umuC uvrB* to UV. Similarly, although the resistance of strains TK702 *umuC* and TK501 *umuC uvrB* to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pregrowth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

The biological effects of electromagnetic radiation have been reviewed extensively (Adey 1981; Davey & Kell 1990). However, the evidence published about potential damage to DNA, which is of special interest because of possible carcinogenic or teratogenic effects (Pool 1990), is contradictory. For example, Bauchinger et al (1981) found no increase in chromosomal abberations or sister chromatid exchanges in peripheral nucleocytes from switchyard workers, while Nordström et al (1983) reported a significantly higher incidence of congenital malformations in children whose fathers worked in high voltage switchyards.

Electric fields, which may be generated by direct, pulsed or alternating current, are used therapeutically (Bassett 1989) and to enhance transdermal drug delivery (Burnette 1989; Parasrampuria & Parasrampuria 1991). There is little evidence of structural damage induced in the skin by this technique (Gangarosa et al 1980), but genotoxic effects have not been studied in depth. Bacteria are used extensively as predictive models for eukaryotic genotoxicity (Mason et al 1990; Ashby 1991), and any increase in mutation frequency induced by electromagnetic radiation will indicate potential toxicity to eukaryotic tissue. Moore (1979) found that growth in electromagnetic fields had no effect on spontaneous mutation in two strains of *Salmonella typhimurium*. We report the effects of applied fields on DNA repair-proficient and DNA repair-deficient strains of *Escherichia coli*, using both spontaneous and induced mutation frequencies, and recovery from DNA damage produced by exposure to ultraviolet (UV) light or mitomycin C as indicators of genotoxicity.

## Materials and Methods

### Bacterial strains and plasmid
The *Escherichia coli* K-12 strain AB1157 (Bachmann 1972),

which is wild-type with respect to DNA repair and its derivatives TK702 *umuC* (Kato & Shinoura 1977), deficient in error-prone repair, and TK501 *umuC uvrB* (Kato & Shinoura 1977), lacking both error-prone and excision repair, were used. Plasmid pKM101 conferring ampicillin resistance and carrying *mucAB* genes, which are analogous to chromosomal *umuDC* genes (Walker 1984), was transferred into strains by conjugation.

### Growth and counting of cultures
Strains grown overnight in nutrient broth (Oxoid No. 2, code CM67) at 37°C were diluted into nutrient broth at 37°C and incubated for 3 h to give late exponential phase cultures of approximately $10^8$ cells mL$^{-1}$, which were used as inocula in all experiments. Viable counts were performed by diluting in nutrient broth at 37°C and plating on nutrient agar (Oxoid Blood Agar Base, code CM55). Unless otherwise stated, colony counts were performed after 16–24 h incubation.

### Exposure of cells to electric fields
Exponentially-growing cells were diluted 1 in 5 for a 1 h exposure or 1 in 100 for a 16 h exposure into nutrient broth in a 30 mL glass screw-capped bottle maintained at 37°C in a water bath. The bottle cap was modified to carry two stainless steel electrodes (area 1 cm$^2$, separation distance 1 mm), which extended into the broth. After each experiment, the cap and electrodes were decontaminated by washing with 70% ethanol, and a fresh bottle of inoculated medium screwed on for each exposure.

Alternating voltages were generated by a dielectric spectrometer (Dielectric Instrumentation, Worcester, UK) using a frequency of 1 Hz. Field strengths of 3 kV m$^{-1}$ were applied for 1 h (3 generations) or 1 kV m$^{-1}$ for 16 h (12 generations to stationary phase). Control cultures were grown under the same conditions, but without exposure to electrical current.

### Exposure of cells to UV irradiation
Cultures were diluted in nutrient broth and placed on overdried nutrient agar. As soon as samples had soaked into

the agar, plates were exposed to a Hanovia model 12 low pressure mercury lamp (Hanovia Lamps Ltd, Slough, UK), which emitted light at 254 nm. The dose rate was $1 \ J \ m^{-2} \ s^{-1}$ for the AB1157 and TK702 strains, and $0\cdot2 \ J \ m^{-2} \ s^{-1}$ for the TK501 strain, determined with a Blak-Ray UV meter model J-225 (Ultraviolet Products Inc., San Gabriel, CA, USA). Plates were then incubated in the dark, together with unirradiated controls, for up to 48 h at 37°C.

## Determination of mutation frequencies

Frequencies of mutation to rifampicin resistance were determined by plating cells on nutrient agar containing inhibitory concentrations of rifampicin (Sigma) and counting the number of colonies that grew after 48 h incubation. Stock solutions of rifampicin were prepared in dimethylsulphoxide at $1 \ mg \ mL^{-1}$ and suitable volumes added to molten nutrient agar. Controls showed that at the concentrations used, dimethylsulphoxide was not inhibitory to cell growth. Mutation frequencies are expressed as mutants per viable cell plated. Frequencies of chemically-induced mutation to rifampicin resistance were determined after cells had been grown for 16 h in the presence of sub-inhibitory concentrations of mitomycin C (Sigma).

## Results

### Effect of pregrowth in charge on mutation frequency

Mutation frequency to rifampicin resistance decreased with increasing concentrations of rifampicin on which cells were plated (Figs 1, 2). However, pre-exposure of cells growing in nutrient broth to a field of $3 \ kV \ m^{-1}$ for 1 h (3 generations), or $1 \ kV \ m^{-1}$ for 16 h (12 generations to stationary phase) before plating on rifampicin-containing media did not significantly affect mutation frequencies (Figs 1, 2).
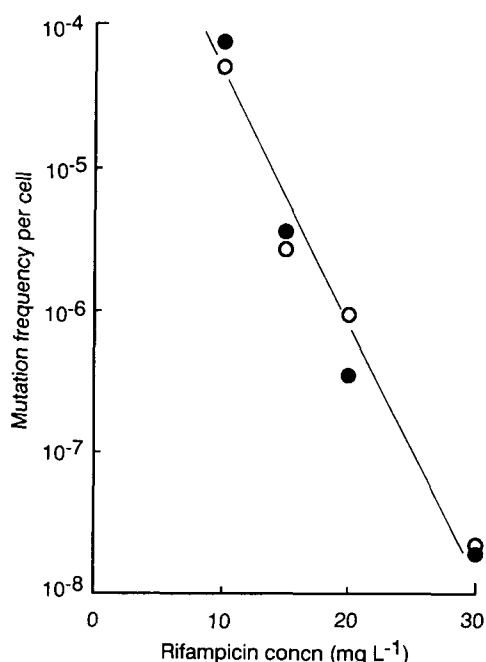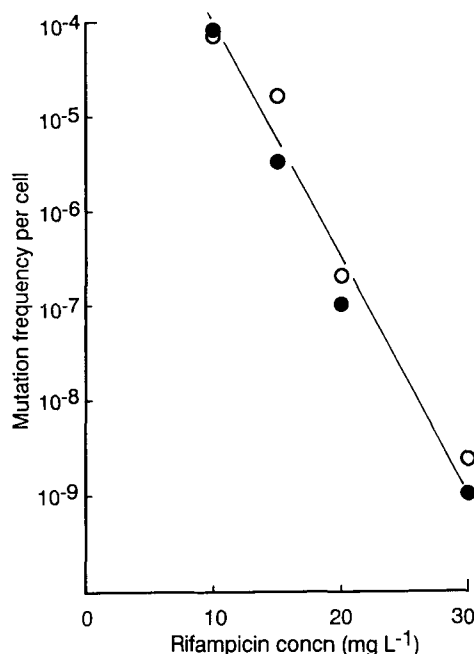
Fig. 2. Effect of exposing growing cultures of *E. coli* for 16 h to a field of $3 \ kV \ m^{-1}$ (frequency 1 Hz) on mutation to rifampicin resistance. ● Exposed cultures, ○ unexposed, control cultures.

### Testing for the induction of mutagenic DNA repair

Radiations and many mutagenic chemicals induce an error-prone (mutagenic) DNA repair pathway in bacteria (Walker 1984). We reasoned that if low frequency electromagnetic fields damage DNA, then this type of DNA repair would be
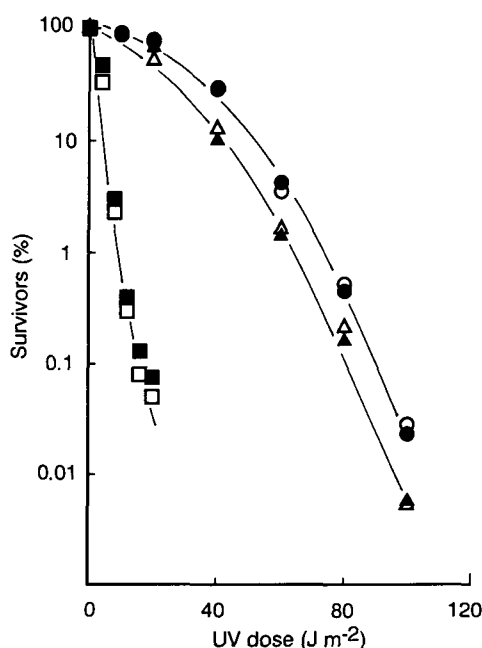
Fig. 1. Effect of exposing growing cultures of *E. coli* for 1 h to a field of $3 \ kV \ m^{-1}$ (frequency 1 Hz) on mutation to rifampicin resistance. ● Exposed cultures, ○ unexposed, control cultures.

Fig. 3. Comparison of the UV sensitivities of *E. coli* strains AB1157 $umuC^+ \ uvrB^+$ (●,○), TK702 $umuC \ uvrB^+$ (▲,△) and TK501 $umuC \ uvrB$ (■,□) grown for 1 h in the presence (closed symbols) or absence (open symbols) of a field of $3 \ kV \ m^{-1}$ (frequency 1 Hz).

induced. Therefore, cells grown in the presence of such fields should be more resistant to the DNA-damaging effects of UV light than cells grown in the absence of electromagnetic induction.

Late exponential phase cultures of *E. coli* strains AB1157 *umuC*[+] *uvrB*[+], TK702 *umuC uvrB*[+] and TK501 *umuC uvrB* were therefore diluted in nutrient broth and exposed to a field of 3 kV m⁻¹ whilst undergoing three further rounds of replication. Cells were then plated on nutrient agar and exposed to UV. Strain AB1157 is fully proficient in all DNA repair pathways and was the most resistant of the three strains to UV (Fig. 3). Strain TK702 lacks error-prone DNA repair and was slightly more sensitive, whereas strain TK501, which lacks both error-prone and excision repair was by far the most sensitive strain (Fig. 3). However, when the UV sensitivities of cultures of the three strains grown in the presence of the electromagnetic field were compared with the sensitivities of control cultures grown in the absence of the field, it was found that the field made no difference to the respective sensitivities of the strains (Fig. 3). This indicates that DNA repair was not being induced by the field, which suggests that the level of electromagnetic radiation used was not damaging bacterial DNA.

Plasmid pKM101 carried *mucAB* genes, which are analogous to the chromosomal *umuDC* genes and suppress deficiencies in error-prone DNA repair in both *E. coli* (Upton & Pinney 1983) and *Salmonella typhimurium* (Little et al 1989). Transfer of plasmid pKM101 into the *umu*-deficient strains TK702 and TK501 therefore resulted in the expected increase in UV resistance due to expression of error-prone DNA repair (Figs 4, 5). However, once again, there was no difference in sensitivities to UV of cultures grown in
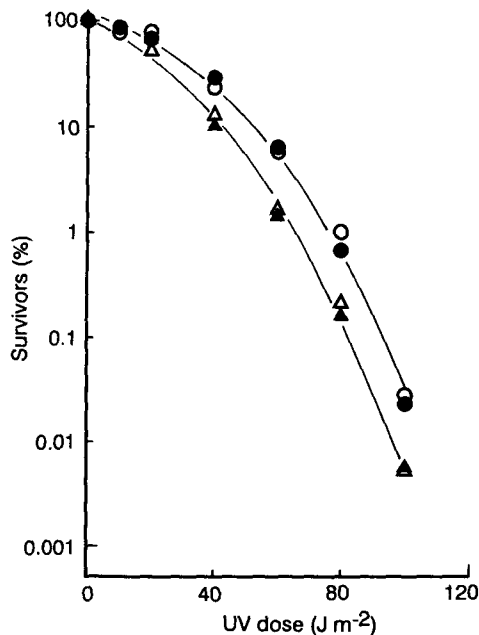


Fig. 5. Effect of growth in a low frequency field (1 Hz, 3 kV m⁻¹ for 3 generations) on UV resistance of *E. coli* strain TK501 *umuC uvrB* with (●,○) and without (■,□) plasmid pKM101. Closed symbols represent exposed cultures, open symbols are results obtained with control, unexposed cultures.
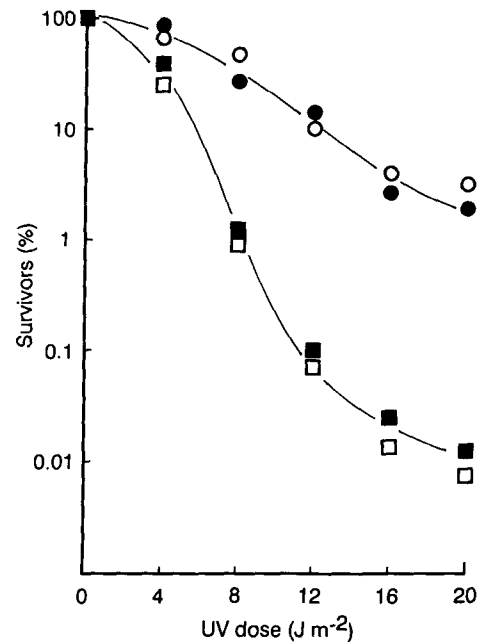
the presence or absence of the electromagnetic field (Figs 4, 5).

The *umu*- and *muc*-encoded DNA repair pathway increases cell survival after DNA damage at the expense of



Fig. 4. Effect of growth in a low frequency field (1 Hz, 3 kV m⁻¹ for 3 generations) on UV resistance of *E. coli* strain TK702 *umuC uvrB*[+] with (●,○) and without (▲,△) plasmid pKM101. Closed symbols represent exposed cultures, open symbols are results obtained with control, unexposed cultures.
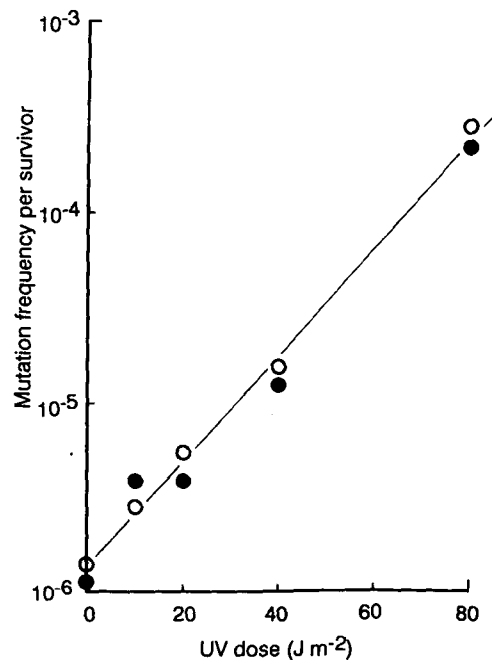


Fig. 6. Comparison of UV-induced mutation frequencies to rifampicin resistance (20 mg L⁻¹) of cultures of *E. coli* strain AB1157 grown in the presence (●) or absence (○) of a low frequency field (1 Hz, 3 kV m⁻¹) for 3 generations.
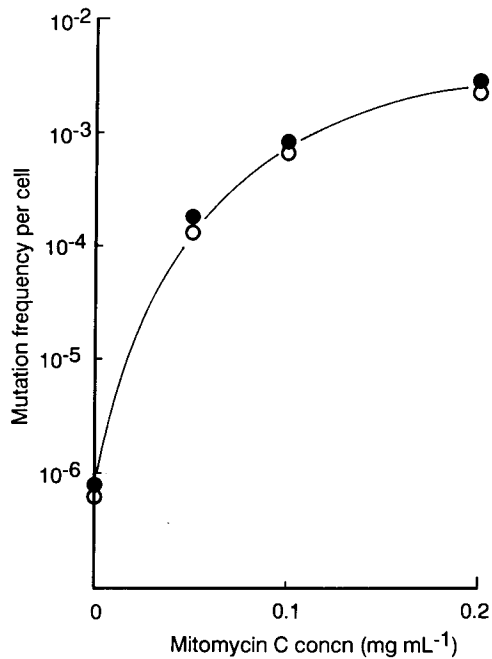
FIG. 7. Effect of growth in a low frequency field (1 Hz, 1 kV m$^{-1}$) for 16 generations on mitomycin C-induced mutation to rifampicin resistance (20 mg L$^{-1}$) in *E. coli* strain AB1157. ● Exposed cultures, ○ unexposed, control cultures.

introducing errors into the repaired DNA (Walker 1984). It would therefore be expected that if electromagnetic fields damage DNA, and thus induce error-prone DNA repair, such exposed cultures should not only exhibit higher survival levels after DNA damage by UV, but should also display higher UV-induced mutation frequencies. Cells grown for 1 h in a field of 3 kV m$^{-1}$ were therefore plated onto nutrient agar containing 20 mg L$^{-1}$ rifampicin and exposed to various doses of UV. Similar cultures were also irradiated on nutrient agar without rifampicin to determine cell survival. UV-induced mutation frequencies per survivor were found to increase logarithmically with increase in UV dose (Fig. 6). However, pre-growth of cells in the electromagnetic field before plating and UV irradiation made no difference to the frequency of UV-induced mutation (Fig. 6).

*Testing for the effect of growth in the presence of low level electromagnetic fields on the activity of a chemical mutagen*
Using the anticancer drug mitomycin C, we investigated whether growth in the presence of charge (16 h, 1 kV m$^{-1}$) increased the mutagenic activity of sub-inhibitory concentrations of mitomycin C against *E. coli* strain AB1157 (minimum inhibiting concentration 0·5 mg L$^{-1}$). Fig. 7 shows that as the concentration of mitomycin C increased, the mutation frequency to rifampicin resistance also increased. However, growth in the presence of the field made no significant difference to the mutation frequency at each concentration of mitomycin C.

## Discussion

Results presented in this paper indicate that growing *Escherichia coli* cells in the presence of low frequency electric fields does not increase mutation frequency, induce error-prone

DNA repair or increase the mutagenic effects of UV irradiation or mitomycin C. We conclude that these prokaryotic tests for the prediction of genotoxicity show no evidence that mutation or mutagenic DNA repair are induced by exposure to electromagnetic radiation. The negative results are encouraging as they indicate that, under the experimental conditions used, there is no evidence of potential genotoxicity induced by exposure to low frequency fields. These studies should be extended to include a wider range of frequencies, field strengths and exposure times, using both other prokaryotic tests and eukaryotic systems. This will help to establish the range of electromagnetic fields to which biological tissues may be safely subjected.

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