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Low-Frequency Electromagnetic Fields Alter the Replication Cycle of MS2 Bacteriophage

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Abstract. The effect of exposure to 60-Hz electromagnetic fields (EMFs) on RNA coliphage MS2 replication was studied. EMF exposure commenced when the bacterial cultures were inoculated with the phage (t = 0). In 12 experiments in which the strength of the field was 5 G, a significant delay in phage yield was found in the EMF-exposed cultures 45–65 min after inoculation, compared with control cultures. However, the EMF did not alter the final phage concentration. Experiments at 25 G (N = 5) suggested that the stronger field resulted in both impeded phage replication and increased phage yield. No differences between test groups were found in experiments involving sham-EMF exposure, thereby indicating that the results obtained with the EMFs were not due to systematic error. It appears that MS2, which codes for only four proteins, is the simplest biological system in which an EMF-induced effect has been demonstrated. The MS2 system is, therefore, conducive to follow-up studies aimed at understanding the level and nature of the underlying interaction process, and perhaps to biophysical modeling of the interaction process.

Many different biological effects due to exposure to electromagnetic fields (EMFs) have been reported, including effects involving bacteria [7, 15], eukaryotic cells [9, 14], animals [13, 19], and human subjects [1, 2, 18]. The complexity of the systems studied thus far has made it difficult to identify the mechanism of interaction between EMFs and cells. Consequently, although various theories describing EMF interactions have been proposed [3, 5, 6, 10], a generally accepted biophysical analysis of EMF/tissue interactions has not been developed, and the ability of EMFs to directly alter protein expression remains in dispute [8, 16].

Recognizing these difficulties, we chose the bacteriophage MS2 for study as a model system [17, 20]. The MS2 genome directs the same basic machinery for protein production found in eukaryotic cells but codes for only four proteins. MS2 does not make use of RNA polymerase, and thus the effects of EMF exposure on translation can be studied in the absence of potential confounding effects on transcription. Further, since the infection cycle of MS2 is rapid, it is possible to take samples at different points in the cycle and thereby characterize the time-dependent response. Finally, since the infection cycle consists of well-known events including attachment, penetration, translation, genomic duplication, assembly, and egress, which occur in a specific order [17, 20], it is possible to isolate the events in relation to the presence of the EMF.

We report here that EMFs affect MS2 replication, thereby raising the possibility that MS2 could be a useful model system for resolving questions regarding mechanisms that have proved to be difficult to resolve in more complex biological systems.

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Materials and Methods

Apparatus. The EMF exposure apparatus consisted of two identical solenoids wound on acrylic formers by use of 15-gauge polyester/polyamideimide-coated magnet wire. Each solenoid was 12.7 cm in height with an internal diameter of 5 cm, and had a resistance of 7.8 Ω and an inductance of 219 mH; they were mounted with a center-to-center distance of 25 cm. The virus–bacteria mixture was located in the horizontal mid-plane of the solenoids at a radius of 1 cm from the axis. One solenoid was powered at 60 Hz by an autotransformer mounted outside the incubator, and the second solenoid was short-circuited and used to provide sham exposure. In most experiments, a magnetic field of

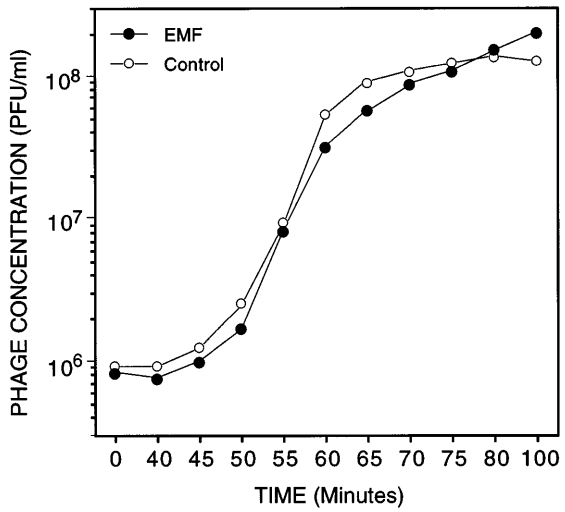

Fig. 1. Data from a representative experiment depicting growth curves for EMF-exposed and control cultures.

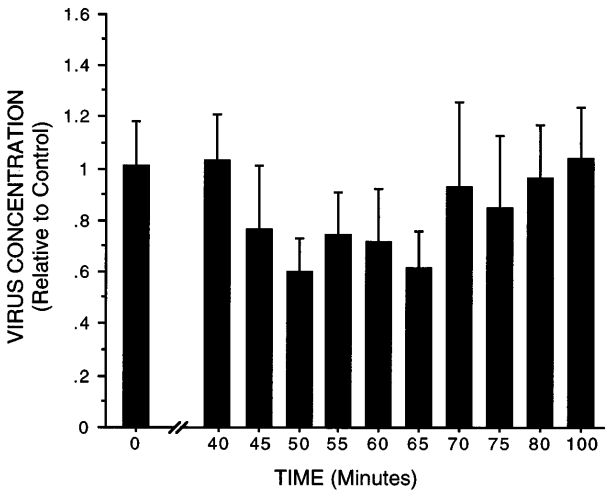

Fig. 2. Effect of EMF on MS2 growth (mean ± SE). The results shown are from 12 experiments in which the applied EMF was 5 G, 60 Hz field. Two significant differences were found with the Wilcoxon signed rank test ($t = 50$, $P < 0.008$; $t = 60$, $P < 0.002$).

5 G (±8%) was used because power-frequency fields of higher strength are unlikely to be encountered in the environment [4]; this resulted in a power dissipation of 5.6 mW but no detectable temperature change. The field (fringing plus background) at the location of the sham-exposure solenoid was about 10 mG.

Bacterial and bacteriophage cultures. The stock culture of *Escherichia coli* (ATCC 15597) used for all experiments was derived from a single isolated colony that was grown in broth, then stored in glycerol (10% final volume) at −70°C. For each experiment, an ampule of *E. coli* stock was thawed and 300 µl added to 5 ml of warmed tryptic soy broth (TSB). The newly seeded bacterial cultures were incubated (37°C) for 2 h, after which 1.5 ml was transferred to 50 ml of TSB and incubated (approximately 2 h) until reaching an OD_{660} of 0.22. The bacteria were kept on ice until mixed with bacteriophage. Stock cultures of coliphage MS2 [ATCC 15597-B1; 10^9–10^{10} plaque-forming units (pfu) per ml] were derived from plaque-purified virus. Stock MS2 was filtered and stored in TSB at 4°C (stable for several months) or −70°C. Bacteria and virus were mixed at a mean multiplicity of infection (MOI) of 1.1 (range 0.1–3.2) pfu per bacterium. The virus–bacteria mixture was maintained in an ice bath for 15 min to permit virus adsorption. The quantity of unadsorbed virus was reduced by subjecting the mixture to four rounds of pelleting by low-speed, refrigerated centrifugation, decanting, and resuspension in chilled TSB. The last virus–bacteria pellet was resuspended in warmed (37°C) TSB, and the mixture was distributed into two to four glass tubes that were randomly (always in equal numbers) placed into EMF- or sham-exposure conditions. Bacterial concentrations and virus titers were determined at $t = 0$ and at 5-min intervals beginning at $t = 40$ min after commencement of exposure. After resuspending the mixture thoroughly with a Pasteur pipet, samples (100 µl) were removed from each tube, dispersed directly into 0.9 ml of chilled TSB, and then filtered into sterile vials (elapsed time, 3–4 min). The bacteria-free, virus-containing filtrate was assayed for phage titers by plaque assay.

Procedure. Both the phage and bacteria in the EMF- and sham-exposed cultures came from the same stock pools. All cultures were uniformly exposed to the magnetic field, which was highly localized in the region of the exposed cultures. The solenoid used to produce the field generated negligible amounts of heat and vibration. The cultures were physically housed in the same incubator and hence experienced the same ambient conditions. The infected cultures were randomized to the conditions (EMF vs. sham) that they received.

Statistical analysis. When the results from the first several experiments were analyzed, the data suggested that the final phage concentrations ($t = 70$–100 min) did not differ between the groups, but that a reduction in phage concentration occurred in the EMF group at $t = 50$–60 min. Comparisons at $t = 45$–65 min were therefore chosen for statistical analysis. It could be argued that the samples were not independent and that the performance of five tests with a comparison-wise error rate of 5% resulted in an unacceptably high family error rate. We therefore employed the Bonferroni procedure [12] and required 5%/5 = 1% for each comparison. On this basis, even 1 significant difference justified the conclusion that EMF exposure altered virus concentration.

Results

In 12 experiments, *E. coli* and MS2 were mixed and exposed to 5 G at 60 Hz. The mixture was sampled at the commencement of exposure and at 5-min intervals beginning at $t = 40$ min. Representative growth curves for virus released from infected bacteria into the surrounding medium are presented in Fig. 1. Under the conditions of study, the bacteriophage were in the eclipse phase during the initial 40 min of incubation; the logarithmic phase of growth occurred at 40–70 min.

The number of infectious phage in the EMF-exposed cultures was consistently less than that in the control cultures between 45 and 65 min (the log phase of phage production), as shown in Fig. 2. The magnitude of the reduction varied and ranged from 15% to 40% of the mean of the controls. After 70 min, there was no significant difference between the exposed and control cultures in the quantity of released phage. Thus, a significant lag in phage production in the EMF-exposed

cultures was seen, but the final phage concentrations did not differ. At t = 70 min, the phage concentration was approximately 115 times the initial level. The rate of replication of uninfected bacteria was identical in EMF- versus sham-exposed cultures.

As an additional control for the possibility of a systematic error, the experiment was repeated (N = 8) without the application of an EMF (sham/sham), and no differences were found between the two sham groups.

To explore the role of the field strength, five experiments were conducted at 25 G, 60 Hz (Fig. 3). Although the individual comparisons at each time point were not statistically significant, a lag in phage production at 45–55 min and an increased yield were apparent.

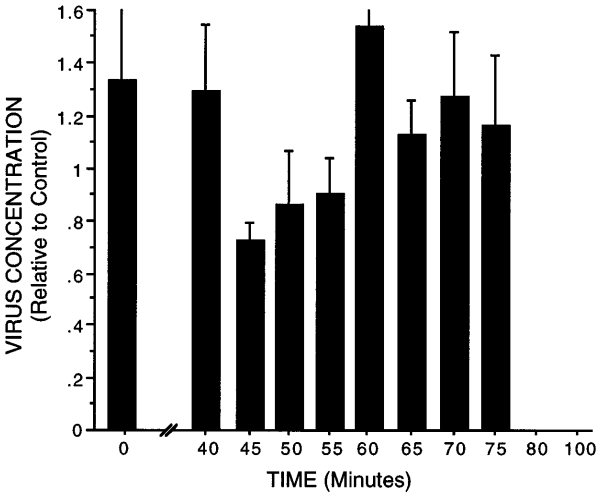

Fig. 3. Effect of 25 G, 60 Hz on phage production (N = 5).

Discussion

The physiochemical processes profiled in phage replication include protein–protein interactions (attachment, assembly), protein–nucleic acid interactions (translation, genomic duplication, assembly), nucleic acid–nucleic acid interactions (secondary folding, replicative intermediates), and protein–membrane interactions (lysis). Some of these processes, such as the binding of coat protein to genomic RNA, require only phage components, whereas other processes, such as translation or genomic duplication, include host factors (for example, elongation factors EF-Tu and EF-Ts). It is the temporal and sequential expression of phage factors, coupled with their interactions with host factors, that culminate in phage production that is quantified by plaque assay. The purpose of this investigation was to determine whether EMF affected phage production. A demonstration of this effect is the prerequisite for determining EMF influence on phage-host factor interactions.

In the adopted experimental design, the phage adsorbed to their bacterial hosts at 4°C for 15 min, and after four successive washings (to remove unadsorbed phage) the infected cultures were placed into the solenoid. Since attachment of the phage to the bacterium occurred prior to commencement of exposure, the effect of the EMF could not have occurred at the attachment step. However, any subsequent reaction—beginning with the entry of the viral RNA into the conjugative pilus, including subsequent interactions with host and viral factors, and culminating in virus progeny—could have been the target of the EMF.

The cultures exposed to 5 G returned to baseline conditions (Fig. 2). Since phage replication is essentially an ordered series of active and passive biochemical reactions commencing with phage binding to the bacterial pilus and terminating with bacterial cell lysis, perhaps the most parsimonious explanation is that one or more reaction rates were affected by the EMF, resulting in a delay in the evolution of the system, but no change in its final state. This is the expected result because effects of low-frequency EMFs less than about 5 G typically are reversible or adaptive in nature. Thus, the observation of a transient but no steady-state effect due to EMF exposure is evidence in favor of the view that one or more reaction rates were affected by the field, but that no irreversible changes were induced.

The experiments performed with 25 G (Fig. 3) suggested that the EMF resulted in increased phage yield, compared with the control cultures. Thus, it appears that increasing the field strength by a factor of 5 was associated with a change in the response of the system from reversible to irreversible.

It has not been possible thus far to establish the characteristic of time-varying magnetic fields that is generally responsible for the biological effects induced by the fields [3, 5, 6, 10]. The possibilities include magnetic field strength, electric field strength, and induced current, among many others. This difficulty has led to presently intractable problems regarding dosimetry and scaling. For example, the change in dose associated with the 5-fold increase in magnetic field strength cannot be predicted precisely because fundamental information regarding interaction mechanisms has not been developed.

The issue of scaling is similarly unresolved. For example, the final liquid volume in our cultures was 4.5 ml. If the strength of the field mediated the observed changes in phage production, then similar effects would be expected with a different liquid volume (because the strength of the field at the location of the cells in the liquid is determined by the current in the solenoid and is unaffected by the liquid volume). On the other hand, the

electric field and induced currents caused by the magnetic field depend on the geometry of the conducting object in a magnetic field (liquid volume, in the present case) [11]. Thus, the absence of information regarding interaction mechanisms presently prevents an unambiguous determination regarding how the results should be scaled.

Consideration of both the dosimetry and scaling problems suggests that standardization of the physical aspects of the experimental procedure (employing an EMF having a fixed strength, for example) while testing biological hypotheses may be the optimal method to study the mechanisms responsible for EMF-induced bioeffects. Implementation of this idea is what motivated the design of this study.

The MS2 system is not simply a model system in which a field-induced effect could be demonstrated (of which there are so many), but a model system of sufficient simplicity to support follow-up studies. The results indicate that a reversible effect occurred at 5 G, and perhaps that an irreversible effect occurred at 25 G. Thus, it is now possible to exploit the (relative) biological simplicity of the MS2 system to test hypotheses involving, for example, field-induced effects on replication and translation.

In conclusion, we have demonstrated an effect of a 5-G, 60-Hz magnetic field in a well-studied viral-replication system whose simplicity is conducive to follow-up studies aimed at understanding the level and nature of the underlying interaction process. In this regard, it is far superior to model systems previously employed to study the effects of EMFs.

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