Vol. 198, No. 1, 1994
January 14, 1994
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 40-44

AN UPPER LIMIT FOR THE EFFECT OF 60 Hz MAGNETIC FIELDS ON BIOLUMINESCENCE FROM THE PHOTOBACTERIUM *Vibrio fischeri**

MICHAEL P. GREENBAUM

Oak Ridge High School, 127 Providence Road, Oak Ridge, TN 37830

Received October 26, 1993

Abstract—Bioluminescence from *Vibrio fischeri* was measured in the presence and absence of 60 Hz magnetic fields. The peak value of the field was ~1.3 mT, a value approximately 13 times the Earth's background static field and comparable to the AC field near heavy-duty electrical equipment such as generators. The objective of this work was a search for causality between the applied magnetic field and a basic biological function at the biochemical, membrane or cellular level based on the direct linkage of bioluminescence to many of the cells mandatory functions such as enzyme (luciferase) activity, electron transport, proton translocation, iron uptake, oxidative metabolism, and cellular communication via the autoinducer N-[3-oxohexanoyl] homoserine lactone. A variation in the activity of any one of these functions will cause a change in bioluminescence. The key result of this work is that, for a signal to noise ratio of 1:1, an effect, if present at all, must be less than 1% of the baseline level of continuously monitored bioluminescence.

As reviewed by Frankel[1], the effect of magnetic fields on biological systems has a long and controversial history. One source of controversy are the conflicting scientific reports that have appeared in the published literature. Working with a wide variety of organisms and magnetic fields, both positive[2,3,4] and negative[5,6,7] results have been published. These conflicting claims have special significance in the context of alleged adverse EMF health effects[8,9,10,11].

If 60 Hz magnetic fields have an adverse effect on a cellular system, there must be a clear cause and effect relationship. Just as any known carcinogen, such as ionizing radiation, has a demonstrable effect on the biochemistry of living systems, reasoning by analogy 60 Hz electromagnetic fields should affect the cell at the biochemical, membrane or cellular level. Bioluminescence is a real-time noninvasive and nondestructive method for observing a broad spectrum of biomolecular and cellular events. Bioluminescence is linked to oxidative metabolism, electron transport, proton translocation, ATP synthesis, and the luciferase enzyme system[12,13,14]. Moreover, it has recently been shown by Dunlap that iron uptake controls bioluminescence in *V. fischeri*[15]. Bacterial bioluminescence also involves chemical communication at the cellular level. Hastings and coworkers termed one of these controls autoinduction, a unique mechanism in which the *lux* operon effectively controls its own transcription[16,17]. For example, in *V. fischeri* induction of bioluminescence is controlled by the cell density-dependent accumulation of the specific autoinducer, N-[3-oxohexanoyl] homoserine lactone[18,19].

*Presented at the 21st Annual Meeting of the American Society for Photobiology, June 26-30, 1993, Chicago, IL, Session on Photosensory Biology/ Luminescence, Abstract No. MPM-E9, *Photochem. Photobiol.* 57 (Supplement), 62S-63S (1993).

Abbreviations: ATP, adenosine-5'-triphosphate; EMF, electromagnetic fields; RNA, ribonucleic acid; mRNA, messenger RNA.

0006-291X/94 $5.00

The present experiments are believed to be the first studies of the effect of 60 Hz magnetic fields on a living bioluminescent system. There have, however, been previous related experiments. Zubkova, Zhuravlev, and Abramov[20] and Bochkova, Yermolin, and Rodichev[21] studied the effect of microwave electromagnetic fields on the intensity of *Photobacterium Krissi* sp. and *V. harveyii* respectively. Not surprisingly, thermal effects were observed at these high frequencies; it is well known that microwave frequencies correspond to molecular absorption transitions. Further work was done by V. N. Petushkov[22] using a static magnetic field. This work did not involve living organisms: the field was applied to an *in vitro* enzyme extract and associated reactants. Petushkov's results indicated that a static magnetic field had no effect on the production of light.

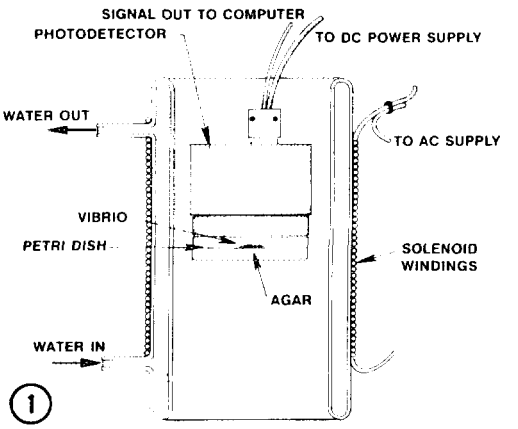
The biological system investigated for this research was *Vibrio fischeri*, a bioluminescent marine bacterium. In nature it is often a partner in a symbiotic relationship with certain fish. The molecular aspects of *V. fischeri* bioluminescence have been studied in detail. This luciferase enzyme catalyzed reaction uses reduced flavin mononucleotide, molecular oxygen, and a long chain aldehyde.

MATERIALS AND METHODS

A schematic illustration of the experimental apparatus used for this research is presented in Fig. 1. A cylindrical water jacket served as both the temperature control and support for the solenoid windings. The cylinder was 17.0 cm in length, from the start of the winding to the end, and had a radius of 4.1 cm. The thickness of the insulated wire used allowed 265 turns per meter. Water was circulated through the jacket using a Lauda Model RM6 heating/cooling circulator.

The formula for a finite solenoid[23], $B = \frac{1}{2}\mu_o nI(\cos\alpha_2 - \cos\alpha_1)$, was used to calculate the magnetic field strength. In this formula μ_o is the permeability of free space, whose value in SI units is $4\pi \times 10^{-7}$ henry/meter; n is the number of turns per meter, I is the current in amperes, and α_2 and α_1 are the angles subtended by a point on the axis of the cylinder by its respective edges. The current was 4.4 A (using a room dehumidifier as the AC load.) This was measured using an Amprobe Instrument model RS-3 ammeter. In related experiments, a static field was generated with a Hewlett-Packard 6286A variable DC power supply. With the DC power supply it was possible to use a wide range of currents.

A culture of *V. fischeri* was purchased from Carolina Biological Supply Co., Burlington, NC, catalog no 15-5723. The bacteria were centered in a transparent Petri dish in order to observe their light emission. A platform was constructed to support the Petri dish in the center of the solenoid. Small variances from this position due to a possible change in agar thickness, for example, made little difference in the experiment because of the broad maximum of the magnetic field as shown in Fig. 2. A Hamamatsu (Middlesex, NJ) model HC220-01 silicon photodetector was centered on top of the Petri dish and



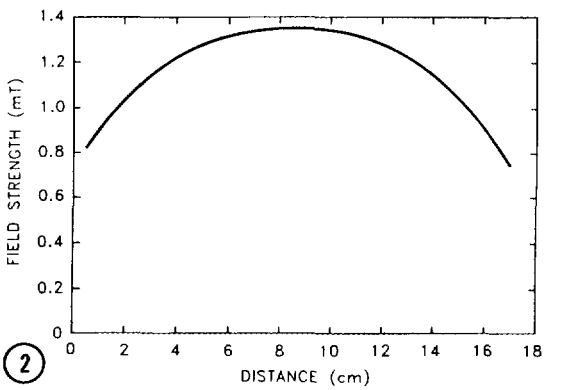


Figure 1. Schematic illustration of experimental apparatus. The photodetector was placed flush with a petri dish containing *V. fischeri* which were positioned at the center of the solenoid. The analog output signal went to a computer. The wires for the solenoid were wrapped around the water jacket which was used for active temperature control.

Figure 2. Field strength as a function of distance within the solenoid. The field strength was calculated from the formula for a finite solenoid, $B = \frac{1}{2}\mu_o nI\ (\cos\alpha_2 - \cos\alpha_1)$. See reference 23 for a detailed description of the use of this formula and a schematic illustration defining the geometry to which it applies. Symbols are defined in the text.

the two ends of the solenoid were made light-tight with layers of opaque, black, plastic sheets. The photodetector was powered by an Albia Electronics DM-6 variable DC power supply. The analog signal produced by the photodetector was sent to a Keithley Metrabyte (Taunton, MA) DAS-8/PGA data acquisition board which was housed in a IBM PC/XT compatible computer. The DAS-8 card is capable of analog to digital conversion with 12-bit resolution. The card was controlled by a program written in Microsoft QuickBasic 4.5.

RESULTS

The preliminary results of these experiments displayed significant response to current in the solenoid as shown in Fig. 3a. For these first experiments, there was *no* method of temperature control (Fig. 3a.) Using a mercury-in-glass thermometer, a temperature increase of 10°C was observed. Almost immediately after the solenoid was turned on the light emission began to rise; however, within 10 minutes it peaked and then fell to zero. The time needed to recover from zero depended on the time the solenoid was on. These data are characteristic thermal responses of light emission by *V. fischeri*. In fact, the same effect was observed using the DC power supply with the same current. This demonstrated that the 60 Hz magnetic field was not a factor in the light emission profiles of Fig 3a. The prolonged decrease in bioluminescence may be due to heat shock of the bacteria. Despite the lack of a gross 60 Hz magnetic effect, the possibility of a smaller effect

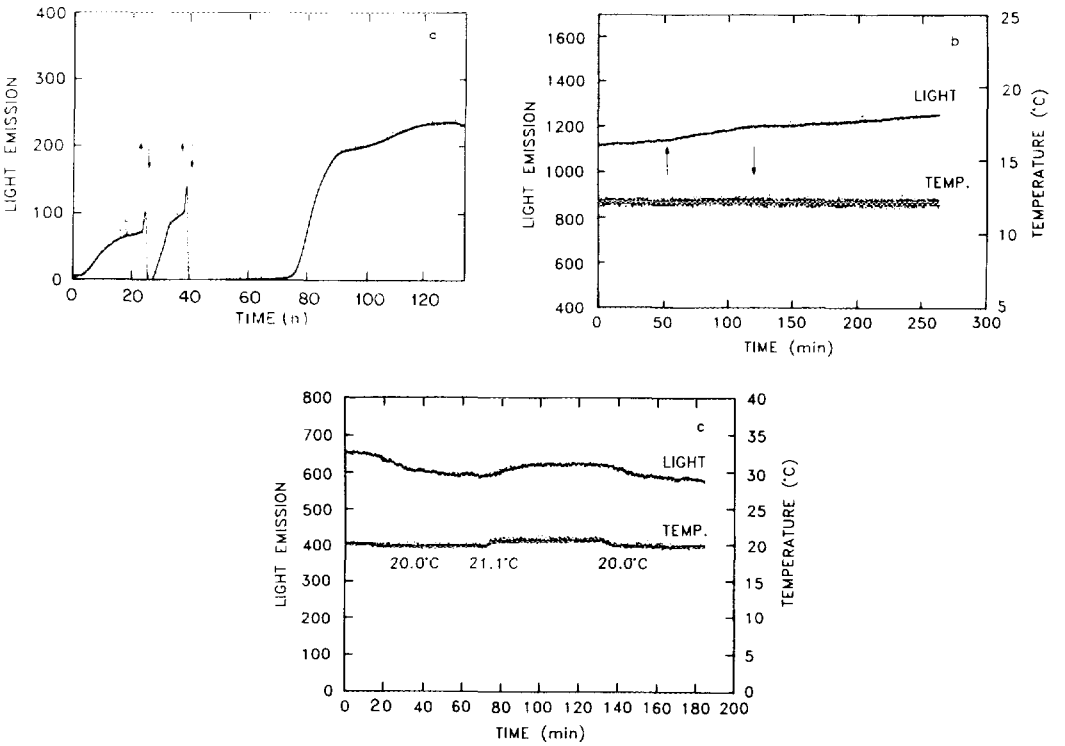


Figure 3. Experimental data of *V. fischeri* bioluminescence vs. time in the presence of 60 Hz magnetic fields. (a) No water cooling employed. Up arrows indicate field on and down arrows indicate field off. In this data up arrows also indicate the start of joule heating from the solenoid. Using a mercury-in-glass thermometer the measured temperature change was ≈ 10°C. (b) This data was similar to Fig. 3a; however, temperature was controlled at 12°C with the use of a water jacket as indicated in Fig. 1. (c) There were no external fields in the data of Fig. 3c. The temperature coefficient of this system in the absence of external fields can be seen in the data of Fig. 3c in which deliberate step functions of +1.1 and -1.1°C are introduced by adjusting the temperature of the circulating bath.

embedded in the large thermal effect of Fig. 3a has been noted[24]. Therefore, additional experiments using active temperature control were performed.

The data in Fig. 3b incorporated a water jacket to stabilize the temperature under the load of Joule heating from the solenoid coils. At 20°C there was no discernable change at all in the level of bioluminescence during 60 Hz magnetic field exposure. However, for T=12°C which corresponds to the data of Fig. 3b a slight effect was observed. In Fig. 3b the upper trace corresponds to light emission; the lower trace is the temperature monitored by a Keithley digital thermometer. The circulating bath temperature for this experiment was set to 12°C. A slight change in the level of bioluminescence can be seen in the upper trace of Fig. 3b. Is this a 60 Hz magnetic field effect? The answer is unambiguously no because the same slight effect could be observed with a static magnetic field produced by a DC current equal to the rms AC current that generated the 60 Hz field. The most likely explanation for the slight effect in Fig. 3b is the inability of the cooling system at 12°C to maintain the temperature as well as it did at 20°C. Seven different experiments were done and only the ones done below room temperature showed this slight effect. The data of Fig. 3b also indicate that the bacteria continued to grow and emit light well beyond the period of 60 Hz magnetic field exposure. Based on the level of light emission and the noise level of the signal, any 60 Hz magnetic field effect, if it were present at all, was less that one percent of the continuous baseline level of light emission. The temperature coefficient of this system in the absence of external fields can be seen in the data of Fig. 3c in which deliberate step functions of +1.1 and -1.1°C are introduced by adjusting the temperature of the circulating bath.

DISCUSSION

Adair[25] has provided a comprehensive and lucid discussion of the constraints on biological effects of weak extremely-low-frequency electromagnetic fields. For the purposes of the results reported here three aspects of Adair's analysis apply: static magnetic fields, changing magnetic fields, and resonance. For the first case, the characteristic energy of magnetic interaction of atoms and molecules, 10^{-7} kT, is much smaller than the characteristic Boltzmann thermal energy, kT (=0.025 eV). For the second case of changing magnetic fields, the energy of electric field interaction (via Faraday's Law of Electromagnetic Induction) is once again small compared to thermal energies. Finally, the possibility of a resonance phenomenon, in which a repetitive small amount of energy becomes significant due to its reinforcing, rhythmical pattern is also ruled out by Adair's analysis.

On the other hand there are reports of measurable effects of varying magnetic fields on biological systems. For example, in a series of studies by Goodman and coworkers[26,27,28] it was concluded that weak pulsing magnetic fields can affect biological processes. When dipteran salivary gland cells or human cells where exposed to low frequency electromagnetic fields a pronounced increase in transcription was measured. Changes in protein synthetic pattern also occurred. In addition, Phillips and McChesney[29] demonstrated a time-dependent alteration, usually an increase, in both total RNA and mRNA synthesis in a cellular system exposed to a pulsed 72 Hz electromagnetic signal. Finally, Marino[30] has reviewed the field of environmental electromagnetic effects from the perspective of public health. The conclusion of his analysis was that "the existence of a link between electromagnetic fields in the environment and disease has been established despite the fact that many important details regarding it remain undiscovered."

The experimental results of the present investigation place constraints on those details. Using a real-time noninvasive and nondestructive measuring technique, the effect of 60 Hz magnetic fields on fundamental biochemical, membrane, and cellular functions such as enzyme activity, electron transport, proton translocation, oxidative metabolism, ion transport, and cellular communication as measured by bioluminescence in *V. fischeri* is less than 1%, if it exists at all. Extrapolating the results of the present work to other systems suggests that we must look elsewhere for an understanding of the reported effects. This understanding at the molecular level is a challenging problem that remains to be solved.

Acknowledgments— I thank J. Jacoby, F. W. Holtzclaw, and D. A. Bilbrey, my teachers in physics, biology, and chemistry; B. D. Faison, J. M. Norman, and H. I. Adler for instruction in microbiological technique; C. D. King for preparing the technical illustrations; D. J. Weaver for secretarial support; J. Trubatch and P. C. Gailey for providing technical information

in the early stages of this research; and E. Greenbaum for instruction in light detection. I also thank the Chemical Technology Division, Oak Ridge National Laboratory for loan of selected equipment. By use of this equipment this project was partially supported by the Office of Basic Energy Sciences, U.S. Department of Energy. Oak Ridge National Laboratory is managed by Martin Marietta Energy Systems, Inc. for the U.S. Department of Energy under contract DE-AC05-84OR21400.

REFERENCES

1. Frankel, R. B. in *CRC Handbook of Biological Effects of Electromagnetic Fields*, C. Polk and E. Postow, Eds. (CRC Press, Boca Raton, Florida, 1986), pp. 169-196.
2. Moore, R. L. (1979) Biological effects of magnetic fields. *Can. J. Microbiol.* **25**, 1145-1151.
3. Gerencer, V. F., M. F. Barnothy and J. M. Barnothy (1962) Inhibition of bacterial growth by magnetic fields. *Nature* **196**, 539-541.
4. Schaarschmidt, B., I. Lamprecht and K. Müller (1974) Influence of a magnetic field of the uv sensitivity in yeast. *Z. Naturforsch* **29b**, 447-448.
5. Blatt, F. J. and Y. Kuo (1976) Absence of biomagnetic effects in *Nitella*. *Biophys. J.* **16**, 441-444.
6. Wiessbluth, M. in *Magnetic Field Effects in Biological Systems*, T. Tenforde, Ed. (Plenum Press, New York, 1979) p. 44.
7. Gaffey, C. T. and T. S. Tenforde (1980) Electrical properties of conducting frog rana-pipiens sciatic nerves exposed to high direct current magnetic fields. *Bioelectromagnetics*, **1**, 208.
8. Florig, H. K. (1992) Containing the costs of the EMF Problem. *Science*, **257**, 468-492.
9. Stone, R. (1992) Polarized debate: EMFs and Cancer. *Science*, **258**, 1724-1725.
10. Hutchinson, F. (1992) ELF-field health effects: biological or illogical? *Phys. Today*, **45**, 13.
11. Richards, B. (1993) Elusive threat: electric utilities brace for cancer lawsuits though risk is unclear. *Wall Street Journal*, February 5, 1993, p. A1.
12. Hastings, J. W. (1986) Why Luminous bacteria emit bright light - and sometimes do not. *Proc. IV ISME*, p. 249-252.
13. Eberhard, A. and J. W. Hastings (1972) A postulated mechanism for the bioluminescent oxidation of reduced flavin mononucleotide. *Biochem. Biophys. Res. Comm.* **47**, 348-353.
14. Tu, S.-C. and H. I. X. Mager (1991) in *Photobiology: The Science and its Applications*, E. Riklis, Ed. (Plenum Press, New York), p. 319-328.
15. Dunlap, P. V. (1992) Mechanism for Iron Control of the *Vibrio fischeri* luminescence system: Involvement of Cyclic AMP and Cyclic AMP Receptor Protein and Modulation of DNA Level. *J. Biolumin. Chemilumin.* **7**, 203-214.
16. K. H. Nealson (1977) Auto induction of bacterial luciferase occurrence mechanism and significance. *Arch. Microbiol.* **112**, 73-79 (1977).
17. K. H. Nealson, T. Platt, and J. W. Hastings (1970) Cellular control of the synthesis and activity of the bacterial luminescent system. *J. Bacteriol.* **104**, 313-322.
18. A. Eberhard (1972) Inhibition and activation of bacterial luciferase synthesis. *J. Bacteriol.* **109**, 1101-1105.
19. A. Eberhard, A. L. Burlingame, C. Eberhard, G. L. Kenyon, K. H. Nealson, H. J. Openheimer (1981) Structural identification of auto inducer of photo-bacterium-*fischeri luciferase*. *Biochemistry* **20**, 2444-2449.
20. S. M. Zubkova, A. I. Zhuravlev and V. M. Abramov (1972) Study on the effect of cm-range electromagnetic oscillations on the intensity of luminescence of luminous bacteria. *Trudy Moskovskogo Obshchestva Ispytateley [Transactions of the Moscow Society of Naturalists]* **39**, 116-120.
21. G. B. Bochkova, S. V. Yermolin and B. S. Rodichev (1985) The influence of an SHF electromagnetic field on the bioluminescence of *V. harveyi*. *Radiobiologiya* (Radiobiology) **25**, 362-366.
22. V. N. Petushkov (1985) Absence of influence of a magnetic field on the intensity of bacterial luminescence. *Biofizika* **30**, 380-382.
23. H. L. Anderson and E. R. Cohen, in *A Physicist's Desk Reference*, H. L. Anderson, Ed. (American Institute of Physics, New York, 1989) p. 32.
24. M. P. Greenbaum, paper presented at the 21st Annual Meeting of the American Society for Photobiology, Chicago, IL, 26-30 June 1993.
25. R. K. Adair (1991) Constraints on biological effects of weak extremely-low-frequency electromagnetic fields. *Phys. Rev A* **43**, 1039-1048.
26. R. Goodman, C. A. L. Basset and A. S. Henderson (1983) Pulsing electromagnetic fields induce cellular transcription. *Science* **220**, 1283-1285.
27. R. Goodman, L.-X. Wei, J.-C. Xu and A. Henderson (1989) Exposure to human cells to low-frequency electromagnetic fields results in quantitative changes in transcripts. *Biochim. Biophys. Acta* **1009**, 216-220.
28. R. Goodman and A. S. Henderson (1991) Transcription and translation in cells exposed to extremely low frequency electromagnetic fields. *Bioelectrochem. Bioenerget.* **25**, 335-355.
29. J. L. Phillips and L. McChesney (1991) Effect of 72 Hz pulsed magnetic field exposure on macromolecular synthesis in CCRF-CEM cells. *Cancer Biochem Biophys* **12**, 1-7.
30. A. A. Marino, "Environmental electromagnetic energy and public health," in *Modern Bioelectricity*, A. A. Marino, Ed. (Dekker, New York, 1988), p. 965-1044.