Bioelectromagnetics 16:231–240 (1995)

Applied AC and DC Magnetic Fields Cause Alterations in the Mitotic Cycle of Early Sea Urchin Embryos

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This study demonstrates that exposure to 60 Hz magnetic fields (3.4–8.8 mT) and magnetic fields over the range DC-600 kHz (2.5–6.5 mT) can alter the early embryonic development of sea urchin embryos by inducing alterations in the timing of the cell cycle. Batches of fertilized eggs were exposed to the fields produced by a coil system. Samples of the continuous cultures were taken and scored for cell division. The times of both the first and second cell divisions were advanced by ELF AC fields and by static fields. The magnitude of the 60 Hz effect appears proportional to the field strength over the range tested. The relationship to field frequency was nonlinear and complex. For certain frequencies above the ELF range, the exposure resulted in a delay of the onset of mitosis. The advance of mitosis was also dependent on the duration of exposure and on the timing of exposure relative to fertilization.

Key words: ELF, RF, cell division time, developmental effects, mitosis

INTRODUCTION

Biological effects of applied magnetic fields have been observed with a variety of field strengths, pulse shapes, and frequencies. Cellular, physiological, and behavioral effects have been described [Persinger, 1974; Chiabrera et al., 1984; Hansson, 1984; Polk and Postow, 1986]. Although the literature is mixed on this issue, several epidemiological studies exist that link exposure to ambient ELF (DC-1 kHz) fields to the incidence of various kinds of cancer [for review, see Polk and Postow, 1986; Stone, 1992].

There are several studies showing that magnetic fields influence the cell division rate in embryonic cells. A 100 μT, 100 Hz pulsed EM field promoted cell proliferation in induced and uninduced embryonic carcinoma cells [Akamine et al., 1984], and AC magnetic fields induced DNA synthesis in mature frog erythrocytes [Grattarola et al., 1985]. Growth of chick embryonic cells in vitro is inhibited by 100 μT, 60 Hz EM fields [Chen, 1974], whereas pulsed EM fields can accelerate or inhibit embryonic chick growth depending upon the pulse shape [Rooze et al., 1982; Saha et al., 1982]. Evolutionarily distant organisms have shown alterations in the cell cycle resulting from exposure to ELF fields. In earlier studies, 200 μT, 75 Hz fields produced alterations in the timing of the mitotic cycle of the slime mold *Physarum polycephalum* [Goodman et al., 1976; Chiabrera, 1984], and 100 μT, 60 Hz fields produced significant delays in Medaka fish egg development [Cameron et al., 1985].

Direct teratogenic effects of these fields have also been observed [for review, see Cameron et al., 1993]. Certain frequencies tend to produce a large percentage of various developmental abnormalities in the embryonic chick [Juutilainen and Saali, 1986]. Pulsed fields with very specific pulse shape geometries produce anatomical abnormalities in developing chicks [Ubeda et al., 1985], exhibiting a window effect with respect to field strength, ruling out the involvement of induced heating. These fields altered the formation of the truncal nervous system and foregut and produced alterations in cell-cell contact behavior in walls of developing blood vessels. One hundred Hertz EM fields result in a lack of morphogenesis in developing chicks [Delgado et al., 1982]. ELF medium-strength fields increase mortality of larvae and decrease egg viability of *Drosophila* [Ramirez

Received for review September 4, 1994; revision received October 25, 1994.

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et al., 1983]. Developmental abnormalities have also been reported for *Drosophila* following exposure of the embryos to weak static magnetic fields [Ho et al., 1992].

Because of the ease in maintaining adults and in obtaining large quantities of developmentally synchronous embryos, the sea urchin is a well studied developmental system. The presence of magnetic field effects reported here affords an opportunity for the study of biochemical and biophysical mechanisms of such effects. There have been a few experimental studies of applied ELF field effects on sea urchin embryos. Falugi et al. [1987] showed that there was an acceleration of early development in the sea urchin *Paracentrotus lividus* resulting from the application of a pulsed (square wave) ELF field. Cameron et al. [1993] have reported that extremely weak magnetic fields cause developmental delay in *Strongylocentrotus purpuratus* sea urchin embryos at the morula stage. Our initial studies [Levin et al., 1991] showed that medium-strength DC fields can significantly affect early urchin development. In studies presented here, we demonstrate that AC and DC fields alter the timing of mitosis in the early *Strongylocentrotus purpuratus* sea urchin embryo. The regulation of mitotic timing is a rapidly expanding field; an understanding of magnetic field effects may add to our understanding of the process.

MATERIALS AND METHODS

Experimental Design for AC Field Exposure

A 10-cm-tall, 3.5-cm-radius copper wound cylindrical coil (resistance 4.2 Ω, inductance 0.018 H), driven by an Industrial PowerTech 5900 AC generator, was used to produce a homogenous (to within 5% at full coil diameter) AC electromagnetic field (Fig. 1). The field magnitude and homogeneity were checked with a gaussmeter (Walker Magnetics model MG-4D). The field strengths reported in this paper are RMS values. The field wave-form characteristics were checked using an oscilloscope and were determined (by inspection) to be pure sine waves.

Extensive controls were used to ensure that any observed effects were due to the fields alone and not to the ohmic heating of the coil. The current generator was outside of the incubator. Insulation was used to prevent the heating of the coil from influencing the cultures. The temperature for all experiments was 12 °C and did not vary by more than ±0.5 °C between the test and control cultures as determined by continuous monitoring with a Quiksite Red Liquid thermometer (VWR Scientific). Test and control cultures were both incubated as described below in 250 ml beakers in the same incubator. To guard against differential heating effects due to the motors, styrofoam blocks were used to insulate the stirring motors from the cultures. The magnetic environment of the entire enclosure of the incubator was investigated with a gaussmeter and was determined not to be different from the ambient DC and AC fields (within 50 μT). The stirring motors were the same in all experiments and were found not to produce any detectable stray fields (detection level 10 μT). This rules out possible effects from magnetic fields generated by the incubator.

Embryo Culture

Embryos of the sea urchin *Strongylocentrotus purpuratus* were cultured with stirring in Millipore-filtered sea water at 12 °C in 250 ml beakers [Pittman and Ernst, 1984]. Successful fertilization was determined by elevation of the fertilization membrane generally within 90 s of sperm addition. Fertilization was greater than 95% in all of the experiments. Eggs from several females were pooled to ensure that individual differences would not bias the results.

Sampling

The sampling procedure was carried out according to Falugi et al. [1987], with slight modification. During the continuous experiments, the field was left on during sampling. Samples of about 200 embryos each were taken approximately every 15 min during the time of first and second cell divisions, fixed with 3% formaldehyde, and scored for the number of blastomeres in each embryo.

The number of cell divisions per 100 embryos was plotted against time to obtain the time course of the development of a given culture. Curves were fitted to the individual points using a low-pass interpolation algorithm (Matlab; Mathworks, Inc.). The difference in division time between the control and treated cultures was determined from the 50% division time on each curve (D_{50}), computed as the average of the differences between the curves on evenly spaced intervals in the middle third of each of the two division phases. Calculations were done with a *Matlab* script written specifically for this purpose on a DEC Vax-11. Plots were constructed using a combination of Matlab, custom C, and Fortran routines written by M. Levin. To normalize results from one experiment to the next, a batch of eggs was fertilized and divided into control and field-exposed cultures immediately following fertilization. The division time of the control culture is set at 100% for each experiment, and the difference between the control and field-exposed cultures is reported as percentage advanced (or delayed) relative to control. Reproducibility was demonstrated by performing five replications of a study with sham and real exposures to a 60 Hz, 4.8 mT magnetic field (see below).

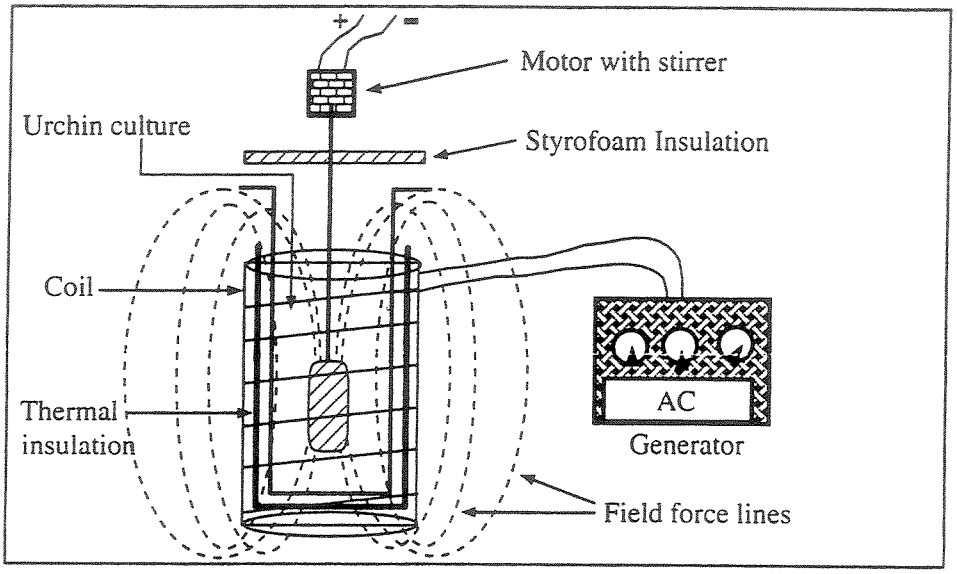

Fig. 1. Apparatus for AC field exposure of embryo culture.

RESULTS

Natural Variation of Cell Division Rate in Identical Nonexposed Embryo Cultures

Control experiments were done to determine the natural variation between two cultures of embryos separated after fertilization that were not under the influence of an applied field. The time course of the first and second cell divisions was quantified by taking samples of about 200 control and 200 experimental embryos approximately every 15 min for calculation of the number of divisions per 100 embryos for each culture.

Data for one experiment, shown in Figure 2, demonstrate that the natural time difference between the curves

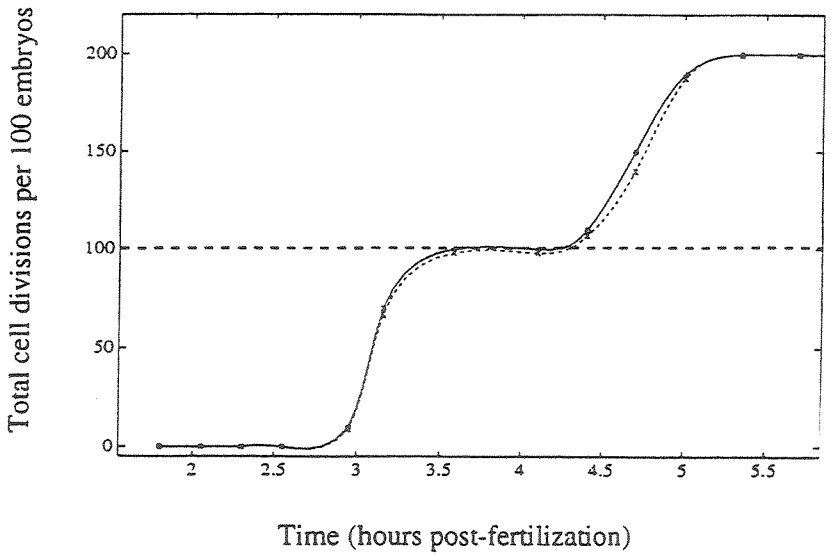

Fig. 2. Natural variation in the timing and scoring of the first two cell divisions of two identical cultures. Eggs were fertilized, and the culture was divided within 5 min of sperm addition. Both cultures were incubated and samples were taken every 15 min. The curve was fit to the points as described in Materials and Methods. There was, at most, a ±3 min difference between the curves.

and the uncertainty of the scoring procedure average less than ±3 min (based on five experiments). The time course of cultures of different experiments cannot be compared directly, since eggs from individual females begin divisions at different times (as shown in Fig. 9).

Advance of the Time of First and Second Cell Divisions as a Function of Field Strength

In this series of experiments, exposure began at fertilization and was maintained throughout the experiment. The 60 Hz magnetic induction varied from 1.7 to 8.8 mT in separate experiments. Figure 3A shows data for embryos exposed to 8.8 mT. In this experiment, the first cell division was advanced by 60 min. The relationship between the advance of the first and second cell cycle relative to controls and the field strength for this experiment (Fig. 3A) and several others are shown in Figure 3B, where each point represents a separate experiment and is calculated from graphs similar to that shown in Figure 3A. The lowest field strength, 1.7 mT, did not produce a measurable effect on the timing of the first or second cell divisions, whereas, at 3.4 mT, the exposed embryos showed an advance in the time of both the first and second cell divisions. Exposed embryos were 12% ahead of the controls for the first cell division and were 14% ahead of the controls for the second cell division. At the highest field strength tested, 8.8 mT, the first cell cycle was advanced 32% relative to the control and the second cell cycle was advanced 35% relative to the control culture. At a frequency of 60 Hz, the advance of the first and second cell divisions was essentially linear with field strength up to 8.8 mT (Fig. 3B). The shortening of the cell cycle appeared to result from premature entry into mitosis or from an advance of the time of mitosis, rather than from a shortening of mitosis, for two reasons: 1) The slopes of the cell division curves were generally the same for control and exposed cultures (for example, see Fig. 3A), and 2) the actual time of mitosis represents only a small portion of the total cell cycle, whereas the time between divisions accounts for the majority of the cycle (Fig. 2).

Effect on Gastrulation and Effect of Exposure of Sperm

In all of the experiments in which an advance was seen at the first two cell divisions, the embryos also reached gastrulation before their corresponding controls (approximately 36 h), indicating that some advance was maintained (data not shown). However, since later divisions are much more difficult to score precisely, all counts were done on the first and second cell divisions only. To determine whether the sperm or the eggs were affected by the field, several experiments were performed wherein the sperm alone were exposed to fields used in this study for 1 h and were then used to fertilized nonexposed eggs. In all these experiments, exposure of sperm had no effect on the timing of cell division (data not shown).

Alterations in the Timing of the First and Second Cell Divisions as a Function of ELF Frequency (0–420 Hz)

A 4 mT magnetic field of various ELF frequencies or a static field was applied to separate cultures of embryos immediately after fertilization. The field was maintained throughout the course of the experiment. The results are summarized in Figures 4 and 5, where each bar represents a single experiment. Figure 4 shows the effect on the length of the cell cycle as a result of exposure to various frequencies. Surprisingly, in embryos exposed to a static field, the first cell division occurred 11% ahead of the first division in the controls, and the second division was 9% ahead of controls at the second division. Each frequency tested between 0 and 420 Hz produced an advance of the first and second cell division in all treated samples relative to the control. However, the relationship between the magnitude of the effect and the frequency was complex. An advance in the time of the first two cell divisions by at least 10% relative to the cell division times of control samples was observed in embryo cultures exposed to 60 Hz, 240 Hz, and 360 Hz fields. At these frequencies, the time of first cell division was 10–12% ahead of the controls, and the time of the second division was approximately 12–13% advanced relative to nonexposed cultures. In contrast, at the same field strength (4 mT), field frequencies of 100, 150, and 420 Hz produced barely detectable (≤2%) advances for the first cell division and produced only 4–6% advances for the second division. With a static magnetic field, the advance of the timing of the first cell division was greater than the advance seen at the second division. In contrast, it was observed that, for all ELF fields tested, the advance at the second cell division is greater than or approximately equal to that at the first, suggesting an additional effect at the second division (Fig. 4).

The data were also plotted as relative intermitotic duration instead of percentage advanced (Fig. 5). The relative intermitotic duration was the time between the point at which 100% of the embryos had reached the two-cell stage to the point at which they started to enter the four-cell stage in exposed, relative to control, cultures. In Figure 5, it can be seen that the time between the first and second divisions is shortened as a result of exposure to a 4.0 mT static magnetic field and at frequencies from 25 to 420 Hz. Since the time between divisions accounts for the majority of the cell cycle (Fig. 2), these results (Fig. 5) in comparison to Figure 4 demonstrate

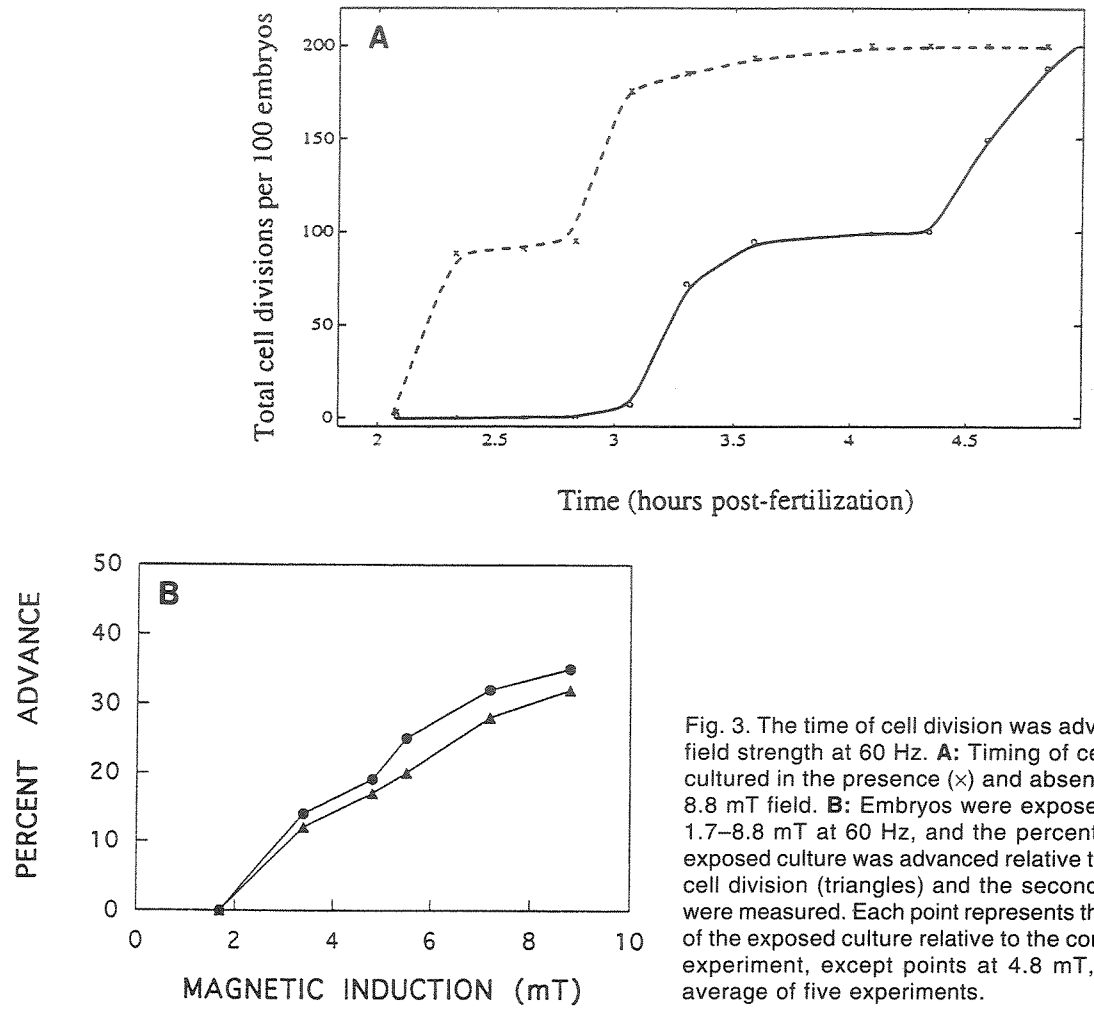

Fig. 3. The time of cell division was advanced as a function of field strength at 60 Hz. **A:** Timing of cell division of embryos cultured in the presence (×) and absence (circles) of a 60 Hz 8.8 mT field. **B:** Embryos were exposed to field strengths of 1.7–8.8 mT at 60 Hz, and the percentage of time the field-exposed culture was advanced relative to the control at the first cell division (triangles) and the second cell division (circles) were measured. Each point represents the percentage advance of the exposed culture relative to the control culture in a single experiment, except points at 4.8 mT, which represent the average of five experiments.

that it is the shortening of the time between divisions that accounts for the advance in the time of cell division and not the time of the actual division itself.

Alterations in the Timing of the First and Second Cell Divisions as a Function of a Wide Range of Frequencies (0–600 kHz)

A 2.5 mT AC magnetic field of various frequencies (in the range of 0–600 kHz) in and above the ELF range was applied to a culture of embryos immediately after fertilization to determine the effects of higher frequency magnetic fields on the timing of the first and second cell divisions (Fig. 6). A 2.5 mT field was used, rather than the 4.0 mT field used in the previous experiments, because of the limitations of the generator. The field was maintained throughout the course of the experiment. Over this wide range of frequencies, a 2.5 mT field altered the time of mitosis ≤10% relative to control cultures (Fig. 6). Interestingly, the greatest alteration was an advance in the timing of both the first and the second cell divisions produced by a static magnetic field. At 0.6 kHz, the field also produced an advance in the time of cell divisions as was seen in previous experiments. However, at the higher frequencies, the embryos in the exposed cultures consistently showed a delay in the time of cell division relative to controls. Over the wide frequency range, the effect is exponentially related to field frequency, reversing itself (becoming a delay) by 6 kHz, and, apparently, saturating by about 60 kHz.

Alterations in the Timing of the First Two Cell Divisions as a Function of Exposure Duration

In all the experiments described so far, field exposure was constant from fertilization to the time of sampling. To determine the relationship between the time of the first and second cell divisions and the length of time the embryos were exposed to the EMF, a series of experiments was performed at 6.5 mT, 240 Hz. Individual cultures were exposed to the field from fertilization to a stop point at selected times from 30–300 min

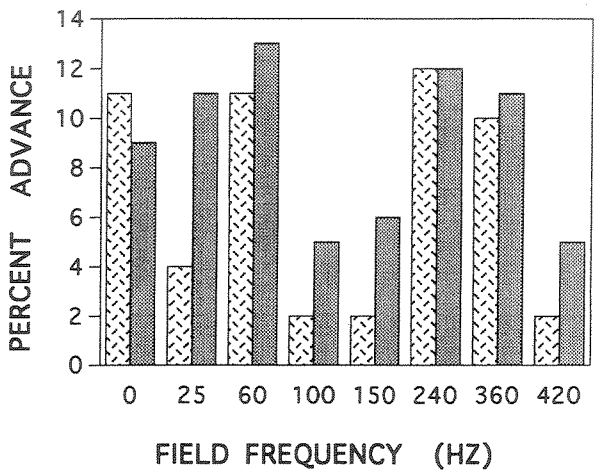

Fig. 4. Advance of the time of the first two cell divisions as a function of ELF frequency at 4.0 mT. The percentage advance of the exposed cultures relative to the controls is shown for the first (hatched bars) and the second (shaded bars) cell divisions. Note that the 0 Hz point corresponds to an applied DC field.

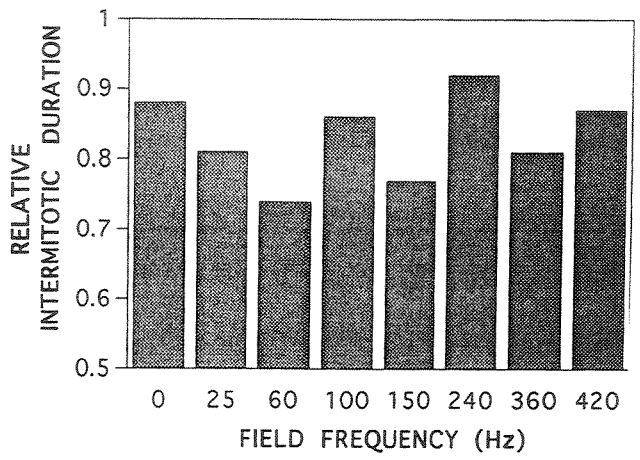

Fig. 5. Duration of the two-cell stage of the exposed embryos relative to that of controls as a function of ELF at 4.0 mT. The relative intermitotic duration is the time when all exposed embryos completed the first cell division until the time they started the second division, relative to those in the control culture.

postfertilization. In Figure 7, the duration of exposure as a percentage of the first cell cycle is plotted against the percentage of the advance of the first and the second cell division times in field-exposed embryos compared to controls. Embryos exposed immediately following fertilization for approximately 15% of the cell cycle showed a slight advance in the time of their first division, 5% ahead of the control culture. Interestingly, there was no detectable effect on the time of the second division. It appeared that this short exposure advanced the first division, but, by the second division, the embryos went back to their "normal" time of division. At all stop points, exposure to the field was terminated by the end of the first cell cycle. However, when embryos were exposed to the field for 20% or more of the first cell cycle, the timing of the first cell cycle was not

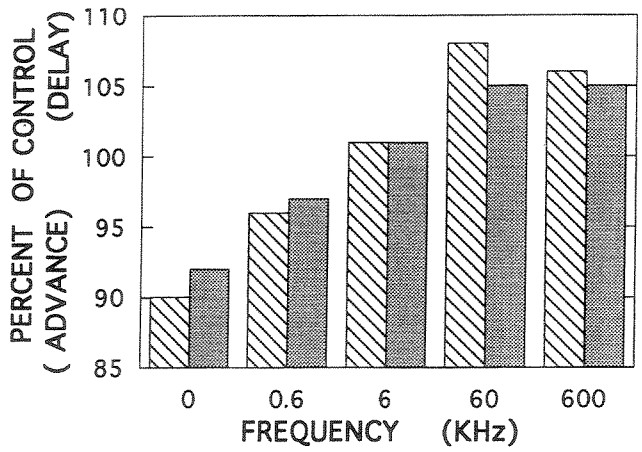

Fig. 6. Timing of the first two cell divisions as a function of frequency at 2.5 mT. The hatched bars represent advanced or delays in the time of cell division in exposed cultures relative to controls at the first cell division, and the stippled bars represent the difference at the second division. Ordinate values greater than 100% indicate slower (delayed) exposed cultures, while ordinate values less than 100% represent faster (advanced) cultures relative to controls.

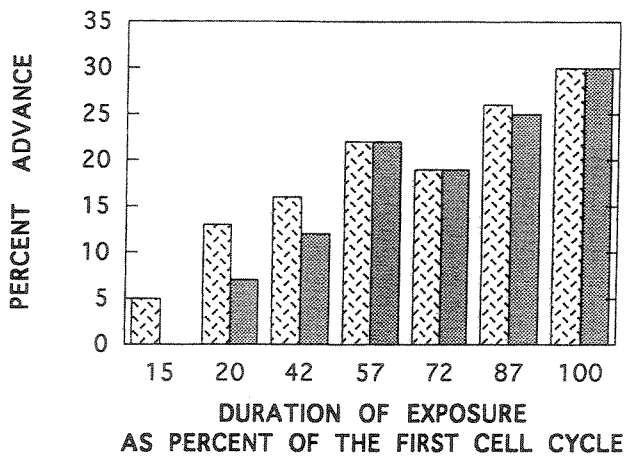

Fig. 7. Advance of the time of first two cell divisions as a function of duration of exposure as a percentage of the control first cell cycle. Embryos were exposed to a 6.5 mT, 240 Hz field for varying portions of the first cell cycle of the control. For each time point, the first bar shows the advance of the first cell cycle, and the second bar represents the advance in the time of the second cell division.

corrected, and, when embryos were exposed for at least 57% of the first cell cycle, there was an additional effect on the timing of the second cell division. The progressive increase in the percentage advance of the time of the first and second cell divisions relative to the duration of EMF exposure suggests that the effect is cumulative (Fig. 7).

Effect of Delayed Exposure on the Second Cell Division

Another experiment was done in which embryos were exposed for 1 h to an AC field of 6.5 mT, 240 Hz starting just after the first division. Exposure produced an advance of 11 min for the time of the second cell division (Fig. 8).

Endogenous Variation in Division Initiation Time: Controls and Selected Magnetic Field-Exposed Embryos

The population of urchins used in these experiments shows a significant variation in the time of the initiation of the first cell division, a commonly observed phenomenon (Ernst, unpublished). In contrast, it was observed that the exposed embryos showed less variation than equivalent nonexposed cultures. To quantify this relationship, the times of the initiation of first division were plotted for 26 control cultures for the experiments shown here and for other experiments in the series (Fig. 9A). Figure 9A shows the times of the initiation of the first cell division in 16 experimental cultures from this study, each exposed for at least 1 h to an EMF and selected for an advance in the time of the first cell cycle by at least 5% of the control cell cycle. It can be seen that the range of the initiation times of control cultures varies by 65 min, whereas that of the selected exposed embryos extends over only 23 min.

DISCUSSION

Effects on the Timing of the First Two Cell Divisions

We have demonstrated that sea urchin embryos exposed to ELF AC fields exhibit dramatic alterations in the timing of the first and second cell divisions following fertilization. At 60 Hz, field strengths of 3.4 mT produced a significant advance in the timing of both the first and second cell divisions. We found no response at 1.7 mT and a linear response between 3.4 and 8.8 mT. In higher organisms, the length of the cell cycle during the cleavage stage of embryogenesis is greatly reduced relative to the length of the cell cycle in later embryogenesis and in adult cells. In some organisms, such as *Xenopus* and *Drosophila*, the entire cell cycle is reduced to alternating M (mitosis) and S (DNA synthesis) phases, both of which occur at maximal rates, with the complete elimination of G1 (gap 1) and G2 (gap 2) [Gilbert, 1991]. In sea urchins, M and S are also reported to occur at maximal possible rates [Hinegardner et al., 1964]. However, in sea urchins, there are also short G1 and G2 phases [for review, see Whitaker and Patel, 1990]. Results of our studies demonstrate that the time between

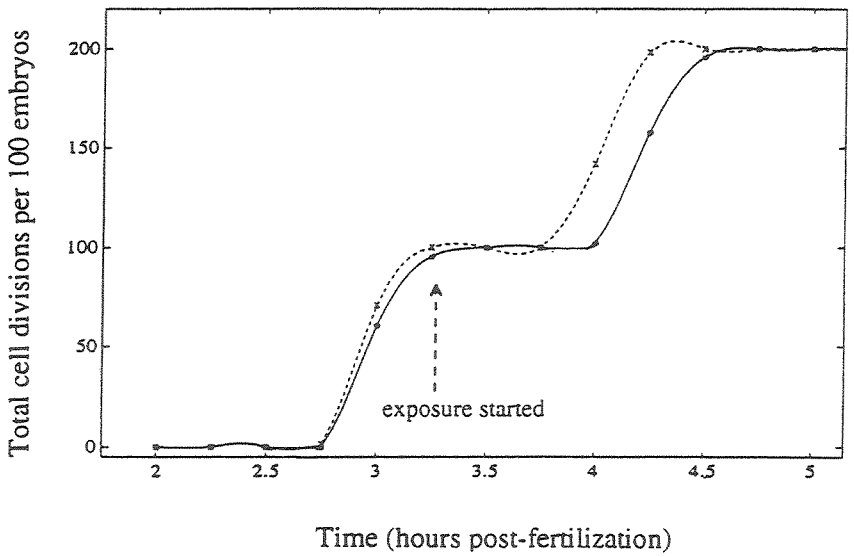

Fig. 8. Advance of the second cell division due to exposure to a 6.5 mT, 240 Hz field at the start of the second cell cycle. Cell divisions for control embryos (circles) and for embryos exposed at start of the second cell cycle (×) are plotted.

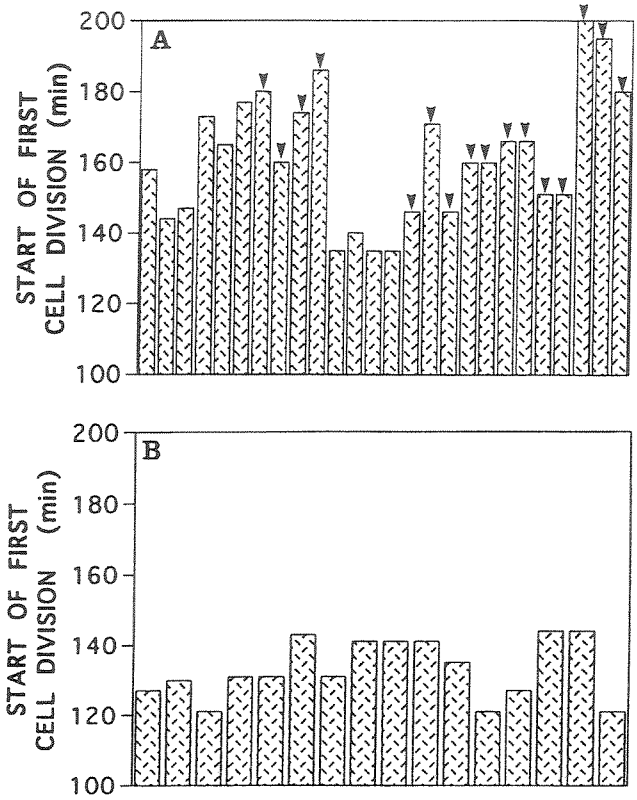

Fig. 9. Variation in the time of the initiation of the first cell division. **A:** Time of the initiation of the first cell division in 26 control fertilizations. The range is from 135 to 200 min, with an average division time of 162 min. An arrowhead over a bar indicates that it is a control for a specific experiment in B. **B:** Time of the initiation of the first cell division in 16 cultures in this study that were exposed to an electromagnetic field that advanced the time of the first cell division by at least 5% of the cell cycle. The range is from 121 to 144 min, with an average division time of 133 min.

consecutive mitotic events (intermitotic duration) is shortened as a result of exposure to the fields in this study. The shortening of the cell cycle appears to be pushing the sea urchin more towards the *Drosophila* and *Xenopus* situation, where M and S phases alternate without intervening G1 and G2 phases.

The data presented in Figure 9 show that this population of sea urchins exhibits a considerable natural variation in the time of the first cell division. Each culture represents eggs from at least two females, and females were not used more than once. The average time for division of the selected set of exposed embryos was 133 min, and the range was 23 min, from 121 to 144 min. However, the earliest division time seen in fertilized eggs from all 26 control cultures was 135 min, and the longest time before division was 200 min; the average division time was 162 min. The exposed embryos, representing 16 fertilizations, never took longer than 144 min to divide. These results suggest that there may be a minimal length of time required for each cell cycle. Otherwise, the variation among the control cultures and the exposed cultures would have been the same, but the average division time for the exposed embryos would have been shorter.

Taken together, the results of the two studies performed on embryos exposed to the same field strength but at different frequencies revealed a complex relationship between frequency and the effect on the timing of the first two cell divisions (Figs. 4–6). Numerous studies in the literature in which field strength is held constant and frequency is varied report the occurrence of window effects on diverse biological processes [Adey, 1981; McLeod et al., 1987; Blackman et al., 1988; Liboff et al., 1989]. The data in Figures 4 and 5 underscore the necessity for studying a wide range of frequencies. Advances in the time of cell division of 10% or greater for both the first and second cell division were observed at 60, 240, and 360 Hz. In addition, an advance of greater than 10% of the first cell cycle was seen in the culture exposed to a static field, and an advance of greater than 10% in the second cell cycle was seen in the culture exposed to 25 Hz (Fig. 4). The greatest effect on the relative intermitotic duration was seen at 60 Hz (Fig. 5).

Exposure to static 2.5 and 4 mT fields produced about a 10% advance in the time of both the first and second cell divisions (Figs. 4, 6). The observation that static fields are about as effective as EMF in advancing the time of the first two cell divisions in the sea urchin embryo was unexpected and has been investigated further [Levin et al., in preparation].

Interestingly, the time of the first cell division was advanced approximately 5% even when the exposure to a 6.5 mT, 240 Hz magnetic field was confined to the first 30 min following fertilization, representing 15% of the control cell cycle (Fig. 7). The magnitude of the advance is steadily increased, as the duration of exposure represents a progressively greater portion of the cell cycle, suggesting that the cellular conditions necessary to record and transmit the EMF effect responsible for premature entry into mitosis are present throughout the majority of the cell cycle. These results also suggest, but do not necessitate, a cumulative effect.

Even though the magnetic fields did not extend beyond the end of the first division in this series of experiments (Fig. 7), the second cell division was also advanced when the field was present for at least 20% of the first cell cycle. However, we observed that, for a 30 min exposure, the exposed batch is ahead for the first cell division, but, then, it is exactly even with the controls for the second cell division. This suggests that the field may be affecting a cellular clock that counts real time from fertilization rather than cell cycle time. That

this mechanism is actually able to delay the cell division process in order to bring the time of second division back to that of the control is a very surprising result and needs to be studied further.

Other investigators have also observed effects of exposure to electromagnetic fields on the timing of developmental events of sea urchin embryos. Cameron et al. [1993], using much lower strength magnetic fields than those employed in this study, demonstrated that a rotating 60 Hz, 50 μT magnetic field delayed, but did not arrest, late cleavage stage embryos. They reported no measurable effects on the timing of the first or other early cleavage stage divisions. Falugi et al. [1987] exposed eggs and embryos of another species of sea urchin, *Paracentrotus lividus*, to a pulsed electromagnetic field, where the magnetic field intensity varied from 0 to 2 mT with a mean value of around 1 mT. They observed advances in the timing of the first and second cell divisions and also observed continued developmental advance at a later stage of development (pluteus). Results from these two labs and from those reported here suggest that exposure to magnetic fields of as little as 50 μT can significantly delay the time of cell divisions in later cleavage stages of sea urchin embryos, and exposure to magnetic fields of greater than 1 mT appear to advance the time of cell divisions throughout embryogenesis.

Possible Mechanisms

Currently, we do not know the mechanism responsible for the effects reported here of EMF on the timing of the first two cell divisions in sea urchin embryos. Also, the relationship between the DC and AC field effects is unknown. Possible mechanisms are discussed below.

Results from other studies found evidence for ion cyclotron resonance (ICR) effects as a possible explanation for why certain frequencies have much greater effects than other frequencies at the same field strength [McLeod et al., 1987; Liboff et al., 1989]. We analyzed our data for possible ICR effects and found that they do not match the expected ICR peaks for any common ions. Similarly, Parkinson and Hanks [1989] found no evidence for ICR in studies on rat osteosarcoma cells. However, more frequencies would have to be studied before conclusively ruling out the ICR effect.

Possible candidates for mechanisms at the biochemical level can be proposed. EM fields could modify the behavior of important ions in a number of ways. For example, EM fields can modify the amount of time an ion spends within a critical distance of membrane receptors [Grattarola et al., 1985]. Effects on calcium, a major regulatory ion that plays a role in sea urchin fertilization and in initiation of cell division [Silver, 1989, 1990; Steinhardt, 1990; Whitaker and Patel, 1990], could be manifest at many levels. The fields may be interfering with calcium ions sequestering, perhaps enabling, the calcium concentration rise to occur earlier, thus, allowing the cells to enter the mitotic cycle earlier.

Several studies indicate that applied ELF magnetic fields can increase the rates of DNA and protein synthesis and induce or alter the rates of production of individual mRNAs and proteins [Liboff et al., 1984; Goodman and Henderson, 1988; Litovitz et al., 1990; Cameron et al., 1993]. Goodman et al. [1983] proposed that these effects may be due to an increase of available Ca^{2+}. This could account for the effect we observed by increasing the rate of the synthesis of cyclin or other cell cycle regulatory proteins.

Electric fields have been shown to shorten the G_2 phase of the cell cycle in mammalian cells in vivo [Mamontor and Ivanova, 1971]. The induced electric fields within the urchin may be an example of such an effect. Cone's laboratory has shown [Cone, 1970, 1974; Cone and Tongier, 1973] that a good correlation exists between cell membrane potential and the mitotic activity of many different types of cells. It is even possible to cause a mature neuron to undergo mitosis by artificially changing membrane potential in the appropriate way.

CONCLUSIONS

We have obtained the following results. 1) AC electromagnetic fields produce an advance in the time of the early cell divisions of sea urchin embryos. The advance is a result of an effect on the fertilized egg and not the sperm alone, and it is linearly dependent on the field strength. 2) The extent of the effect is dependent on, but not proportional to, ELF frequency, and the frequencies that elicit maximum effect do not, under the conditions of our study, match those predicted by the ICR theory for any common biological ion. The effect is exponentially dependent on frequency over a wide range of frequencies. It reverses (becoming a delay) after a certain frequency and, eventually, saturates. 3) The effects of magnetic exposure push the embryos towards a lower limit of how soon after fertilization cell division occurs.

ACKNOWLEDGMENTS

We thank Jorge L. Gonzalez for his assistance in some of the experiments and for many helpful discussions. We are grateful to Mr. Joe Monroe of Industrial Test Equipment Company for the use of the generator and Drs. Barry Trimmer and Ronald Goldner for critically reviewing the paper and for helpful discussions. We also thank the reviewers and editor for their helpful suggestions in reviewing this paper. This work was supported in part by the U.S. Environmental Protection

Agency through the Tufts University CEM, a Biomedical Research Support grant, and the Howard Hughes Medical Institute's Initiative in Undergraduate Education.

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