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# Environmental Magnetic Fields: Influences on Early Embryogenesis

**Ivan L. Cameron, W. Elaine Hardman, Wendell D. Winters, Selma Zimmerman, and Arthur M. Zimmerman**

Departments of Cellular and Structural Biology (I.L.C., W.E.H.), and Microbiology (W.D.W.) University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284; Division of Natural Science, Glendon College, York University, Toronto, Ontario, Canada M4N 3M6 (S.Z.); Department of Zoology, University of Toronto, Toronto, Canada M5S 1A1 (A.M.Z.)

**Abstract** A 10-mG, 50 to 60-Hz magnetic field is in the intensity and frequency range that people worldwide are often exposed to in homes and in the workplace. Studies about the effects of 50- to 100-Hz electromagnetic fields on various species of animal embryos (fish, chick, fly, sea urchin, rat, and mouse) indicate that early stages of embryonic development are responsive to fluctuating magnetic fields. Chick, sea urchin, and mouse embryos are responsive to magnetic field intensities of 10–100 mG. Results from studies on sea urchin embryos indicate that exposure to conditions of rotating 60-Hz magnetic fields, e.g., similar to those in our environment, interferes with cell proliferation at the morula stage in a manner dependent on field intensity. The cleavage stages, prior to the 64-cell stage, were not delayed by this rotating 60-Hz magnetic field suggesting that the ionic surges, DNA replication, and translational events essential for early cleavage stages were not significantly altered. Studies of histone synthesis in early sea urchin embryos indicated that the rotating 60-Hz magnetic field decreased zygotic expression of "early" histone genes at the morula stage and suggests that this decrease in early histone production was limiting to cell proliferation. Whether these comparative observations from animal development studies will be paralleled by results from studies of human embryogenesis, as suggested by some epidemiology studies, has yet to be established. 

**Key words:** electromagnetic fields, embryogenesis, chick, sea urchin, fish, mouse, mitosis, histones

Electromagnetic fields surround any source that carries an electric current and the intensity of the EMF falls off with distance. The EMFs contain two different components, i.e., a magnetic component measured in Tesla or Gauss units and an electric component measured in volts per meter. The electric component can be screened easily by almost any type of conducting material while the magnetic field easily penetrates through most materials without losing intensity. As illustrated in Figure 1, the horizontal intensity of magnetic fields depends on their source and distance from the source. For example, the intensity of the magnetic field surrounding appliances decreases with distance more precipitously than does the intensity of the magnetic field surrounding transmission lines.

Table I and Figure 1 provide information of use in estimating magnetic field exposures in the home and in the workplace. Table I indicates that several sources produce ELF–EMF that are $\geq$ 10-mG magnetic field intensity, and most people are exposed to magnetic fields $<$ 10 mG for sustained periods. Two notable exceptions are users of electric blankets, which some manufacturers have recently redesigned to emit lower intensity fields, and the few who live within 100 meters of a high-voltage transmission line.

Uncertainty has existed about the potential effects of electromagnetic fields on developing embryos. Of particular interest and concern are the biological effects of electromagnetic fields created as a consequence of generation, transport and use of electricity [Pool, 1990; Tenforde, 1992].

Abbreviations: EB, early blastula; EG, early gastrula; ELF, extremely low frequency; EMF, electromagnetic field; MB, mesenchyme blastula; mG, milligauss (measure of magnetic field in CGS system); Mor, morula; mRNA, messenger RNA; PMF, pulsed magnetic field; rms, root mean square; μT, micro Tesla; V/m, volts per meter; VLF, very low frequency

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Address reprint requests to Dr. Ivan L. Cameron, Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, TX 78284.

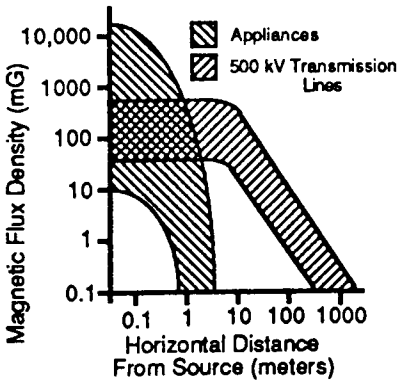


Fig. 1. Magnetic field intensity varies depending on the source and the distance from the source (redrawn from Pool, 1990).

Delgado and co-workers [1982] were first to report that a low-intensity ELF pulsed magnetic field increased the incidence of abnormal development in early chicken embryos. The Delgado report on chicken embryos was followed by several other studies confirming that early developmental stages of chicken embryos were sensitive to pulsed low-intensity μT range (1 μT = 10 mG) ELF-EMF exposures [Leal et al., 1982, 1984; Trillo et al., 1983, 1986; Ubeda et al., 1983, Juutilainen et al., 1987; Martin 1988]. It should be noted, however, that several other investigators were unable to find a similar sensitivity of early chicken embryos to pulsed low-intensity ELF–EMFs [Maffeo et al., 1984, 1988; Sisken et al., 1986; Sandström et al., 1986]. These opposing experimental findings lead to a meeting of several interested scientists for the purpose of designing a carefully controlled experiment to test the effect of a specific pulsed-low intensity ELF–EMF on the development of chicken embryos. This research project has subsequently been referred to as "the hen house project." The design of the experiment called for identical equipment, protocols and embryonic evaluation by investigators in six independent laboratories in four countries [Berman et al., 1990].

## INTERNATIONAL STUDY OF A WEAK PULSED EMF ON CHICKEN EMBRYOGENESIS

In brief, the experimental design of this international project called for supply of two identical incubators to each of the participating laboratories. Pulse generators for the incubators developed a EMF of unipolar form (magnetic field-500-μs pulse duration, 100 pulses per second, 10-mG peak intensity and 2-μs rise and fall

**TABLE I. Sources and Approximate Intensity of Commonly Encountered Magnetic and Electric Fields**

| Source[a] | Frequency | Magnetic flux intensity | Electric field intensity |
|---|---|---|---|
| Transmission power line 115–230 kV, ground beneath[b] | ELF | 10–200 | 800–3,000 |
| Electric blanket | ELF | 75–100 | 100–2,500 |
| Electric shaver | ELF | 1,000–10,000 | 100–400 |
| Toaster | ELF | 70–200 | 100–400 |
| Distribution line | ELF | 2–12 | 50–100 |
| Center of living room | ELF | 0.5–8 | 2–9 |
| Video display unit 30 cm from screen[c] | ELF | 0.6–6 | NR |
| | VLF | 0.2–6 | NR |
| Urban corners[d] | | | |
| Within 46 m of transmission line 23 kV | ELF | 12.9 ± 0.15 | NR |
| Within 46 m of three phase primary transmission line, thick wire | ELF | 6.4 ± 0.30 | NR |
| Home-front door | ELF | 0.10–4.7 | NR |
| Front side walk | ELF | 0.10–13.1 | NR |

[a]ELF, extremely low frequency (30–300 Hz); VLF, very low frequency (300–3,000 Hz).
[b]Data from Morgan et al. [1990].
[c]Data from Tofani and D'Amore [1991]; NR, not reported.
[d]Data from Dlugosz et al. [1989].

times of the current pulse). The magnitude of the orthogonal components of the electric field were 0.05, 0.10, and 0.12 V/m in the vertical, horizontal, and axial directions, respectively. Fertilized hen eggs were exposed to this ELF–EMF field or to a control field (in an incubator with an inactivated pulse generator) for 48 h at 37.6–38.0°C prior to evaluation of the eggs for fertility, stage of development, and presence of abnormalities. Evaluations were performed by trained personnel without knowledge of the treatment conditions of each embryo.

The design of the experiment allowed statistical analyses of significant differences in measured endpoints that could be attributed to the PMF exposure condition; the laboratory site where the experiment was done and any interaction between these two factors. The analyses of pooled results of each of five endpoints from the six laboratories in the study showed a significant increase in abnormal embryos due to exposure to a 10-mG pulsed magnetic field ($P < 0.001$). The mean incidence of abnormal embryos was 25% for the PMF condition and 19% for the sham control condition. The factor or factors causing the significant interlaboratory differences remain(s) unknown.

The overall conclusion from this ambitious multi-laboratory study was that a pulsed EMF–EMF of 10 mG can have a significant effect on development and on incidence of abnormalities in chicken embryos.

## ROTATING AND PULSED ELF-EMFs ON DIFFERENT EMBRYONIC SYSTEMS

Table II lists examples from three types of animal embryos; Japanese rice fish, purple sea urchin, and laboratory mice, that have been reported to be responsive to a rotating 60-Hz magnetic field. Based on the data listed in Table II and reports of effects of pulsed EMF on early embryonic development in domestic chicken [Berman et al., 1990], fruit fly [Ramirez et al., 1983], sea urchin [Falugi et al., 1984], and laboratory rat [Zusman et al., 1990], it can be concluded that the embryos of these animal species are responsive to weak EMF.

**TABLE II. Summary of Embryonic Systems Reported Responsive to 60-Hz Electromagnetic Fields**

| System | Condition, effect, and reference |
|---|---|
| Japanese rice fish[a] | 60-Hz electric 300 mA/m$^2$, exposed for 48 h postfertilization, no delay of development [Cameron et al., 1985] |
| | Rotating 60-Hz magnetic 1,000 mG (rms), exposed for 48 h postfertilization, delay of development [Cameron et al., 1985] |
| | 60-Hz combination of above two field exposed for 48 h postfertilization, delay of development [Cameron et al., 1985].[a] |
| Purple sea urchin | Rotating 60-Hz magnetic field, 1,000 mG (rms), exposed for 23 h postfertilization, delay of development [Zimmerman et al., 1990] |
| Laboratory mouse | Rotating 60-Hz magnetic field 100–500 mG, 2-cell stage exposed for 48–68 h, delay of development (see Fig. 4) |

[a]It was concluded from the combined studies on Japanese rice fish embryogenesis that the delay in development was due to the magnetic field, and not to the electric field.

## LOWEST EFFECTIVE MAGNETIC FIELD INTENSITY

Juutilainen et al. [1987] indicated that a 13-mG (rms) field caused a significant increase in chicken embryo abnormalities. Juutilainen et al. found that all field intensities tested above 13 mG (to 1,300 mG) caused increased abnormalities. On the other hand, combined findings from several studies in another laboratory [Leal et al., 1984, 1986; Ubeda et al., 1983, 1985] suggested that the effect of field intensity on the incidence of chicken embryo abnormalities was nonlinear. Ubeda et al. and Leal et al. showed that peak field intensity between 10 mG and 140 mG caused increased abnormalities, while field intensities within the 250- to 1,000-mG range were ineffective in causing abnormalities. Based on the results from these two laboratories, the question of "window of sensitivity" to magnetic field intensity remains unclear but exposure to a field intensity of 10–140 mG induced increased abnormalities in both laboratories. Once again, it is necessary to point out that other investigators [Maffeo et al., 1988; Siskin et al., 1986; Sandström et al., 1986; Martin 1992] have failed to find a significant increase in abnormalities in chicken embryos exposed to PMF.

Magnetic field parameters other than field intensity must be taken into account when making comparisons between different studies. These other parameters include the waveform or "shape" of the pulsed wave, the pulse frequency and the orientation of the egg in the field [Juutilainen et al., 1987; Berman et al., 1990; Saha et al., 1983; Martin 1992]. In addition, questions of different experimental conditions must always be considered as a source of variable results.

## ROTATING 60-Hz MAGNETIC FIELDS ON SEA URCHIN EMBRYOGENESIS

The sea urchin provides a favorable embryonic system to answer questions concerning stages in development with greater or lesser degrees of sensitivity to ELF–EMF and mecha-

nisms of action. Sea urchin embryos were selected to assess the effects of a rotating 60-Hz magnetic field because of (1) ease of handling; (2) synchrony of embryonic development in mass cultures; (3) permeability to protein and nucleic acid precursor molecules; and (4) because much is already known about genomic transcription processes in sea urchin embryos. The specific field was designed to stimulate the magnetic field parameters associated with high-power electric transmission lines [Lucus and Johnson 1984; Winters 1986]. In brief, the exposure chambers used consisted of two identical magnetic field exposure chambers each equipped with two pairs of Helmholtz coils 5 feet in diameter. The control (sham-exposure) chamber did not have a locally-generated rotating 60 Hz magnetic field because the coils were not energized. The experimental (field-exposed) chambers included a rotating 60-Hz magnetic field produced by energization of the two pairs of coils, with one pair orthogonal and 90 degrees out of phase with the other. The two exposure chambers were 10 m apart, so the sham exposure chambers received some stray field from the field-exposure apparatus. For example, when the field exposure apparatus was at 500 mG, the sham exposure area was at 0.3 mG. The experiments were performed under local geomagnetic field strengths, earth's "DC" magnetic field, of 0.438G vertical and 0.256G horizontal.

A summary of two recent reports on the effects of 60 Hz ELF-EM fields is presented here [Zimmerman et al., 1990; Cameron et al., 1992]. The purple sea urchins (*Strongylocentrotus purpuratus*) were obtained from Pacific Bio-Marine Laboratories, Venice, CA. Gamete release was stimulated by intracoelomic injections of 0.5 M KCl. Eggs were collected in ice-cold artificial seawater (Instant Ocean, Aquarium Systems, Inc., Wickliffe, OH) and washed twice before insemination. Sperm were collected and kept over crushed ice; minimal sperm concentrations were used for egg fertilization. All batches of eggs used in these experiments showed at least 98% fertilization as judged by fertilization membrane elevation within 60 s after the addition of sperm. Throughout the experiments, fertilized eggs were maintained in 20-ml of seawater in 250-ml beakers. Beakers with fertilized eggs (2 min postinsemination) were placed in the 60-Hz magnetic field exposure chambers or in a control chamber in which there was no applied magnetic field and were maintained at 18°C (range 17.5–18.5°C). Magnetic-field exposure of the embryos was continuous and samples of eggs were collected from experimental and control beakers and observed at various times during the experiment.

The field exposure chamber was located 10 m from the control chamber and the eggs in the control chamber received about 0.1% of the time varying magnetic-field exposure stemming from the experimental chamber. Thus, for example a 1,000-mG field generated in the field exposed chamber was found to cause a 1.0-mG field in the sham-exposed chamber, a 100-mG field in the field-exposed chamber caused a 0.2-mG field in the sham-exposed chamber and any field less than 100 mG in the field-exposed chamber caused a field of $<0.2$ mG in the sham-exposed chamber (lowest level detectable with our Gauss meter).

Independent experiments were run to help determine the magnetic field intensity responsiveness of sea urchin embryos [Cameron et al., 1992]. Assessment of the developmental progress of embryos was made by counting the number of cells in each embryo. Morula (Mor), early blastula (EB), mesenchyme blastula (MB), and early gastrula (EG) stages of development were scored according to Morell [1986]. There was a significant, $P < 0.01$, linear decrease in mean number of cells per embryo when magnetic field intensity was correlated with the mean number of cells per embryo. These results indicate that a rotating 60 Hz magnetic field of greater than 0.3 mG caused a magnetic field intensity dependent delay in early development (at the morula stage) which was reflected in a decrease in mean number of cells per embryo.

Figure 2 illustrates the effect of a rotating 60-Hz, 500-mG magnetic field on sea urchin development as scored at 10, 16, and 22 h postfertilization (top to bottom of Fig. 2 respectively). The frequency distributions of embryos at different stages of development revealed no significant delay in development due to the 500-mG field exposure up to 10 h post-fertilization.

Moreover, by 16 h postfertilization more than 40% of the embryos in the sham-exposed chamber had progressed to the early blastula stage and a few had progressed to the mesenchyme blastula stage. At this same time, embryos from the same batch of fertilized eggs that had been exposed to the 500-mG magnetic field accumulated at the morula stage, and only 2% had progressed to the early blastula stage. This rep-

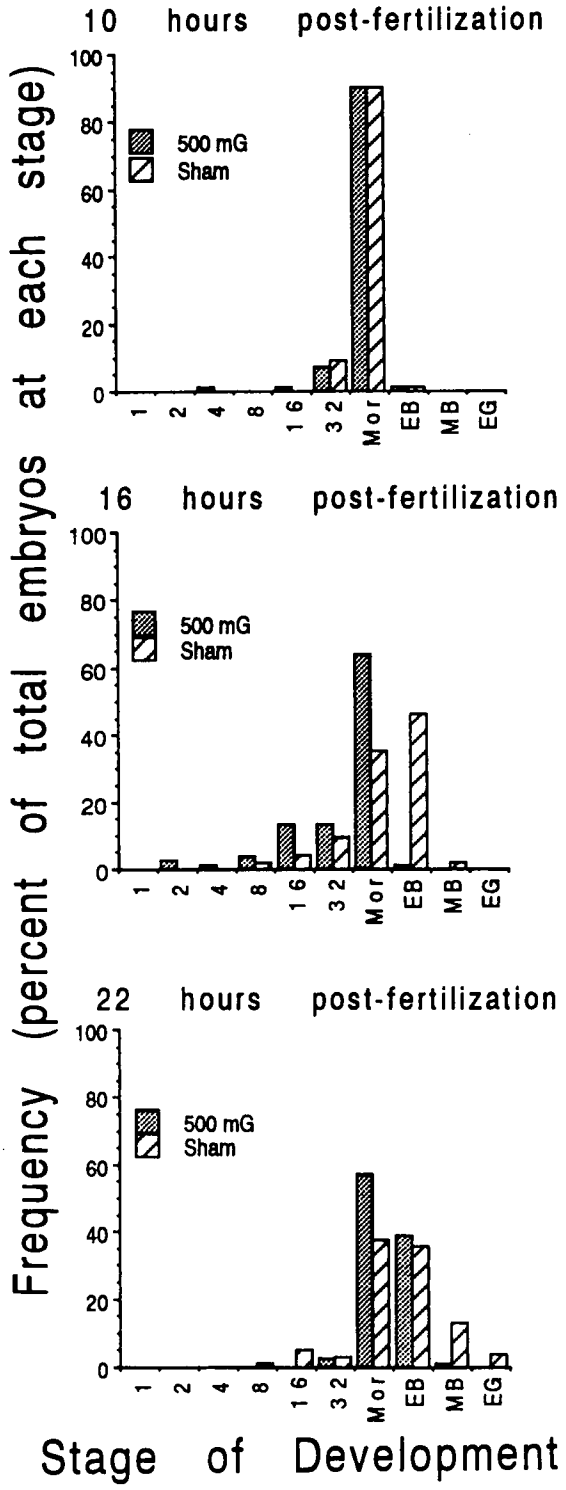


Fig. 2. Effect of exposure to a rotating 60-Hz magnetic field (500 mG) or to a sham-exposed "control" field, no applied magnetic field, on development of newly fertilized sea urchin eggs cultured for 10 h (top), 16 h (middle), and 22 h (bottom). One hundred embryos were scored in a "blind" manner for each distribution.

resents a significant delay in development due to exposure to the 500-mG magnetic field. The delay must therefore have occurred at 10–16 h postfertilization.

Observations on embryonic development made at 22 h postfertilization showed that exposure to the 500-mG field caused a delay at the morula stage but not a permanent arrest at the morula stage as many blastula stage embryos were now observed. Similar results were observed in four independent experimental runs using a different batch of fertilized eggs from a different female for each run. These findings indicated that a rotating 60-Hz magnetic field, like those in the environment, temporarily delayed development of sea urchin embryos at the morula stage. This fact when taken together with other published information on the gene activity of early sea urchin development [Davidson 1986] suggests that this slowing of cell multiplication at the morula stage may be due to inhibition of zygotic gene expression. The rationale for this idea is explained next.

## EFFECTS OF ROTATING 60-Hz MAGNETIC FIELD ON HISTONE EXPRESSION DURING EARLY EMBRYOGENESIS

It is known [Davidson, 1986] that cleavage stage sea urchin embryos synthesize histone proteins using maternal messenger RNAs (mRNA) and that the translation of this maternal mRNA is adequate to produce the histones needed for assembly with newly synthesized DNA and to allow replication of chromatin for about 32–64 cells. However the overall rate of histone synthesis normally increases greatly per embryo starting at about the 16-cell stage. Indeed, histone synthesis normally amounts to more than 30% of total protein synthesis as the embryo reaches the morula stage. This increased synthesis of histones beyond the 16-cell stage is increasingly due to translation of histone mRNA transcribed from the zygotic genome. Davidson points out that in sea urchin embryos the period of most rapid increase in cell number (the morula stage) coincides with the period of rapid increase in histone synthesis. Histone synthesis is just adequate for assembly with newly replicated DNA at the morula stage. Thus, any interference with histone synthesis, either transcription or translation, at the morula stage would be expected to interfere with increase in number of cells in the developing embryo.

A prediction from these facts is that interference in the transcription and/or translation of the "early" zygotic histone genes starting at the morula stage (9–10 h postfertilization) would be expected to slow cell reproduction and delay development until adequate amounts of histone could be synthesized. To test this prediction, sea urchin embryos, half of which were exposed to a rotating 60-Hz magnetic field of 500-mG intensity, while the other half were sham exposed were incubated with equal quantities of a mixture of radioactive $^{14}$C-labeled amino acids for 1 h at 10 or at 16 h postfertilization in order to label newly synthesized proteins. The nuclear proteins of both batches of embryos were then extracted [Nevins, 1988] and equal amounts of extracted proteins were loaded in SDS–PAGE gel slots, and the proteins were separated by electrophoresis. The autoradiograph of the dried electrophoresis gel was then prepared, and the lanes were subjected to identical densitometry scans.

Briefly, the autoradiograph of the gel of the control sham-exposed, embryos demonstrated evidence of synthesis of histones at 10 h postfertilization and showed increased synthesis of histones at 16 h postfertilization. Embryos exposed to the 500-mG magnetic field showed little if any histone synthesis at either 10 or 16 h (Fig. 3). These results support our prediction that the rotating 60-Hz magnetic field interferes with expression of "early" zygotic histone genes at the morula stage. Further study is needed to determine whether the magnetic field is inhibiting at the transcriptional level and how the morula stage embryo is able to overcome the inhibition of cell replication brought about by the magnetic field.

The observation that the rotating 60-Hz magnetic field caused but a temporary delay in sea urchin development at the morula stage also requires an explanation. One speculative explanation is that the eventual synthesis of another "late" set of zygotic histone genes, that are not totally inhibited by the rotating 60-Hz magnetic field, are eventually expressed to fulfill the need for histones.

It is worth noting that the cleavage stage of sea urchin development was refractory to the rotating 60-Hz magnetic field as judged by timing of the first and later cleavage stage cell divisions [Zimmerman et al., 1990; Cameron et al., 1992]. The lack of delay of cell cycles in the early cleavage stages or lack of delay of development to the morula stage would seem to rule out significant interference by the rotating 60-Hz magnetic field on many cellular processes, i.e., ionic surges, DNA replication, and translational events, all of which are known to be essential for cleavage stage development. This further suggests that the rotating 60-Hz magnetic field may act to alter gene activity at the transcriptional level at the morula stage of embryonic development in the sea urchin. Goodman et al. [1983, 1986, 1987, 1989] has previously reported that a ELF–EMF can effect cellular transcription. These facts indicate the need for study of the effects of rotating 50- and 60-Hz magnetic fields on the gene transcription process.

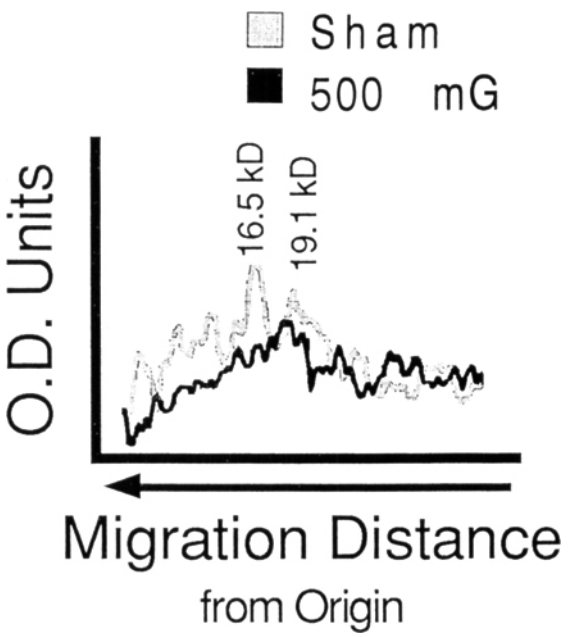


Fig. 3. Effects of continuous exposure of newly fertilized sea urchin eggs to a rotating 60-Hz magnetic field on synthesis of histone proteins as determined following a one hour terminal exposure to $^{14}$C-labeled amino acids at 16–17 h postfertilization. The dark scan line shows evidence of less radioactivity indicating comparatively little synthesis of $^{14}$C-labeled proteins in the MW range of histones in the embryos that were exposed to the 500 mG vs. the embryos in the sham exposure chamber.

## ROTATING 60-Hz MAGNETIC FIELDS ON MOUSE EMBRYOGENESIS

We exposed 2-cell stage mouse embryos, that had been cultured for 36 h postfertilization at 37°C, to rotating 60-Hz magnetic fields of either 500- or 100-mG intensity or to the sham-exposure chambers for 48–68 h. The field-exposed embryos demonstrated developmental delay as revealed by counting the number of cells in the embryo using a phase-contrast mi-

crosope. The results from two of two independent experiments, one using 100 mG and the other 500 mG, showed significant delays in embryonic development when tested by the normal approximation of the binomial distribution test ($P < 0.025$ and $P = 0.01$, respectively). Thus, exposure of 2-cell stage mouse embryos to rotating 60-Hz 100- to 500-mG magnetic fields caused interference with the cell multiplication process (Fig. 4).

The fertilized mouse embryo is only at the 2-cell stage by 36 h postfertilization. By 60 h postfertilization, the mouse embryo is at a morula stage of 8 cells; by 84 h, the embryo compacts and begins the formation of a blastocoel (cavity) with but 32 cells. The newly fertilized mouse egg has twice the rate of histone synthesis as does the mouse 2-cell embryo, and the mass of histone mRNAs declines quickly at the 2-cell stage [Davidson, 1986]. However by the 8-cell stage the mass of histone mRNAs from the zygotic genomes are greatly increased [Davidson 1986]. The fertilized mouse egg and the fertilized sea urchin egg would therefore appear to have a similar pattern of early decline in maternally stored histone mRNAs followed by an increase in histone mRNAs produced by transcription of the zygotic genome. The timing of interference in cell multiplication by exposure to the 60-Hz magnetic field in both the sea urchin and the mouse, suggests that the magnetic field may somehow function to alter early gene activity (possibly by interference with transcription of "early" zygotic histone genes), in both species.

The interference with development of the early (preimplantation) mouse embryo by a rotating 60-Hz magnetic field of 100-mG field intensity indicates that an early stage mammalian embryo was not immune to the effects of ELF magnetic fields. Just how sensitive mammalian embryos are and what other stages of development may be influenced by EMFs remains to be determined.

Whether or not EMFs in the home or in the workplace pose a real or serious health problem to human embryogenesis has yet to be established. There have been reports that ELF-EMFs associated with electrically heated beds or ceiling-cable electric heat are associated with increased spontaneous abortions [Wertheimer and Leeper, 1986]. It would seem from our observation on mouse embryos that harmful EMF effects on the preimplantation mammalian embryo might

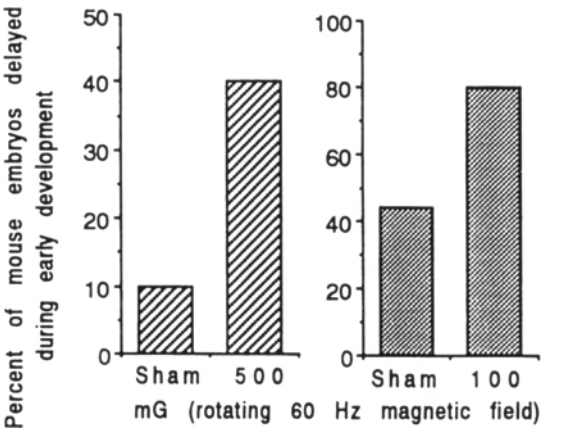


**Fig. 4.** Delay in development of 2-cell mouse embryos exposed to different magnetic field strengths. Half of the 2-cell mouse embryos were exposed to 100- or to 500-mG magnetic fields, for 48 or 68 hrs, respectively, in a 37°C circulating water-warmed field exposure chamber. Other 2-cell mouse embryos were incubated at 37°C in a similar water-warmed sham "control" exposure chamber (no applied magnetic fields). Nine or 10 embryos were scored for delay in development, defined as less than eight cells per embryo, for each condition.

be manifested more as a fertility problem than as a spontaneous abortion or teratogenic problem.

## CONCLUSIONS

Our data on the effects of rotating 60-Hz magnetic fields, designed to simulate the magnetic fields produced by power lines, demonstrate stage specific delays in early embryonic development in the sea urchin and in the mouse. These data confirm that EMF fields previously regarded as too weak to have biological effects, do have biological effects. Our preliminary findings suggest that the rotating 60-Hz magnetic field preferentially effects expression of zygotic genes responsible for early histone synthesis in sea urchin embryos. Further research is needed to determine how the 60-Hz magnetic field works to cause the biological effects.

Our current data on the delay of early development in sea urchin and mouse embryogenesis certainly do not provide adequate information to judge if exposure of fertilized human eggs to rotating 50- to 60-Hz magnetic fields would or would not adversely effect their development. At this time it is premature to draw any firm conclusions to refute or to support any contention that human embryos exposed to everyday EMF fields are or are not at increased risk of harmful effects.

Additional research is required to deal with increasing public concern about the possibility of adverse effects of EMF exposure associated with the use of electrical power. In view of our society's absolute dependence on electrical power, a thorough understanding of these issues is of critical importance.

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