

Effect of Ambient Levels of Power-Line-Frequency Electric Fields on a Developing Vertebrate

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Fertilized eggs of *Gallus domesticus* were exposed continuously during their 21-day incubation period to either 50- or 60-Hz sinusoidal electric fields at an average intensity of 10 Vrms/m. The exposure apparatus was housed in an environmental room maintained at 37°C and 55–60% relative humidity (RH). Within 1.5 days after hatching, the chickens were removed from the apparatus and tested. The test consisted of examining the effect of 50- or 60-Hz electromagnetic fields at 15.9 Vrms/m and 73 nTrms (in a local geomagnetic field of 38 µT, 85°N) on efflux of calcium ions from the chicken brain. For eggs exposed to 60-Hz electric fields during incubation, the chicken brains demonstrated a significant response to 50-Hz fields but not to 60-Hz fields, in agreement with the results from commercially incubated eggs [Blackman et al., 1985a]. In contrast, the brains from chicks exposed during incubation to 50-Hz fields were not affected by either 50- or 60-Hz fields. These results demonstrate that exposure of a developing organism to ambient power-line-frequency electric fields at levels typically found inside buildings can alter the response of brain tissue to field-induced calcium-ion efflux. The physiological significance of this finding has yet to be established.

Key words: ELF fields, calcium ions, electromagnetic fields, brain tissue, developing organism, chicken

INTRODUCTION

Many studies have been performed to evaluate the potential biological consequences of exposure to electric fields generated by electric-power distribution systems [Repacholi et al., 1984]. These studies have generally used electric field intensities equivalent to or larger than those normally found in the vicinity of high-voltage transmission lines, i.e., above 1 kV/m. Based on toxicological principles, higher intensities were thought to produce a larger effect that could be more easily detected by various tests. In fact, high-intensity electric fields have been reported to cause biological changes due to field-induced movement of vibrissae or animal hair [Kaune

Received for review November 5, 1986; revision received July 20, 1987.

These results were first presented at the Eighth Annual Meeting of the Bioelectromagnetics Society, June 1–5, 1986, in Madison, WI.

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et al., 1978]. This rationale to focus studies on high intensities (above 1 kV/m) has not received universal acceptance because it is known that some biological species are sensitive to electromagnetic (EM) fields only within a certain range of intensities; e.g., the honeybee may only use magnetic fields for guidance when the density is between 0.1 and 10 Gauss [Walker and Bitterman, 1985; Towne and Gould, 1985]. European robins held at a local geomagnetic field density of 0.46 Gauss can no longer use this field to orient if it is lowered to 0.34 Gauss or raised to 0.68 Gauss. However, orientation ability returns if the robins are kept in the new field for more than 3 days [Wiltschko, 1972]. It is possible that other sensitive biological systems may not respond to intensities that are too high. Thus an alternative approach in the search for possible biological effects caused by electric fields emanating from power lines is to use those much lower intensity values found in the home. These values commonly fall between 1 and 20 V/m [Sheppard and Eisenbud, 1977]. The use of these intensities would test the exposure situation that many humans normally experience.

This study is designed to examine the consequences of exposure of an organism during the time when rapid and coordinated changes are occurring in development. In order to minimize uncertainties in defining electric-field exposure intensities, a geometrically and experimentally simple biological model, the chicken egg, was selected. We have previously reported on dosimetric considerations of a generalized model of the exposure situation [Joines et al., 1986]. Following exposure during the entire 21-day incubation period, the newly hatched chickens were tested with a standard procedure in our laboratory to evaluate the response of brain tissue to EM fields. This procedure, which assays for changes in efflux of calcium ions from the brain tissue *in vitro*, has been used to study the interaction of EM fields with the central nervous system [see Adey, 1981; Blackman, 1985, 1988].

MATERIALS AND METHODS

In this study, fertilized eggs are exposed to either 50- or 60-Hz electric fields for their entire 21-day incubation period. Due to limitations on the number of chickens that could be analyzed at any one time after hatching, the chickens were also exposed on the average of an additional 36 h. The influence of this additional exposure of the chickens were tested in an ancillary experiment. Brain tissues were removed from the hatched chickens and examined for the ability of 50- or 60-Hz EM fields to cause enhanced release of calcium ions. We have had extensive prior experience using this endpoint and have found it to be a sensitive indicator of tissue interaction with EM fields [see Blackman, 1985, 1988]. Because the size of the egg exposure apparatus was limited by the dimensions of the incubator room, only 72 eggs could be exposed simultaneously to 50- and 60-Hz electric fields. In order to obtain a suitable sample size for each treatment and analysis condition, additional eggs were exposed under identical conditions at another time, thereby providing data from two separate hatchings. The results of the tests of brain tissues from the two hatchings were analyzed to determine if they could be combined. These data, collected in the categories shown in Table 1, composed one experiment. Details of this procedure are provided below.

Exposure Apparatus

(1) Eggs of *Gallus domesticus* (chicken) were exposed to one of two frequencies in a parallel plate apparatus (Figure 1), consisting of one ground plate between two

TABLE 1. Model for Experiments 1-3

Egg exposure	50 Hz		60 Hz	
Brain exposure	50 Hz	60 Hz	50 Hz	60 Hz
Hatch 1	X ^a	X	X	X
Hatch 2	X	X	X	X

^aX denotes data collection categories.

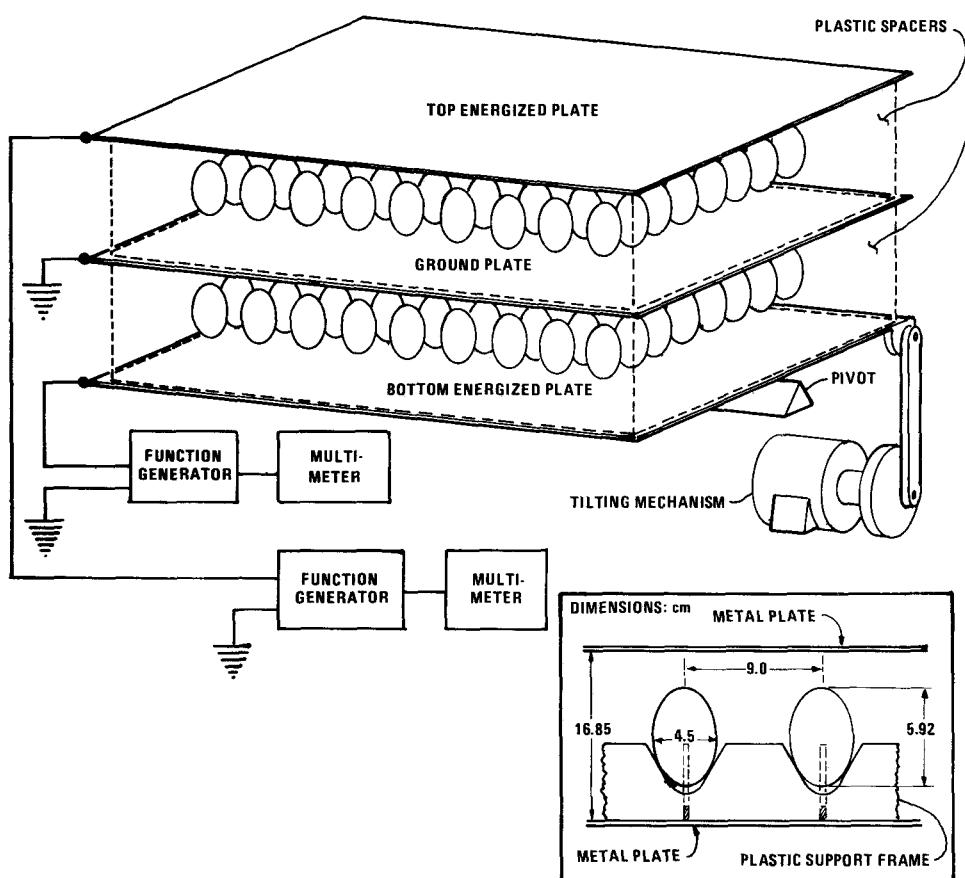


Fig. 1. Parallel plate apparatus. Eggs are supported by plastic frames (inset) midway between metal plates.

energized plates. The plates were 0.765 m × 0.675 m, and were separated by 0.1685 m. Plastic frames suspended the eggs midway between the plates in grids with a unit cell of 0.09 m². There were nine rows of eight eggs in each tier between the two energized plates and the ground plate. The plates were energized by function generators (Wavetek, San Diego, CA, Model 182A) with either 50- or 60-Hz sine waves (total harmonic distortion measured to be less than 0.28%). The signals were monitored by multimeters (Fluke, Everett, WA, Model 8060A) for both frequency and voltage. The intensity of the leakage fields of one frequency into the space occupied by the eggs exposed to the other frequency was down by at least 43 dBV as measured

by a spectrum analyzer (Hewlett-Packard, Atlanta, GA, Model 3582A) connected to the opposite plate and the ground.

The intensity of the sinusoidal electric field was calculated from equations developed by Joines et al. [1986]. There were two considerations. With a vertical plate separation of 0.1685 m and a mean egg height on the long axis of 0.0592 m, the field would be enhanced 1.043 times the applied field. However, because the horizontal center-to-center egg separation is 0.09 m and the mean egg diameter on the short axis is 0.045 m, the field would be reduced to 0.875 of the applied field. Combining these effects the mean net field is actually 0.959 of the applied field; the applied voltage was adjusted so that the eggs were exposed to a calculated mean intensity of 10 Vrms/m. With an applied field of 10 Vrms/m in air, the current flow generated inside an egg (along the 0.0592 m long axis) is approximately 0.2 nA at 60 Hz. For a mean diameter of the egg across the short axis of 0.045 m, the corresponding current density is 0.126 $\mu\text{A}/\text{m}^2$.

The entire apparatus was mounted on a pivot which allowed the eggs to be automatically tilted through 66° once an hour. The apparatus was housed in a walk-in temperature-controlled room maintained at 37 °C and 55–60% relative humidity (RH). The local geomagnetic field (LGF) in the egg exposure apparatus was 40 μT with an inclination of 55° N, which was approximately perpendicular to the axis of tilt. The ambient 60-Hz magnetic field was less than 70 nT.

(2) The basic exposure system for the brain tissue has been described in detail [Blackman et al., 1982]. It consisted of a transmission line exposure chamber (Instruments for Industry, Farmingdale, NY, Model 105s) terminated with a 50-ohm load, a function generator (Wavetek, Model 186) to provide the ELF signal, and associated instrumentation to monitor the frequency and intensity of the signal. This system was used to expose the brain-tissue samples to an AC field composed of both an electric and a magnetic component [Blackman et al., 1982, 1985a,b]. The vector of the LGF in the brain-tissue exposure system was 38 μT , inclined at 85° to the horizontal plane of the AC electric and magnetic components.

Biological Preparations

(1) The fertilized chicken eggs were obtained from the Poultry Science Department of North Carolina State University. In many cases they were up to 7 days old but were stored below 15.6 °C (60 °F) to keep them viable. There was no reliable difference in the percent hatch between hatchings of those eggs exposed to 60-Hz electric fields ($55.5\% \pm 3.9\%$ SE, $n=8$ hatchings) compared to 50-Hz electric fields ($54.9 \pm 3.8\%$ SE, $n = 8$ hatchings), nor between those eggs exposed in the top tier of the exposure apparatus ($53.1 \pm 3.9\%$ SE, $n = 8$ hatchings) compared to the bottom tier ($57.2 \pm 3.7\%$ SE; $n = 8$ hatchings). Further, there was no obvious difference between either the 50- or the 60-Hz exposed eggs nor the eggs exposed in the top tier compared to the bottom tier when evaluated by other indicators of abnormal gestation, e.g., percentage deformed or stillborn, or in the forebrain weight of hatched chickens.

Eggs were randomly selected from the supplier trays and placed (small end down) in the upper and lower tiers of the exposure apparatus to eliminate any bias that might have been introduced before we received the eggs.

After hatching, the chickens stayed in the exposure apparatus for an average of 1.5 days before being tested. During that time, the chickens were electrically isolated

from the plates. In separate experiments, it was found that the response of brain tissue was not affected by exposure of the chicks during this period.

The basic procedure used in this study was to expose simultaneously 72 eggs to 60-Hz electric fields and another 72 eggs to 50-Hz electric fields for 21 days. Following the assay of the brain tissue from the first hatching, a second set of eggs was identically exposed and assayed. These results were identified as data from a second hatching. The data from these two hatchings were combined into one data set, which constitutes one experiment (see Figure 1), and were analyzed for a replication effect.

To test for the influence of position in the upper or lower tiers of the exposure apparatus, the frequencies used in the two locations were reversed for one experiment.

(2) The brain tissues were assayed as described in Blackman et al. [1985a]. Brain tissues were removed from randomly selected chickens hatched from eggs exposed during incubation to either 50- or 60-Hz electric fields. The brain tissues were separated along the midline and labeled for 30 min at 37 °C with radioactive calcium ions (New England Nuclear, Boston, MA, NEZ 013; specific activity of 1 μ Ci/ml) in a physiological salt solution containing 155 mM NaCl, 5.6 mM KCl, 2.16 mM CaCl₂, 2.4 mM NaHCO₃, and 11.1 mM glucose at pH 7.8. The brains were then rinsed and placed in individual plastic tubes containing 1 ml of nonradioactive medium. The tubes were sealed with latex stoppers and placed in the exposure chamber at 37 °C while the paired halves, designated controls, were placed in a 37 °C water bath for the 20-min treatment. Four tubes containing individual brain halves along with six tubes containing medium to an equivalent column height were treated at a time as described previously [Blackman et al., 1985b].

Exposure and Assay

The exposure, sampling, and assay for radioactivity were performed as described by Blackman et al. [1982], except no sham exposures were conducted. The brain-tissue samples were exposed to EM fields of 15.9 Vrms/m (45 Vpp/m) and 73 nTrms at either 50 or 60 Hz. For each exposure frequency during incubation of the eggs, the corresponding brain tissues were tested at both 50 and 60 Hz. After a 20-min exposure, a 0.2-ml aliquot of solution was removed from each tube and assayed for the amount of radioactive calcium ions. Radioactivity measurements were made of the paired control halves in the water bath, and ratios were calculated of the counts per minute in the exposed samples to the corresponding counts for the control samples (V_t/V_c). Four brain tissues were tested at a time at one of the four combinations of exposure conditions for the brains, followed in order by four more brain tissues tested at one of the other combinations until all the tissues were examined from a given hatching. The identical procedure was repeated for a second set of eggs, and the data were combined and analyzed as one experiment. There were 36 brains in each of the 4 exposure conditions for the main experiment, and either 32 or 40 brains in those conditions for each of the 2 replicates.

Statistical Analysis

The basic design of the study is shown in Table 1. One hundred forty-four eggs were incubated together at the same time (one hatch). Half of these eggs were exposed to 50-Hz fields and half to 60-Hz fields. Half of the brains from chicks hatched at

each exposure were randomly selected and exposed to 50 Hz and the brains from the other half were exposed to 60 Hz. This procedure was replicated for a second hatch.

The experiment is a 2×2 factorial experiment with replication (hatchings). The two factors are egg exposure (E) during incubation with the two levels, 50 and 60 Hz, and brain exposure (B) after birth with the two levels, 50 and 60 Hz.

An analysis of variance is conducted for the full model. This analysis tests for effects due to egg exposure (E), brain exposure (B), and their interaction ($E \times B$), and hatchings (H) and interactions with hatchings ($E \times H$, $B \times H$, and $E \times B \times H$). Should, as expected, an effect due to hatchings and interactions with hatchings not reach statistical significance ($P > .05$), an analysis of variance for the reduced model without effects due to hatchings or interactions with hatchings can be used to examine the E and B main effects and their interaction ($E \times B$). Conclusions about a main effect are not possible when an interaction involving the main effect is significant. In that event, Bonferroni t-tests can be performed on the six possible pairs of means in order to uncover the nature of the interaction.

RESULTS

The data analyses from experiment 1 and its replicate, experiment 2, are shown in Tables 2 and 3, respectively. In both experiments, eggs exposed to 50 Hz during incubation were in the top tier of the parallel plate exposure system and those exposed

TABLE 2. Analysis of Brain Tissue From Chickens Exposed In Ovo (Experiment 1)

Source	D.F.	Mean square	F	P
Part A: Full Model Analysis of Variance				
Egg exposure (E)	1	2.0026		
Brain exposure (B)	1	1.4616		
$E \times B$	1	.6504		
Hatchings (H)	1	.0513	0.70	.405
$E \times H$	1	.0092	0.13	.724
$B \times H$	1	.0693	0.94	.333
$E \times B \times H$	1	.2035	2.77	.098
Error	136	.0735		
Total	143			
Part B: Reduced Model Analysis of Variance				
Egg exposure (E)	1	2.0026	27.16	< .001
Brain exposure (B)	1	1.4616	19.82	< .001
$E \times B$	1	.6504	8.82	.004
Error	140	.0737		
Total	143			
Egg exposure	Brain exposure	N	Mean	SE
Part C: Means and SE				
50	50	36	1.025	.032
	60	36	0.958	.035
60	50	36	1.395*	.067
	60	36	1.059	.037

* $P < .01$, Bonferroni-adjusted t-tests.

TABLE 3. Analysis of Brain Tissue From Chickens Exposed In Ovo (Experiment 2)

Source	D.F.	Mean square	F	P
Part A: Full Model Analysis of Variance				
Egg exposure (E)	1	1.9067		
Brain exposure (B)	1	1.4629		
E × B	1	2.0135		
Hatchings (H)	1	.0002	0.00	.960
E × H	1	.2043	3.05	.082
B × H	1	.0719	1.07	.302
E × B × H	1	.1487	2.22	.138
Error	152	.0669		
Total	159			
Part B: Reduced Model Analysis of Variance				
Egg exposure (E)	1	1.9067	28.09	< .001
Brain exposure (B)	1	1.4629	21.55	< .001
E × B	1	2.0135	29.66	< .001
Error	156	.0679		
Total	159			
Egg exposure	Brain exposure	N	Mean	SE
Part C: Means and SE				
50	50	40	1.005	.047
	60	40	1.038	.029
60	50	40	1.448*	.052
	60	40	1.032	.032

*P < .01, Bonferroni-adjusted t-tests.

to 60 Hz were in the bottom tier. Part A of each table shows the results of the analysis of variance for the full statistical model; part B is the reduced model; and part C shows the means and standard errors for each exposure combination.

In both cases, the terms for hatchings and interactions with hatchings in the full model were not significant; however, the E × B interaction term in the reduced model was significant. Thus, Bonferroni t-tests were done on the six possible pairs of means in each table (part C) in order to uncover the nature of the interaction. The conclusion for both experiments is that brain tissue from chickens exposed in ovo to 60 Hz and subsequently exposed to 50 Hz has significantly higher calcium-ion efflux ratios than the other three combinations ($P < .01$ for each experiment). The mean differences were approximately 40%.

In order to test for a position effect inside the egg exposure apparatus, another experiment, composed of two hatchings, was performed with reversed frequencies: the top tier exposed eggs to 60 Hz and the bottom tier to 50 Hz. The data analysis is shown in Table 4. As with the results in the previous two experiments, the lack of significant contributions from hatchings or interactions with hatchings allowed a collapse of the data to the reduced model, which showed a significant interaction between egg and brain exposures. A Bonferroni analysis of the six possible pairs of means (Table 4, part C) showed a result identical to the two earlier experiments, i.e., that brain tissue from chickens exposed in ovo to 60 Hz which were then exposed to 50 Hz has a significantly higher calcium-ion efflux ratio than the other three combi-

TABLE 4. Analysis of Brain Tissue From Chickens Exposed In Ovo With Positions Reversed (Experiment 3)

Source	D.F.	Mean square	F	P
Part A: Full Model Analysis of Variance				
Egg exposure (E)	1	1.1217	17.90	
Brain exposure (B)	1	.6151	9.81	
E × B	1	1.4229	22.71	
Hatchings (H)	1	.0132	0.21	.647
E × H	1	.1352	2.16	.144
B × H	1	.0602	0.96	.329
E × B × H	1	.0461	0.74	.393
Error	120	.0627		
Total	127			
Part B: Reduced Model Analysis of Variance				
Egg exposure (E)	1	1.1217	17.89	<.001
Brain exposure (B)	1	.6151	9.81	.002
E × B	1	1.4229	22.69	<.001
Error	124	.0627		
Total	127			
Egg exposure	Brain exposure	N	Mean	SE
Part C: Means and SE				
50	50	32	0.986	.042
	60	32	1.059	.047
60	50	32	1.385*	.049
	60	32	1.035	.039

*P < .01, Bonferroni-adjusted t-tests.

nations ($P < .01$; mean difference is approximately 36%). Thus, there appears to be no positional effect confounding the results.

DISCUSSION

The basic question posed by this study is: Can exposure to electric fields of frequency and intensities found in the average home cause biological changes in developing organisms? The results obtained indicate that eggs exposed during the incubation period to a 60-Hz electric field produced animals with brain tissue that was affected by a 50-Hz but not a 60-Hz EM field; this was true in three separate, independent experiments. These findings replicate and confirm our previous result [Blackman et al., 1985a] obtained with chickens incubated at the supplier, who used 60-Hz power-line frequency to energize the incubators, thus producing stray 60-Hz fields in the vicinity of the eggs. Conversely, those eggs incubated at 50 Hz produced chickens with brain tissues that were not affected at either 50 or 60 Hz, again in three independent experiments. A logical corollary of this finding is that the frequency used to treat the incubating eggs can affect the subsequent response to EM fields of the brain tissue from the chickens after hatch. Further, the results were not the outcome of an artifact generated by different nonelectrical conditions in the two tiers of the egg exposure system, nor were they due to exposure of the newly hatched chickens.

The same basic results described here were also obtained in a pilot experiment (data not shown) using a more primitive capacitive-plate system that exposed the eggs to 50- and 60-Hz electric fields at intensities ranging from 3 to 50 Vrms/m (measured with an electric field probe). It appears that unlike the tests of brain tissue which demonstrated different results at various combinations of frequency and intensity [Blackman et al., 1985a], the exposure of eggs which elicits the changes is not highly dependent on the exact electric field intensity used. Thus, these results indicate that the ambient electrical environment commonly found in homes is biologically active for at least one species during development, which is a time of embryogenesis, organ development, and organ maturation.

We have estimated the current density in a spheroid model of an egg to be $0.126 \mu\text{A}/\text{m}^2$. On a larger scale, a seated person has a height-to-width ratio of approximately 4.5 (0.9 m/0.2 m). If this person is within a 60-Hz, 10-Vrms/m field, the internally generated current density along the torso is about $0.67 \mu\text{A}/\text{m}^2$, as determined from homogeneous, spheroidal model calculations presented in Joines et al. [1986]. This current density is not a function of tissue type. Hence, with all other factors constant, the current density is $0.67 \mu\text{A}/\text{m}^2$ within a spheroid of muscle tissue, fatty tissue, or physiological saline solution. To estimate the current density within a human fetus, e.g., more elaborate models would need to be developed.

It is also apparent from these results that the physiologically significant sensitivity window in bird orientation observed by Wiltschko [1972], which was based on relatively minor changes in the ambient magnetic flux density, supports the use of ambient values in designing future experiments to test biological sensitivity to artificial fields. Thus, the results of experiments to test egg development at electric field intensities substantially higher than those used in this study [Graves et al., 1985] may not have relevance to ambient exposure levels in homes [Sheppard and Eisenbud, 1977]. The impact of this "windowing" of dose response on the establishment of exposure standards awaits an understanding of the underlying mechanism of action. This observation has been made before in connection with exposures to modulated radiofrequency radiation [Blackman et al., 1979].

What can be said or inferred from the biological assay about the physiological significance of the change induced during development? Although the biological assay, an EM field-induced change in calcium-ion efflux from chick brain tissue, has been used by two independent laboratories to test various radiological and biochemical parameters that are essential for the phenomenon [for reviews, see Adey, 1981; Blackman, 1988], the data do not yet allow for any firm interpretation of the physiological significance of the phenomenon for the intact organism [Blackman, 1985]. It is unlikely that these data can be fully appreciated and extrapolated to other species before the underlying mechanism of action is established or a health consequence of an increased calcium efflux from brain tissue is identified. Thus, these data presently do not support any suggestion that such exposures constitute a health hazard.

These data do have fundamental implications for research designed to investigate the biological consequences of exposure to EM fields. Both control and exposed biological test systems should be characterized for EM field exposure during development, and more broadly from conception to the beginning of the testing regime, in order to distinguish between possible sensitive subpopulations caused by differences in prior ambient exposure conditions. One of the causes of the so-called "Cheshire cat" phenomenon [Graves et al., 1979] could be because the biological systems had

different exposure histories that were not always replicated. We recently demonstrated that the density of the local geomagnetic field during exposure to AC fields might contribute to this "Cheshire cat" phenomenon as well [Blackman et al., 1985b].

It is presently impossible to establish a complete mechanism of action for either the electric field effect on developing chickens *in ovo*, demonstrated here, or the EM field effect on brain tissue *in vitro* for chicken [see Blackman, 1988], cat [Bawin et al., 1975; Adey et al., 1982], rat [Lin-Liu and Adey, 1982], or human being [Dutta et al., 1984]. For the effect on brain tissue *in vitro*, we have proposed a three-step process involving transduction of the EM signal to a physicochemical change, amplification of the chemical change, and secondary reactions resulting from the amplification [Blackman, 1988]. One key to the process is the initial transduction step that occurs in a frequency-specific manner between the EM field and the chemical entity. Identification of the chemical transducers would greatly assist model building and extrapolation activities [see Blackman et al., 1988, for a more detailed discussion]. Another key to the process is the amplification step that utilizes energy already stored in the biochemical system rather than requiring the EM field to provide all the energy necessary for the biochemical changes. Adey [1975] first suggested that a cooperative transition may be involved to provide that energy; we agree with this suggestion and believe that a phase transition in membrane subunits may also be involved.

How then could an exposure to electric field of specific frequency during development cause the results that we have observed here? This question cannot be answered without further investigations. In this study eggs were exposed to low-intensity electric fields and the brain tissue was exposed to EM fields for analysis. It would be useful to study the possible differential roles played by the electric and magnetic components of the oscillating fields and to test whether the local geomagnetic field is involved. It would also be useful to investigate whether any specific time in the incubation period, e.g., embryogenesis, organ development, or organ maturation, might be a particularly sensitive time for these field-induced changes to occur. For example, Ubeda et al. [1983] showed that eggs are particularly sensitive to low-intensity, pulsed magnetic fields during the first 48 h of incubation, the time of embryogenesis. Among other changes, they reported alterations in the glucopolysaccharides on the surface of nervous tissue. This result is consistent with the hypothesis that the calcium-ion efflux phenomenon is measuring changes in cell surface responses [see Adey, 1981; Blackman, 1988]. Recently, we have shown that the neutral sugars, mannitol and sucrose, can replace calcium ions as a monitor for field-induced changes in chick brain tissue exposed at 16 Hz and 40 Vpp/m, and under the conditions described in this study [Blackman et al., 1987]. These results are all consistent with field-induced alterations in the boundary layer at the surface of cells composed of glucopolysaccharides, proteins, and other components on or in the membrane structures. It is possible that metabolically induced changes in these components are directly or indirectly involved in the frequency sensitivity of the initial transducing species, altering sensitivities to either the frequency or the intensity of signals in the ambient environment [see discussion in Blackman et al., 1988]. These changes in either the transducing or the amplifying system are one way a physical system could manifest a long-term alteration in the physiological systems studied here. Should this hypothesis be true, it remains to be seen whether the changes induced in cells of the central nervous system by exposures during development are permanent or reversible, and what physiological significance the changes have.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Berman, Elder, Hall, and Phillips for their helpful comments during this work and during the preparation of the manuscript. We also wish to recognize and thank Mr. Killough of Northrop Services, Inc., for his enthusiasm and expert craftsmanship in the fabrication of the parallel plate apparatus, as well as for its schematic in Figure 1. Mr. Summey, of Northrop Services, Inc., and Dr. Ali are thanked for their contributions to measurements of fields and of distortion in signals used to expose the eggs.

DISCLAIMER

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of tradenames or commercial products constitute endorsement or recommendation for use.

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