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Chick Embryo Development in a 26-KHz Electromagnetic Field

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This paper discusses an experiment designed to examine the effects of a nonionizing electromagnetic field on chick embryo development. Sixty-two test eggs were exposed for 19 days to a 26-kHz, 160- gauss peak-to-peak a-c field. Seventy unexposed eggs were maintained under the same environmental conditions as the controls. The percent of eggs hatched from the initial number was 64.5 for the exposed and 48.5 for the controls. The hypothesis that embryos in the electromagnetic field have less chance of hatching than the controls is rejected at the 5% significance level.

Introduction

MAN HAS BEEN EXPOSED for decades to a broad region of the electromagnetic (EM) spectrum. While the biological effects and hazards of ionizing radiation have received extensive study, the nonionizing portion is a relative newcomer to scientific scrutiny. Of the nonionizing radiation, exclusive of ultraviolet and visible light, the microwave region has merited most of the attention—namely, the wavelength fields between 1 cm and 10 meters (30 GHz to 30 MHz). The interest in these fields was generated by the introduction of varied EM applications such as radar, microwave ovens, and medical diathermy units. These earlier activities have been reviewed and presented in the four Tri-Service Conferences.[1,2] The Soviet electromagnetic field (EMF) work has been summarized by Presman[3] and Gordon.[4] The latest United States state-of-the-art microwave theory and technique survey was compiled in 1970 and issued in February of 1971 by the Institute of Electrical and Electronics Engineers.[5] In general, these surveys agree that the experimental results suggest thermal damage as the primary effect of excessive exposure to microwave radiation.[6] Nonthermal effects have been postulated[3,7] but have not been experimentally demonstrated to a degree where the majority of investigators in the United States will accept their existence.[8-10] In the Soviet Union, the nonthermal effects have been associated with neurophysiological responses, asthenic manifestations, and general autonomic nervous system reactions.[3,4] Many of these responses are speculative and unproved; however, it must also be said that neither have these nervous responses been disproved. In the United States literature, nervous stimulation has been attributed to local thermal stimulation,[11] general body temperature response,[12] and nonthermal nerve activation.[7]

The majority of United States EMF work has been in the megahertz range and above, while some of the Soviet studies have also investigated the kilohertz fields, at which level biological responses have been shown. The only very-low-frequency work found in the United States, performed by the Hazleton Laboratories in 1970, showed essentially no biological effects to 45-Hz and 75-Hz fields.[13]

The low- and medium-frequency fields of 3- to 300-kHz radio waves and frequencies of certain induction heaters have been virtually neglected, although man has been frequently exposed to these EM fields.

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This paper presents the effects of a 26-kHz, 160-gauss peak-to-peak a-c field on developing chicken embryos. This specific field was selected for study because this frequency is used for induction heating in industry and in broadcasting, thus exposing the occupational, nonmilitary person to this EM spectrum.

Experimental Design

The fertilized egg was selected as the test system. It has been felt by some investigators that electromagnetic field forces, especially the megahertz frequencies and higher, act at subcellular levels, influencing enzyme reaction rates, the mitotic spindle, and other vital molecular and/or subcellular activities.[6,7,14,15] If any of these suspicions are correct for low-frequency radio waves, then the developing egg, going through its many levels of cellular differentiation and growth, should demonstrate abnormalities and increased embryo lethality. The fertilized egg is a very sensitive, rapidly developing, complete biological unit which, in 21 days, can show stress effects on the total embryonic development. It was for these reasons that the chick embryo was chosen.

Fertilized chicken eggs (White Leghorns) were purchased from a commercial poultry farm. This establishment supplies fertilized eggs and embryos at specified ages for the area's medical and biological laboratories. The test criterion was the hatchability of eggs exposed to a constant EMF during the first 19 days of incubation compared to that of controls in the same environment sans EMF.

Physical Setup

Incubator

The incubator measured 68.5 cm (27 inches) in width, 48 cm (19 inches) in depth, and 57 cm (22½ inches) in height, and was covered on the outside by plastic to maintain a constant humidity. Air channels were added to ensure proper air circulation. This incubator contained two electromagnetic-field-generating solenoid coils, two capacitors, a large water-evaporating dish for moisture, a thermostat to control the temperature, and a 100-watt light bulb as the heat source. In addition, space was provided for the control eggs outside the EMF generated by the solenoids. The incubator layout diagram is shown in Figure 1. The relative humidity was maintained at

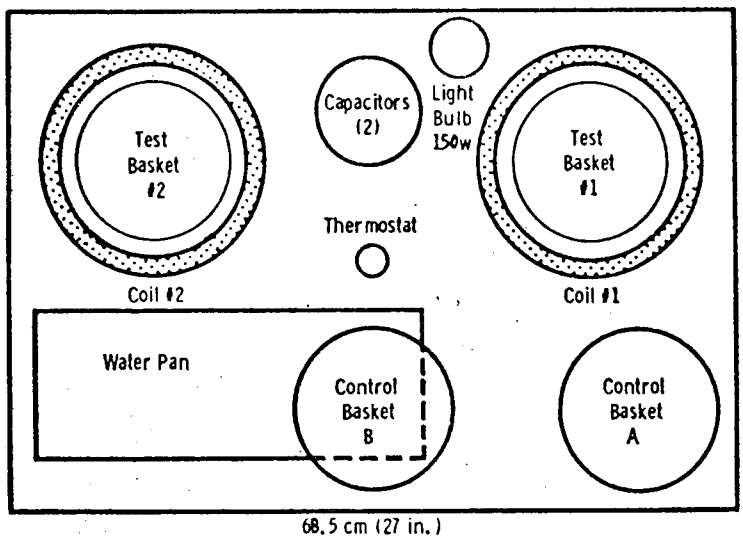

Figure 1.—Incubator diagram for exposing fertilized chicken eggs to 26-kHz, 160-gauss peak to peak a-c field.

50 ± 5% as measured by a continuous recorder.

The electromagnetic-field-generating solenoid coil produced temperatures of 45° to 46°C inside the plastic cylinder, which was to house the test egg baskets. To control the required egg-incubating temperatures of 38° to 39.5°C, it was necessary to provide, by means of a fan, a continuous air circulation; therefore, each cylinder was mounted on a fan. To prevent excess dehydration of the eggs, each cylinder and fan was supported in an individual evaporating dish.

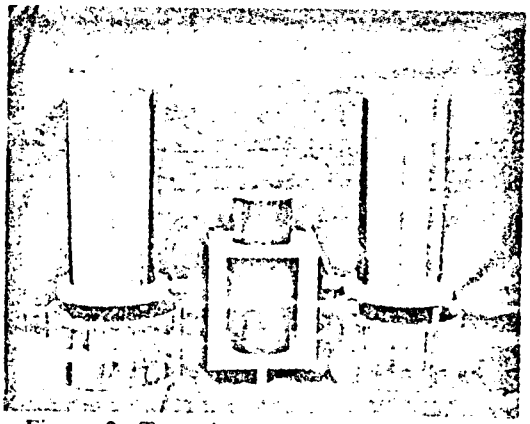

Figure 2. Two electromagnetic-field-generating solenoid coils wound on plastic cylinders and mounted over cooling fans. Each unit sits in a water dish to maintain moisture around the eggs within the cylinders. Between the cylinders are two capacitors.

The cylinder, fan, and evaporating dish assembly is shown in Figure 2.

Electromagnetic Field

A sinusoidal magnetic field was generated by using a coil (a single-layer, closely wound, 398-turn cylindrical solenoid) 27.9 cm in length and 12.7 cm in diameter. The magnitude of the magnetic field was 160 gauss (peak to peak). The frequency was 26 kHz. The field was uniform to within ±7% throughout the innermost 17.7 cm of the coil. The coil was operated with a Sangamo type 293, 0.005-MFD capacitor as part of a series-tuned resonant circuit. The tuned circuit was driven by a Class B complementary transistor amplifier and oscillator whose frequency was adjusted to the resonant frequency of the circuit.

This study involved the near-field exposure; therefore, the power density and power flux, as used in microwave field definition, could not be used as EMF measurement.[9,16-18] The exposure field here is basically defined by its geometry, the frequency, and the a-c magnetic field measurement.

Egg Handling Procedure

The fertilized eggs were randomly divided into test and control groups and numbered. Twelve eggs were then placed horizontally into each basket. The basket was supported 7 cm above the bottom of the coil and filled up to 5 cm from top of coil to ensure the most uniform (<10%) field strength variation—that is, using only 17.7 cm (7 inches) of the 27.9 cm (11inch) coil field, as shown in Figure 3.

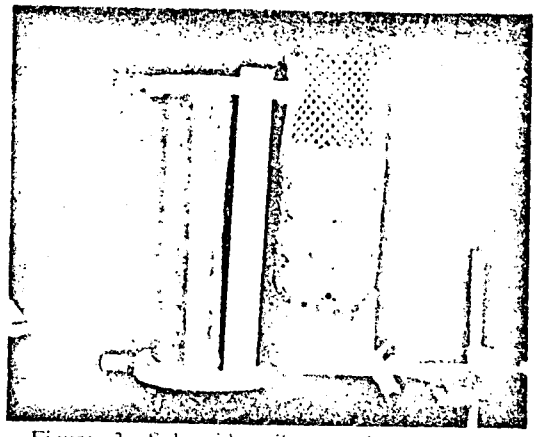

Figure 3. Solenoid coil wound on a plastic cylinder with a basket of twelve eggs demonstrating the location of the eggs inside the cylinder.

To ensure that the EMF did not produce any heating effects,[19] the eggs were tested in the coil with a copper-constantan thermocouple. Three thermocouples per egg were employed—one inside the yolk, one on the shell, and the third 2 cm above the egg in the ambient air inside the solenoid coil. To avoid thermocouple heating by the electromagnetic field, no readings were taken with

the field on. The field was kept on for 15- and 30-minute periods, then turned off; the thermocouple circuit was immediately completed, and the voltage was read. This procedure was repeated for a total of sixteen recordings. Over several hours of testing, no temperature difference was detected between the egg yolk, the outside shell temperatures, and the ambient air.

All eggs were candled daily; the weight loss of each twelve-egg basket and the date of any embryo death were recorded. The weight loss (water loss) averaged 0.4 gm per egg per day. The test eggs were kept continuously in the EMF except for approximately 60 minutes daily during the handling and examination period. At the end of the nineteenth day, all live exposed and control eggs were transferred to a 53-cm-diameter incubator (Brower Manufacturing Company, Quincy, Illinois, Catalog No. 846, Model 1670-3, 155 watts, 120 volts, 60 cycle a-c). Hatching took place in this incubator because the physical stacking of eggs in the baskets made normal hatching impossible.

At the end of 21 full days, the hatched chicks were counted, and all unhatched eggs were re-examined for viability. If a dead chick had broken the egg shell but had not emerged from it, it was counted as dead.

Several chicks of the test and control groups were sent to a farm. The others were observed for 36 hours prior to being euthenized and discarded. Any gross abnormalities were recorded.

Results

The summary of the experimental results is presented in Table I. All dead chick embryos were examined for gross abnormalities and teratology. None was found. Following hatching the chicks were observed for 36 hours prior to euthenasia. All exposed and control chicks appeared normal.

Although not part of the original study, twenty exposed test chicks and sixteen control chicks were sent to a farm. These birds have grown normally in appearance and have had a 50–50 sex ratio. No mortality or abnormal behavior has been reported in a 6-month period. No weight comparisons on growth rates were made.

Statistical Analysis

The test unit used was the fertilized egg. The test parameter was the development of the embryo to full term and hatching. Embryo development and hatching were used as the criteria for success. The total 21-day incubation period was divided into two phases. During phase I, the eggs are continuously kept in the electromagnetic field (EMF). This phase was 19 days long. Phase II took place outside the EMF in an incubator until hatching. This phase was 2 days long.

The hypothesis to be tested was that the EMF-exposed embryos have a poorer survival and hatching prospect than the controls. If p_C and p_T denote the true population survival probabilities for control and test embryos, respectively, during the first 19 days, then the hypothesis is

$$H_0: p_T - p_C < 0$$

Let n_T and n_C denote the initial count of fertilized eggs, and x_T and x_C the survival counts.

A point estimate of $p_T - p_C$ is obtained as follows when it is assumed that the test units (eggs) constitute a random sample from the population of interest:

$$\hat{p}_T - \hat{p}_C = \frac{x_T}{n_T} - \frac{x_C}{n_C} = \frac{47}{62} - \frac{43}{70} = 0.145$$

To assess quantitatively the degree to which this statistic contradicts the hypothesis H_0, we use the following results: If it is assumed that $p_T - p_C = 0$, then the statistic $\hat{p}_T - \hat{p}_C$ is to a close approximation normally distributed with mean zero and standard deviation.

$$\sigma(\hat{p}_T - \hat{p}_C) = \sqrt{p(1-p)\left(\frac{1}{n_T} + \frac{1}{n_C}\right)}$$

TABLE I

Fertilized Egg Exposure to Electromagnetic Field (EMF) of 26-KHz, 160-Gauss Peak-to-Peak A-C Field

Initial Number of Eggs	Number of Eggs Alive at Each Given Day																			Embryos Dead in 19 Days		Number of Eggs to incubator on 19th Day	Embryos Died in the Incubator		Eggs Hatched From Initial Number		From Incubator Number	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	No.	Percent		No.	Percent	No.	Percent	No.	Percent
Test 62 in EMF	—	—	62	61	61	61	60	59	58	55	51	51	51	50	48	48	47	47	47	15	24.2	47	7	14.8	40	64.5[a]	40	85.1
Controls 70	—	—	67	67	66	65	62	53	52	51	49	47	46	46	45	45	43	43	43	27	38.6	43	9	20.9	34	48.5[a]	34	79.1

[a]The normal hatching rate at the poultry farm where eggs were purchased is 72 to 77%, using commercial incubators. Under less-ideal conditions of temperature, turning of the eggs, size of the eggs, and the position of the eggs the hatching rate can be below 50%.

TABLE II

Initial Number of Fertilized Eggs	Embryos Alive in 19 days and Transfered to Incubator				Eggs Survived and Hatched Based on Those Transferred on Day 19 to Incubator				Based on the Initial Number of Fertilized Eggs			
	No.	Percent	Z-statistic[a]	Probability[b]	No.	Percent	Z-statistic	Probability	No.	Percent	Z-statistic	Probability
Test 62 in EMF	47	75.8	1.79	3.67	40	85.2	0.77	22.06	40	64.5	1.85	3.22
Controls 70	43	61.4			34	79.1			34	48.5		

[a]Z-statistic, explained in text.

[b]Probability that given standard normal variable will be exceeded in percent.

where p is the survival probability common to both test and control embryos under our assumption (note $\hat{p} = 90/132$). Thus, the statistic $(\hat{p}_T - \hat{p}_C)/\hat{\sigma}(\hat{p}_T - \hat{p}_C)$ is essentially standard normal in distribution, where $\hat{\sigma}(\hat{p}_T - \hat{p}_C)$ denotes the estimated standard deviation, obtained by using $\hat{p}$. We compute:

$$Z = \frac{\hat{p}_T - \hat{p}_C}{\hat{\sigma}(\hat{p}_T - \hat{p}_C)} = \frac{\frac{47}{62} - \frac{43}{70}}{\sqrt{\left(\frac{90}{132}\right)\left(\frac{42}{132}\right)\left(\frac{1}{62} + \frac{1}{70}\right)}} = 1.79$$

This result completes the test of the hypothesis H_o, as follows:

1. If the hypothesis is true in the specific form of $p_T - p_C = 0$, as assumed above, then the probability of obtaining a value for Z as large or larger than $+1.79$ is less than 0.05 (the actual value being 0.0367 from probability tables).
2. If the hypothesis is true in the form of $p_T - p_C \leq 0$, then the corresponding value of Z increases and the probability of obtaining as large or larger a value decreases.

Therefore, the hypothesis that embryos in the EMF have less of a chance of survival than the controls is rejected at the 5% significance level. A summary of the statistical data is presented in Table II.

Discussion

The biological effects of microwave irradiation have been thoroughly discussed and published in the American[6,12,20-22] and Soviet literature.[3,4,22] Biological response information in the low-kilohertz fields is almost nonexistent. Presman[3] has presented several pieces of research in this range which deals with golden hamster avoidancy action (1 to 10 kHz, 9 volts/cm), mouse reteaching response (100 Hz, 300 to 470 oersteds), rabbit carbohydrate metabolism, especially sugar level increases (9.5 to 9,500 kHz), immunity suppression to tick encephalitis virus in mice, rats, and rabbits (7000 oersteds and 50 Hz, 200 oersted), and malignant cell culture inhibition (99 to 1000 Hz, 1.1 to 1.7 volts/meter). In some of these experiments, the magnetic field was several hundred gauss, and this may have been the biologically significant field vector. No specific induction heating equipment or low-frequency radio wave biological response reports were found. In the field of electromagnetic radiation exposure standards, the USSR is the first to propose standards for low frequencies. The lowest frequency is for medium waves of 100 kHz to 3 MHz of 20 volts/meter, which the USSR considers as having no effect on the human body.[23] The reported test frequency of 26 kHz is below frequencies covered by these Soviets standards.

Although there is no theoretical reason to believe that a 26-kHz, 160-gauss peak-to-peak a-c field will produce a biological response, the possibility, however remote, cannot be ignored. Presently there do not appear to be any available data on frequency-specific effects in cell systems. However, dielectric dispersion studies on proteins, peptides, and amino acids have shown that characteristic relaxation times cover the range from 3.3 GHz to 63 kHz. This covers almost the whole EM spectrum and approaches the low frequencies of radio waves, some induction heaters, and therefore the frequency selected for this study.

Schwan[10,20] has made physical calculations and determined theoretically that, for typical biological cells, field strengths of more than 100 volts/cm are needed to affect macromolecular or chromosomal rearrange-

ment in the microwave frequency range of continuous fields. In other words, biologically significant force effects cannot be expected at field strength levels which are thermally insignificant. In this study, no thermal changes could be recorded in the fertilized and the developing egg.

The fertilized eggs developed normally in the EM field; therefore, this field did not adversely affect the early differentiation of the organ systems and the fundamental processes of body formation common to all groups of vertebrates. The biochemical (enzyme, protein, peptide, amino acid, and carbohydrate) processes also do not appear to have been significantly influenced because the development, growth, and subsequent chick survival was within the bounds of the control group.

In this study, no attempt was made to measure nervous responses. From the observed results, the development of the nervous system did not appear to be anatomically or functionally affected. Behavioral changes were beyond the scope of this study and the biological system employed. Chromosomal aberration, observed for microwave fields, will be examined in the mouse and reported subsequently.

This study sought to find developmental changes in avian fertilized eggs in search for possible biological effects of a 26-kHz, 160-gauss peak-to-peak a-c field. No changes or significant hatchability effects were noted in the chick embryos.

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