Ultrasound analysis of the eye of chick embryos exposed to low-frequency magnetic fields

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SUMMARY

Ultrasonography was used to measure different ocular parameters in chick embryos exposed to low-frequency (50Hz) magnetic fields with flow intensities of 1 µT, 0.1 mT, 0.5 mT, or 1 mT throughout their incubation. In comparison with unexposed control embryos, a significant reduction was observed in corneal thickness and in the anteroposterior diameter of the lens at 21 days of incubation (hatching) during exposure to the highest intensities (0.5 mT and 1 mT). By contrast, there was an increase in the anteroposterior diameter of the anterior eye chamber in embryos exposed to the lower magnetic field intensities (1 µT and 0.1 mT). The anteroposterior diameter of the eye was smaller at 21 days of incubation in all the embryos exposed to magnetic fields as compared with the controls. The anteroposterior diameter of the lens was significantly smaller in embryos exposed to intensities of 0.5 mT and 1 mT in comparison with the controls.

Key Words: Magnetic fields – Eye – Chick embryo – Development

INTRODUCTION

Increased electrification in modern societies has immersed the individual in a veritable pollution of electromagnetic waves, both in the general environment and at work.

The low-frequency magnetic fields to which humans are frequently exposed have always been considered to pose no health risks, but concerns are now growing that these fields act as promoters or co-promoters of alterations in the organism. Indeed, recently this issue has received wide coverage in the mass media. Many epidemiological and experimental studies have been published on the carcinogenic action of magnetic fields, above all regarding children living near high-voltage power lines (Olsen et al., 1994; Knave, 1994; Feychting et al., 1995; Kraut et al., 1996; Tynes et al., 1997; Michaelis et al., 1998; Ly et al., 1998; etc.), office personnel (Juutilainen et al., 1993), and people working with devices that emit electromagnetic waves, such as train-drivers (Floderus et al., 1994; Alfredsson et al., 1996; etc.) and electric utility workers (Theriault et al., 1994; Armstrong et al., 1994; Miller et al., 1996; Guenel et al., 1996).

Some epidemiological and experimental researchers have proposed that magnetic fields are harmful to health, whereas others have failed to find any association between magnetic fields and biological aggression, or at least none of sufficient significance for the authors to take a position on the question (Myers et al., 1990; Verkasalo et al., 1993; Preston Martin et al., 1996; Petridou et al., 1997; Kheifets et al., 1999; Feychting et al., 2000).

Regarding the action of magnetic fields on the eye, in 1980 Strzhizhovskii et al. reported that one hour of exposure to a magnetic field inhib-

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ited the mitotic activity of the corneal epithelium in rats, and that activity was recovered when the exposure ended.

Workers exposed to magnetic fields ranging from 112 to 190 Gauss, depending on their place of work, were examined daily in an epidemiological study and were found to suffer from general weakness, abdominal pains, thirst, and conjunctivitis (Lankosz et al., 1983).

In 1985, Skrinnik showed that pulsed magnetic fields of 8.5 mT and 50 Hz, with a squared pulse length of 0.02 seconds, caused increased corneal permeability. In contrast, Kues et al. (1999) observed no ocular alterations in rabbits or primates after exposure to 10mW/cm^2 from a 60 GHz source. However, magnetic fields of extremely low frequency (1 Hz) has been shown to induce a reduction in human visual cortex excitability (Boroojerdi et al., 2000).

The confusion created by these conflicting data prompted us to conduct an experimental study on the action of magnetic fields on the embryonic development of the eye.

Material and Methods

We used fertilized eggs of *Gallus domesticus* (White Leghorn variety) in view of their short embryonic period and their self-sufficiency in development, thus avoiding possible maternal interactions.

Fertilized eggs were incubated at a temperature of 37.8 ± 0.4º C and a relative humidity of 60-70% in a model 65 Masalles incubator equipped with forced ventilation and automatic voltage (1 volt/h). Half of the incubator (occupied by the eggs) was placed centrally between two Helmholtz coils in parallel, separated by a distance equal to their radius (70 cm), so that the trays containing the incubating eggs would receive a uniform magnetic field.

We used 400 fertilized eggs, subdivided into five groups of 80 eggs each. Four groups were exposed to 50 Hz magnetic fields of 1 µT, 0.1 mT, 0.5 mT, or 1 mT, respectively. The fifth group was incubated separately and formed the control group.

Two extractions were performed in each experimental group at 15 days of incubation and at 21 days (hatching). The choice of day 15 was based on our previous experience that the impact of a teratogenic element can be best observed after this period of development. Day 21 was selected because the hatched chick reveals the final result of any possible teratogenic action. On each extraction day, approximately 20 randomly selected eggs were studied from each group.

After the extractions had been performed, we used a 15-500 A/B Scan Humdex ultrasound apparatus to study the anteroposterior diameters of the cornea, anterior chamber, lens, and the whole eye.

Because of the volume occupied by the samples, we used two Heltmholtz coils, arranged as described above. Each coil had a diameter of 1.40m, with 500 turns of 2 mm diameter copper wire, a resistance of 12Ω, and self-induction of 0.6 H.

Because the intensity of magnetic fields is directly proportional to current intensity, a high voltage was necessary; this was supplied by integrating the coils in a second-order RLC electric circuit in series, incorporating a bank of condensers. The magnetic fields were measured with two digital Teslameters: Phywe model 13610.93 and Chauvin-Arnoux model C.A 40.

Results

Anteroposterior diameter of the cornea (Table 1)
The anteroposterior diameter (thickness) of the cornea of exposed embryos showed no significant differences *versus* the controls at 15 days of incubation. However, at 21 days, embryos exposed to fields of higher intensity (0.5mT and 1mT) showed a smaller corneal thickness than the controls ($p<0.01$), whereas embryos exposed to 1µT and 0.1mT showed no differences in this measurement.

Table 1. - Anteroposterior diameter of the cornea (mm).

	Day 15 of incubation	Day 21 (Hatching)
Controls	1.1286±0.1309 (N=21)	1.2000±0.0000 (N=17)
Treated with 1µT	1.2000±0.09733 (N=20) n.s.	1.1714±0.09024 (N=21) n.s.
Treated with 0.1mT	1.0650±0.1531 (N=20) n.s.	1.1571±0.1134 (N=7) n.s.
Treated with 0.5mT	1.0833±0.1823 (N=18) n.s.	1.0773±0.1510 (N=22) P<0.01
Treated with 1mT	1.1550±0.1468 (N=20) n.s.	1.0950±0.1468 (N=20) P<0.01

All values are means ± SD. N, number of sample; n.s., not significant.

Anteroposterior diameter of the anterior chamber (Table 2)

This parameter represents the distance between the posterior surface of the cornea and the anterior surface of the lens, and was affected differently according to the intensity of magnetic field and the day of development studied. At 15 days of development, embryos exposed to magnetic fields of 0.1mT or 1mT showed a significant increase in this parameter *versus* the con-

trols ($p<0.001$ and $p<0.05$, respectively), whereas those exposed to fields of 0.5mT or 1µT showed no significant differences. At 21 days, this measurement was increased *versus* controls in the embryos exposed to fields of 1µT, 0.1mT ($p<0.001$) and 1mT ($p<0.05$), but not in those exposed to 0.5mT.

Table 2.- Anteroposterior diameter of the anterior chamber of the eye (mm).

	Day 15 of incubation	Day 21 (Hatching)
Controls	0.9000±0.09487 (N=21)	0.9000±0.0000 (N=17)
Treated with 1µT	0.9150±0.06708 (N=20) n.s.	1.1429±0.1207 (N=21) p<0.001
Treated with 0.1mT	1.0650±0.1531 (N=20) p<0.001	1.1571±0.1134 (N=7) p<0.001
Treated with 0.5mT	0.9667±0.1645 (N=18) n.s.	0.8591±0.1919 (N=22) n.s.
Treated with 1mT	1.0200±0.1795 (N=20) p<0.05	0.9600±0.1231 (N=20) P<0.05

All values are means ± SD. N, number of sample; n.s., not significant.

Anteroposterior diameter of the lens (Table 3)

At 15 days of incubation, this parameter only showed significant differences from the controls in embryos exposed to 0.5mT ($p<0.05$). However, at 21 days, the embryos exposed to fields of 0.5mT or 1mT had a smaller anteroposterior lens diameter as compared with the controls ($p<0.05$ and $p<0.001$, respectively).

Table 3.- Anteroposterior diameter of the lens (mm).

	Day 15 of incubation	Day 21 (Hatching)
Controls	1.0714±0.1793 (N=21)	1.1824±0.07276 (N=17)
Treated with 1µT	0.9750±0.1333 (N=20) n.s.	1.1571±0.1076 (N=21) n.s.
Treated with 0.1mT	1.0950±0.1468 (N=20) n.s.	1.2000±0.1732 (N=7) n.s.
Treated with 0.5mT	1.2667±0.3646 (N=18) p<0.05	1.0545±0.2064 (N=22) p<0.05
Treated with 1mT	1.0350±0.2059 (N=20) n.s.	1.0200±0.1508 (N=20) P<0.001

All values are means ± SD. N, number of sample; n.s., not significant.

Anteroposterior diameter of the eye (Table 4)

This parameter represents the distance from the anterior surface of the cornea to the retina. Although at 15 days of incubation there were no significant differences between the exposed and unexposed embryos, at 21 days the embryos exposed to 1µT and 0.5mT showed significant reductions in this measurement ($p<0.05$ and $p<0.01$, respectively) as compared with the controls.

Table 4.- Anteroposterior diameter of the eye (mm).

	Day 15 of incubation	Day 21 (Hatching)
Controls	9.1143±0.3214 (N=21)	9.5294±0.5145 (N=17)
Treated with 1µT	9.1200±0.3820 (N=20) n.s.	9.1571±0.3627 (N=21) p<0.05
Treated with 0.1mT	8.9250±0.3878 (N=20) n.s.	9.2143±0.4488 (N=7) n.s.
Treated with 0.5mT	8.9333±0.4058 (N=18) n.s.	9.0409±0.4748 (N=22) p<0.01
Treated with 1mT	9.2700±0.4758 (N=20) n.s.	9.4350±0.3829 (N=20) n.s.

All values are means ± SD. N, number of sample; n.s., not significant.

DISCUSSION

We observed a reduction in corneal thickness and the anteroposterior diameter of the lens in chick embryos exposed to magnetic fields throughout their development (21 days). This reduction was statistically significant in embryos exposed to the highest intensities (0.5 mT and 1 mT) as compared with controls. By contrast, the anteroposterior diameter of the anterior chamber was increased with the lower intensities (1µT, 0.1mT), both at 15 and 21 days of incubation. The anteroposterior diameter of the eye was smaller in all the embryos exposed to magnetic fields after 21 days in comparison with the unexposed embryos.

On the basis of the present data, we propose that magnetic fields can affect the morphology of the eye, although much research remains to be done before definitive conclusions can be drawn. To date, we have been unable to find any study in the literature with which to compare our findings.

REFERENCES

ALFREDSSON L, HAMMAR N and KARLEHAGEN S (1996). Cancer incidence among male railway engine drivers and conductors in Sweden, 1976-90. *Cancer Causes Control*, 7: 377-381.

ARMSTRONG B, THERIAULT G, GUENEL P, DEADMAN J, GOLDBERG M and HEROUX P (1994). Association between exposure to pulsed electromagnetic fields and cancer in electric utility workers in Quebec, Canada and France. *Am J Epidemiol*, 140: 805-820.

BOROOJERDI B, PRAGER A, MUELLBACHER W and COHEN L (2000). Reduction of human visual cortex excitability using 1-Hz transcranial magnetic simulation. *Neurology*, 54: 1529-1531.

FEYCHTING M, FLODERUS B and AHLBOM A (2000). Parenteral occupational exposure to magnetic fields and childhood cancer (Sweden). *Cancer Causes Control*, 11: 151-156.

FEYCHTING M, SCHULGEN G, OLSEN JH and AHLBOM A (1995). Magnetic fields and childhood cancer –a pooled analysis of two Scandinavian studies. *Eur J Cancer*, 31A: 2035-2039.

FLODERUS B, TORNQVIST S and STENLUND C (1994). Incidence of selected cancers in Swedish railway workers, 1961-79. *Cancer Causes Control*, 5: 189-194.

GUENEL P, NICOLAU J, IMBERNON E, CHEVALIER A and GOLDBERG M (1996). Exposure to 50-Hz electric field and incidence of leukemia, brain tumors, and other cancers among French electric utility workers. *Am J Epidemiol*, 144: 1107-1121.

JUUTILAINEN J, MATILAINEN P, SAARIKOSKI S, LAARA E and SUONIO S (1993). Early pregnancy loss and exposure to 50-Hz magnetic fields. *Bioelectromagnetics*,14: 229-236.

KHEIFETS LI, SUSSMAN SS and PRESTON-MARTIN S (1999). Childhood brain tumors and residential electromagnetic fields (EMF). *Rev Environ Contam Toxicol*, 159: 111-129.

KNAVE B (1994). Electric and magnetic fields and health outcomes--an overview. *Scand J Work Environ Health*, 20 Spec NoP 78-89.

KRAUT A, TATE R and TRAN N (1996). Residential electric consumption and childhood cancer in Canada (1971-1986). *Arch Environ Health*, 49: 156-159.

KUES HA, D'ANNA SA, OSIANDER R, GREEN WR and MONAHAN JC (1999). Absence of ocular effects after either single or repeated exposure to 10mW/cm(2) from a 60GHz CW source. *Bioelectromagnetics*, 20: 463-473.

LANKOSZ J, TOKARZ J, WESELUCHA P, OCHMANSKI W, GZYL E, BARBARO B, GATARSKI J, DROZDZEWICZ L and KIELAR I (1983). Health status of the workers exposed to strong, constant magnetic fields. *Med Pr*, 34: 65-73.

LI CY, LEE WC and LIN RS (1998). Risk of leukemia in children living near high-voltage transmission lines. *J Occup Environ Med*, 40: 144-147.

MICHAELIS J, SCHUZ J, MEINERT R, ZEMANN E, GRIGAT JP, KAATSCH P, KALETSCH U, MIESNER A, BRINKMANN K, KALKNER W and KARNER H (1998). Combined risk estimates for two German population-based case-control studies on residential magnetic fields and childhood acute leukemia. *Epidemiology*, 9: 92-94.

MILLER AB, TO T, AGNEW DA, WALL C and GREEN LM (1996). Leukemia following occupational exposure to 60-Hz electric and magnetic fields among Ontario electric utility workers. *Am J Epidemiol*, 144: 150-160.

MYERS A, CLAYDEN AD, CARTWRIGHT RA and CARTWRIGHT SC (1990). Childhood cancer and overhead powerlines: a case-control study. *Br J Cancer*, 62: 1008-1014.

OLSEN JH, JENSEN JK, NIELSEN A and SCHULGEN G (1994). Electromagnetic fields from high-voltage installations and cancer in childhood. *Ugeskr Laeger*, 156: 2579-2584.

PETRIDOU E, TRICHOPOULOS D, KRAVARITIS A, POURTSIDIS A, DESSYPRIS N, SKALKIDIS Y, KOGEVINAS M, KALMANTI M, KOLIOUSKAS D, KOSMIDIS H, PANAGIOTOU JP, PIPEROPOULOU F, TZORTZATOU F and KALAPOTHAKI V (1997). Electrical power lines and childhood leukemia: a study from Greece. *Int J Cancer*, 73: 345-348.

PRESTON-MARTIN S, NAVIDI W, THOMAS D, LEE PJ, BOWMAN J and POGODA J (1996). Los Angeles study of residential magnetic fields and childhood brain tumors. *Am J Epidemiol*, 143: 105-119.

SKRINNIK AV (1985). Effect of a pulsed magnetic field on corneal permeability and the sorption properties of tissue structures and refractive media of the eye. *Biull Eksp Biol Med*, 100: 429-431.

STRZHIZHOVSKII AD, GALAKTIONOVA GV and CHEREMNYKH PA (1980). Tissue specificity of changes in mitotic activity induced by strong magnetic fields. *Tsitologiia*, 22: 205-209.

THERIAULT G, GOLDBERG M, MILLER AB, ARMSTRONG B, GUENEL P, DEADMAN J, IMBERNON E, TO T, CHEVALIER A and CYR D (1994). Cancer risks associated with occupational exposure to magnetic fields among electric utility workers in Ontario and Quebec, Canada, and France: 1970-1989. *Am J Epidemiol*, 139: 550-572.

TYNES T and HALDORSEN T (1997). Electromagnetic fields and cancer in children residing near Norwegian high-voltage power lines. *Am J Epidemiol*, 145: 219-226.

VERKASALO PK, PUKKALA E, HONGISTO MY, VALJUS JE, JARVINEN PJ, HEIKKILA KV and KOSKENVUO M (1993). Risk of cancer in Finnish children living close to power lines. *BMJ*, 307: 895-899.