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EFFECTS OF AMBIENT ELF MAGNETIC FIELDS: VARIATIONS IN ELECTROLYTE LEVELS IN THE BRAIN AND BLOOD PLASMA

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Purpose: To determine whether concentrations in the brain and plasma tissues are influenced by ELF magnetic fields.

Methods: Male, 250-300 g guinea pigs were kept under the laboratory conditions of 23 °C, a day and night cycle of 12 hours and an ambient geomagnetic field of 30 μT (microTeslas) in plastic cages. The subjects were divided into two groups: a control group (n=11) and an experimental group (n=24), which was exposed to a 50 Hz, 2 mT field for 4 hours/day for 5 days. The control subjects were handled in an identical manner without being exposed to any magnetic field. After the completion of the experiment, blood samples from the control and exposed animals were collected by cardiac puncture without hemolysis. The animals were sacrificed by ether inhalation in a closed box; then their brains were dissected out immediately. Cu++, Zn++, Ca++ and Mg++ concentrations in both the plasma and brain tissue of guinea pigs were determined by flame atomic absorption. Na+ and K+ concentrations in the plasma were measured by direct application to flame photometry.

Results: Na+, Ca++ and Mg++ concentrations in the blood plasma increased while Zn++ and K+ concentrations decreased with the effect of the magnetic field. The increase in the Ca++ concentration was statistically significant. The Cu++ concentration was not affected by magnetic field exposure. The magnetic field, having a greater effect on Zn++ and Mg++ concentrations, increased Cu++, Zn++, Ca++ and Mg++ concentrations in the brain tissue.

Conclusion: The ELF magnetic field altered the Ca++ concentration in the brain and plasma tissues. It is generally thought that the cell membrane is the first target of external fields, and calcium regulated activity is involved in ELF field coupling to living systems. Our results appear consistent with this hypothesis.

Key Words: ELF Magnetic Fields, Guinea Pigs, Brain Electrolytes, Plasma Electrolytes

ÇEVRESEL ELF MANYETIK ALANLARIN ETKILERI: BEYIN VE PLAZMA ELEKTROLIT SEVIYELERINDE DEĞIŞIMLER

Amaç: Bu çalışmada, ELF frekanslı manyetik alanların beyin ve plazma elektrolit düzeylerini etkileyip etkilemediğini araştırmak amaçlanmıştır.

Yöntem: Erkek, 250-300 g ağırlıkta kobaylar, sıcaklığı 23 °C, geomanyetik alanı 30 μT (mikroTesla) olan laboratuarda plastik kafeslerde ve 12 saat gece-12 saat gündüz koşullarında muhafaza edildi. Kontrol ve deney grubu olmak üzere iki gruba ayrıldı; kontrol grubu kobayları (n=11) aynı laboratuar şartlarında manyetik alana maruz bırakılmadan tutuldu. Deney grubu (n=24) ise günde 4 saat olmak üzere 5 gün boyunca 50 Hz, 2 mT manyetik alana maruz bırakıldı. Maruziyet tamamlandığında tüm hayvanların kan örnekleri enjektör ile doğrudan kalbe girerek alındı, bunu takiben hayvanlar eter ile bayıltıldı ve beyin dokuları çıkarıldı. Plazma be beyin Cu++, Zn++, Ca++ ve Mg++ konsantrasyonları atomik absorbsiyon tekniği ile Na+ ve K+ ise doğrudan plazmanın alev fotometre cihazına uygulanması yoluyla tesbit edildi.

Bulgular: Plazma Na+, Ca++ ve Mg++ düzeylerinin manyetik alan etkisi ile artmış olduğu, Zn++ ve K+ seviyelerinin ise azalmış olduğu belirlendi. Ca++ seviyesindeki artış istatistiksel anlamda önemli bulundu. Cu++ düzeyi manyetik alandan etkilenmedi. Beyin dokusu Cu++, Zn++, Ca++ ve Mg++ konsantrasyonları artmış bulundu, ancak Zn++ ve Mg++'un manyetik alandan daha fazla etkilenmiş olduğu saptandı.

Sonuç: ELF manyetik alanın beyin ve plazmada Ca++ miktarını etkilediği belirlendi. Dış kaynaklı alanların hedefinin hücre membranı olduğu ve ELF alanların canlı sistemlerle etkileşebilmesi için Ca++ tarafından düzenlenen mekanizmalara ihtiyaç duyulduğu yönünde bir genel inanış oluşmuştur. Bizim bulgularımız da bu hipotezi destekler görünmektedir.

Anahtar Kelimeler: ELF Manyetik Alanlar, Kobay, Beyin Elektrolitleri, Plazma Elektrolitleri

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Life developed in the presence of natural electromagnetic fields on Earth, but man-made electromagnetic fields have become increasingly common in the environment. Electromagnetic fields having various frequencies and intensities are produced by high-voltage transmission lines, household appliances, radio and television antennas, radar and industrial equipment in the residential environment (1,2). In recent years, concerns have been raised about the possible health effects of environmental exposure to the ELF range (ELF: Extremely Low Frequency <300 Hz) of the electromagnetic spectrum, which is associated with electrical power transmission, distribution and use (50-60 Hz) (3-7). Epidemiological studies and many laboratory investigations have suggested a link between ELF magnetic fields and cancers, especially childhood leukemia. It was reported that persons living in the vicinity of high voltage transmission lines, who work closely with electric power tools or appliances, or who are exposed to fields from distribution lines may be exposed to an elevated risk of some types of cancer (3-20). ELF magnetic fields have been classified as a "possible human carcinogen" by The International Agency for Research on Cancer (IARC) (4,7). There are many investigations on the effects of electromagnetic fields (EMF) on the proliferation, function and metabolism of cells, tissues and organs for examining basic EMF interactions with living organisms. It has been shown that EMFs affect biomolecular synthesis in cells, the metabolism of carbohydrates, protein and nucleic acids, the kinetics of DNA, RNA and protein production, endocrine functions, immunoresponses to various antigens and lectins, membrane permeability, cellular respiration and growth rate, morphological structure and development in adult animals (20-45). Environmental electromagnetic fields (EMFs) influence behavioral, neurophysiological and chemical responses in the mammalian central nervous system also have a significant effect on the blood and circulation, but the mechanism of these effects is not known (46-51). EMF effects in numerous biological systems have indicated that the cell membrane is central in the interactions with EMFs (52-55).

EMFs commonly found in the environment can be expected to cause biologically significant interactions between transported cations and basic domains of cation channel proteins. Such interactions could trigger functional (gating) conformation changes in channel proteins, leading to altered physiological states of the cell (53,55-58).

EMFs in the 50-60 Hz range can perturb the transmembrane movement of cations such as K+, Na+ or Ca++ through their respective channels; thus they can modify cation flow across biological membranes and alter cell metabolism for producing biological effects (53-55,57-61).

Cells have complex electrical systems sensitive to electric and magnetic changes. An important aspect of understanding the possible effects of ELF electric and magnetic fields on living systems is the analysis of ionic and molecular pathways involved in the interaction of these fields at the cellular and subcellular levels (53,56,57,59,62).

There is a physical obstacle between the plasma and the extracellular environment of the brain, called the blood-brain barrier (BBB). The BBB normally provides resistance to movements of large molecular weight, fat-insoluble substances (e.g., proteins or polypeptides) from the blood vessels into the surrounding cerebral extracellular fluid, presumably to protect the brain from invasion by various blood-born pathogens and toxic substances (63). Some water soluble substances (e.g., sulfate, albumin) are highly excluded, whereas others (e.g., metabolic substrates and many inorganic ions) penetrate by carrier-mediated mechanisms. The impermeability of the BBB effectively uncouples the concentrations of plasma and brain interstitial fluid. This permits the regulation of the brain cell microenvironment, independent of plasma composition. Ionic homeostasis of this microenvironment is of great importance for the central nervous system.

It has been shown under in vitro and in vivo conditions that EMF alters Ca++ efflux in central nervous system tissues. It is generally thought that the cell membrane and Ca++-regulated activity is involved in bioactive ELF field coupling to living systems (53-62).

The aim of this study was to investigate whether the in vivo exposure of guinea pigs to an ELF magnetic field affects the concentrations in plasma and brain tissues. Variations in the concentrations of Cu++, Zn++, Ca++ and Mg++ in brain tissues and Na+, K+, Ca++, Mg++, Cu++ and Zn++ in blood plasma were determined under the effect of a 50 Hz, 2 mT magnetic field applied for 4 hours/day for 5 days.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University (Report no: 36-7838). A total of 35 male, 250-300 g (10-12-week old) guinea pigs were used. The animals were kept in the laboratory at a room temperature of 23 °C, a day and night cycle of 12 hours and an ambient geomagnetic field of 30 µT. Twenty-four guinea pigs were housed in the centre of the Helmholtz coils, 2 per plastic cage, and were exposed to a 50 Hz, 2 mT magnetic field for 4 hours/day for 5 days. To control possible variations in responses due to circadian rhythm the exposure period was chosen between 8:00 and 12:00 a.m. Eleven subjects were handled in an identical manner without being exposed to any magnetic field and used as controls.

Twenty hours after exposure, blood samples from the control and exposed animals were collected into tubes. After centrifugation, the plasma was separated. All of the animals were sacrificed by ether inhalation in a closed box. Their brains were dissected out immediately. They were shocked with liquid nitrogen and stored in a deep freeze at -70 °C until the electrolytes were analysed.

Analyses of electrolytes were performed by atomic absorption spectrophotometer and emission flame photometer. All experiments were run blind, i.e. the researchers performing the electrolyte analysis did not know the exposure conditions the animals were being subjected to. Exposure Apparatus

Circular coil pairs of a Helmholtz configuration were used vertically. The magnetic field generated by the vertical coils was classified as vertical (field lines perpendicular to the bottom plane of the animal's cage).

A pair of circular coils of either 42.75 cm diameter or 21.375 cm clearance was constructed from insulated copper wire and consisted of 154 turns. The electrical parameters of each were as follows: resistance, 1.2 ohms; and inductance, 19.6 mH.

Sinusoidal current pulses of frequency 50 Hz were generated at the output of the clipper circuit, which was driven by the specially designed variable transformer, 2.7 KVA in power, to feed the coils.

The magnetic field was measured with a Hall-Effect Gaussmeter (Yokogawa 3251, Japan) with an axial probe.

The frequency and waveform of the magnetic field were monitored on an oscilloscope (ITT Instruments OX 722 Metrix, France) by the current flowing in the field coils' connection with the gaussmeter.

Analysis of Electrolytes in the Blood Plasma

Blood was collected by cardiac puncture without hemolysis by disposable syringe into a glass tube with heparin as anticoagulant for Ca++, Mg++, Cu++ and Zn++. Having a gel as an anticoagulant a disposable glass tube was also used for Na+ and K+.

Plasma was separated immediately by centrifugation and stored at -20 °C until the determination of the electrolytes.

Ca++, Mg++, Cu++ and Zn++ concentrations in blood samples from magnetic field exposed and control animals were determined using flame atomic absorption (64,65). Heparinized plasma from each animal (3-5 ml) was applied to the atomic absorption spectrophotometer (AAS) (Varian 30/40, Australia) for determination of the Ca++, Mg++, Cu++ and Zn++ concentrations. The working conditions of the AAS used for determining the electrolytes are given in Table 1.

Analyses of Na+ and K+ were performed by direct application of approximately 1 ml of plasma to the emission flame photometer (Instrumentation Laboratory-943) without any processing.

Table 1: Premixed Flames and Wavelength of Electrolytes.

Electrolyte	Wavelength (nm)	Flame
Ca++	422.7	Nitrous Oxide - Acetylene
Mg++	285.2	Air-Acetylene
Cu++	324.7	Air-Acetylene
Zn++	213.9	Air-Acetylene

Analysis of Electrolytes in the Brain Tissue

Ca++, Mg++, Cu++ and Zn++ concentrations in the brain tissues of animals exposed to the magnetic field and control animals were determined using flame atomic absorption.

Brain samples were digested with nitric acid-hydrogen peroxide in a microwave digestion unit (Milestone, Mega 1210, Italy) and then they were used for determining the concentrations of electrolytes with AAS (Varian 30/40, Australia).

Statistical Analysis

The Mann-Whitney U test was applied for the statistical analysis.

RESULTS

Na++, Ca++ and Mg++ concentrations in blood plasma increased, while Zn++ and K+ concentrations decreased with the effect of the magnetic field (Table 2, Table 3). The increase in Ca++ concentration was statistically significant. Cu++ concentration was not affected by magnetic field exposure (Figure 1, Figure 2). With a greater effect on Zn++ and Mg++, concentrations of Cu++, Zn++, Ca++ and Mg++ in the brain tissues of guinea pigs exposed to the magnetic field were increased (Table 4, Figure 3).

Table 2: Ca++, Mg++, Zn++ and Cu++ Concentrations of Blood Plasma.

GROUPS	Ca++	Mg++	Zn++	Cu++
Control (C)	118.6±3.6 (n_C=11)	28.5±1.4 (n_C=11)	1.30±0.09 (n_C=11)	0.50±0.03 (n_C=11)
Exposed (E)	126.4±2.3 (n_E=24)	30.7±1.3 (n_E=24)	1.20±0.04 (n_E=22)	0.50±0.02 (n_E=22)

* Concentrations are measured by atomic absorption spectrophotometry and given as mean ± sem (standard error of mean)

Table 3: Na+ and K+ Concentrations of Blood Plasma.

	CONCENTRATIONS**	(mg/ml)
GROUPS	Na+	K+
Control (C)	136.0±3.5 (n_C=11)	9.4±1.1 (n_C=8)
Exposed (E)	137.5±2.8 (n_E=24)	8.7±0.9 (n_E=12)

** Concentrations are measured by Emission Flame Photometry and given as mean ± sem (standard error of mean)

Table 4: Concentrations of Brain Electrolytes.

	CONCENTRATIONS*		**(mg/g wet weight)**	
GROUPS	**Ca++**	**Mg++**	**Zn++**	**Cu++**
Control (C) (n_C=8)	249±42	137±10	13.9±0.9	1.67±0.13
Exposed (E) (n_E=11)	254±47	144±11	15.1±1.2	1.77±0.09

* Concentrations are given as mean ± sem (standard error of mean)

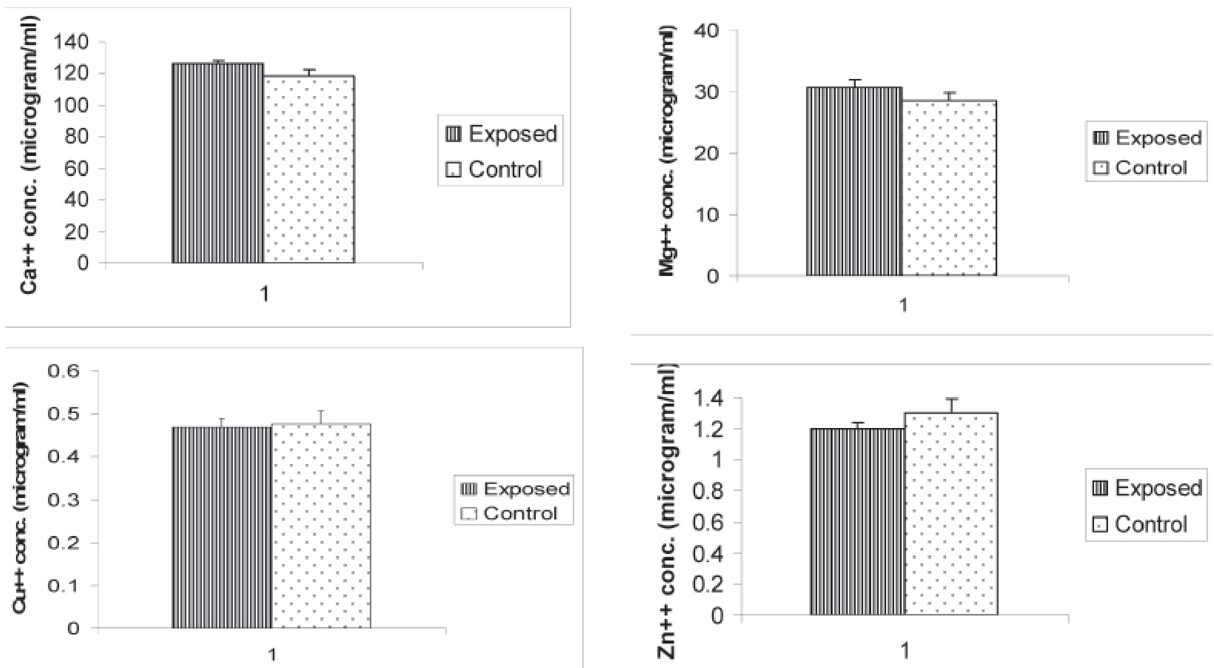

Figure 1. Ca++, Mg++, Cu++ and Zn++ concentrations in plasma of exposed and control animals

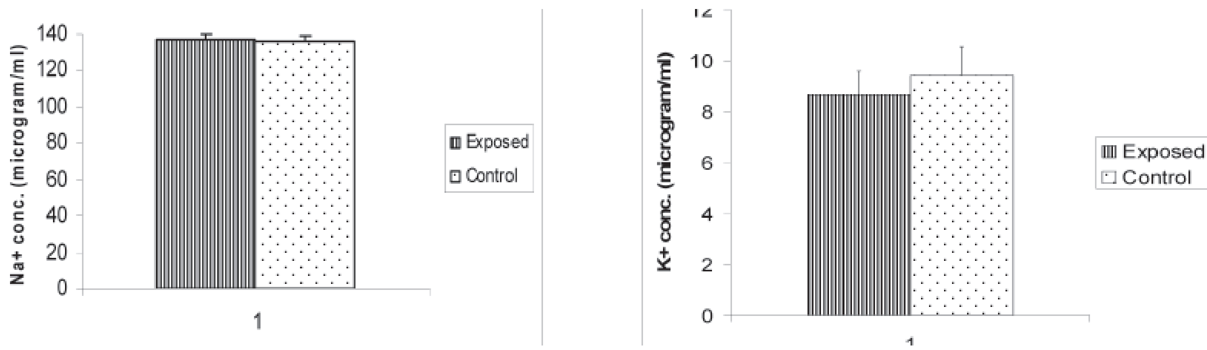

Figure 2. Na+ and K+ concentrations in plasma of exposed and control animals

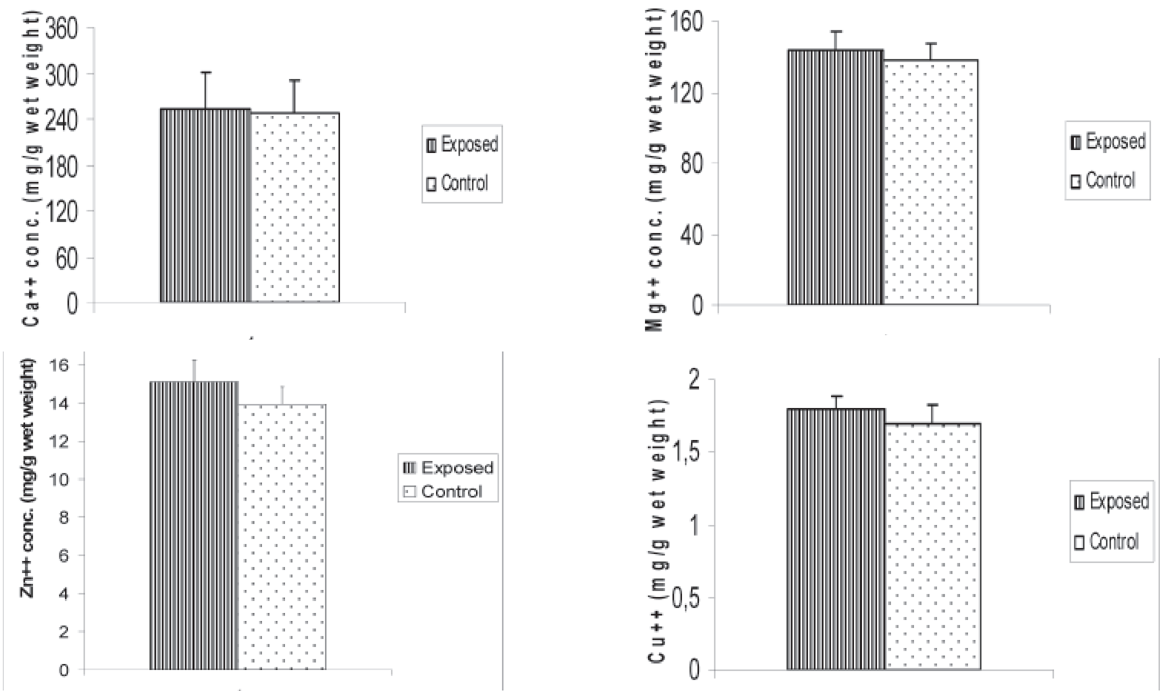

Fig. 3. Brain electrolytes concentrations of exposed and control animals.

DISCUSSION

Increases in Ca++, Mg++, Cu++ and Zn++ concentrations in the brain tissues and in Na+, Ca++ and Mg++ concentrations in the plasma of animals exposed to a magnetic field were observed with respect to the controls. K+ and Zn++ concentrations were decreased in the plasma in this study.

Gmitrova et al. studied electrolyte concentrations of plasma and brain tissue in guinea pigs (66). They used a 50 Hz, 3 mT magnetic field for 1 h/day for 5-6 days and they found that Ca++ and Mg++ concentrations were increased in the plasma, whereas the Ca++ concentration was increased and the Mg++ concentration was decreased in the brain. The results of the Ca++ concentrations of plasma and brain tissue are consistent in the two studies. The Mg++ concentration is also consistent in the plasma but is contradictory in the brain tissue. It has been observed that changes in plasma Na+ and K+ concentrations are not consistent.

Eraslan et al. studied blood electrolyte concentrations under the effects of 5 mT magnetic fields with frequencies of 60 Hz and 90 Hz. Magnetic fields were applied for 8 h/day (67). In all groups, it was found that [Na+] and [Ca++] decreased while [K+] increased. Eraslan et al. also reported the effects of a 90 Hz, 5 mT magnetic field applied for 12 h/day for 15, 30 and 45 days on blood electrolytes (68). The Na+ concentration was increased while Ca++ and K+ concentrations were decreased throughout the study. Variations in the concentrations of Na+ and K+ are consistent with those in our study.

It is suggested that the effects of EMF were highly specific regarding the frequency and amplitude of the applied field. Discrepancies between these results may be due to the experimental parameters such as field parameters, exposure periods, application periods during the day and methods of analysis (62).

Ionic processes involved in the cell metabolism might have been affected by the 50 Hz magnetic field. It was suggested that the cell membrane and Ca++-regulated activity are involved in bioactive ELF field coupling to living systems (31). Ca++ is an essential ion in transductive coupling of a wide

IONS	Our experiment 50 Hz, 2 mT 4 h/days, for 5 days		Gmitrova's experiment 50 Hz, 3 mT 1 h/day, for 5-6 days		Eraslan et al.'s experiment 8 h/day, for 1 day		Eraslan et al.'s experiment 12 h/day for 15, 30, 45 days
	Plasma	Brain	Plasma	Brain	60 Hz, 5 mT	90 Hz, 5 mT	90 Hz, 5 mT
Na^+	↑	not studied	↓	↓	↓	↓	↑
K^+	↓	not studied	↑	↓	↑	↑	↓
Ca^{++}	↑	↑	↑	↑	↓	↓	↓
Mg^{++}	↑	↑	↑	↓	not studied	not studied	not studied
Cu^{++}	No effect	↑	not studied	not studied	not studied	not studied	not studied
Zn^{++}	↓	↑	not studied	not studied	not studied	not studied	not studied

range of immunological, endocrinological, and neurological events at cell membrane surfaces (53,55,61).

Ionic homeostasis of the brain's microenvironment is of great importance for the central nervous system. The BBB, composed of a vascular endothelial cell layer and a layer of glial end feet, isolates the brain from fluctuations in plasma concentrations by separating the plasma and the extracellular environment of the brain. The electrical resistance of the cerebral endothelium reflects its ionic permeability. The permeability of the BBB increases in response to chemicals that cause inflammation, such as histamine, bradykinin, arachidonic acid and free oxygen radicals (63,69). Furthermore, BBB permeability was increased by a low intensity EMF, depending upon the pulse characteristics (16).

Increased BBB permeability may result in increased Ca++, Mg++, Cu++ and Zn++ concentrations in the brain. Additionally, disturbing the barrier functions may alter the ionic distribution of the body by altering metabolic activities related to Na+, K+, Ca++, Mg++, Cu++ and Zn++ ions. Increased Mg++ concentrations both in the plasma and in the brain may reflect induced phosphorylation and related enzymatic reactions.

Actually an ELF EMF itself could lead to apoptosis (27). Cell lysis and/or cell apoptosis may result in an increase in the Ca++ concentration. Zinc may affect the immune status of animals and humans. Allen et al. reported that low serum zinc and high urinary zinc excretion in lung cancer patients were significantly associated with depressed T cell phytohemagglutinin response. The cytotoxic functions of natural killer (NK) cells were reduced to a great extent in zinc-deficient rats (70). NK cytotoxic activity has also been studied in our laboratory and reduced NK activity was observed in the splenocytes of guinea pigs exposed to a 50 Hz, 2 mT magnetic field applied for 5 days for 4 h/day (30). A reduced Zn++ concentration in plasma is consistent with reduced NK activity.

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