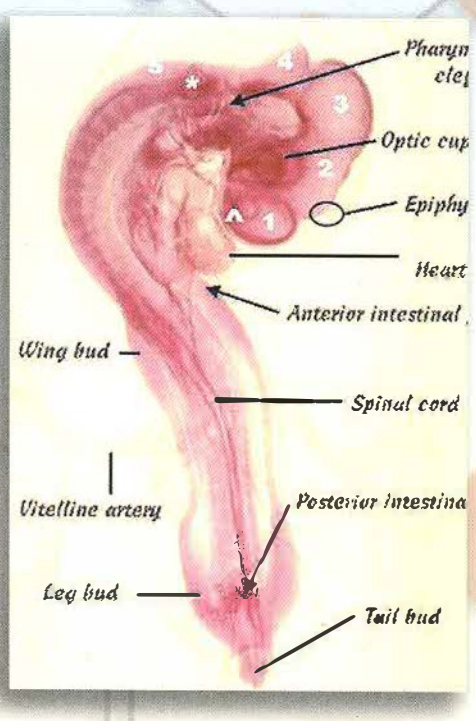

EFFECTS OF MAGNETIC AND ELECTROMAGNETIC FIELDS ON EMBRYOS

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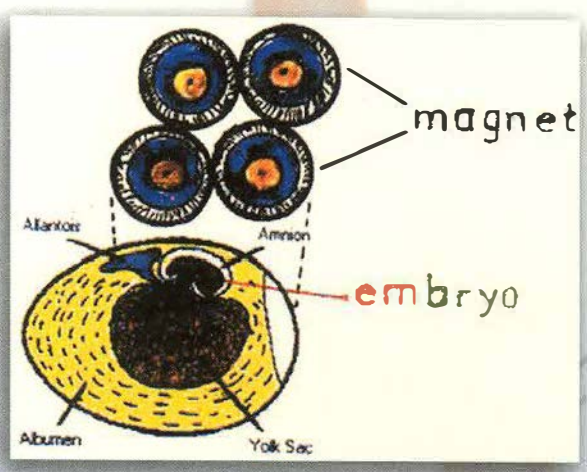

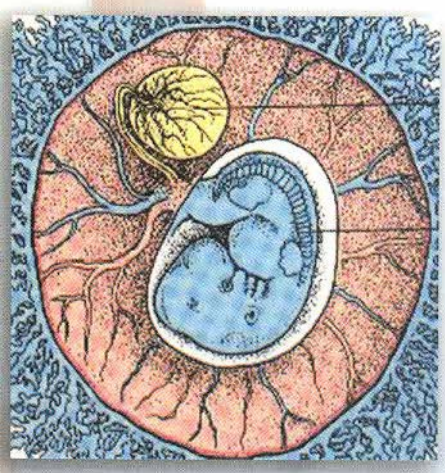

Scientific and Applied Research Center (SARC)
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EFFECTS OF MAGNETIC AND ELECTRO-MAGNETIC FIELDS ON EMBRYOS

تأثيرات المجالات المغناطيسية والكهرومغناطيسية على الأجنة

EFFECTS OF MAGNETIC AND ELECTRO-MAGNETIC FIELDS ON EMBRYOS

Project No. (1/7-99)

تأثير المجالات المغناطيسية والكهرومغناطيسية على الأجنة

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CONTENTS

PREFACE

The effects of magnetic and electro-magnetic fields on embryos is an increased interest subject related to the increasing use and practice of magnetic devices in alternative clinical medicine and research. In fact, human is living surrounded by many magnetic impacts, some are naturally occurring like earth magnetism which directed from north to south, others are artificial such as those generated by the electric power lines or even some home appliances.

Some epidemiological studies which have established in last decades showed that even households in the high-wire code categories which have higher capacity wiring or closer to power stations and high energy transmission lines show approximately 1.5 fold increase in childhood leukemia over households with low capacity wiring or those further away from power sources. As a matter of fact, electro-magnetic fields are of direct impact on the human body and their effects on embryos are of great importance to investigate. This is the main aim of the current study.

ACKNOWLEDGEMENTS

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SUMMARY

SUMMARY

The current project concerns with the effects of magnetic and electro-magnetic fields on embryos. The chick embryos were selected as a an experimental animals and the time of study was extent from March 2000 up to March 2001. Four groups of fertilized eggs were incubated and selected for developmental stages of 5, 10, 15 days and immediately after hatching. These groups were exposed to two types of magnetic fields which are commonly used in medical therapy. The first was static magnetic field with 80 mT and the second alternating with 20.000 mT. The effects were observed on blood, haemoglobin contents, osmotic activity of cellular membranes and histological structure of pineal gland. There was an evidence in the results indicating some effects of these types of magnetic fields on erythrocytes (Red blood cells) and also on osmotic activity rates of its plasma membrane. However, no effect was observed on the pineal gland histological structure. The work team recommend the extension of this interesting study in the future to comprise another physiological effects specially melatonin which is the main secretion of pineal gland and plays an remarkable role of inhibition of some types of breast cancer.

INTRODUCTION

INTRODUCTION

Ever since the discovery of lodestones – naturally magnetized rocks – well over 2000 years ago, man has been intrigued by the possible relationship between the health of the human body and magnetism and electricity. Science has developed many uses for electricity and magnetism in the area of health care. These types of energy are especially useful in helping to diagnose disease. Their use in the treatment of disease. A number of purported health care methods claim that much of our disease is the result of a malfunction of the electrical or magnetic currents of the body (Macklis and Roger, 1993).

1-1. MAGNETIC AND ELECTRO-MAGNETIC FIELDS:

The electromagnetic fields (EMF) surround any source that carries an electric current and the intensity of it falls 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 (Cameron *et al.* 1993).

Power lines, electrical wiring, and appliances all produce electric and magnetic fields. EMFs are invisible lines of force that surround any electrical device. Electric and magnetic fields have different properties and possibly different ways of causing biological effects.

It must be noted that while electric fields are easily shielded or weakened by conducting objects such as trees, buildings and even human skin, magnetic fields not. However, both electric and magnetic fields weaken with increasing distance from the source.

The electromagnetic spectrum covers an enormous range of frequencies. These frequencies are expressed in cycles per second (i.e. Hz). Electric power which is normally 50-60 Hz is in the extremely – low – frequency range, which includes frequencies below 3000 Hz. The higher the frequency, the shorter the distance between one wave and the next, and the greater the amount of energy in the field. Microwave frequency fields, with wavelengths of several inches, have enough energy to cause heating in conducting material. Still higher frequencies like X-rays cause ionizations and the breaking of molecular bonds which damages genetic material. In comparison, power frequency fields have wavelengths of more than 3100 miles (5000 Km) and consequently have very low energy levels that do not cause heating or ionization. The earth also produces EMFs mainly in the form of DC (also called static fields). Electric fields are produced by thunderstorm activity in the atmosphere. Magnetic fields are though to be produced by electric currents flowing deep within the earth's molten core. The DC magnetic field averages around 500 milligauss (mG). This number is larger than typical AC electric power magnetic fields, but DC fields do not create currents in objects in the way that AC fields do.

1-2. EPIDEMIOLOGICAL ASPECTS OF EMFs:

Epidemiology is concerned with the studies related to the patterns and possible causes of diseases in human populations. Several epidemiologic studies have looked for EMF effects on Cancer, Leukemia, pregnancy outcomes and general health. Some studies have found a relation between cancer and people living near power lines. Most studies have analyzed a possible association between proximity to power lines and various types of childhood cancer. The first study to report an association between power lines and cancer was conducted in 1979 in Denver by Dr. Nancy Wertheimer and Ed Leeper . They found that children who had died from cancer were 2 to 3 times more likely to have lived within 40 m of a high – current power line than were the other children studies. Exposure to magnetic fields was identified as a possible factor in this finding (NIEHS, 1998). More recent studies were also significant associations between living near high-current power lines and childhood cancer incidence like leukemia.

There is also some epidemiological evidence for an association between EMFs exposure and breast cancer, but studies have also reported evidence to the contrary. In 1994, Loomis and others (NIEHS, 1998). examined death records of female workers and found that women employed in electrical occupations were slightly more likely to have died of breast cancer than were other working women. However, because the study could not control for factors such as diet, fertility and family history which are known to affect breast cancer risk. The results are considered to be preliminary, not conclusive. In 1994 a study was done in Norway and reported

an excess risk of breast cancer among female radio and telegraph operators aboard ships. However, A 1993 Danish study found no association between occupational EMF exposure and female breast cancer. The reason for the recent interest in EMFs and breast cancer has less to do with epidemiology than with biology-laboratory evidence concerning the role of EMFs and melatonin in the developed and suppression of breast cancer. (NIEHS, 1998).

1–3. BIOLOGICAL ASPECTS OF EMFS EFFECTS:

Several kinds of biological effects have been reported in studies of electric and magnetic fields. A biological effect is a measurable change in some biological factor. It may or may not have any bearing on health. Overall, effects attributed to EMFs have been small and difficult to reproduce. Very specific laboratory conditions are usually needed for effects of EMFs to be detected. It is not known how EMFs actually cause these effects. Laboratory studies to date have not answered questions about possible human health effects. These studies are, however, providing clues about how EMFs interact with basic biological processes. The cell membrane may be an important site of interaction with induced currents from EMFs.

Zecca et al. (1998) have studied the biological effects of prolonged exposure to ELF electromagnetic fiekmlds in rats. Groups of adult male rats were exposed for 8 months to EMFs of two different field strength combinations: 5 mT- 1kV/m and 100 mT – 5 kV/m. A third group was sham exposed. Field exposure was 8

hrs/day for 5 days/week. Blood samples were collected for hematology determinations before the onset of exposure and at 12 weeks intervals. At sacrifice, liver, heart mesenteric lymph nodes, bone marrow and testes were collected for morphology and histology assessments, while the pineal gland and the brain were collected for biochemical determinations. At both field strength combinations, no pathological changes were observed in animal growth rate, in morphology and histology of the collected tissues specimens (liver, heart, mesenteric lymph nodes, testes, bone marrow) and in serum chemistry. An increase in norepinepherine levels occurred in the pineal gland of rats exposed to the higher field strength. The major changes in the brain involved the opioid system in frontal cortex and parietal cortex. From this study, it may be hypothesized that EMF may cause alteration of some brain functions.

1- 4. EFFECT OF EMFs ON PINEAL GLAND AND ITS EFFECT ON CANCER.

The pineal gland which in human is located near the anatomical center of the brain, is normally responsive to visible electro-magnetic fields like light since the eyes are functionally connected to the pineal gland by a series of neurons. Normally, the pineal gland produces low amounts of melatonin during the day and high amounts at night; this rhythm is reflected in the blood melatonin concentrations which are higher at night than during the day. In both man and lower mammals, their exposure to light at night is followed by a drop in pineal melatonin production and blood melatonin levels. Likewise, exposure of non-human mammals to

sinusoidal electric and / or magnetic fields as well as pulsed static magnetic fields often reduces pineal melatonin production. Melatonin has many functions in the organisms and any perturbations (not only electromagnetic fields) which causes levels of melatonin to be lowered than normal may have significant physiological consequences. Melatonin, because it is a potent antioxidant, may provide significant protection against cancer initiation as well as promotion. However, it is pre-mature to conclude that the alleged increase cancer risk reported in individuals living in higher than normal electromagnetic environments related to reduced melatonin levels caused by such field exposures (Reiter, 1993).

In 1980s, scientists found that in rats exposed to 60-Hz electric fields, nighttime melatonin levels were reduced. Other studies have since reported that AC and DC magnetic fields can also affect melatonin levels in rats and hamsters. These experiments are very delicate and depend on a combination of factors such as age of the animals and length of day. Melatonin levels were not affected in sheep raised for nearly a year in the EMFs directly beneath a 500-kV transmission line. Experiments with baboons also showed no changes in melatonin. Grota et al. (1994) have proved that electric field exposure alters serum melatonin but not pineal melatonin synthesis in male rats.

1-5 . EFFECT OF EMFs ON EMBRYONIC DEVELOPMENT:

Studies about the effects of 50- to 100-Hz EMFs on various species of animal embryos like fish, chick, fly, sea urchin, rat and mouse embryos indicate that early stages of embryonic development are responsive to fluctuating magnetic fields. Chick, sea urchin and mouse embryos are responsive to magnetic fields intensities of 10-100 mG. Result from studies on sea urchin embryos indicate that exposure to conditions of rotating 60-Hz magnetic field (similar to those in our environment) interacts with cell proliferations at the morula stage in a manner dependent on field intensity. The cleavage stages prior to the 64-cell stages are not delayed by this rotating 60-Hz magnetic field suggesting that the ionic surges, DNA replication, and translation 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 during morula stage and suggests that this decrease in early histone production was limited 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. (Cameron et al., 1993).

Extremely low frequency (ELF) magnetic fields have been reported to disturb the development of chick embryo (Juutilainen 1987 and Ubeda et al. 1994). Occupational and residential exposure to ELF magnetic fields is considered to be a potential health hazard. Martin,1992 stated that a reproducible and

statistically significant number of malformations have revealed in live chicken embryos that had been exposed during the first incubation to a pulsed magnetic field (unipolar pulses, 100-pps, 1-mT peak density). In reverse contrast, no adverse effect was seen following similar exposure to 60-Hz, bipolar, unipolar waves at 3 mT.

Six independent experiments of common design were performed in laboratories in Canada, Sweden and USA. Fertilized eggs of domestic chickens were incubated in a pulsed magnetic fields (PMF) ; embryos were then examined for developmental abnormalities, blind of development and stage of maturity. Most of exposed embryos exhibited structural anomalies than did controls. When all data from all six laboratories are pooled, the difference in incidence of abnormalities in PMF exposed embryos (about 25%) and that of controls (nearly 19%), although is highly significant, as is the interaction between incidence of abnormalities. (Berman et al., 1990).

1-6 . EFFECT OF EMFs ON BLOOD AND CIRCULATORY SYSTEM:

In (2002), Dasdag, .et al. studied the effects of extremely low frequency electromagnetic fields on hematologic and immunologic parameters in welders. The study was carried out on 16 male welders and 14 healthy males from the same geographical areas and with similar life styles. The following hematologic and immunologic parameters measured in both groups: red blood cells; hemoglobin, hematocrit, platelets, total WBCs, neutrophils, lymphocytes and others. The results indicated that some of the hematologic and immunologic parameters under investigation

were similar in both groups. The hematocrit levels of the welders were found to be higher than those of the control. However, the differences observed were not clinically significant.

The effect of low – frequency electromagnetic field on the coagulant and fibrinolytic properties of blood and tissues of cardiovascular system was studies by Kuksinskii (1978). Phase characters of changes in the coagulative properties of blood as well as in those of the heart and aorta was established. Irradiation of rats for 8 days is attended by an increase in the coagulating and fibrinolytic activity of blood and simultaneous inhibition of the thromboplastic compounds of the heart and vascular wall. Increase of exposure to 20 days leads to inactivation of tissue and plasma blood coagulation factors. Lino, 1997 studied the effects of a homogenous magnetic field on erythrocyte sedimentation and aggregation. A magnetic field of 6.3 T in the vertical direction only slightly enhanced ESR in saline solution, which was consistent with an effect on cell orientation. The magnetic field greatly enhanced ESR in plasma. A morphological examination and the nonlinear time course of the sedimentation in plasma indicated that the magnetic field increased cell aggregation and thereby enhanced ESR in plasma.

The influence of 50-Hz electric and magnetic fields on human blood pressure was investigated by Korpinen and Patanen (1996). Electrocardiograms (ECG) and the blood pressure of 41 male volunteers were recorded using ambulatory methods. When analyzing the blood pressure, it could not be shown that the fields affected diastolic or systolic blood pressure. Blood pressure

regulations were studies also by Gmitrov et al. (1990) who stated that the local action of a permanent magnetic field (PMF) with an intensity of 0.2 T on the rabbit carotid area has a hypotensive effect under normotonic conditions. The fibrinolytical processes activated by magnetic field were studied by Gorczynska (1988), who exposed the rabbits to a constant magnetic field of 0.005 T, 0.1 T and 0.3 T induction for one hour per day each day for a period of four weeks. It was found that the magnetic field increases the rate of fibrinolytical processes. A decrease in fibrinogen concentrations, an increase in the level of fibrinogen degradation products and a considerably shorter time of fibrinolysis in plasma were all noted. The magnitude of these processes was proportional to duration of exposure to the magnetic field in action

The effects of interaction between a magnetic field and the haemodynamics of the arterial system have been studied by Sud and Sekhon (1989). An analysis of a supine subject has been carried out in the presence of an externally applied magnetic field. Time – dependent hematological changes were studied in workers exposed to electromagnetic fields emanating from radars and high frequency radio and the effects of this exposure on the blood. Statistically significant correlations between increasing white blood cell count and average daily exposure to EMFs were found. Changes in cell count were within the normal range, and thus their relation to epidemiological studies linking EMFs and leukemia, if any, is unclear. Results suggest that the time of exposure may be an additional factor (along with field strength and perhaps frequency) in ascertaining the safety of EMF exposure (Marino, 1995).

1-7. EFFECT OF EMFs ON IMMUNE SYSTEM:

Exposure of animals to electric fields does not appear to affect the immune system. In a comprehensive investigation of the immune system, no effects of exposure at low field strengths (150-250 V/m) in mice or rat were observed. In contrast to the apparent lack of E-field influence in vivo on the immune system, magnetic fields are reported to strongly affect system responses to mitogens and antigens. As a result of the different measures of immunity used in various studies, and the very inconsistent results obtained, it is difficult to come to any firm conclusions regarding the potential health implication of reported actions of EMF on the immune system. Exposure of whole animals to sinusoidal fields of the type generated by power lines has not, in general, produced major overall immunological effects. It seems more likely that EMF exposure may produce rather selective and subtle alterations in individual components of the immune system changes which vary depending on many characteristics of both the EMF exposure and the biological status of the immune system. Studies carried out in vitro have generally shown modest but conflicting effects on various components of the system. These have included both stimulation and inhibition of lymphocyte activation and proliferation, NK and LAK cell activity, and interleukin production. Reasons for this conflict include possible existence of "window effects" occurring at specific combinations of frequency and intensity, causing responses that may differ in direction under different exposure conditions (Creasy, 1994).

1-8. EFFECT OF EMFs ON BRAIN AND NERVOUS SYSTEM:

Many of the biological effects which have been reported in animals or humans exposed to EMFs appear to be associated with the nervous system. Such system responsiveness to fields might be anticipated since the nervous system plays a basic role in the interaction of animals with their environment. Indeed, other biological systems may be influenced indirectly by EMFs exposure through neural or hormonal functions. Reported nervous system effects from EMFs exposures include changes in behavioral and activity response, chemical changes in nerve cells; changes in excitability of nerves; altered neurotransmitter and neurohormone levels and disruption of biological rhythm.

Biological and morphological effects on the brain after exposure of rat to a 1439 MHz TDMA field were investigated by Tsurita et al. (2000). This field of Time division Multiple Access (TDMA) of 1439 is normally used in cellular phone. The morphological changes in the cerebellum were investigated by assessing the degeneration of Purkinje cells and the cell concentration in the granular layer. No significant changes were observed in the groups of rats exposed to HF-EMWs for two or four weeks. Averaged body masses were not affected by HF-EMWs exposure. In conclusion, a 1439 MHz TDMA field did not induce observable changes in permeability of the Blood brain barrier, morphological changes in the cerebellums, or body mass changes in rats, as evaluated by the conventional methods.

There is evidence clearing that electromagnetic fields can affect the brain catecholamine in fetal mice. Jiang et al. (1994) stated that brain catecholamine content in all the exposed groups of mice was lower than that in controls. Both electric and magnetic fields could decrease brain catecholamine content in mice, but electric fields had greater effects. The neurochemical effects of magnetic fields were also investigated by Dimberg (1995) who used 20 KHz magnetic field and studied its effects on the central nervous system in prenatally exposed mice. He found a significant difference was found in the whole brain weight between exposed and control animals. However a significant decrease in the whole brain detected in some exposed animals. No significant differences were found in the weight of cortex, hippocampus, septum or cerebellum on any of the sampling occasions. The amount of DNA was significantly reduced in magnetic field treated cerebellum and enzyme activities still enhanced in MF- treated cortex compared to controls. Most of the effects of MF-treatment during the embryonic period were similar to those induced by ionizing radiation but much weaker. However, the duration of the exposure required to elucidate the response of different markers to MF seems to be greater and effects appear later during development compared to responses to ionizing radiation

1-9. PHYSIOLOGICAL EFFECTS OF EMFs:

The physiological effects of EMFs are greatly varied and controversial. It showed be noted that the most reports of general physiological studies status appear to be relatively unaffected by exposure to ELF fields. It is also generally recognized that

exposure to such fields does produce responses in specific biological system, however, health implications for humans and animals have yet to be determined. Where the experimental affects are demonstrated, the mechanisms of interaction between the field and the organisms remain largely unknown. For instance, it is not known whether observed effects result from fields acting at the surface of the body, Electric field induced in the interior of the body or from magnetic fields penetrating the body.

Farrell et al. (1998) have shown that the application of a weak (4 mT) 60 Hz magnetic field (MF) can alter the magnitudes of the ornithine decarboxylase activity peaks which occur during gastrulation and neurulation of chick embryo. There was also evidence shown that the electrogenic Rb+ transport of (Na, K)-ATPase can be activated by electric fields. Serpersu and Tsong, (1984) have presented the evidence that transport of Rb+, Na+ and K+ were carefully monitored during the voltage stimulation. It was shown that the electric field stimulated only the ouabain-sensitive influx of Rb+, and this uptake was against a chemical concentration gradient. The applied electric field could polarize the membrane to provide membrane potential required for the electrogenic transport of Rb+. The data of this study also show vanadate at 180 microM completely inhibited the ATP-dependent Na+ and Rb+ pumping activities of the enzyme, but only reduced the voltage-stimulated Rb+ uptake to 50% level. This represents the first systematic study of the activation of a transport ATPase by an externally applied electric field.

Studies concerning the effects of extremely low frequency EMFs on plasma membrane-associated enzymes were carried out by Moses and Martin (1992). They investigated the changes in levels of 3 enzymes: 5- nucleotase (5,NT), Acetylcholinesterase (AchE) and Alkaline phosphatase (ALP) in early chick embryos. They found that enzyme levels were significantly higher in embryos with various anatomic malformations than in those with no visible abnormal characteristics. The presence of the electromagnetic field was associated with a marked reduction in enzyme activities in abnormal embryos.

EMFs and its effects on pituitary, thyroid and adrenocortical hormones were also investigated by Selmeaui et al. 1997 who studied the possible effects of acute exposure to 50-Hz linearly polarized magnetic fields (10 mT) on the hormones of pituitary, thyroid and adrenocortex of young men. They stated that no significant differences were observed between exposed and control men for any of the parameters measured: thyroid-stimulating hormone, follicle stimulating hormone, luteinizing hormone, triiodothyroxine, thyroxine, free triiodothyroxine, free thyroxine, thyroxine binding-globulin, cortizol, 17-hydroxycorticosteroids, and TBK. Their results suggest that acute exposure to either continuous or intermittent 50-Hz linearly polarized magnetic fields of 10 mT dose not affect these endocrine functions or their circadian rhythmicity in healthy young men.

The magnetic field influence on the concentration of serum K+, Na+ and Cl- was tested by Gorczynska and Wegrzynowicz (1986). The guinea pigs were exposed to the static magnetic field for six

weeks 1 hour a day, 7 days a week. Magnetic field of induction 0.005T- 0.3 T produced progressively an increase in Na+ concentration and decrease in chlorides concentration in the serum. The range of observed changes was dependent on the duration of exposure to the magnetic field. No changes in K+ serum concentration was observed following magnetic field exposure.

1-10. THERAPUTIC ASPECTS OF EMFs:

Science has developed many uses for electricity and magnetism in the area of health care. These types of energy are especially useful in helping to diagnose disease. Their use in the treatment of disease, however, has not been found useful unless sufficient energy is applied to cause heating or actual stimulation of nerve or muscle impulses. At Present, there is a wide promotion of magnetic pads for the treatment of a multitude of diseases. Health magnets come in the form of rings, bracelets, belts, shoe inserts, pads of various shapes and pads inserted in mattresses. Marvelous claims are made for their use. Up to 80-90 % of a variety of illnesses are claimed to be benefited within as little as ten minutes. It is stated that double-blind studies confirm these results, some are considered them as miraculous when describing results and others say that these devices have no evidence of benefits. (Barker and Antony, 1994)

Some promoters theorize that these low dosage magnets work by creating new electrical currents in the tissues, causing some heat production that dilates the blood vessels resulting in improved

circulation. Some theorize that they decrease pain by interfering with nerve transmission. Some are promoting the belief that the magnetic field of the earth is diminished resulting in a magnetic deficiency syndrome. They claimed that ,magnetic magnetic therapy works because it replenishes the magnetic deficiency that we all suffer from. (Hacmac and Edward, 1991).

There are many methods of health care that purport to diagnose and/or treat disease through the electrical currents of the body or through magnetism (Sparks and Vernon, 1996). Many of these health caremethods have strong associations age movements. Many new agers are quite frank about their rejection of standard anatomy and physiology textbooks. They say that we need an alternative model, one that is based on energy rather than matter Therefore, illness is not seen by New agers as a physical problem, but as an imbalance or deficiency of electrical energy. Cure thus focuses on the manipulation of or the replenishing of this purported energy, to remove its blockage and to balance its flow.

Several methods have developed over the years for the use of magnetism and/ or electricity in health care and therapy that are explainable as operating in harmony with natural law. Examples are Magnetic Resources Imaging (MRI) for demonstrating the structure and form of the various body tissues and organs, and methods for evaluating as well as stimulating the nerve and muscles. Yet, not one of these rational methods has been shown to work because the external electricity is providing additional electrons needed by the body or by providing healthy electrons to replace sick electrons. Neither has science found that external

currents serve to balance the currents of the body. Electricity is free electrons. The body has no clearly demonstrated disease conditions caused by a deficiency of electrons. Man's interest in the use of electricity and magnetism in the treatment of the human body has increased greatly over the last few decades. A search for a physiological explanation of acupuncture and other modalities frequently associated with Eastern religions has fueled part of the interest (Barker and Antony, 1994).

There were also some evidence showing that the electro-magnetic fields cab be used treat inflammatory diseases. While it is well known that EMFs can induce repair of non-healing bone fractures. Nindle et al. (2000) have found that clearly demonstrate that EMFs can regulate lymphocyte proliferation, since T cells are key modulators of inflammations, the development of EMFs based on therapeutic devices to regulate their activity can be expected to provide important tools to treat numerous human inflammatory diseases such as psoriasis and arthiritis.

METHODOLOGY

METHODOLOGY

A total number of "96" fertilized eggs [Rhode Island Red] were incubated at 37-39 C in an incubator (**N**ovital, **I**taly) under standardized experimental conditions . The fertilized eggs were divided into 4 experimental groups each of "20" eggs and all were exposed to static magnetic fields of 80 mT "800 G" magnets . Eggs of the experimental exposed (e) groups were collected after 5 days (group I), 10 days (group II), 15 days (group III) of incubation and directly after hatching (group IV). Another group of "16" unexposed eggs served as control groups "c" for all experimental groups (Table 1).

Table (1)

Magnetic Field applied	***No. of exposed Eggs***	***Experimental groups***			
		5-days	10-days	15-days	hatched
EXPOSED	4 x 20	Ie	IIe	IIIe	IVe
CONTROL	4 x 4	Ic	IIc	IIIc	IVc

There are many medical belts, containing static magnets of different powers for magneto-therapy, available in the pharmacies, they are used for relief of human back pain. We used the same magnets originally found in one of these commercial belts (Magnetic Therapy Ltd., UK). Four permanent magnets were fixed perpendicularly to each egg intimately over the embryo. The static magnets used were disc-shaped with a diameter of 15 mm and 4 mm thick. Magnets were oriented as outside direction of the two left side discs were the North & South poles with opposite orientation on the right hand outside direction. This could be clearly shown in figure (1-A).

Blood samples were collected from chick embryos after the incubation period of each experimental and control group as well. Embryos were sacrificed and blood samples were quickly collected in heparinized capillary tubes. Blood films of all samples were spread on glass slides, dried in air, fixed with methanol and stained with Wright stain for 5 minutes. The slides were rinsed with phosphate buffer solution until acquired pinkish color, rinsed with tap water and dried at room temperature. The blood smears were photographed using a photo-micrographic system (Olympus, Japan), all figures were taken under oil immersion condition with standard magnification of 1000 X.

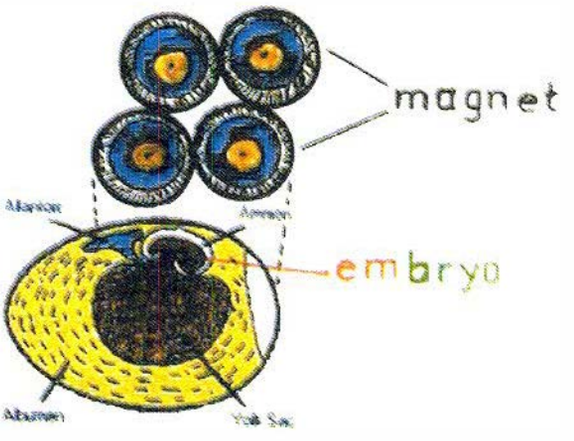

Fig. (1-A): The exposure mode of chick embryos to the 80 mT static magnetic fields.

Another method of exposure has carried out during the study. Rhode Island Red fertilized eggs with a total number of "96" eggs were used within 24 hours after they had been received. The eggs were incubated at 37-39°C in an incubator (Novital, Italy) under standardized experimental conditions. The fertilized eggs were divided into four experimental groups namely A, B, C, and D, each of 12 eggs. Groups A, B, C and D were exposed to alternating magnetic fields (20,000 μT), Embryos from group A were collected after 5 days [Ae] , those of group B were collected after 10 days [Be] while embryos of group C collected after 15 days [Ce] and Group D young's were collected directly after hatching [De]. Another four groups each of 12 eggs were used as control for each experimental group, would be identified as Ac, Bc, Cc and Dc respectively. They were also incubated under the same experimental conditions and their embryos were collected after 5, 10, 15 days and directly after hatching (e=exposed &c=control). Alternating-pole magnets (20,000 μT), that are currently used as therapy products and made from a sheet of magnetic material with north and south magnets arranged in an alternating pattern (Commercial Medical Magnet Use " Magnetotherapy " & dimensions 3 x 4 cm), so that both north and south pole facing the skin alternatively. Therapy products were applied perpendicularly to each egg where the embryo lies (Figure 1-B).

A small drop of blood was collected from a vein of each embryo from all the exposed and control groups using heparinized Micro-Haematocrit–tubes. All films were prepared within a few minutes following blood withdrawal. Blood films were stained using Wright stain method and examined using an oil immersion lens.

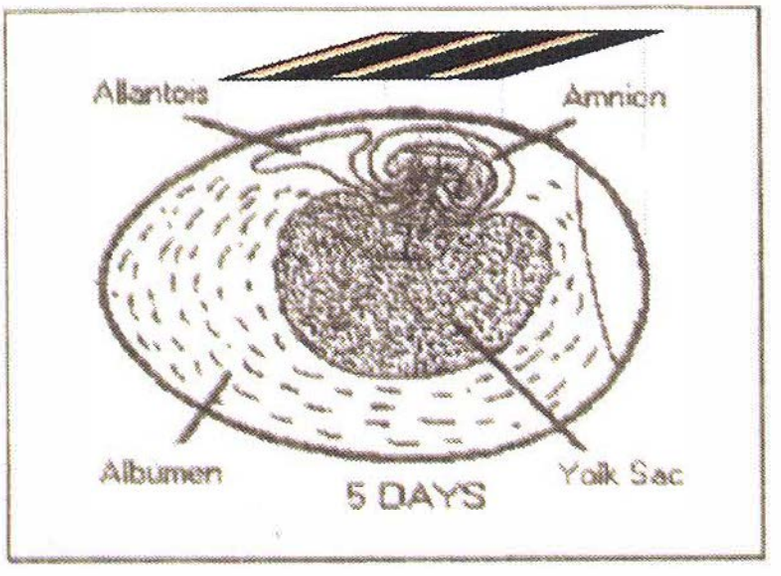

Figure (1-B) The magnetic applied design.

RESULTS
AND
DISCUSSION

PART I. EFFECT OF 80 MT (800 G) STATIC MAGNETIC FIELD ON THE BLOOD OF DEVELOPING CHICKEN.

PART II. DEVELOPMENT OF CHICK EMBRYOS EXPOSED TO 20.000 MT MAGNETIC FIELDS.

PART III. EFFECT OF 80 MT (800 G) STATIC MAGNETIC FIELD ON THE PINEAL GLAND OF DEVELOPING CHICKEN.

PART 1

EFFECTS OF 80 mT (800 G) STATIC MAGNETIC FIELD ON THE BLOOD OF DEVELOPING CHICKEN

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Key words: Static magnet, chick embryo, blood, Erythrocytes, Leukocytes.

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ABSTRACT

The controversial effects of magnetic fields on the experimental biological systems, together with magnetotherapy, are still remain a big question since their claimed benefits are unconvincing. Blood cells, of developing chick embryos exposed to 80 mT static magnetic fields, were used as a model to investigate these effects after incubation periods of 5, 10 & 15 days and directly after hatching as well. Embryos exposed to the applied magnetic fields exhibited many erythrocytic deformations and condensed nuclei. These changes can not be observed as long as the incubation period increased. This was partly attributed to the relatively high membrane fluidity and also to the fragility of the cell endoskeleton in this early stage of the developing blood. It was also proposed that 80 mT static magnetic fields might increase thrombogenesis. It was concluded that the acute exposure pattern applied had really affected blood cells especially in the early developmental stages.

INTRODUCTION

Magnetism is of direct impact on the human body due to the lines of force of the earth on which we live . Our body is invested with magnetism and enveloped in a magnetic field . The human brain creates the strongest magnetic field while we sleep, sleeping with our head to the north facilitates the easy flow of the magnetism created by the earth through our body and thus inducing sound sleep mode.

Regarding the " magnetotherapy ", the story of magnetism goes back a long way to many centuries before Christ when magnet was used as a healing agent, to preserve the Mummies of the ancient Egyptians and to relieve aches & pains but all were used unwittingly. Later on, at the beginning of the 16th century, the Swiss alchemist & physician, Paracelsus, discovered the healing power of magnets and other researches were initiated after that . However, the " father " of the modern magnetotherapy is Dr. Samuel Hahneman who recommended its use and in the present, it is widely recognized and has scientific support . Magnetotherapy needs no medicines, no injections and no tonics. There are two types of artificial magnets used : 1. *Electro-magnets,* and, 2. *Permanent magnets* , they are used for treatment as disc-shaped magnets of medium power " 150 mT or 1500 G ", to high power " 300 mT or 3000 G " (Nagar, 2000).

In spite of that, the chicken heart is " Four " chambered and the circulation is the same as the mammalian heart, the heart rate is about 200 – 300 / minute but in human it is about 70 / minute only !. Blood volume of an adult chicken is about 6 % of body weight and only 4 % can be obtained by bleeding out . Cells of the chicken blood are different from that of mammals and little is known of the functions of these various cells (Riddell, 1998) . Blood chemistry differs also from that of mammals especially in the high level of Ca " 10 – 30 milligrams / 100 ml " which is mainly attributed to the egg production when estrogen results in the production of phosphoprotein which binds Ca and raises its level in chicken blood. Chicken blood exhibits many developmental variations during embryogenesis. According to Patten (1946) and Romanoff (1960) [after Lillie, 1919 & 1952; and cited by Gilbert, 2001], erythroblasts are usually developing by the end of the first day and will differentiate into erythrocytes at the 12^{th} day of incubation. Thromboblasts, however, are developing through the 3^{rd} &4^{th} day of incubation. Leukocytes are often appear by the 2nd day of incubation – primordial lymphoid cells- or at the 3^{rd} day , immature granulocytes; other leukocytes appear between day 12 up to post hatching. Adult chicken blood is generally composed of erythrocytes " 3 million / cubic mm " and characterized by elongated shape and large nucleus . Thrombocytes are only " 30 thousand / cubic mm " and also nucleated . Total leukocytes are 20 – 30 thousand / cubic mm , majority of which - 65 : 80 % - are lymphocytes which are of granular cytoplasm with rounded nuclei . Monocytes , however , are only of about 5 % , they are large cells

with round nuclei and abundant cytoplasm . Other leukocytes " Heterophils , Eosinophils and Basophils " represent 15 – 30 % , characterized by their polymorphonuclear shape . Chicken plasma however, contains various electrolytic ions and negatively charged proteins of total 4 g / 100 mmof blood (Campbell, 1988) .

The chicken erythrocyte is oval in shape with an almost flattened oval nucleus to which cytoplasm and hemoglobin is tightly packed (Romer & Parsons, 1977) . The surface of an erythrocyte is covered with negatively charged polysaccharides (Shiga et al, 1996). According to the same author, erythrocytes have a unique feature, i.e. , changing the valence and the spin state can vary the magnetic susceptibility of hemoglobin. The deoxygenated erythrocytes are paramagnetic due to ferrous deoxy-hemoglobin (S = 2), while the oxygenated erythrocytes are diamagnetic due to oxy-hemoglobin (S = 0); and the erythrocytes containing high & low spin (ferric) methemoglobin are both paramagnetic (S = 5/2 0r ½ , respectively) but with different magnetic susceptibility.

The static magnetic field has been known to interact with the cellular components of blood, e.g., the magnetic separation of phagocytes loaded with ferromagnetic material (Levine, 1955), the orientation of sickled erythrocytes in a uniform magnetic field of 0.35 T (Murayama, 1965 & Brody et al, 1985) .

An external magnetic field may affect the blood flow even in the normal state through three distinct physical mechanisms :

(1) The magnetohydrodynamic action :

When charged particles pass at a high speed through a strong magnetic field, a force against the flow develops (Lorentz force). This force is predicted to increase the pressure drop by 10 % in the ascending aorta under a uniform magnetic field of 5 T (Chen & Saha, 1984); but, no experimental proof has been given yet.

(2) The diamagnetic interaction :

Which affects the orientation of erythrocytes in an uniform magnetic field with normal " discocytes " at several Tesla values (Yamagishi et al, 1992).

(3) The paramagnetic interaction :

This acts only on the pa ramagnetic erythrocytes in an unhomogeneous magnetic field with a strong spatial gradient. The first trial to trap the Malaria-infected erythrocytes from flowing blood was not satisfactory (Heidelberger et al, 1946), but later on, the idea was successfully applied to the magnetic separation of paraagnetic erythrocytes as stated by others,(Melville, 1975)

Effects of magnetic fields on biological systems have become more and more important in the world today as they have become increasingly ubiquitous in the environment, medicine, research and even industry. Magnetic fields are not only became an important tool for scientists to use in their research work, but also they have become necessary for us to

understand how safe they are?, and; what long-term effects it could have on our environment?.

In the present study, the effects of static 80 mT (800 G) magnetic fields on blood cells of the different stages of developing embryos and directly hatched chicken were investigated.

MATERIALS & METHODS

A total number of "96" fertilized eggs [Rhode Island Red] were incubated at 37-39 C in an incubator { Novital, Italy } under standardized experimental conditions . The fertilized eggs were divided into FOUR experimental groups each of "20" eggs and all were exposed to static magnetic fields of 80 mT "800 G" magnets . Eggs of the experimental (e) groups were collected after 5 days (group I), 10 days (group II), 15 days (group III) of incubation and directly after hatching (group IV). Another group of "16" unexposed eggs served as control (c groups) for all experimental groups [Table "A"].

Table (A)

Magnetic Field applied	No. of exposed Eggs	Experimental groups			
		5-days	*10-days*	*15-days*	*hatched*
EXPOSED	*4 x 20*	*Ie*	*IIe*	*IIIe*	*IVe*
CONTROL	*4 x 4*	*Ic*	*IIc*	*IIIc*	*IVc*

There are many medical belts, containing static magnets of different powers for magnetotherapy, available in the pharmacy, they are used for relief of human back pain. We used the same magnets originally found in one of these commercial belts { Magnetic Therapy Ltd., UK }. Four permanent magnets were fixed perpendicularly to each egg intimately over the embryo. The static magnets used were disc-shaped with a diameter of 15 mm and 4 mm thick. Magnets were oriented as outside direction of the two left side discs were the North & South poles with opposite orientation on the right hand outside direction. This could be summarized through Figure 1-A (see page 39)

Blood samples were collected from chick embryos after the incubation period of each experimental and control group as well. Embryos were sacrificed and blood samples were quickly collected in heparinized capillary tubes. Blood films of all samples were spread on glass slides, dried in air, fixed with methanol and stained with Wright stain for 5 minutes. The slides were rinsed with phosphate buffer solution until acquired pinkish color, rinsed with tap water and dried at room temperature. The blood smears were photographed using a photomicrographic system { Olympus, Japan }, all figures were taken under oil immersion condition with standard magnification of 1000 X.

RESULTS

Both the control and exposed embryos did not exhibit significant morphological changes neither the early embryological stages (5 & 10 day embryos) nor the late stages (15 days embryos & directly hatched). The changes observed were minimal and not characteristic to the magnetic fields applied since they are also noticed on the control group individuals.

The mortality rate, however, was almost normal since it was about 99 % independent of the treatment with magnetic fields. This rate also had no relation to the embryological stage either early or late. Blood smears of controls after 5 days incubation period " group Ic ", exhibited the typical chicken blood cells (Fig. 2, a) at this early stage. Erythroblasts (Er) were of a relatively disc-shaped pattern with well-defined rounded nuclei. Most of cells appeared large in size but some of them were smaller or not typically rounded with any clear-cut differences among them and thromboblasts (Tr).

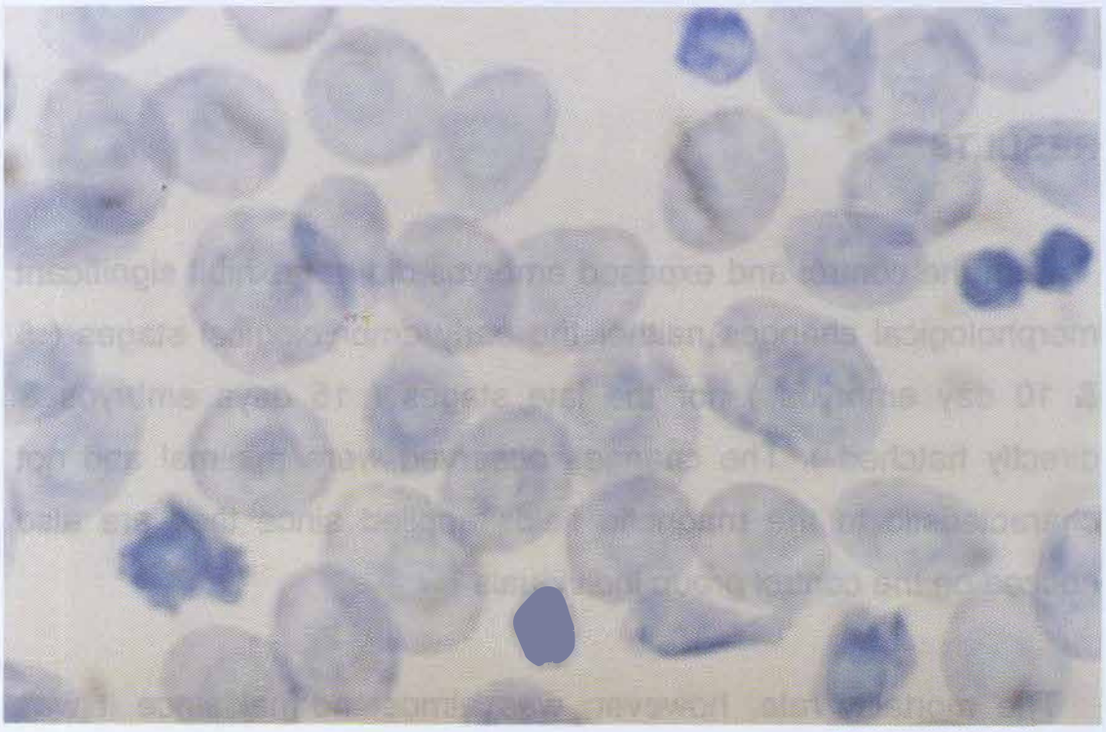

Fig. (2 a): Blood of the control group after incubation for 5 days.

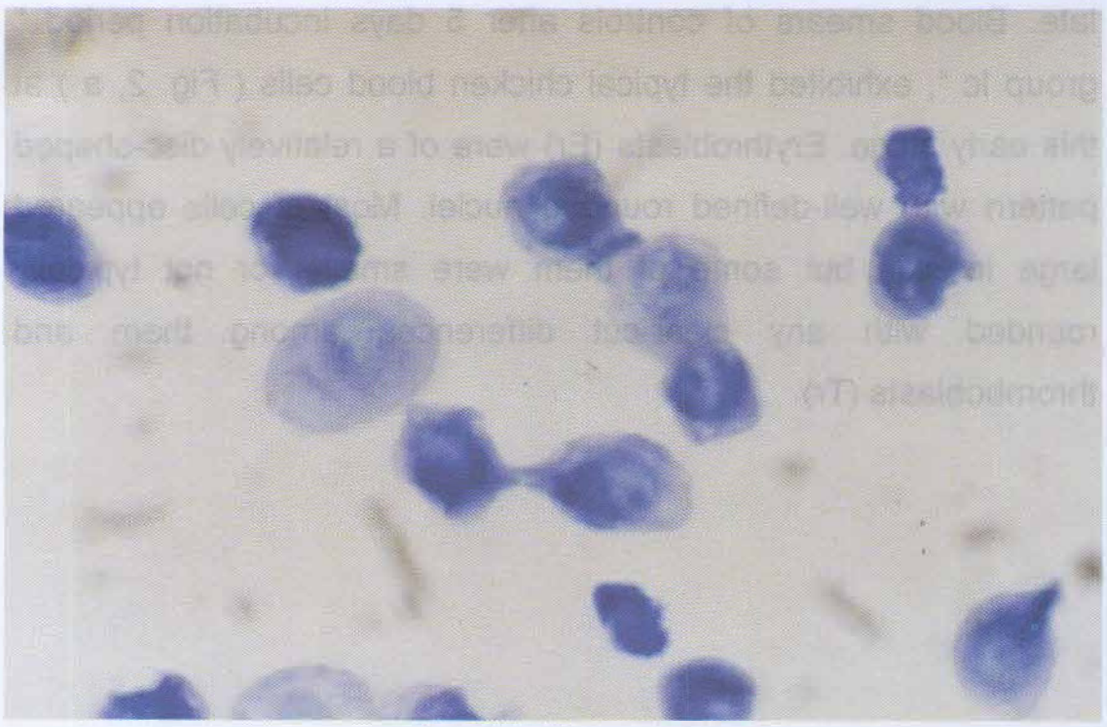

Fig. (2 b): Blood of the exposed group after incubation for 5 days.
The deformed erythrocytes with their denser nuclei.

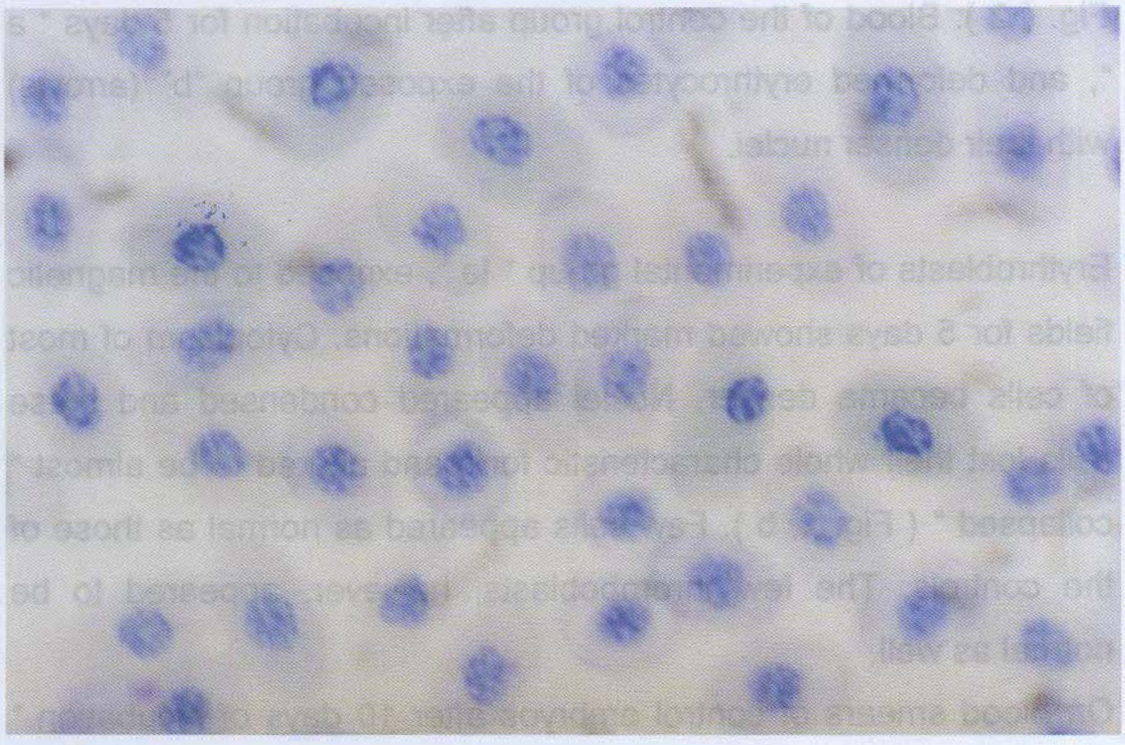

Fig. (3 a): Cells with blue nuclei which are more elongated than those with the violet nuclei of 10-days incubation control group.

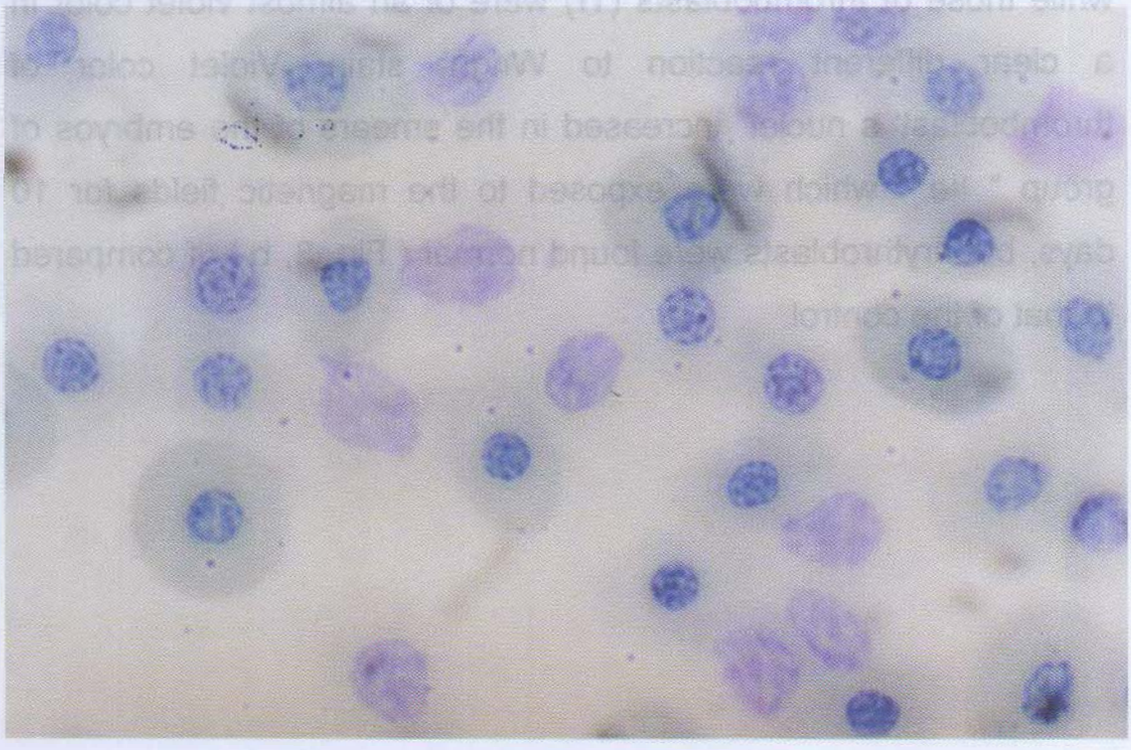

Fig. (3 b): No sharp differences appeared in the blood of exposed embryos after 10-days incubation.

Fig. (2): Blood of the control group after incubation for 5 days " a ", and deformed erythrocytes of the exposed group "b" (arrows) with their denser nuclei.

Erythroblasts of experimental group " Ie ", exposed to the magnetic fields for 5 days showed marked deformations. Cytoplasm of most of cells became denser. Nuclei appeared condensed and these cells lost their whole characteristic form and altered to be almost " collapsed " (Fig. 2, b). Few cells appeared as normal as those of the controls. The few thromboblasts, however, appeared to be normal as well.

On blood smears of control embryos after 10 days of incubation " group IIc ", many erythroblasts tended to be elongated rather than disc-shaped (Fig. 3, a). Nuclei of erythroblasts (Er) appeared blue while those of thromboblasts (Tr) were of an almost violet color in a clear different reaction to Wright stain. Violet color of thromboblast' s nuclei increased in the smears of the embryos of group " IIe " which were exposed to the magnetic fields for 10 days, but erythroblasts were found normal (Fig. 3, b), if compared to that of the control.

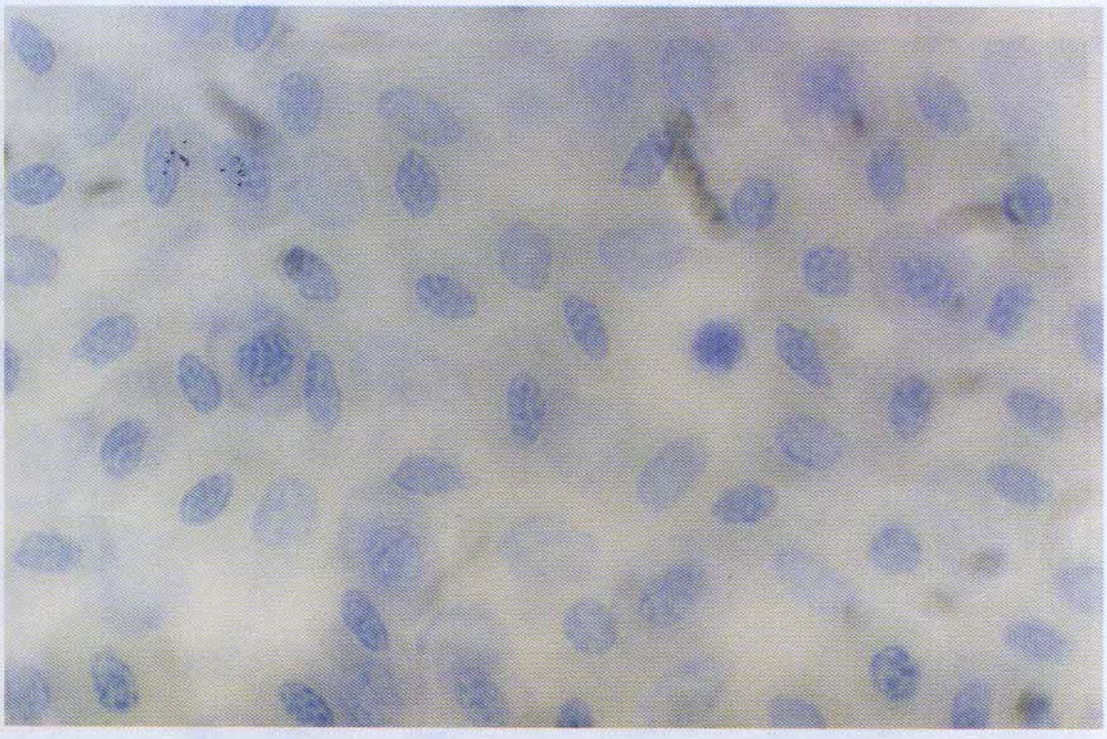

Fig. (4 a): No clear-cut changes appeared in the control blood cells after incubation for 15 days.

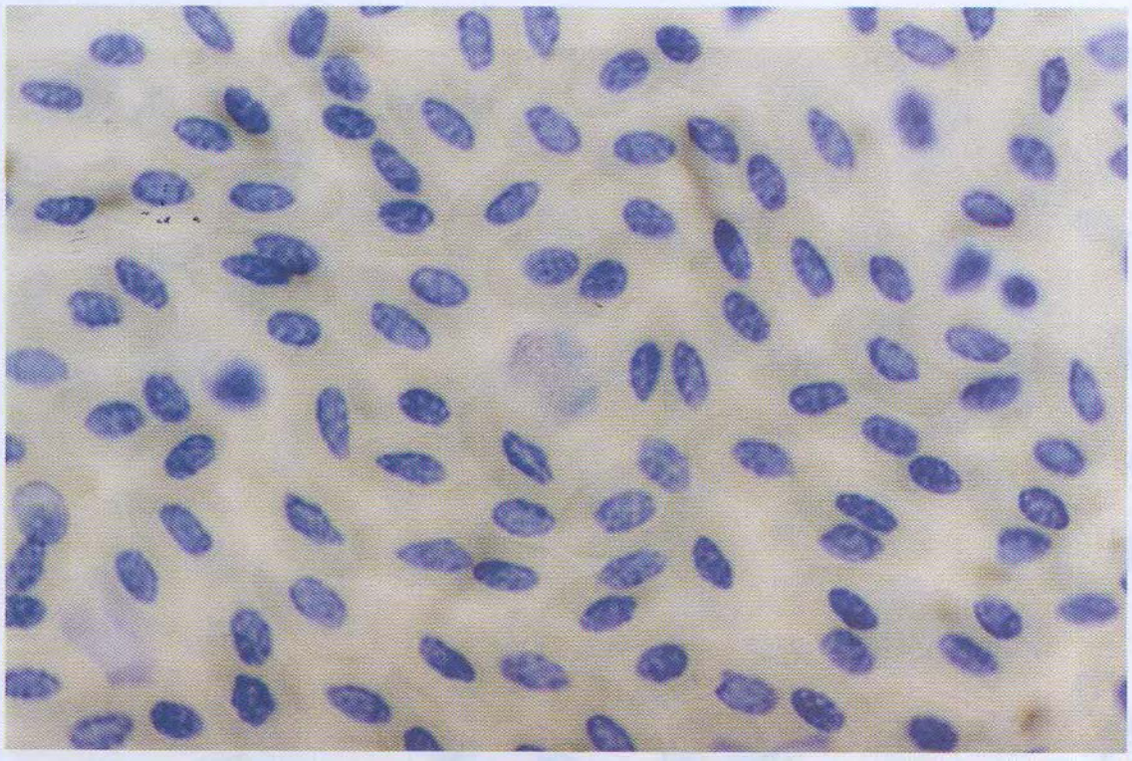

Fig. (4 b): No clear-cut changes appeared in the exposed blood cells after incubation for 15 days.

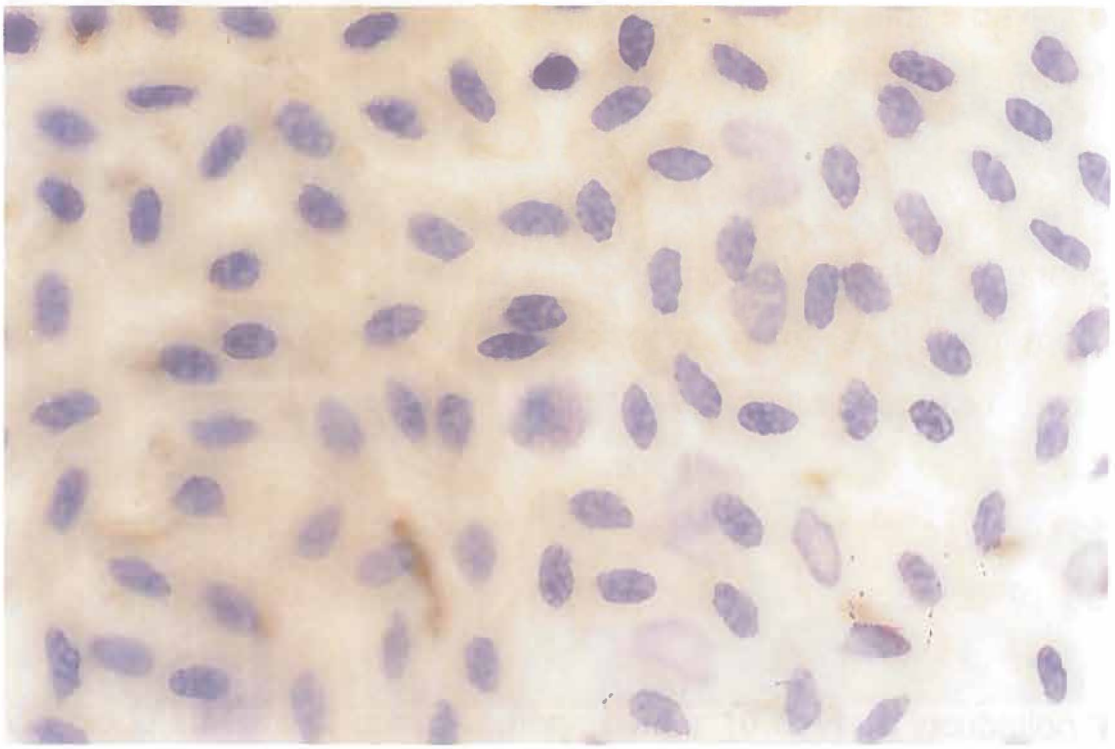

Fig. (5 a): Typical avian blood elements were observed in blood smears of control groups directly after hatching.

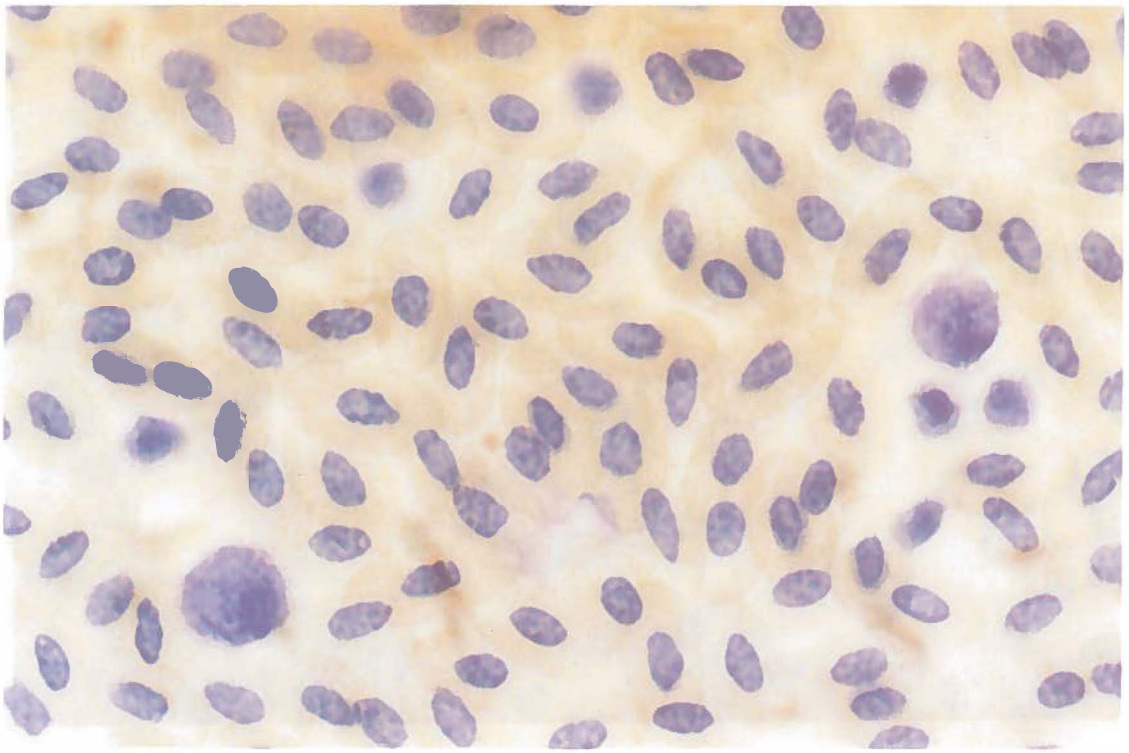

Fig. (5 b): Typical avian blood elements were observed in blood smears of exposed groups directly after hatching.

Fig. (3): Cells with blue nuclei (Er) are more elongated than those with the violet nuclei (Tr) of 10-days incubation control group " a ". In " b ", no sharp differences appeared in the blood of exposed embryos.

In the late stage before hatching, embryos after 15 days of control incubation " group IIIc ", only very small number of late erythrocytes appeared less elongated, (Fig. 4, a), while the majority of cells exhibited the uniformly avian and/or chicken red blood cells with almost typical appearance. Nuclei of both erythrocytes [elongated] and thrombocytes [rounded], reflected the same tendency to the stain. Erythrocytes and leukocytes exhibited well-differentiations. No clear-cut changes observed in the blood cells of the embryos exposed for 15 days " group IIIe ". On the other hand, nuclei of thrombocytes appeared circular and of darker blue color - compared to that of erythrocytes - (Fig. 4, b), rather than the controls.

Fig. (4): No clear-cut changes appeared in the control " a " and exposed " b " blood cells after incubation for 15 days.

Directly after hatching of controls, group " IVc ", typical avian blood elements were observed, as stated by Riddell, 2000. RBCs and throbocytes appeared elongated but it is clear that erythrocytes are much more in number. Together with the different stain pattern of their nuclei, thrombocytes appeared with rounded ends rather than the elongated ends of erythrocytes. Leukocytes appeared with clear typical pattern (Fig. 5, a). The static magnetic fields of 80 mT (800 G), often made no physical changes to the blood of the directly hatched exposed individuals " group IVe " (Fig. 5, b). It was clear that blood elements are close to the control of the same group.

Fig. (5): Typical avian blood elements were observed in blood smears of both control " a " and exposed " b" groups directly after hatching.

DISCUSSION

The minimal morphological abnormalities recorded may be attributed partly to either technical or experimental reasons, and/or to the individual variations among different embryos. It is well known that physical factors – except radiation – could not affect too much the embryogenesis if compared to chemicals and hence the latter may have more effects rather than magnetism.

Almost, the same attribution could be given to the normal mortality rate observed in the different embryological stages independent of magnetism. It may be also due to other incubation conditions e.,g., torsion of the fertilized eggs.

Elements of chicken blood of the control group "Ic" - 5 days incubation – agreed with that described by Lillie, 1919. On the other hand, increased density of the erythroblastic cytoplasm and the condensed nuclei of the blood cells in the experimental group "Ie" exposed for five days, would be understandable since at this early stage, cells are still fragile and easily affected by static magnetic fields. Plasma membrane is not too rigid yet or its fluidity is higher than in the other late embryological stages. Membrane deformation and fragility of erythroblasts are in an agreement with that of Changjun et. al,2000 applying an electromagnetic fields. It may be also due to that the microtubules and microfilaments of the cell endoskeleton, however, could not keep the cell shape

against the magnetic effects on the iron molecules included in the blood hemoglobin as stated by Nagar, 2000. Supporting this argument, presence of few cells in the exposed blood smears appeared normal which might be described, as they are younger than the affected cells and hence exposed for shorter duration. Lillie, 1919; typically fit the control erythroblasts of this stage, "Ic" group, that described, a finding which is still cited through most of the recent researchers.

Regarding the blood smears of the "IIc" group exposed for 10 days, its control erythroblasts - which appeared to become more elongated rather than the disc-shape pattern of the previous stage – with its blue stained nuclei agreed with the same data of Riddell, 2000. Our findings for the less elongated and violet stained nuclei, the thrombocytes, were supported by the same author. The increased nuclei' s violet color of thromboblasts in the exposed blood smears of this group – IIe - may indicate that static magnetic fields of 80 mT may increase its affinity to the stain, a finding which is in need for further investigations. Normal erythroblast number and appearance may agree with our guess that cells in this relatively late stage started to resist the effects of the magnetic fields.

Both well-differentiated erythrocytes and thrombocytes of blood smears taken from embryos incubated for 15 days "IIIc", are expected findings considering that this is a late developing stage compared to Ic and IIc groups. At this pre- adult mode for the blood cells, most of them appeared more close to the ordinary chicken blood. Smears of the exposed embryos in this developing stage "IIIe", did not exhibit clear-cut observations, which may support the idea based on that the cell resistant to the magnetic fields was increased more in this embryonic stage.

The typical avian blood structure obtained in the blood smears of group IVc of the directly hatched embryos confirms again the findings of Riddell, 1998; which is normal. Even blood cells of the exposed hatched embryos of this group ,"IVe", did not show any reliable changes which could be explained as they are fully matured cells with maximum resistance and tolerance rate among the experimental groups at all.

Finally, it was stated that magnetism has variable beneficial effects on blood cells of the adult human and animals. These effects varied among many directions, magnetic fields may promote and increase the number of red blood cells resulting in an increase in the oxygen content and removal of cholesterol and other deposits. It may also clean and purify blood preventing internal clotting and normalizes blood

pressure (Nagar, 2000). Evidences given by Schulten, 1982 and Steiner & Ulrich, 1989, attributed magnetotherapy applications obtained are due to the influencing effects of static magnetic fields on the electronic spin rate of the chemical reactions influencing also the cyclical changes in the physical state(s) of water as proposed by Beall, et. al, 1976. However, none of these proposed effects has been demonstrated in biological systems under physiological conditions (Frankel & Liburdy, 1996).

In contrast, other investigators proposed that the use of magnetotherapy is still remain controversial, the uncontrolled reports supporting its benefits still have some serious flaws (Ramey, 1998). He also claimed that these benefits are not approved and moreover, their evidences are unconvincing. The matter does not exceed the placebo effects of the users and most of the claims made by the companies that manufacture magnetotherapy devices cannot be supported based on the current state knowledge.

In conclusion, and considering the previous positive and negative reports on the effects of the static magnetic fields on the blood cells of mature human and even animals, we think that our findings on developing embryos are worthy to be demonstrated. Our experimental design and the acute mode of exposure together with the early stage of development (5 days) may explain the clear cell deformations observed. This may probably urging us to follow up these effects on the post hatching, young, adult and even on the next generation of the exposed chicken in the future.

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PART II

DEVELOPMENT OF CHICK EMBRYOS EXPOSED TO 20,000 μT MAGNETIC FIELDS

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PART II

DEVELOPMENT OF CHICK EMBRYOS EXPOSED TO 20,000 μT MAGNETIC FIELDS

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Development of Chick Embryos Exposed to 20,000 μT Magnetic Fields

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Key words: Chick Embryo – Red Blood Cells – Plasma Membrane – Endoskeleton - Alternating Magnet.

Development of Chick Embryos Exposed to 20,000 μT Magnetic Fields

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Key words: Chick Embryo – Red Blood Cells – Plasma Membrane Endocytosis – Alternating Magnet

ABSTRACT

There is an increasing interest in the use and practice of magnetic devices in clinical alternative medicine, research and industry. In this work, the development of red blood cells (RBCs) of chick embryos exposed to 20,000 μT alternating pole magnet was investigated. It was noticed that RBCs of the embryos that have been exposed for 5 days exhibited marked deformations especially their membranes and the whole morphology. However, RBCs of the embryos which had been exposed to the applied magnetic fields for 10, 15 – days and those that directly hatched, did not show clear changes. This was partly attributed to the capability of the magnetic fields to affect RBCs during this early developmental stage. It was suggested that during the late stages of development, RBCs are matured enough and so, they have more tolerance against magnetic field's effects.

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INTRODUCTION

At this time of heightened public concern about the health impacts of magnetic fields, there is an increasing use and practice of magnetic devices in clinical alternative medicine, research and even in industry. The possible effects of weak magnetic fields on biological systems have attracted considerable attention of many investigators. Some claimed that this had become particularly true after the publication of many epidemiological studies in this topic. For that, it has become necessary for us to understand how safe it is, especially that magnet therapies have become increasingly popular in recent years, chiefly for the pain relief. They have been offered as an inexpensive pain-reliever without side effects (Nagar, 2001).

Human being is living under many magnetic impacts, some are naturally occurring, e. g., Earth's magnetic fields that makes compass needles point north and south. Other magnetic fields are artificial, e. g., those generated by the electric power lines or even due to the electric home and office appliances.

The static magnetic field has been known to interact with the cellular components of blood, e.g. the magnetic separation of phagocytes loaded with ferromagnetic material (levine, 1955), and the orientation of sickled erythrocytes in uniform magnetic field of 0.35 T (Murayama, 1965 and Brody et al., 1985). An external magnetic field may affect the blood flow even in the normal state through three distinct physical mechanisms:

(1) The magnetohydrodynamic action, i.e. when charged particales pass at a high speed through a strong magnetic field, a force against the flow develops [Lorontz Force], (Chen and Saha, 1984); but no experimental proof has been given yet.

(2) The diamagnetic interaction, which affects the orientation of erythrocytes in an uniform magnetic field with normal discocytes at several Tesla values (Yamagishi et al., 1992).

(3) The paramagnetic interaction, which acts only on the paramagnetic erythrocytes at an inhomogeneous magnetic field with a strong spatial gradient (Shiga et al., 1996).

Laboratory studies have identified cell membranes as the primary tissue sites of interaction with environmental electromagnetic (EM) fields. They have determined major sequences in the coupling of cell surface signals to a cascade of high energy enzymatic mechanisms inside cells, including mechanisms regulating cell growth (Ross, 1996). He also stated that these studies point to joint actions of chemical cancer promoters and EM fields at cell membranes as key steps in tumor formation. Biophysical models and experimental data support the role of free radicals in first detection of EM fields at a thermal level.

Several mechanistic approaches attributed the electromagnetic-induced phenomena to an interaction with the electrically charged double layer of the membrane (Blank and Findl, 1987). It is generally accepted that the primary site of interaction is at the level of the plasma membrane (Carpenter and Ayrapelyan, 1994).

Blood, like all tissues, contains electrically charged ions. When a magnetic field with a series of alternating North and South poles is placed over a blood vessel, the influence of the field will cause positive and negative ions (for example, Na^+ and Cl^-). This is to bounce back and forth between the sides of the vessel, creating flow currents in the moving blood.

The combination of the electromotive force, altered ionic pattern, and the currents causes blood vessel dilation with a corresponding increase in blood flow (Porter, 1997).

Avian blood is generally composed of erythrocytes (red blood cell), leukocytes (white blood cell) and thromboblasts, all suspended on plasma that contains various electrolytic ion and negatively charged proteins. The surface of erythrocyte is covered with negatively charged polysaccharides (Shiga et al., 1996). Since Lillie, 1919, described the chicken blood cells, Gilbert (2001), cited Lillie's description as the standard form of the chicken blood. The chicken red blood cells (RBC's) are usually developing by the end of the first day of incubation and it could be called as erythroblasts and during their development, they are almost rounded. However, mature RBC's appear by the day 12 and then they are known as erythrocytes, they are oval in shape and nucleated with 2.4 - 4 million/cubic mm in number. Erythrocytes are altered to be reticulocytes during certain late stage of age depending on the species. Thrombccytes are the avian equivalent of mammalian platelets. They are oval and nucleated with clear cytoplasm, measuring about 20,000 - 30,000/cubic mm. Thromboblasts are usually developing during the 3rd and 4th days. Leukocytes, includes eosinophils, heterophils, lymphocyles and monocytes, are developing later. Eosinophils and heterophils are developing in the day 7, eosinophils are similar in size and shape to the heterophils and founded as 800-1, 600/cubic mm. The heterophils are the second most numerous WBCs in most avian species. These are fairly large and rounded cells with lobed nuclei, they represent about 3,000-12,000/cubic mm. Lymphocytes developing at 17th days, these are mononuclear cells possessing a single, large, rather round nucleus. It is classified as non-granular WBC, founded as almost 40,000-80,000/cubic

mm. Monocytes appear directly after hatching, it is the largest circulating blood cell. Monocytes, like lymphocytes, are considered mononuclear cells possessing a single, large, rather round nucleus and classified as non-granular WBC. The nuclei of these cells are large with an irregular indentation, its number is 300-1700/cubic mm (Birrenkott & Wiggins, 1995).

The use of magnetic fields in clinical medicines is still disputatious since the U.S. Food and Drug Administration (FDA) have not approved magnetic therapy, but the National Institutes of Health is investigating the phenomenon (Smith, 1999).

In the present study, the effects of alternating magnetic fields (20,000 μT) on the blood of different stages of the developing embryos and directly hatched chicken were investigated.

MATERIALS & METHODS

Rhode Island Red fertilized eggs with a total number of "96" eggs were used within 24 hours after they had been received. The eggs were incubated at 37-39°C in an incubator (Novital, Italy) under standardized experimental conditions. The fertilized eggs were divided into four experimental groups namely A, B, C, and D, each of 12 eggs. Groups A, B, C and D were exposed to alternating magnetic fields (20,000 μT), Embryos from group A were collected after 5 days [Ae] , those of group B were collected after 10 days [Be] while embryos of group C collected after 15 days [Ce] and Group D young's were collected directly after hatching [De]. Another four groups each of 12 eggs were used as control for each

experimental group, would be identified as Ac, Bc, Cc and Dc respectively. They were also incubated under the same experimental conditions and their embryos were collected after 5, 10, 15 days and directly after hatching { e = exposed & c = control }.Alternating-pole magnets (20,000 μT), that are currently used as therapy products and made from a sheet of magnetic material with north and south magnets arranged in an alternating pattern (Commercial Medical Magnet Use " Magnetotherapy " & dimensions 3 x 4 cm), so that both north and south pole facing the skin alternatively. Therapy products were applied perpendicularly to each egg where the embryo lies (Figure 1-B), see page (41).

A small drop of blood was collected from a vein of each embryo from all the exposed and control groups using heparinized Micro-Haematocrit-tubes. All films were prepared within a few minutes following blood withdrawal. Blood films were stained using Wright stain method (Dacie and Lewis 1995) and examined using an oil immersion microscope lens (Olympus, Japan) under standardized magnification of 1000 X for all exposures.

RESULTS

Chick embryos of all exposed and control groups exhibited normal mortality rate since about 1 % only was recorded dead through the different developmental stages. However, both exposed and control embryos did not exhibit marked morphological changes neither at the early nor at the late embryonic stages. There were some insignificant variations among all embryos, especially the weight, in each group independent on the exposure.

Blood smears of embryos incubated for 5 days as control group [Ac], showed a typical RBCs appearance (Figure 2a). In this embryonic stage, cells exhibited a rounded shape appearance and are characterized by large and well developed nuclei. However, some cells appeared smaller and/or oval a little. On the other hand, blood cells of exposed embryos [Ae] are almost altered to be either elongated or spindle shape (Figure 2b), while their nuclei did not exhibit any significant changes. Some cells appeared close to the control, i.e. no sharp cut changes at all.

The majority of RBCs are either transformed or going into the ordinary oval pattern of the avian RBCs on the smears of the control group [Bc] incubated for 10 days (Figure 3a). It was clear that the affinity of the nuclei towards wright stain was different since some nuclei appeared blue in colour while others were violet. In the exposed blood film [Be], the number of these rounded cells with the violet nuclei increased in the blood smears (Figure 3b).

Most cells appeared typically oval in the blood of the control group [Cc] after 15 days incubation (Figure 4a) and little were kept round. Affinities of the nuclei to the stain are uniform since the nuclei exhibited some tendency

to the oval shape and blue colour pattern. No critical changes recorded on exposing embryos at this stage [Ce] to the magnetic fields (Figure 4b).
Control blood smears collected at the end of the incubation period directly after hatching [Dc], showed a typical form and structure of the oval avian RBCs (Figure 5a). In addition, exposed blood smears of this group [De] did not show obvious changes (Figure 5b) if referred to the control.

DISCUSSION

The normal mortality rate could be attributed to the incubation conditions and also to the normal individual variations among the incubated fertilized eggs. Almost, the same attribution would be given to explain the minor morphological variations recorded for some embryos along the different embryonic stages independently on the exposure to the magnetic fields.

RBC's of the embryos exposed for 5 days were deformed in shape and are altered to be elongated to spindle in shape especially their cytoplasm. These morphological deformations observed may be partly attributed to certain changes in the membrane rigidity and fluidity. It could be also explained as a process of re-arrangement for cell endoskeleton (especially microtubules and microfilaments) resulted after exposure of these cells to the alternating magnetic fields. These membrane deformations may cause changes in the cellular metabolic activities through different processes, it is believed that charges in cell surface membrane are major determinants in the membrane transport mechanisms (Parola, 1982).

In blood smears of 10 days, 15 days incubation and directly hatched there are no changes appeared in the erythrocytes that appeared with blue nuclei and thrombocytes with its violet nuclei. Our findings on the RBC's which are not clearly affected during these late stages of incubation and directly after hatching could be attributed to that cells have more tolerance and resistancy to the alternating magnetic fields applied. On the other hand, absence of any significant morphological changes for the RBCs in these late incubations (10, 15 – days; and hatched young) does not mean that no membrane structure occurred but it is probable that certain changes may resulted but could not be recorded using the light microscope investigations.

Our membrane and cellular deformations described, especially after exposure of the embryos for the first 5 days of incubation, are in an agreement with other laboratory studies. (Ross, 1996). Moreover, Carpenter & Ayrapelyan, 1994; stated that cell membranes are the primary sites of interaction with electromagnetic fields which may determine or regulate cell growth. Moreover, electromagnetic field's induction phenomenon could be explained as an interaction of these fields and the electrically charged double layer of the plasma membrane (Blank & Findle, 1987). A large number of cellular phenomena, including alterations in growth rate, gene expression and macromolecular synthesis, have been reported to occur in response to fields of moderate to weak intensity (Tenforde, 1992). There is a rapidly growing body of information that implicates the cell membrane as a primary site of electromagnetic fields (ELFs) interactions (Tenforde, 1992; Adey, 1990 a, b and Tenforde & Kaune, 1987). A wide variety of cell membrane structural and functional properties have been reported to be altered in response to ELF fields.

In conclusion, the main effects of alternating magnetic fields on blood of the 5 days developing chicken were found to be concentrated on the cellular membranes. However, focused research is still in need to confirm mechanisms of interaction between alternating magnetic fields and biological systems. Also, the best role of alternating magnetic fields should be founded. It could be recommended that alternating magnet therapy might be risky for kids and pregnant women, then it should be avoided.

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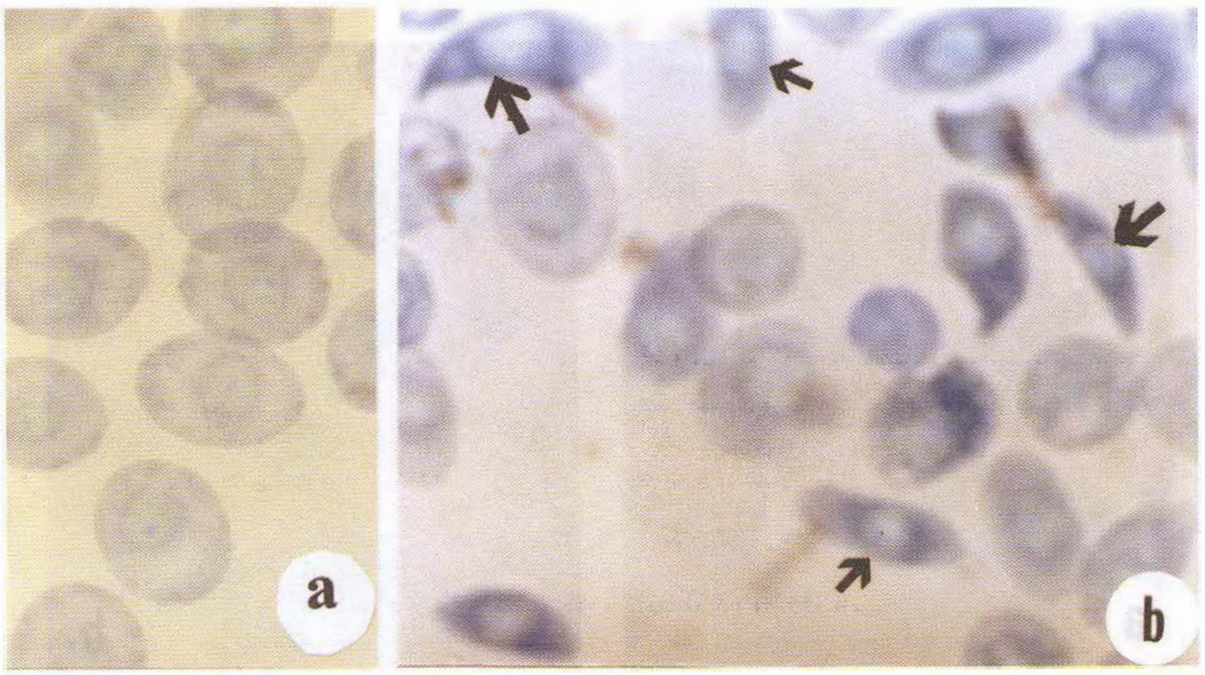

Figure (2) : Photomicrographs of blood films showing 5 days, control and exposed.

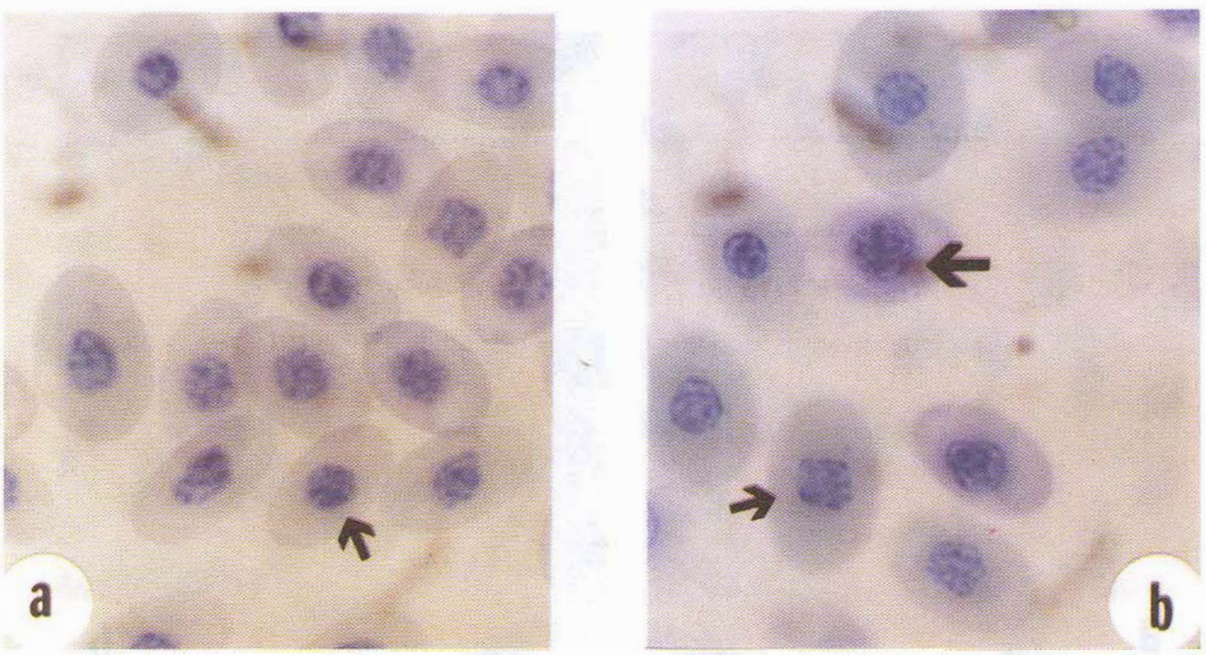

Figure (3) : Photomicrographs of blood films showing 10 days, control and exposed.

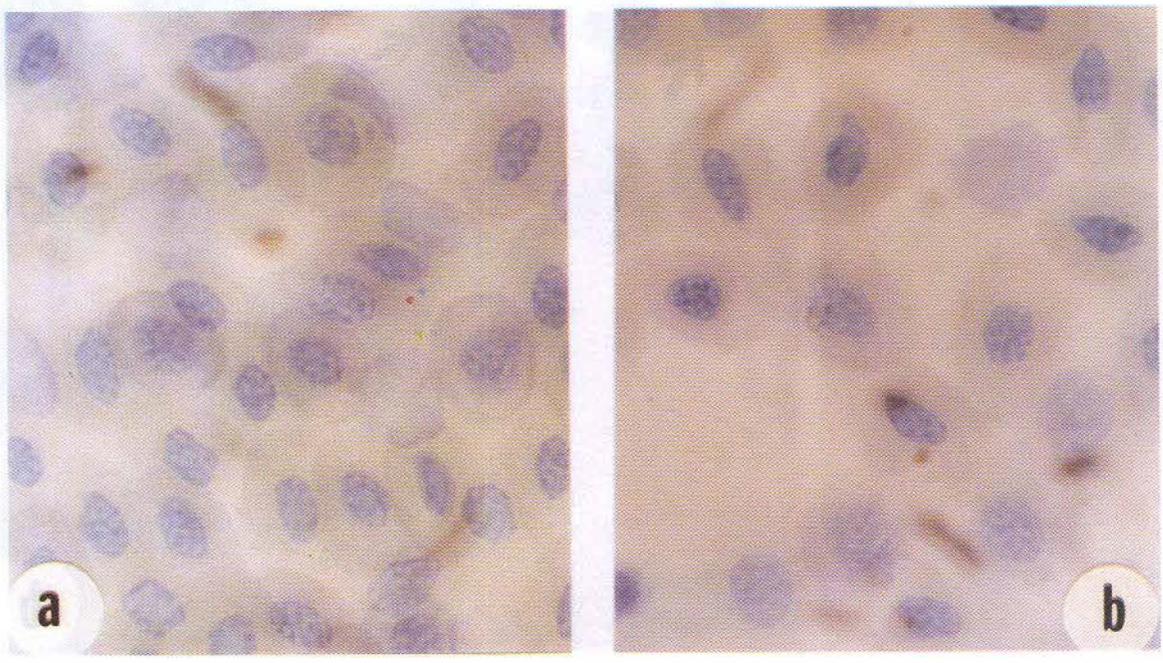

Figure (4) : Photomicrographs of blood films showing 15 days, control and exposed.

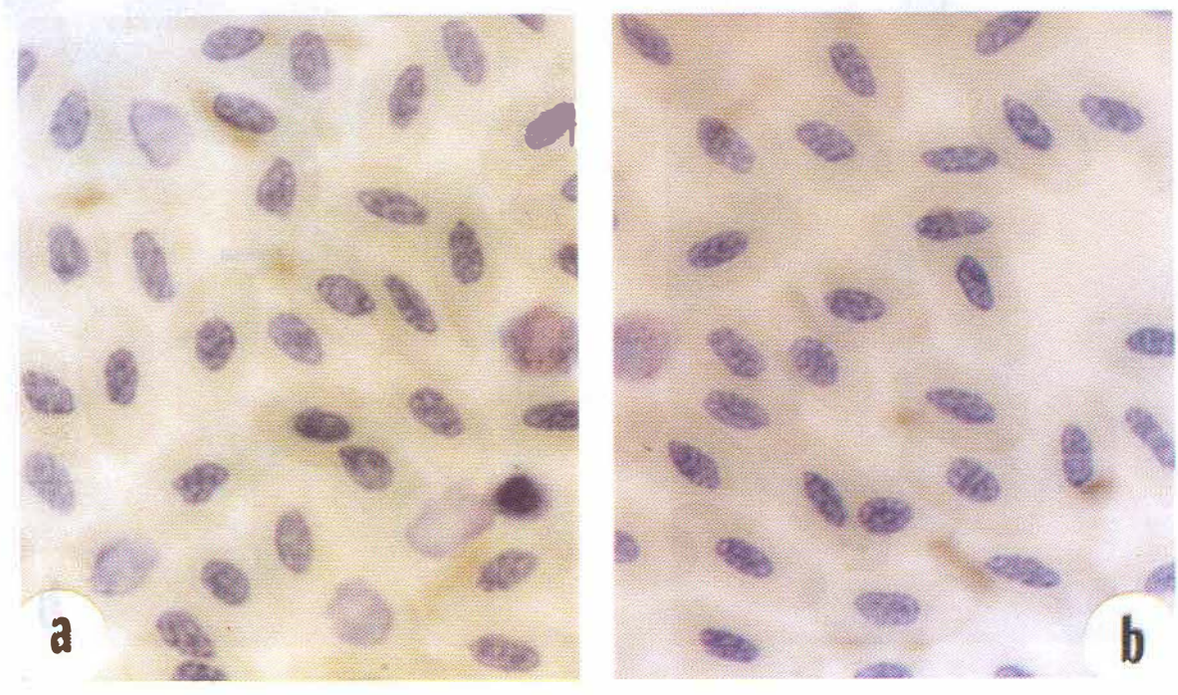

Figure (5) : Photomicrographs of blood films showing directly after hatching, control and exposed.

PART III

EFFECT OF 80 mT (800 G) STATIC MAGNETIC FIELD ON THE PINEAL GLAND OF DEVELOPING CHICK

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Keywords: Electromagnetic field, static magnet, chick embryo, pineal gland.

ABSTRACT

The effect of exposure to static electromagnetic field at 80 mT (800 G) were in 80 fertilized chicken eggs. 16 control eggs were incubated in the same conditions as the experimental eggs. Chick embryos were dissected and pineal gland removed and prepared for histological examination after 5, 10, 15 days of incubation and after hatching. In conclusion, the static electromagnetic field of 80 mT did not induce observable changes in the histological structure of pineal gland exposed to it.

INTRODUCTION

Several kinds of biological effects have been reported in studies of electric and / or magnetic fields. A biological effect is a measurable change in some biological factors. It may or may not have any bearing on health. Overall, effects attributed to EMFs have been small and difficult to reproduce. Very specific laboratory conditions are usually needed for effects of EMFs to be detected. It is not known how EMFs actually cause these effects.

The pineal gland which in human is located near the anatomical center of the brain, is normally responsive to visible electro-magnetic fields like light since the eyes are functionally connected to the pineal gland by a series of neurons. Normally, the pineal gland produces low amounts of melatonin during the day and high amounts at night; this rhythm is reflected in the blood melatonin concentrations which are higher at night than during the day. In both man and lower mammals, their exposure to light at night is followed by a drop in pineal melatonin production and blood melatonin levels. Likewise, exposure of non-human mammals to sinusoidal electric and / or magnetic fields as well as pulsed static magnetic fields often reduces pineal melatonin production. Melatonin has many functions in the organisms and any perturbations (not only electromagnetic fields) which causes levels of melatonin to be lowered than normal may have significant physiological consequences. Melatonin, because it is a potent antioxidant, may provide significant protection against cancer initiation as well as promotion. However, it is pre-mature to

conclude that the alleged increase cancer risk reported in individuals living in higher than normal electromagnetic environments related to reduced melatonin levels caused by such field exposures (Reiter, 1993 and Mevissen et al. 1996).

For many decades, the developing chicken embryo has been considered an ideal model for investigating a large number of vital processes in different areas of biological sciences. In particular, studies of the interactions between growing tissues and organs of the embryo and chemical compounds or physical factors have attracted the interest of scientists (Veicsteinas et al. 1996 , Selmaoui et al. 1997). The effect of static electromagnetic fields on the development of the chick embryo pineal gland has been studied by Jove et al. (1999) They stated that static electromagnetic fields affect the development and growth of embryos unequally, and that they can depend not only on the intensity of the static electromagnetic fields, but also on the length of exposure and the organ which is developing

MATERIALS AND METHODS

A total number of "96" fertilized eggs [Rhode Island Red] were incubated at 37-39 C in an incubator (Novital, Italy) under standardized experimental conditions . The fertilized eggs were divided into FOUR experimental groups each of "20" eggs and all were exposed to static magnetic fields of 80 mT "800 G" magnets . Eggs of the experimental (e) groups were collected after 5 days (group I), 10 days (group II), 15 days (group III) of incubation and directly after hatching (group IV). Another group of "16" unexposed eggs served as control (c groups) for all experimental groups Table (1), see page (37).

There are many medical belts, containing static magnets of different powers for magneto-therapy, available in the pharmacy, they are used for relief of human back pain. We used the same magnets originally found in one of these commercial belts (Magnetic Therapy Ltd., UK). Four permanent magnets were fixed perpendicularly to each egg intimately over the embryo. The static magnets used were disc-shaped with a diameter of 15 mm and 4 mm thick. Magnets were oriented as outside direction of the two left side discs were the North & South poles with opposite orientation on the right hand outside direction. This could be Shown as designed in figure (1-A), see page (39).

Embryos of control and exposed groups were sacrificed and pineal gland was excised, fixed in Bouin's stained with Haematoxylin, countered with eosin and mounted in Canada Balsam. The slides were photographed using a photomicrographic system (Olympus, Japan), all figures were taken under oil immersion condition with standard magnification of (1000 X).

RESULTS

After careful investigation of specimens, we have found that both the control (Figure 1) and the exposed (Figure 2) did not reveal any significant morphological changes specially one day after hatching which the specimens were taken. No effects were detected on the follicular layer which represents the most core inside in the body inside of the gland. Also, the para-follicular zone that surrounds and separates the follicles from the connective walls was also unchanged after the long period of exposure which extended along all incubation period and exceeds one day after incubation. It is important to emphasize that the white area seen in follicular layer in specimens of figure (1) are due to artifacts during the processing of the slides.

It was clearly shown that in the current study, the pineal gland is not affected by the exposure to 80 mT (800 G) static magnetic field. However, some studies stated that the exposure to static magnetic field (SMF) can affect the development and growth of embryos unequally (Jove et al. , 1999).

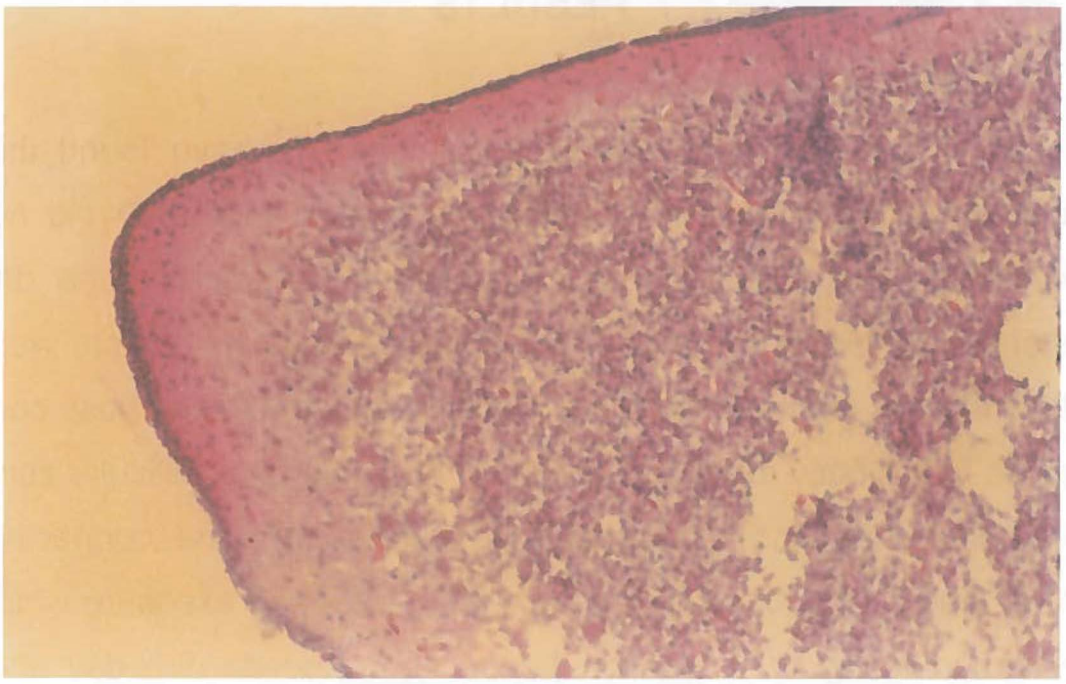

Figure (1). Photomicrograph of the histological picture of the pineal gland of the control chick embryo one day after hatching.

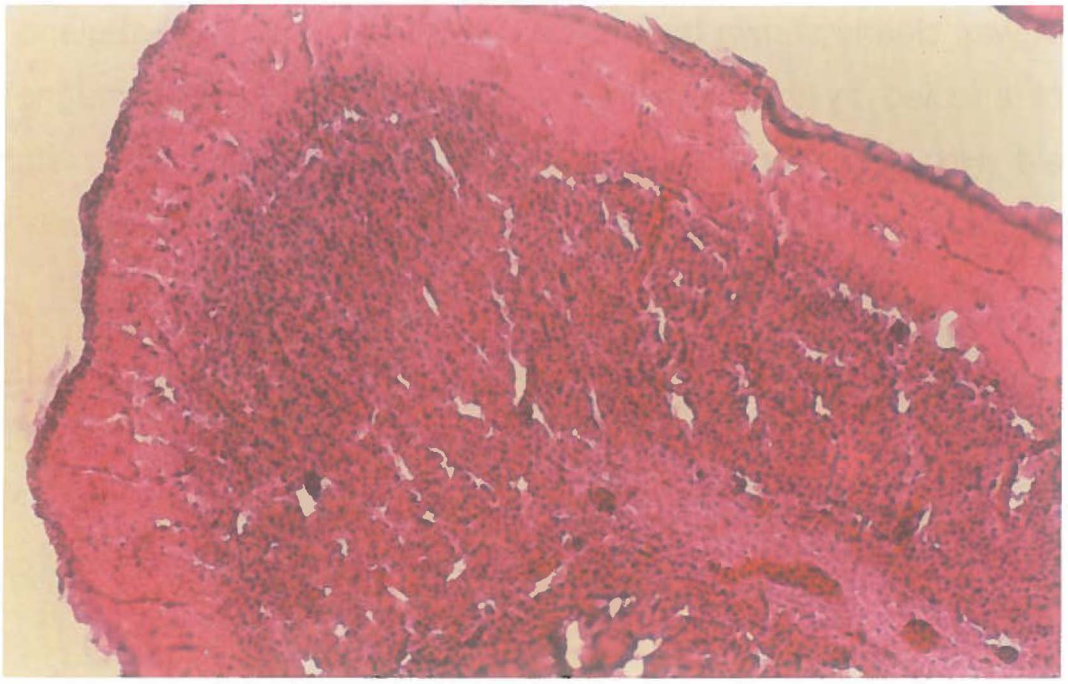

Figure (2). Photomicrograph of the histological picture of the pineal gland of the exposed chick embryo one day after hatching.

DISCUSSION

The pineal gland develops in the early stages from the diencephalons of the brain and remains attached to the posterior part of the diencephalic roof. In birds, secretory cells - pinealocytes – which represent the ancestral photoreceptive cells are located in pineal gland and act as light receptors. As a result, light may be received directly in birds through these cells instead of neural connections of the eye present in mammals (Zimmerman and Tso, 1975). The pineal gland acts as an endocrine gland which secretes mainly hormone melatonin which supports rhythmic action of the body acting as a biological clock (Fevre-Montage et al. 1978).

One hypothesis states that visual signals (Mammals) or photoreception signals (birds) received by the pineal gland allow it to determine day length and lunar cycles. Day-length information helps to keep daily and seasonal cycles on time reproductive cycle in birds). Melatonin, the principal pineal secretion inhibited by the presence of sunlight. Melatonin is considered to be a marker of circadian rhythms, and abnormalities in its secretion are associated with clinical disorders, including fatigue, sleep disruption, mood swings, impaired performance and depression which are consequences of desynchronisation (Selmaouli et al. 1996).

The current study revealed that there was no effect of static EMFs on histological structure of chick embryo pineal gland. The same result was established by Jove et al. (1999). However, in recent years, some epidemiologic studies have suggested that extremely low frequency magnetic and electric fields might affect

human health and in particular that the incidence of certain types of cancer depression and miscarriage might increase among individuals living or working in environments exposed to such fields. In contrast, some studies suggested that acute exposure to either continuous or intermittent 50-Hz linearly polarized magnetic fields of 10 mT does not affect the endocrine functions (including luteinizing hormone which affected by melatonin) or their circadian rhythmicity in healthy young men (Selmaouli et al., 1997).

Since, the pineal gland produces low amounts of melatonin during the day and high amounts at night; this rhythm is reflected in the blood melatonin concentrations. Reiter, 1993 found that the exposure to sinusoidal electric and / or magnetic fields as well as pulsed static magnetic fields often reduces pineal melatonin production. Melatonin, because it is a potent antioxidant , may provide significant protection against cancer initiation as well as promotion (Reiter, 1993). Another evident to correlate pineal function and cancer was studied by Mevissen et al ,1996 who found a correlation between pineal function of rats and DMBA – induced cancer during exposure to magnetic field. They demonstrated a tumor-co-promotion effect of magnetic field with DMPA mammary carcinogenesis but appear to be dose dependent. However, it is premature to conclude that alleged increased cancer risk reported in individuals living in higher than normal electromagnetic environments relate to reduced melatonin levels caused by such field exposures. We hope that these important and very interesting studies well extending in the soon future specially related to pineal gland and its function specially melatonin secretion.

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RECOMMENDATIONS

RECOMMENDATIONS

The work team wishes to present the following recommendations:

1- The study must be continued to follow other effects specially on developmental germ cells of the embryos.

2- The team suggests study the effects of EMFs on enzymes activity of the developing embryos.

3- The accumulation effects of exposure to EMFs must be considered specially in the following generations of the exposed parents.

4- The hormone melatonin is too important to be investigated carefully through the study of pineal gland activity and its related level in serum.

5- These studies will considered as the corner stone for establishing a Biophysical lab. On SARC and gradually and step by step, all equipments will be completed.

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تأثير المجالات المغناطيسية والكهرومغناطيسية على الأجنة

روضه خليفة آل ثاني [1]
نوره جبر آل ثاني [2]
جمال الشريف [3]

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[3] قسم العلوم البيولوجية – كلية العلوم – جامعة قطر
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ص. ب: ١٦٧١ الدوحة - قطر