EFFECT OF POWER FREQUENCY MAGNETIC FIELDS ON THE DEVELOPMENT OF AMBYSTOMA MEXICANUM AND ARTEMIA FRANCISCANA KELLOGG

BY

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A Thesis

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ABSTRACT

This thesis investigates the effects of extra low frequency magnetic fields on the development of embryos. The investigation was done by exposing a group of Axolotl (*Ambystoma mexicanum*) embryos to power frequency magnetic fields and comparing the number of healthy developed ones to those of a control group.

Also, brine shrimp (*Artemia franciscana Kellogg*) were exposed to a direct current (dc) magnetic field to investigate its effect on the axis orientation of the developed embryo.

The results of both experiments indicate that above ambient levels of low frequency magnetic fields have no adverse effect on the development of either species, as measured by the parameters used.

ACKNOWLEDGMENTS

The writer wishes to thank Dr. Jane Evans of the advisory committee, my thesis advisor Dr. Richard Gordon and co-advisor Dr. Ed Shwedyk for their patience, guidance and support in the undertaking of this study.

The writer also gratefully acknowledges Natalie K. Björklund for her valuable help with handling and identifying the various stages of embryonic development of the axolotl; Mr. Allan McKay for making the Helmholtz coils and Mr. Mark Gouthro for his help with the brine shrimp.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Thesis Motivation :

Interest in the interaction of electromagnetic fields (emf) with biological systems dates back to the late eighteenth century when Galvani and Volta, among others, experimented with frog's legs. Since that time, many kinds of biological systems have been exposed to a wide variety of emf configurations at various levels of power frequency. In the last two decades particularly, increasing use of electromagnetic devices such as radar and microwave ovens has caused mounting concern about possible hazards produced by the exposure to EM fields. Over the past several years, however, a number of epidemiological studies have suggested a possible link between exposure to power frequency, non-ionizing electric and magnetic fields (emf) and some human health problems.

In 1979, Wertheimer and Lepeer[1] published the results of an epidemiological study that related childhood cancer to emf. The report claimed a significant association between childhood cancer and magnetic fields inside households caused by "high current configuration distribution wiring".

In 1987, Savitz [2] obtained interview data, took magnetic field measurements and classified the homes of cases and controls according to wiring configuration. He found a positive association between wiring code and childhood leukemia and no significant association between measured magnetic fields and childhood cancers, including leukemia.

This study suggested the possible contribution of confounding factors to cancer cases such as smoke inhalation, or other carcinogenic agents.

Other research related to the biological effects of emf has covered a wide range of topics, such as immunology, cancer[1], cell proliferation and differentiation[3], chromosome structure, nucleic acids and protein synthesis[4], reproduction and development[5] and bone healing [6].

Laboratory studies related to reproduction and development have concentrated mainly on chick and mice embryos. The motivation of this thesis is to contribute to this field by examining the possible effect of electromagnetic fields on the development of axolotl and brine shrimp embryos.

Thesis Scope:

This thesis documents investigations into the possible teratogenic effect of emf on the development of **axolotl** and brine shrimp embryos. The axolotl is the neotenous salamander *Ambystoma mexicanum* from lakes Xiechimelo and Chalco in Mexico. The axolotl was chosen because it has been well researched, its developmental stages are well documented and it matures sexually while still in the larval stage. The experiments involved subjecting a group of fertilized eggs to a 60 Hz electromagnetic field from the early stages of development until they hatch. Various field strengths were used from slightly below to as much as triple the ambient values. Various orientations of the exposed eggs with respect to the direction of the field were observed. The hatched embryos were scored and compared to a control group for mortality rate and morphological abnormality.

A second set of experiments involved subjecting brine shrimp eggs to a direct current (dc) magnetic field to investigate its effect on the orientation of the hatched organism.

The fields were generated by Helmholtz Coils in the case of axolotl experiments and by one coil in the brine shrimp (artemia) experiment.

CHAPTER 2

Background Material:

1. Introduction

The effect of electric and magnetic fields on cells, tissues and organisms became a subject of considerable interest as a result of epidemiological studies that suggested their possible adverse health effect, as well as other work that investigated the therapeutic and bone healing

The biological effects of electric and magnetic fields were first investigated in 1873 by Bertholon[7]. In this study, he collected atmospheric charges by means of an antenna arrangement and passed it through plants growing in the field. He reported accelerated growth in treated plants as compared with untreated ones. Since then, other researchers used variety of experimental techniques to study the effect of emf on living organisms.

In vitro studies of low frequency (LF) and extra low frequency (ELF) fields were carried out and have revealed a variety of effects[3,8]. These effects depended mainly on the electrical and physiological state of the exposed organism. In many cases, however, conflicting results have been reported, possibly due to the failure to duplicate the exact experimental conditions.

In general, research conducted, thus far, did not conclusively prove or disprove whether there are significant health risks associated with exposure to 60 Hz emf. This research could be categorized in two main categories:

a) Epidemiology studies[1,2] that were carried out by collecting data on disease occurrence among human populations known to be exposed to higher emf than the

general population. Such subjects included those who lived near, or were engaged in occupations that deal with high voltage transmission lines. They covered areas such as childhood and adult cancer, reproductive abnormalities as well as the association of exposure to general physiological and psychological conditions. In general, these studies indicated the need to conduct more research under controlled conditions to assess the levels of exposure that might cause adverse health effects and to gain understanding of the mechanism by which emf could be interacting with living organisms.

The advantage of epidemiological studies is that they investigate real people in their environment. Their disadvantage is that they require sophisticated statistical techniques in designing experiments, choosing samples, conducting observations and most importantly, drawing meaningful conclusions. These studies take a long time and it is very difficult to identify the effect of confounding factors, such as smoke inhalation or the use of herbicides along the right of way of power transmission lines. Nevertheless, they may reveal effects that need new laboratory approaches for their explanation or may indicate an area of observation which should be examined more closely.

b) Laboratory studies[8] are mainly in vitro studies in which cells and whole organisms are subjected to emf and their effect is monitored. Since the lab conditions can be controlled, these studies allow the establishment of a dose-response relationship better than epidemiological studies. Studies on the cellular level can be helpful in indicating the possibility of physiological malfunction resulting from exposure to an external field. However, they cannot accurately establish a cause-and-effect relationship between exposure and disease occurrence in a living organism. This is due to the fact that a cell in a living organism has a much more complex reaction mechanism to an external agent

than can be observed through in vitro experiments only. Therefore, in vivo experiments need to be performed on the whole animal, though it is still difficult to establish a relationship between risk to animals and to humans. In vitro studies on the cellular level help narrow down the range of in vivo experimental goals.

In this chapter, the basic electric and magnetic field theory is presented. Animal development is reviewed together with some details on the development of chick embryos. Axolotl development is also presented since it is the subject of the present work. Other subjects that relate to the reviewed literature are described. Italicized words are explained in the glossary (Appendix A).

2. Basic electric and magnetic field concepts:

2.1 Forces due to Electric and Magnetic Fields:

Electric and magnetic fields are measured by the forces they exert on charged particles. Electric fields (**E**) interact with matter by exerting forces on the charges in the material. In the absence of a field, a material is usually electrically neutral, that is the amount of positive charge is equal to the amount of negative charge and on the average, the two appear to be superimposed and they cancel each other. When an electric field is applied, it moves the positive charges in one direction and the negative charges in the other direction, producing electric dipoles and thus polarization. A second effect of the electric field is that it exerts forces on the positive and negative charges of the dipoles causing them to line up with **E**. A third effect is that it causes charges that are not tightly bound (called free charges) to move; that is, creating conduction current. A property of material called *permittivity* ε, describes the ease with which polarization occurs. A large ε means a

lot of polarization for a given **E**. The force exerted by an electric field is described by the equation (bold letters refer to vector quantities):

$$\mathbf{F} = q\,\mathbf{E},$$

where:

F is the force,

q is the charge,

E is the electric field,

Magnetic fields (**B**) interact with material by aligning its magnetic dipoles. The property of material that describes the ease with which polarization occurs is called magnetic permeability μ. A large μ means a lot of polarization for a given **B**. The force exerted by a magnetic field is described by the equation:

$$\mathbf{F} = q\,(\mathbf{v} \times \mathbf{B})$$

Where:

v is the relative velocity of the charge in the magnetic field

B is the magnetic flux density.

The force exerted by an electric field is in the direction of the field, whereas that due to a magnetic field is perpendicular to the direction of both the field and the movement of the electric charge. A charged particle such as a positive ion will be accelerated in a straight line by the force of an electric field. Replacing the electric field with a static magnetic field perpendicular to the direction of motion will result in the particle moving in a circle.

If the magnetic field is not at right angles to the particle motion, then the particle will move in a helical path (the sum of straight line motion due to the electric field and circular motion due to the magnetic field). The angular velocity by which the particle moves in a magnetic field is given by the equation

$$\omega = (q/m)\,\mathbf{B}$$

where

ω = angular velocity in radians/second (rad/sec)

m = particle's mass.

The above phenomena has been proposed as an explanation of the efflux of calcium ions from cells through ion channels[9].

The electric field strength is measured in units of volts per meter (V/m). Magnetic flux density is measured in units of Gauss (G) or Tesla (T); where 1 T = 10000 G.

2.2 Low frequency field sources:

Low frequency electric and magnetic fields are either

(i) naturally occurring:

a) caused by the separation of charges between the Earth's surface and the ionosphere and

b) caused by the Earth's magnetic field; or

(ii) artificially generated:

a) near overhead transmission lines

b) near underground conductors

c) fields from home environment (appliances, home wiring, etc.)

Although any source produces both **E** and **B**, at low frequencies, sources can be made to produce mostly **E** with negligible **B**, or vice versa.

A vector field can be completely defined by specifying its curl and its divergence. Curl is a mathematical property that describes how the vector is curling, or circulating at a given point in space, like water circulating around the drain in a bathtub. Divergence is a mathematical property that describes how a vector field is diverging or expanding at a given point in space, like smoke expanding in the atmosphere. Maxwell's two equations for **E** in free space define its curl denoted by $\nabla \times \mathbf{E}$ and divergence denoted by $\nabla \cdot \mathbf{E}$ as:

$$\nabla \times \mathbf{E} = -\partial \mathbf{B}/\partial t$$

$$\nabla \cdot \mathbf{E} = \rho/\varepsilon_o$$

where

ε_o is the permittivity of free space and,

ρ is the charge density in Coulombs per cubic meter.

In other words, the changing magnetic field is a curl-type source of **E**, and the charge density is the divergence-type source of **E**.

Maxwell's two equations for **B** in free space define its curl and divergence as

$$\nabla \times \mathbf{B} = \mu_o(\mathbf{J} + \varepsilon_o \partial \mathbf{E}/\partial t)$$

$$\nabla \cdot \mathbf{B} = 0$$

where

μ_o is the permeability of free space and,

J is the current density in Amperes per square meter

that is, both current density **J** and the time rate of change of **E** are curl-type sources of **B**. The field lines **B** encircle **J** and $\partial \mathbf{E}/\partial t$, which is also called displacement current. Also, the divergence of **B** does not exist, and therefore the field lines of **B** form closed lines.

When the wave length λ is much greater than the size of the object L to which Maxwell equations are applied (low frequencies), the sources of **E** can be either curl-type or divergence-type. When a low frequency source produces mostly charge with a little or no current, the dominating charge is a divergence type source that produces E which begins and ends on charges. On the other hand, if the low frequency source produces mostly current, with little or no charge, the dominating current is a curl-type source that produces primarily **B**. If **B** is time varying, then it produces secondarily **E**.

A familiar source of **E** is the parallel plate capacitor as shown in figure 2.1. A voltage source applied across the plates causes a charge to build up on the two plates which produces **E**. If the voltage is time varying, then **E** will also change with time, which will act as a curl type source of **B**, but at low frequencies, this magnetic field will be negligible.

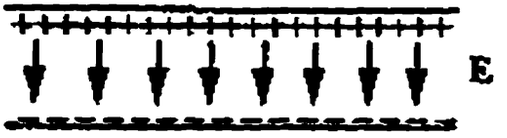

Figure 2.1 The E Field produced by a potential difference between capacitor plates.

A familiar source of **B** at low frequencies is a loop in which the current **I** is uniform as shown in figure 2.2. The figure shows the primary induced magnetic field **B** as well as the secondary electric field **E**.

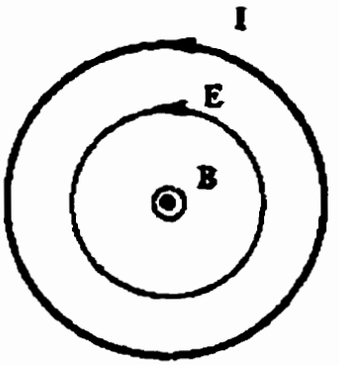

Figure 2.2 The **B** Field produced by line current out of the paper which, as it changes with time, produces **E**.

If the current changes direction halfway around the loop (high frequency), then it becomes non-uniform. In this case, a charge is produced at the top and the bottom of the loop and this charge produces **E** as shown in figure 2.3.

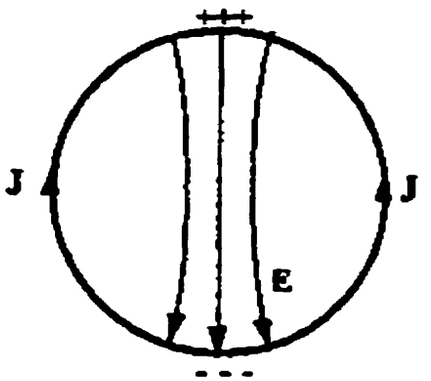

Figure 2.3 A non-uniform current in a loop produces charge at the top and the bottom of the loop. The charge produces **E**, which is much stronger than **E** produced secondarily by the time changing **B**.

If a hoop with a diameter a is placed in the x-y plane then a current I_a flowing in the hoop will generate a magnetic field along the z axis. The field strength at any point z is given by:

$$B_z = \mu_o I_a^2 / 2(z^2+a^2)^{3/2}$$

At low frequencies, **B** can be generated by a pair of current coils which are called Helmholtz coils. A highly uniform field along the axis between the two coils could be generated by making the distance between them equal to the coil's radius a. In this case, the field strength at any point z is given by:

$$B_z = 0.5\ \mu_o I_a^2 \{ [1 / (z^2+a^2)^{3/2}] + [1 / ((z-a)^2+a^2)^{3/2}] \}$$

This arrangement is shown in figure 2.4.

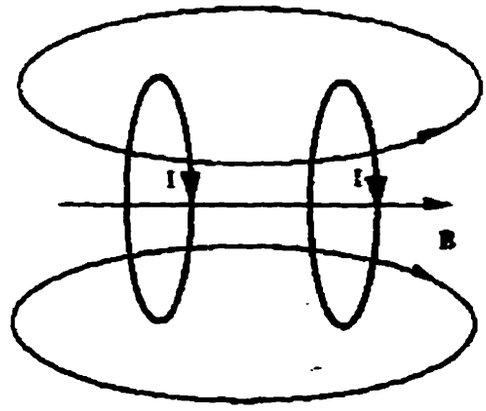

Figure 2.4 Helmholtz coils used to produce a **B** field.

2.3 Magnetic field measurement :

Magnetic fields are measured using a Gauss meter. The meter measures the root-mean-square (rms) value of the sinusoidal wave form. The rms value of a periodic variable x is calculated from the equation:

$$X = \sqrt{\frac{1}{T}\int_0^T x^2 dt}$$

where:

T is the time of one cycle of the variable. For a sinusoidal wave with a peak value X_{max} and a frequency ω rad/sec,

$$x = X_{max} \sin \omega t,$$

the rms value is

$$X = 0.707 * X_{max}$$

2.4 Pulsed waves:

A wave consisting of a train of pulses contains a fundamental frequency component as well as infinite harmonics (multiples of the fundamental frequency). Thus, the Fourier analysis of a function y whose amplitude is a, and it repeats itself every 2π seconds, results in:

$$y = \frac{a}{2} + \frac{2a}{\pi} \sum \frac{\sin(n\omega t)}{n}$$

where n = 1,3,5,... ; is known as the harmonic number.

It should be noted that the magnitude of the harmonic decreases as the harmonic number increases.

3. Biological background:

In this section, the biological and biochemical topics related to the literature surveyed and to this work are reviewed. The main topic of investigation is the effect of power frequency electromagnetic fields on embryonic development. Most of the previous work involved the study of field effects on chicken and rat embryos. The present work investigates the possible effect of emf on axolotl and brine shrimp development.

3.1. Animal development:

Animal development denotes the processes that are involved in the transformation of a fertilized egg into an adult individual. The development takes place in five steps[10]:

3.1.1 Gametogenesis: the transformation of certain cells in the parents into specialized cells called gametes. The process is called oogenesis in the female and the gamete is the egg or ova. In the male, the process is spermatogenesis and the gamete is a spermatozoa.

3.1.2 Fertilization: the fusion of the two gametes (sex cells), a male one and a female one, followed by the joining of their nuclei. This fusion activates the egg so that it

starts to develop, and the joining together of the nuclei results in the endowment of all the cells of the developing new organism with carriers of hereditary properties derived from the maternal and paternal organisms[10].

3.1.3 Cleavage: a series of cell divisions that the egg experiences following fertilization. Cell division involves two distinct processes that often, but not always occur together: division of the *nucleus* and division of the *cytoplasm*. The process by which the nucleus divides to produce two nuclei, each with the same number of chromosomes as the paternal cell's nucleus is called **mitosis**. The process of division of cytoplasm is called **cytokinesis**.

During cleavage, the following events take place:

- The unicellular fertilized egg is transformed by consecutive mitotic divisions into a multicellular complex. No growth takes place during this stage. One of the important events that take place during cleavage is the synthesis of ribonucleic acids (RNA). Although *messenger RNA* (mRNA) is synthesized during cleavage, it remains inactive throughout this process except for maternal mRNA deposited during oogenesis. The function of this particular RNA is to carry the instructions specifying the order of amino acids in new proteins from the genes to the *ribosomes* where protein synthesis take place.

- The general shape of the embryo does not change but as cleavage continues, the newly formed cells (*blastomeres*) begin to pump sodium ions (Na^+) into the center of the mass of cells forming a fluid filled chamber called the *blastocoele*. The embryo at

this stage is termed a **blastula.** Transformation of the cytoplasmic substances (such as nucleic acids bases) into nuclear substance (DNA) take place but the qualitative changes in the chemical composition of the egg are limited.

- A series of complex movements follow and they establish the shape of the organism. This process is termed **morphogenesis**, meaning the genesis of the form.

3.1.4 Gastrulation:

The process of gastrulation begins when a broad depression, or invagination, starts to form at a point on the surface of blastula where the cells are somewhat larger than those on the opposite side. The smaller cells make up the *animal hemisphere* of the embryo. The larger cells make up the *vegetal hemisphere*. The invaginated layer bends farther inwards until it comes to lie against the inside of the outer layer. The embryo at this stage is termed a **gastrula**.

3.1.5 Organogenesis:

This process involves the formation of different body organs.

3.2 Development of chick embryo:

In bird's eggs[11], cleavage does not take place in the yolk due to its large size, and all cell division is restricted to the small cytoplasmic disc, or **blastodisc**, as shown in figure 2.5. The gastrulation process is modified in such eggs and, instead of invagination, outer and inner layers called the **epiblast** and the **hypoblast** are produce by splitting of the blastodisc (figure 2.6A-C). The cells of the epiblast then converge toward the midline, giving rise to a clearly visible line or streak. This is termed the primitive streak (figure 2.6D). Individual cells move downward from this region. Some cells stay between the

epiblast and the hypoblast and give rise to the *mesoderm*, while others insert themselves into the hypoblast and help form the *endoderm*.

The *ectoderm* eventually gives rise to the outermost layer of the body, the nervous tissue and to structures derived from the *epidermis* such as hair, nails, eye lens, the pituitary gland, and the epithelium of the nasal cavity, mouth, and anal canal. The endoderm gives rise to the innermost layer of the body: the epithelial lining of the digestive tract, respiratory passages and the lungs, the liver, the pancreas, the thyroid and the bladder. The mesoderm gives rise to most of the tissues in between, such as muscle, *connective tissue* (including blood and bone), and the *notochord*.

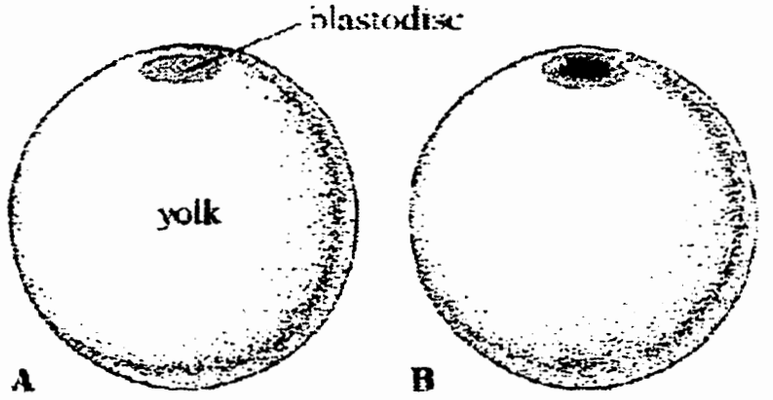

Figure 2.5 (A) a small cytoplasmic disc lies on the surface of a massive yolk. (B) Early cleavage[11].

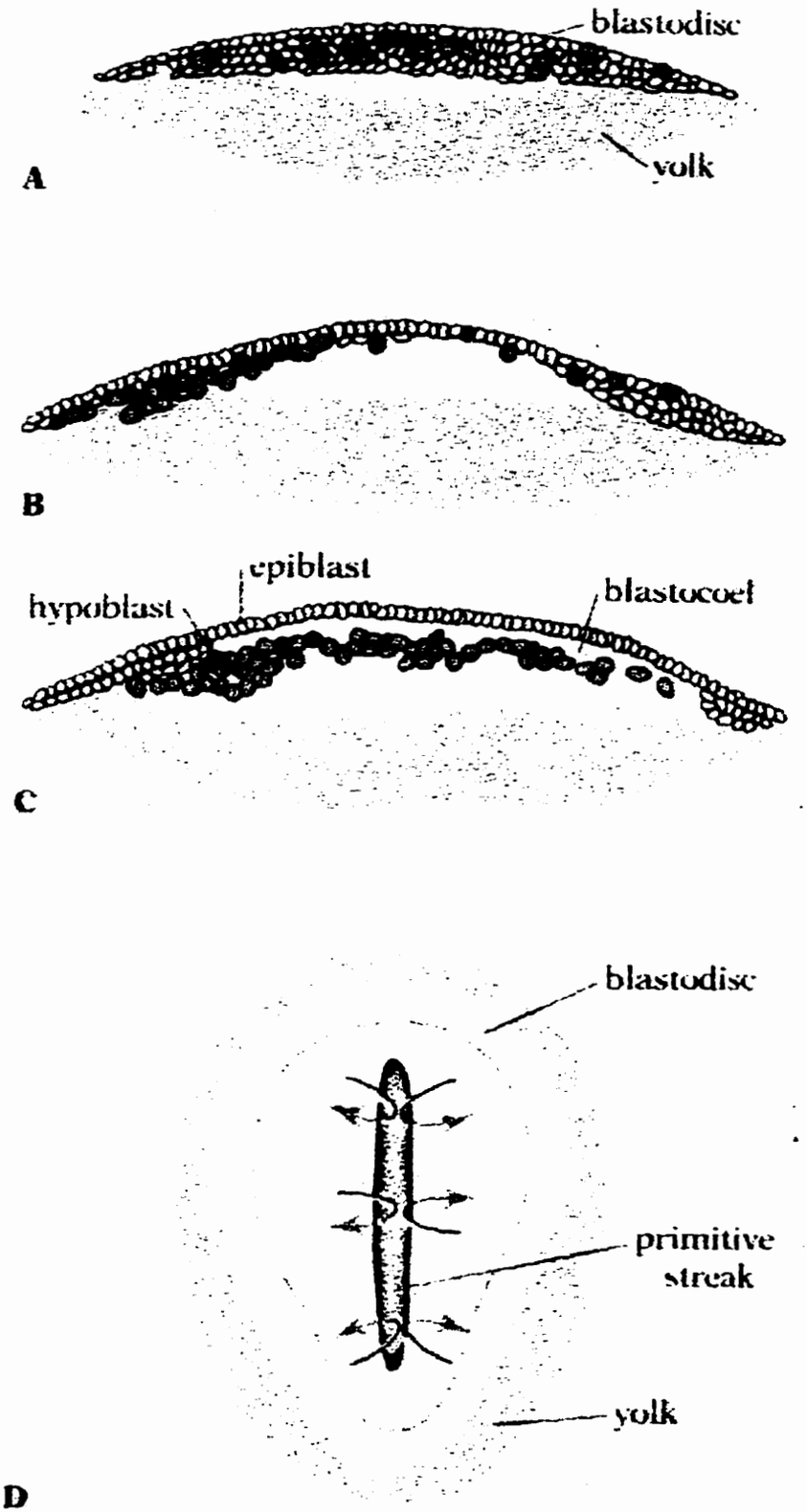

Figure 2.6 Gastrulation in chick embryo[4]

3.3 Development of the Axolotl embryo:

Schreckenberg and Jacobson[12] divided the development of axolotl into 43 stages, based on external morphological features. The size of the egg is 1.85 to 2 mm and it grows to about 11.3 mm by the time the fully developed larvae hatches. At 20°C, it takes the fertilized egg 342 hours to develop into a full organism.

Cleavage begins after 40 minutes of fertilization (stage 2). The early blastula (stage 8) is reached after 16 hours. Gastrulation begins after 26 hours of fertilization (stage 10) and after 33 to 35 hours, a slitlike blastopore is formed. The blastopore then becomes narrow after 36-37 hours and the boundaries of the neural plate are outlined. This stage is termed as "early neurula I" (stage 13). This process ends at hour 72 (late neurula IV, stage 20). The gill region becomes distinct at stage 22 after 48 hours of fertilization. The ears are distinct at stage 26 after 55 hours. The body of the embryo begins to straighten at stage 29 (64 hours), and the body axis is totally straight at stage 35 (80 hours). The development of the embryo then proceeds until the breaking of the mouth occurs at stage 43, 223 hours from fertilization. The stages of development are shown in figure 2.7.

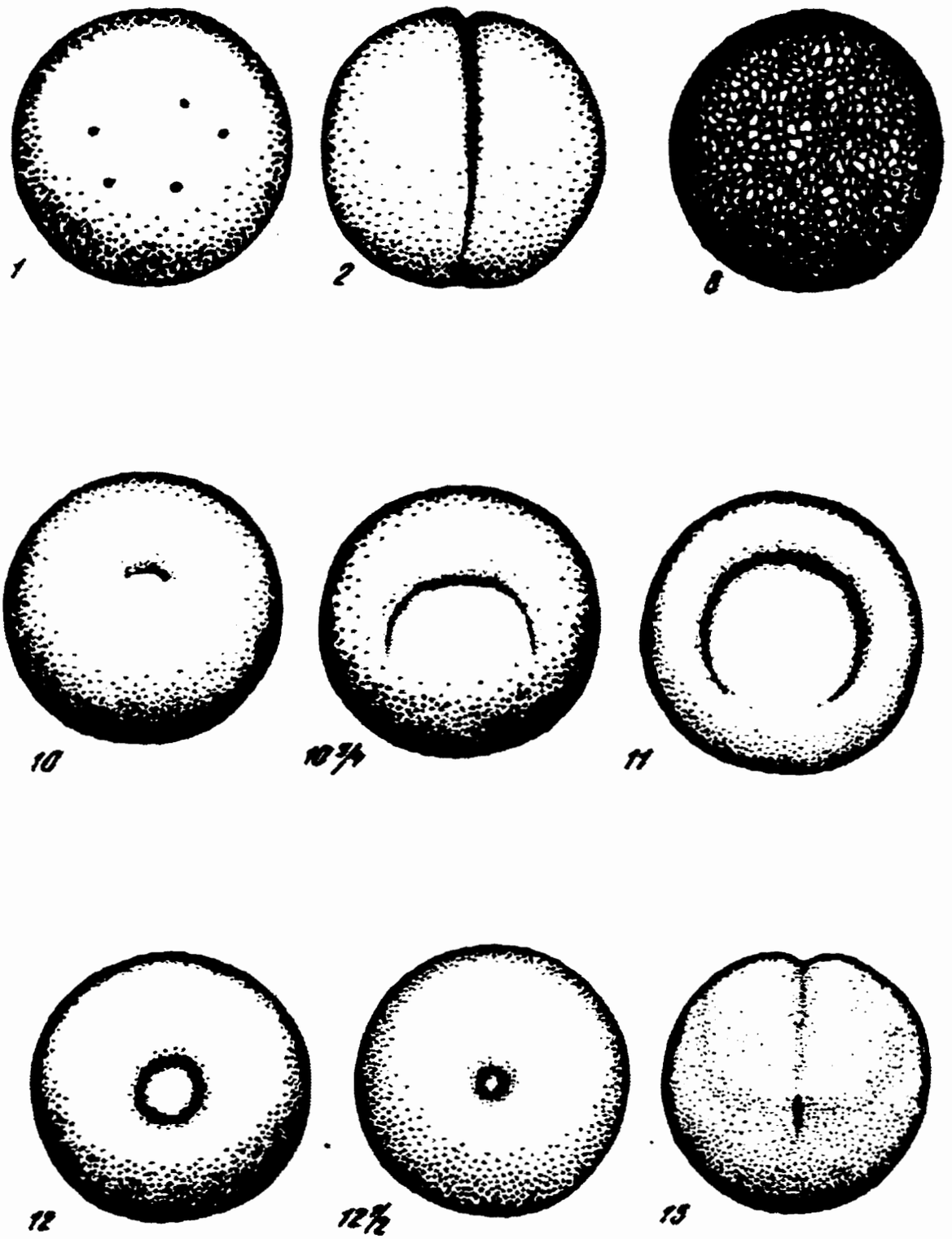

Figure 2.7 Developmental stages of axolotl embryo[12].

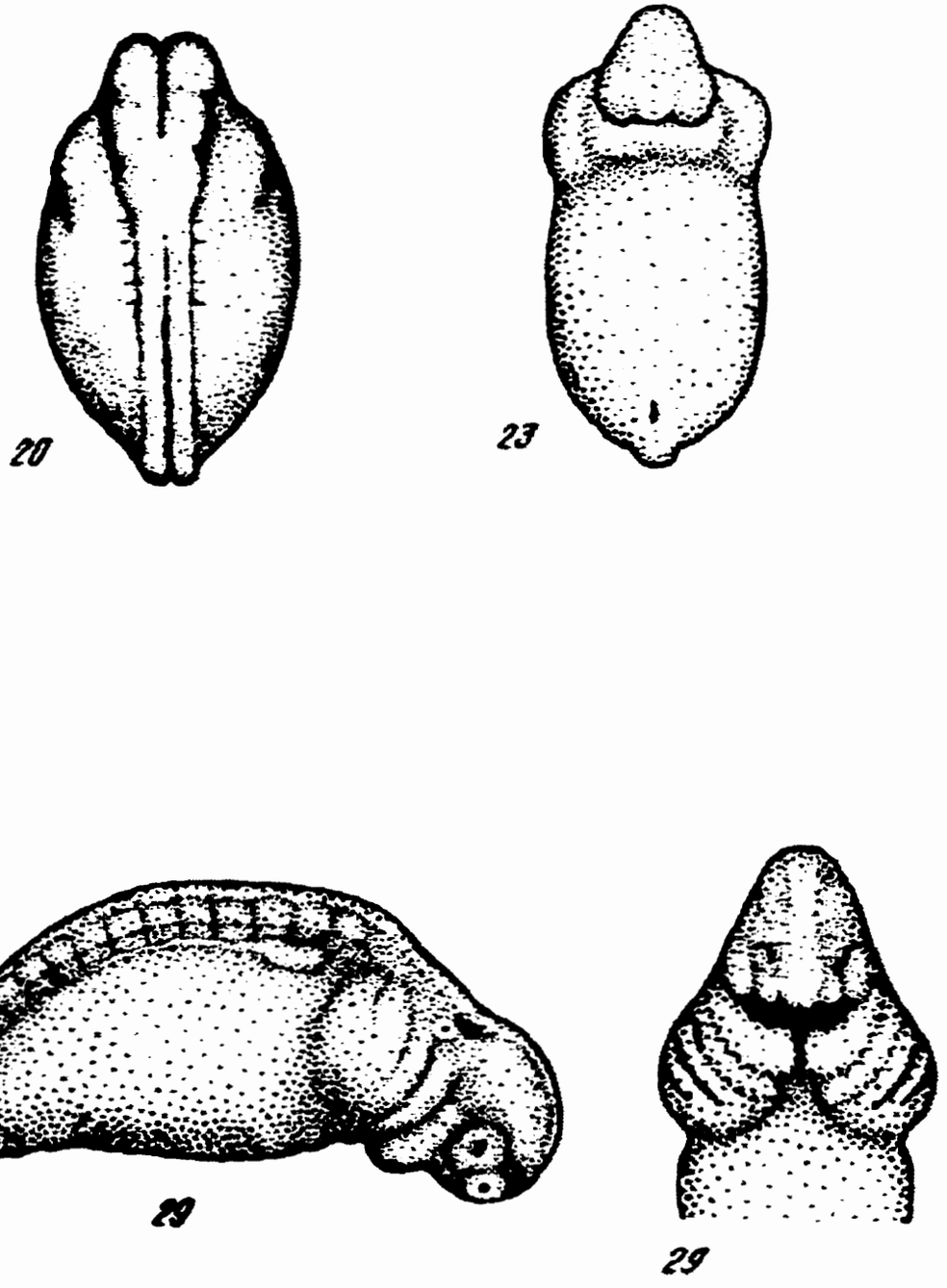

Figure 2.7 (continued) Developmental stages of axolotl embryo.

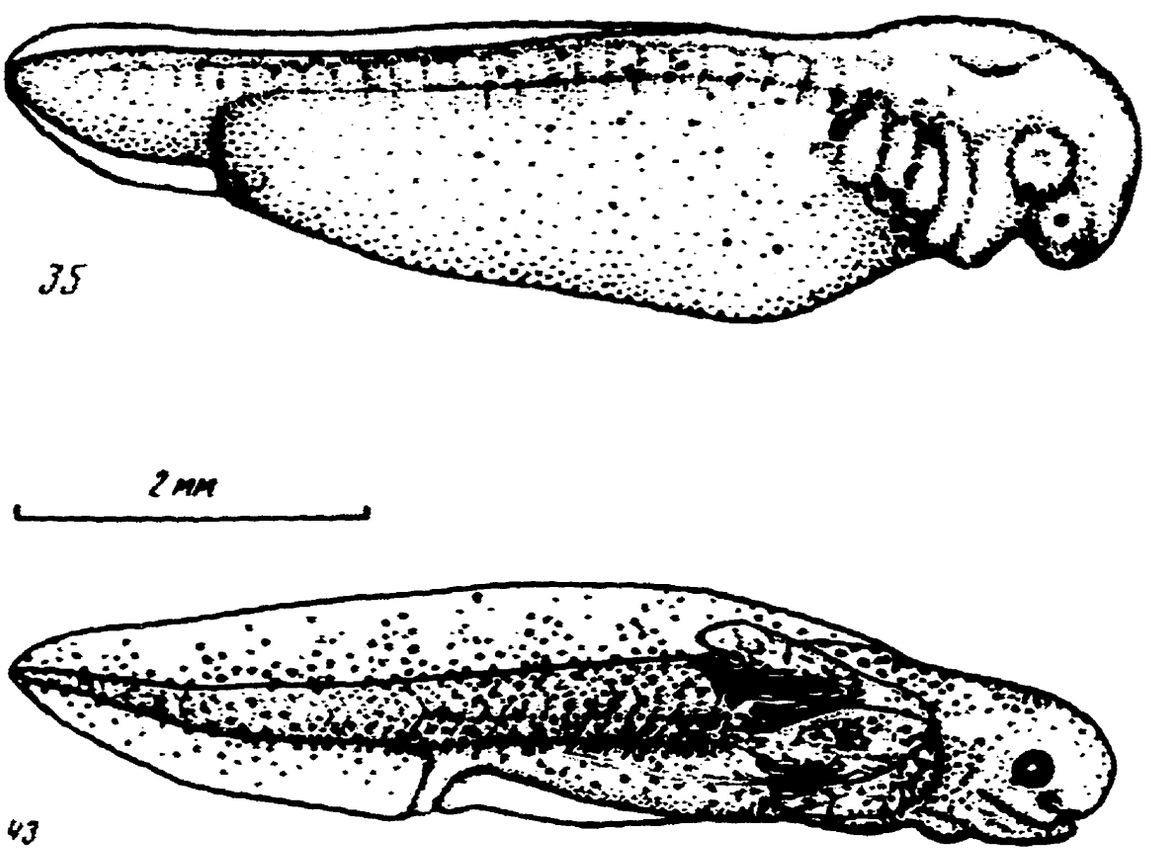

Figure 2.7 (continued) Developmental stages of axolotl embryo.

3.4 Brine Shrimp:

The brine shrimp **Artemia franciscana** forms dormant, blastula-stage embryos in cysts, encapsulated in a thick shell[13]. When these shells are aerated in salt water, *nauplii develop* with their simple eye always aligned upwards. When the embryos were allowed to develop in microgravity aboard the space shuttle, their eye orientation was random[14]. The developing embryo has the same number of cells as the encapsulated one. It has a heart, circulatory and nervous systems and a digestive tract. This makes it an adequate species to examine the effect of external agents on the cell differentiation of the organism in isolation from cell division (mitosis).

4. Ambient Electric and Magnetic Fields:

a) Naturally occurring fields:

The Earth has a static, vertically directed electric field of about 130 V/m near the surface that is caused by the separation of charges between the earth and the ionosphere[15]. Fields of 10 kV/m or higher can occur during thunderstorms.

The geomagnetic field is also essentially static. Its vertical component has a magnetic flux density that averages about 50 μT (500 mG) at middle latitudes. The field peaks at the magnetic poles to about 67 μT, and has a value of zero at the magnetic equator. The horizontal component is 33 μT at the magnetic equator and zero at the magnetic poles.

b) Power frequency fields near overhead transmission lines:

Electric fields near transmission lines are measured or calculated at a height of 1 m above the ground. The maximum calculated electric field underneath a 500 kV transmission line is 7.8 kV/m and it decays to 1 kV/m at the edge of the right-of-way. The peak value of magnetic flux density underneath a double circuit, 500 kV line carrying 2624 A per phase (a total of 5000 MW) is less than 350 mG. This value drops to about 50 mG at the edge of the right-of-way.

Table 2.1 shows 60 Hz electric field levels at the center of various rooms in a typical U.S home, 1974. Table 2.2 shows the levels at 30 cm from 115-V home appliances and table 2.3 shows 60 Hz magnetic fields near various appliances[15].

Location	V/m
Laundry room	0.8
Dinning room	0.9
Bathroom	1.2-1.5
Kitchen	2.6
Bedroom	2.4-7.8
Living room	3.3
Hallway	13

Table 2.1 Electric fields at the center of various rooms[15].

Appliance	V/m
Electric blanket	250
Boiler	130
Stereo	90
Refrigerator	60
Electric iron	60
Hand mixer	50
Toaster	40
Hair dryer	40
Color TV	30
Coffee pot	30
Vacuum cleaner	16
Incandescent bulb	2

Table 2.2 Electric fields at 30 cm from home appliances[15]

Appliance	Magnetic Flux Density, μT		
	3 cm	30 cm	1m
Can openers	1000-2000	3.5-30	0.07-1
Hair dryers	2-2000	0.01-7	< 0.01-0.3
Electric shavers	15-1500	0.08-9	< 0.01-0.3
Drills	400-800	2-3.5	0.08-0.2
Mixers	60-700	0.6-10	0.02-0.25
Portable heaters	10-180	0.15-5	0.01-0.25
Blenders	25-130	0.6-2	0.03-0.12
Television	2.5-50	0.04-2	0.01-0.15
Irons	8-30	0.12-0.3	0.01-0.025
Coffee makers	1.8-25	0.08-0.15	< 0.01
Refrigerator	0.7-0.7	0.01-0.25	< 0.01

Table 2.3 Magnetic flux densities near various home appliances[15].

CHAPTER 3

Literature Review:

Review of the literature indicates that electromagnetic fields tend, in some cases, to induce morphological abnormalities in developing embryos.

As far as the emf effect on embryos is concerned, most of the research has been done on chicken and rat embryos. They are convenient because they are readily available, their stages of development are well documented and hence, the effect of teratological agents can be easily observed. Also they allow the study of many generations in a short period of time. Besides, many of their ontogenetic characteristics are shared by many vertebrates during their development.

This chapter reviews the results of exposing the embryos of various species to electric and magnetic fields of different strengths, wave shapes and frequencies. A note on the healing effects of electromagnetic fields is also presented.

In 1968, Neurath[16] exposed the fertilized eggs of the common leopard frog (*Rana pipiens*) to a high magnetic field (1 T) generated by a permanent magnet. The eggs were held between two holders when the animal-vegetal axis was in a vertical direction. The same spring water was circulated between the exposed and control groups and the temperatures of both holders was kept constant. The author found that sixty eight percent of the embryos subjected to this field stopped developing after gastrulation.

Levengood[17] found that the application of a non-varying (dc) magnetic field with strength varying between 0.63 and 1.77 T to *Drosophila* pupae (fruit fly) results in *morphological* abnormalities as well as retardation of development. These effects continued to occur without further treatment in succeeding generations, implying that the fields resulted in genetic abnormalities. Experiments were also carried out with salamander eggs (*Ambystoma maculatum*). It was found that severe *edema* (or oedema) appeared in the larvae from eggs treated in the early gastrula stages of development. The test larvae died five days after hatching and their size at this time was one third of the control group. The treated larvae also had a severe growth on the abdomen and the head was not completely formed with only an indication of eye spots and rudimentary gills. The author also reported that brief treatment of eggs from the wood frog (*Rana sylvatica*) produces several types of abnormalities, such as severe leg malformations and pronounced alteration in the pattern of *histogenesis* which took the form of subepidermal blistering.

Marino et al.[18] allowed mice to mate, gestate, deliver and rear their offspring for three successive generations while being continuously exposed to a 60 Hz electric field. Mice exposed to a vertical electric field of 150 Volts/cm exhibited decreased body weights and an increased mortality rate for three successive generations. The ones exposed to a horizontal electric field of 100 volts/cm exhibited decreased body weights for two successive generations.

Manmohan et al.[19] subjected chick embryos to a strong direct current (dc) magnetic field (500 G) for one hour. The chicks were incubated to the definitive primitive streak

stage before exposure. They observed that the exposed embryos were considerably malformed, as evidenced by shortening of the embryonic long axis and by other malformations. Fifty six percent of the exposed embryos had poorly developed brains with open *neural tubes*. In 72%, the *somites* were diffused, and in 48% the heart was developed with slight displacement. They also reported that histological observations of the neural structures showed an increase in the number of dividing cells and their dispersed distribution throughout the *neuroepithelium*. The authors attributed the malformations to the stress caused by the magnetic field.

Ubeda et al.[20] stated that there exist "windows of frequencies" in the teratogenic effects of electromagnetic fields. Intensities of 10 to 139 mG of pulsed waves with frequencies varying between 40 and 71 Hz were used for two days. They found that they have only slight, specific effects on the development of chick embryos. The exposure to 10 mG could increase mitotic and metabolic activities of the truncal neural tube. Exposure to 139 mG altered the *organogenesis* of the circulatory system. They also found that pulses with very short rise time (step pulses) have a significant teratogenic effect on the embryos. The field intensity was 40 mG, which is much less than the Earth's magnetic field. This study indicates that the high frequencies contained in the step wave are the main cause of the teratogenic effects.

Based on an epidemiological study conducted in Columbus, Ohio, Wilkins et al.[21] reported an association between paternal employment in jobs linked with exposure to both ionizing and non-ionizing emf and a risk of *neuroblastoma* in offspring.

In a comprehensive study by Berman et al.[22], fertilized eggs of domestic chicken were subjected to 100 Hz, 1 μT peak and 2 μsec rise time pulsed emf. The same experiment was carried out simultaneously in six different countries. In five of the six laboratories, more exposed embryos exhibited structural anomalies than did the controls. In total, the number of the exposed embryos that exhibited abnormalities exceeded the controls by 25%.

Chacon et al.[23] exposed chick embryos to 30 Hz magnetic field in order to produce near resonance conditions for calcium and sodium ions. They found that the field did not increase the number of abnormal embryos as compared to the control groups. However, among the abnormal embryos, the proportion of the ones that did not develop increased significantly, indicating that the field has no effect on healthy organisms but affects the ones which already had some developmental effects.

Martin[24] exposed chicken embryos during the first 48 h of incubation to 60 Hz, bipolar, unipolar, or split-sine waves at 3-μT peak-to-peak. No adverse effect was seen following this exposure. Four experiments were carried out and the differences in the numbers of malformations between control and experimental groups were not statistically significant. Field-free incubation for an additional 72 h after exposure to a bipolar sine wave for 48 h resulted in an increase in normal live embryos in both control and treated groups.

Wiley et al.[25] exposed mated CD-1 mice to 20-kHz sawtooth magnetic fields similar to those associated with video display terminals (VDT). Four groups of animals were continuously exposed from day 1 to day 18 of pregnancy to field strengths of 0, 3.6, 17,

or 200 μT. There were no less than 185 mated dams in each exposure group. On day 18, the dams were sacrificed and assessed for weight gain and pregnancy. The litters were evaluated for numbers of *implantations*, fetal deaths and resorptions, gross external, visceral and skeletal malformations, and fetal weights. There were no less than 140 pregnant females in each group, and there were no significant differences between any of the exposure groups and the sham group (0 μT) for any of the end points. The results of this study do not support the hypothesis that the 20 kHz VLF magnetic fields associated with video display terminals are teratogenic in mammals.

Khomak et al.[26] found that a static electric field with intensity of 150-80 V/cm has no teratogenic or embryolethal effect on rat embryos. However, it provokes disturbance of the morphofunctional state of embryo liver, causes destructive-dystrophic changes in the *parenchyma cells*, decreases the content of *nucleic acids*, induces disturbances of the membrane systems of *mitochondria* and other cell structures.

Huuskonen et al.[27], exposed one group of pregnant rats to 50 Hz, 35.6 μT and a second group to 20,000 pulse per second, 15 μT sawtooth magnetic field continuously from day 0 to day 20 of *gestation*. It was found that both magnetic fields significantly increased the incidence of subtle skeletal malformations such as extra thoracic ribs as well as wavy, partially thickened or displaced ribs. Other abnormalities such as bladder dilatation, irregular nasal cavities and undersized adrenals were similar in all groups including the controls. The authors concluded that prenatal exposure to both fields is not teratogenic to rats but may have a slight effect on ossification.

Frolen et al.[28] investigated the influence of a pulsed sawtooth magnetic field with 45-μsec linear rise time and 5-μsec decay time, peak strength of 15 μT, and frequency 20 kHz) on the embryogenesis of CBA/S mice in five experiments. Sham and field exposures began on day 1 of gestation in two experiments, on day 2 , on day 5, and on day 7 in each of the other experiments. All exposures continued until day 19 post conception. The following variables were then measured:

1. number of implants
2. number of placental resorptions
3. number of living fetuses
4. number of dead fetuses
5. number of malformations in living and dead fetuses
6. length and body mass of living fetuses.

Control dams were sham-exposed concurrently with corresponding, exposed dams. With the exception of experiment 5, in which exposure started on day 7 , all groups of exposed mice had significantly more placental resorptions when compared with concurrent controls. The increased resorption rate was not reflected in a reduction in litter size or in the number of litters. No significant increase in malformed fetuses was observed in any of the exposed groups. Only in the first experiment the number of dead fetuses was affected by the exposure. The effect of field on the implantation rate was not significant. Body mass and length of exposed fetuses were significantly reduced only when the treatment began on day 7.

Moses and Martin[29] reported that the exposure of chicken embryos to a 60Hz, 4 μT electromagnetic field resulted in a significant reduction in the activity level of *nucleotidase* (5' nucleotide) in normal live embryos. Levels of *acetylcholinesterase* and *alkaline phosphatase* were not affected. The effect of the field on 5'NT levels appears to be permanent, as incubation in a field free environment for a further 15 days did not result in enzyme levels returning to control values.

Cameron et al.[30] studied the effect of exposing sea urchin embryos to 60 Hz magnetic fields. They found that the field interferes with cell proliferation at the morula stage in a manner dependent on field intensity. The cleavage stages, prior to the 64-cell stage, were not delayed by the field suggesting that the ionic surges, *DNA replication*, and *translational* events essential for early cleavage stages were not significantly altered. Studies of *histone* synthesis in early sea urchin embryos indicated that the magnetic field decreased zygotic expression of "early" histone genes at the morula stage and suggests that this decrease in early histone production was limiting to cell proliferation.

Santini et al.[31] demonstrated that 50 Hz sinusoidal magnetic fields (with intensities ranging from 1 to 10 mT) induce a nonlinear change in both membrane *conductivity* and *permittivity* of primary chick embryo *myoblasts* in vitro. However, myoblasts exposed to dc-induced static magnetic field of either 1, 3 or 5 mT resulted in no changes in the membrane electrical parameters as compared to the controls.

Ubeda et al.[32] exposed freshly fertilized chicken eggs during the first 48 h of postlaying incubation to 1 μT, 100 Hz , 500 μsec duration pulsed magnetic field. Two different

pulse waveforms were used, having rise and fall times of 85 μsec (PMF-A) or 2.1 μsec (PMF-B). It has been reported that, with 2 day exposure, these fields significantly increase the proportion of developmental abnormalities. Following exposure, the eggs were allowed to incubate for an additional 9 days in the absence of the PMFs. The embryos were taken out of the eggs and studied blind. Each of the two PMF-exposed groups showed an excess in the percentage of developmental anomalies compared with the respective sham-exposed samples. This excess of anomalies was not significant for the PMF-A-treated embryos, but was significant for the PMF-B-exposed group , which showed a particularly high rate of early embryonic death. These results reveal that PMFs can induce irreversible developmental alterations and confirm that the pulse waveform can be a determinant factor in the embryonic response to ELF magnetic fields.

Pafkova and Jerabek[33] studied the influence of power frequency, 10 mT and 6 μT emf on avian and mammalian embryo. They reported that no significant alterations of either avian or mammalian embryogenesis were found after repeated exposures with different orientations.

In another study, Pafkova and Jerabek [34] investigated the combined effects of exposure to magnetic field (MF) 50 Hz 10 mT and X-ray ionizing radiation on developing chick embryos. When chick embryos at early stages were repeatedly exposed to MF prior to X-ray radiation, reduction of X-ray teratogenicity was observed. When MF exposures started immediately after X-ray radiation, the adverse developmental effects of ionizing radiation were observed.

Nagai and Ota[35] examined the effects of a pulsed electromagnetic field on mRNA expression of bone morphogenetic protein-2 and -4 in chick embryonic calvaria. From the beginning of embryogenesis (day 0), chick embryos were incubated in a continuous 2 mT, 15 Hz pulsed electromagnetic field. Control chicks were incubated in a normal magnetic field. It was found that the magnetic field enhanced the expression of both mRNAs. The enhancements were more pronounced in younger chick embryos (day 15 to day 17), and no significant change was observed in the 19-day-old embryos. These results indicate that osteo-inductive effects of the magnetic field were mediated at least in part by bone morphogenetic protein-2 and -4.

Levin and Ernst[36] demonstrated that exposure to 60 Hz magnetic fields (3.4-8.8 mT) and fields in the range of dc to 600 kHz (2.5-6.5 mT) can alter the early embryonic development of sea urchin embryos by inducing alterations in the timing of the cell cycle. Batches of fertilized eggs were exposed to the fields produced by a coil system. Samples of the continuous cultures were taken and scored for cell division. The times of both the first and second cell divisions were advanced by both ac and by static fields. They found that the effect was proportional to the field strength. However, the relationship to field frequency was nonlinear and complex. For certain frequencies above the ELF range, the exposure resulted in a delay of the commencement of mitosis. The advance of mitosis was also dependent on the duration of exposure and on the stage of development.

Narra et al.[37], exposed male and pregnant female Swiss Webster mice to a 1.5-T static magnetic field for 30 minutes. Effects on spermatogenesis in male mice were investigated by counting testicular spermheads and epididymal spermhead shape-abnormalities as a

function of time after exposure. Pregnant female mice were exposed to the field at the two-cell embryo stage, sacrificed immediately, and the ability of these preimplantation embryos to mature into *blastocysts* was examined in vitro. It was found that this exposure caused a statistically significant reduction (15%) in testicular sperm on the 16^{th} and 29^{th} days after exposure. However, the increase in spermhead shape abnormalities above normal control values was minimal. A substantial effect was noted on the development of preimplantation embryos with a survival fraction of 0.56 compared with controls. The authors concluded that 30-minute exposure to a 1.5-T static magnetic field appears to cause some deleterious effects on spermatogenesis and embryogenesis in mice.

In 1996, Pafkova et al.[38] reported that the exposure of chick embryos to magnetic fields influenced the sensitivity of embryonic morphogenetic systems to the subsequently administered chemical teratogens, insulin and/or tetracycline. A protective effect of MF was detected similarly as in the case of indirect interaction with ionizing radiation.

Veicsteinas et al.[39] studied the effects of intermittent exposure (2 h on/22 h off) of fertilized chicken eggs to a 200 μT sinusoidal (50 Hz) magnetic field. Control eggs (sham-exposed) were incubated in the same chamber as the experimental eggs. Chick embryos were examined for developmental anomalies and maturity stage after 48 h of incubation. Immunohistochemical analysis of extracellular membrane components, histological examinations for malformations of brain, liver, and heart; egg fertility and egg weights were evaluated at different periods of incubation. The investigation also measured the body weight of chickens for 90 days from hatching and included histological analysis of body organs. Statistical comparison between exposed and sham-

exposed values did not show significant differences in any of the variables investigated. Moreover, there were no differences in body weight, morphology, or histology of central nervous system, liver, heart, or testis in 90-day-old chickens hatched from the exposed in comparison to sham-exposed eggs.

Greenebaum et al.[40] (1996), stimulated the *dorsal root ganglia* (DRG) of 6-day chick embryo with *nerve growth factor* (NGF) and subjected them to pulsed magnetic fields (PMF). They found that a combination of NGF and bursts of asymmetric, 220 μsecond-wide, 4.0 mT-peak pulses induced significantly greater outgrowth than NGF alone, that fields without NGF do not significantly alter outgrowth, and that, unlike NGF alone, 4.0 mT fields and NGF can induce asymmetric outgrowth. The asymmetry is independent of the orientation with respect to the field. Similar results were found when applying 15 or 25 Hz pulses.

From the above review, it appears that electromagnetic fields affect the developing embryos in the following cases:

1. High frequency.
2. Low frequency pulsed fields, possibly because they contain high frequency components.
3. Very high magnetic field intensities.

Table 3.1 shows a summary of the results of the literature reviewed that is related to the exposure of chicken embryos to magnetic fields. The results are sorted by frequency.

Column 1 lists the field wave shape.

Column 2 lists the reference number.

Column 3 lists the field frequency (Hz)

Column 4 lists the field strength(mG)

Column 5 shows the results: + indicates that the field has an adverse effect on the embryos and '-' indicates no effect.

type	Reference	frequency	strength	results
dc	44	0	20	+
dc	19	0	500000	+
pulsed	35	15	2	-
pulsed	40	15	4	+
pulsed	40	25	4	+
sin	23	30	4.4	-
pulsed	20	40	10	+
sin	31	50	1	-
sin	39	50	2	-
sin	31	50	10	-
sin	33	50	10	-
non-sin	24	60	0.03	-
pulsed	20	71	139	+
pulsed	22	100	0.01	+ & -
pulsed	32	100	0.01	+ & -

Table 3.1: Summary of results on chicken embryos

A Note on the Use of Electric and Magnetic Fields in Bone Healing:

In 1988, Hastings and Mahmoud[6] investigated the electrical effects in bone. They concluded that the bone cellular response is a result of an electrical potential that is generated in the bone as a result of loading. The bone tends to bend when loaded. this results in tension on one side and compression on the opposite side. In vivo studies were performed in which field gradient was measured across the bone from tension to compression side. It was found that negative potential occurs where the bone compression is experienced and positive potential where tension is experienced. The

authors indicated that formation of bone material can be accelerated by inducing electrical current in the bone. The magnitude of this current is in the μAmpere range.

Scott and Korostoff[41] performed tests on 47 human and bovine bone specimens. A wide range of frequencies, from 0.5 to 100 Hz, both sinusoidal and pulsed waves were used. The authors confirmed that electric potentials are responsible for the remodeling process which bone experiences in response to mechanical loading.

Harriga and Hamilton[42] found that bone stimulation could be achieved by applying a 15 Hz emf to the bone.

Rubin[43] performed in vivo investigations using both pulsed and sinusoidal waves, with different frequencies, field strength and exposure duration. The authors concluded that endogenous electric fields serve as a regulatory factor for both bone modeling (formation) and remodeling (maintenance) processes. The authors also found that bone resorption can be prevented and bone formation can be initiated by exogenous induction of a field.

In general, it appears that the formation of bone by electrical stimulation is an established fact. Both osteoporosis and osteopenia can be prevented or even reversed by applying low frequency electric and/or magnetic fields. The fields also accelerate bone healing. These studies however, indicate that the field strength, its frequency and duration and frequency of exposure are not universal and they need to be optimized separately for each case.

CHAPTER 4

4.1 Hypothesis:

Review of literature indicates that adverse effects of electromagnetic fields appear to be caused by high levels of field strength or high frequencies.

It is expected that ambient levels of 60 Hz magnetic fields should have no adverse morphological or behavioral effects on the developing axolotl embryos. The following set of experiments were design to test the validity of this hypothesis.

Methodology and Results:

4.2 Experiment Setup

The present work comprises a number of experiments in which a Axolotl embryos were subjected to 60 Hz electromagnetic fields. The embryos were placed in two identical glass aquaria, sealed with non-conducting aquarium silicone: one was exposed to fields generated by Helmholtz coil, the other was shielded by placing it inside a box-like metal mesh and grounded through a connection to the laboratory's water pipes.

With the exception of the first experiment, the aquaria were filled with an equal amount of the same solution in a temperature controlled environment. The electromagnetic field is generated by a pair of Helmholtz coils. The aquaria were cubic in shape with side dimensions of 15 centimeters.

The setup, including the circuit connection is shown in figure 4.1. Figures 4.2 and 4.3 show the aquarium and the pair of Helmholtz coils. Figure 4.5 shows the control tank.

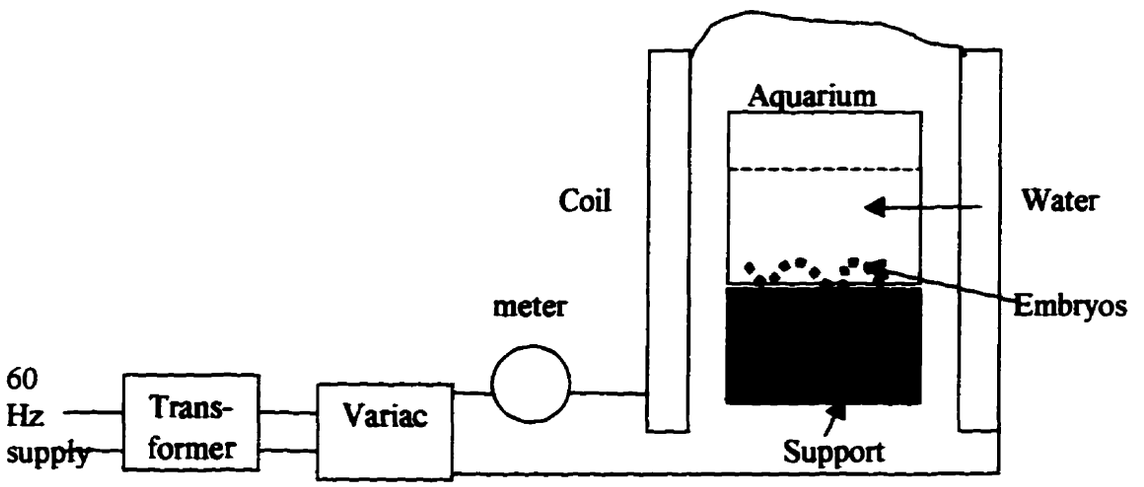

Figure 4.1 Experiment setup

4.3 Design of Helmholtz Coil

As mentioned in section 2.2, in order to generate a uniform field between the two coils, the distance between them must be equal to their radius. Having chosen 15 cm aquariums, the coil radius was chosen to be 16 cm. A spread sheet has been developed to perform the basic calculations. The following are the design data.

Wire size AWG	10
Resistance(Ω/m)	0.00334
Diameter (m)	0.00259
Number of turns	24
Dist. bet. coils(m)	0.160
Inductance (H)	0.000937
Ind. reactance (Ω)	0.353
Resistance (Ω)	0.161
Impedance (Ω)	0.388

The calculated magnetic flux density in mG as a function of current in Amps is shown in figure 4.4.

Figure 4.2 A side view of the aquarium and Helmholtz coils.

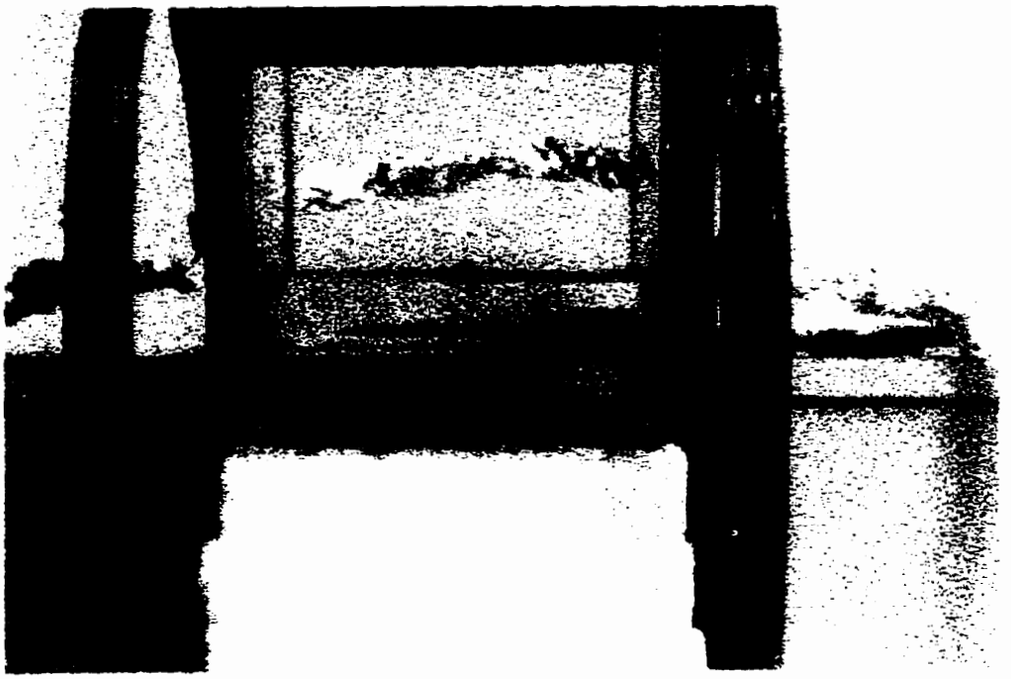

Figure 4.3 A front view of the aquarium and Helmholtz coils

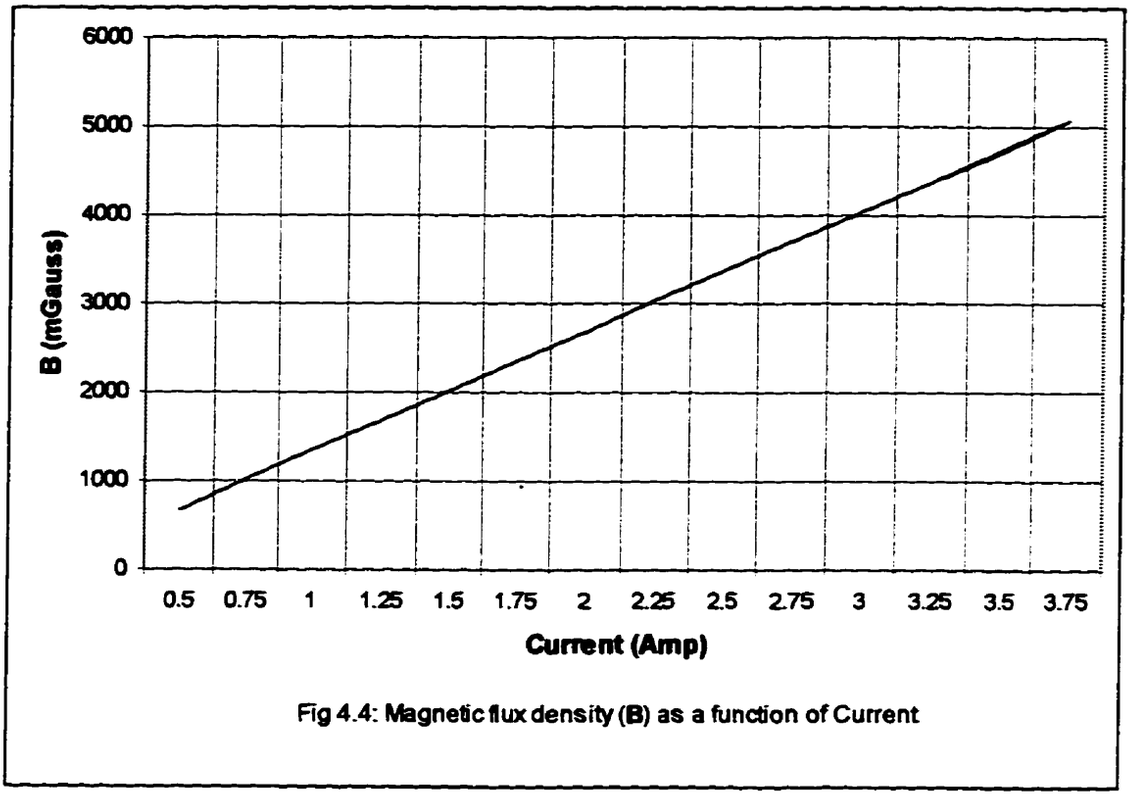

Fig 4.4: Magnetic flux density (B) as a function of Current

4.4 Experiments and results:

The experiment was repeated six times between February 1994 and May 1996. The following are the particulars of each experiment and the results. In all of the experiments, the embryos were in stages of development numbers 4 to 7.

Experiment 1:

The exposed and control embryos were placed in two aquaria of different sizes. Seventy-five axolotl eggs were placed in the aquarium exposed to emf and were subjected to a magnetic field of about 100 mG.

The control aquarium contained 63 eggs and was shielded with commercial Aluminum foil, thus the control embryos were in the dark. Fifty-three exposed embryos (71%) and 39 control embryos (65%) fully developed and hatched.

Experiment 2:

This experiment was carried out under better controlled conditions. Both groups were placed in identical aquaria. The control one was shielded by placing it in a grounded metal mesh cage, thus the embryos were not deprived of light. Forty-four eggs were placed in each aquarium. One aquarium was subjected to a magnetic field of about 100 mG. Four eggs did not develop in each aquarium. Out of the ones that developed, twelve of the exposed and four of the control embryos failed to hatch on their own after developing. However when taken out of their eggs they did not exhibit any abnormalities.

Experiment 3:

Each aquarium contained fifty-five eggs. The exposed group was subjected to a 1500 mG magnetic field. Only one egg in each aquarium did not develop. It was noticed that before hatching, all the embryos had a curl shape and were oriented in a vertical plane. This is shown in figure 4.6 as viewed from above the aquarium.

Experiment 4:

Forty-eight embryos were placed in each aquarium. The field strength was 1500 mG. Six embryos did not develop in the exposed aquarium and four of the control ones failed to develop. The orientation of the embryos was random.

Experiment 5:

Sixty-two embryos were placed in each aquarium. Again the field strength was set at 1500 mG. Twelve embryos failed to develop in the exposed aquarium and thirteen in the control one. The orientation was random.

Experiment 6:

Seventy three embryos were placed in each aquarium. The field strength was doubled to 3000 mG. Five of the control group and seven of the exposed group did not develop. The orientation was again random.

Table 4.1 below summarizes the results of the six experiments.

Experiment #	Field strength mGauss	Number Of Embryos						p value
		# of control	# developed	# of exposed	# developed	% control developed	% exposed developed	
1	100	63	39	75	53	62%	71%	0.2765
2	100	44	40	44	40	91%	91%	0.7123
3	1500	55	54	55	54	98%	98%	0.5173
4	1500	48	44	48	42	92%	88%	0.7379
5	1500	62	49	62	50	79%	81%	0.8177
6	3000	73	68	73	66	93%	90%	0.7611
Total		345	294	357	305	85%	85%	

Table 4.1 : Summary of the Experiments Results

In all of the above experiments, the embryos that developed were free from morphological abnormalities. As a behavioral test, the embryos were fed after hatching and there was no evidence of any behavioral abnormalities. The variability of the number of developed embryos could be due to the parents being different.

Figure 4.5 The control aquarium shielded with a metal mesh screen

Figure 4.6 Embryos alignment in vertical planes in experiment # 3

The 'p values' for all of the above experiments, as measured by the chi-squared test, is greater than 0.05 and hence is not significant. The 'p value' of the pooled data vs experiment # 2 is 0.0156 for the exposed embryos is 0.0156 which is significant whereas that of the control ones is 0.8965 which is insignificant. These calculations exclude experiment # 1 for a number of reasons:

1. The number of control and exposed embryos was not identical.

2. The size of the two aquaria and the amount of water in each of them were different.

3. the control embryos were deprived from light.

4.5 Effect of Magnetic Fields on the Orientation of Brine Shrimp:

The purpose of the next set of experiments was to examine the effect of magnetic fields on the orientation of brine shrimps as they hatch from their cysts. The cysts are decapsulated and are then attached with an adhesive (agar) to a microscope slide and are allowed to develop. The procedure is described in Appendix B.

Two slides were prepared for each experiment; one was placed parallel to the Earth plane (horizontal) at the center of a coil, the other was placed inside a metal cage, 3 meters from the coil. In each experiment, 50 hatched shrimp were randomly chosen and counted on each slide. The coil was connected to a current controlled dc source (a dc power supply), and the following experiments were carried out:

Experiment 7:

The coil's orientation was horizontal (parallel to the Earth's surface); the magnetic field density was 3000 mG.

Experiment 8:

Same as experiment # 7 except that the field direction was reversed by reversing the polarity across the coil.

Experiment 9:

In this experiment, the coil was tilted to make a 45° angle with the slide, and the field density was 3000 mG.

Experiment 10:

Same as experiment # 9 except that the field density was increased to 5000 mG.

Experiment 11:

The coil's orientation was vertical (perpendicular to the Earth's surface), the magnetic field density was 5000 mG.

Table 4.2 shows the results of each experiment and it shows that the orientation of the brine shrimp embryos is not affected by magnetic fields.

Experiment #	Field mG	Number Of Embryos					
		Control		Exposed		%control	%exposed
		Upward	Sideways	Upward	Sideways	Upward	Upward
7	3000	42	8	39	11	84%	78%
8	3000	34	16	36	14	68%	72%
9	3000	40	10	38	12	80%	76%
10	5000	38	12	35	15	76%	70%
11	5000	38	12	40	10	76%	80%
Total		192	58	188	62	77%	75%

Table 4.2 Results of the brine shrimp experiments.

CHAPTER 5

Conclusions

This work investigated the possible effects of ambient and above ambient levels of North America power frequency magnetic fields on the axolotl embryos. Also the effect of non-varying (dc) magnetic field on the orientation of developing brine shrimp embryos (*Armetia franciscana* Kellogg) has been investigated.

The results of the study clearly indicate that exposure of axolotl embryos to 100, 1500 and 3000 mG, 60 Hz sinusoidal magnetic field; from the early cleavage stages until hatching (9 to 10 days), do not adversely affect the developing embryo. The scoring was based on:

1. Failure to develop.
2. Morphological abnormalities.
3. Post hatching abnormal behavior.

The first experiment indicates that deprivation of light could possibly have adverse effect on the developing embryos. The fact that a relatively large number of the embryos, although fully developed, failed to hatch in experiment # 2 and the embryos vertical alignment with the field in experiment # 3 cannot be attributed to the magnetic field since these results could not be reproduced.

The main obstacle encountered was the unavailability of the axolotl embryos at all times and thus the number of experiments were limited.

The results of the second set of experiments indicate that the exposure of brine shrimp to 3000 and 5000 dc magnetic field has no effect on the organism's gravity perception as indicated in table 4.2.

Most of the adverse effects reported in the literature resulted from exposing embryos to high frequency, or to low frequency pulsed magnetic fields which contains high frequency components. This, together with the results of the present study, indicates the need to establish a relationship between possible adverse effects on organisms and the field frequency. Pure fundamental power frequency sinusoidal wave that are free of harmonic contents appeared to have no effect on axolotl and brine shrimp embryos. These results are in agreement with those reported by other researchers such as Chacon et al.[23], Martin et al.[24], Santini et al.[31], Pafkova et al.[33]. Nagai et al.[35] and Veicsteinas et al.[39].

The study performed by Huuskonen[27], and those related to bone healing indicate that the effect of emf on ossification is an established fact. They also emphasize that, in addition to both field frequency and strength, other parameters, mainly the duration and frequency of exposure, play a significant role in affecting the outcome of these studies.

The fact that both positive and negative effects of emf on bone have been reported emphasizes the need to carry out further research in this field.

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Ectoderm: the germ layer lying on the outside of the developing embryo and eventually gives rise to the epidermis and nervous tissue.

Endoderm: the embryonic germ layer that gives rise to the gut system and its associated organs.

Epidermis: the outer layer of the skin derived from embryonic ectoderm.

Gestation: the development of the embryo in the uterus of mammals.

Histogenesis: the formation of tissues and organs from undifferentiated cells.

Histone: a simple protein that regulates DNA functioning in some way.

Implantation: the act of attachment of the mammalian embryo to the uterus of the mother.

Mesoderm: the layer of embryonic cells lying between the ectoderm and endoderm. It gives rise to muscle, the blood system, connective tissues, the kidney, the dermis of the skin and the axial skeleton.

Messenger RNA: (mRNA) a single-stranded type of polynucleotide molecule that carry a coded sequence of instructions about protein structure from DNA to ribosomes where the protein is synthesized.

Mitochondria: an organelle known as the powerhouse of the cell, where ATP (adenosine-tri-phosphate) is produced.

Morphology: the study of the shape, general appearance or form of an organism.

Myoblasts: cells that gives rise to muscle fiber.

Nauplius: the typical crustacean larva which has a single eye, three pairs of limbs and a round transparent body.

Nerve growth factor: a substance that is essential for mitosis of the embryonic sympathetic nerve cells.

Neural tubes: the neural plate folds into a tube which detaches from the general ectoderm and becomes the nervous system (brain, spinal cord and nerves).

Neural plate: the part of ectoderm which sinks below the surface of the developing vertebrate embryo and forms the spinal cord.

Neuroblast: any embryonic cell which develops into a nerve cell or a neuron; an immature nerve cell.

Neuroblastoma: a malignant tumor of the nervous system composed chiefly of neuroblasts

Neuroepithelium: simple columnar epithelium made up of cells specialized to serve the sensory cells for the perception of external stimuli.

Notochord: the longitudinal axial support of the embryos of chordates.

Nucleic acids: a molecule that is composed of the string of nucleotides forming the polynucleotide chain.

Nucleotidase: an enzyme that catalyses the hydrolysis of a nucleotide to a nucleoside and orthophosphate.

Nucleus: an organelle inside the cell that is bound with a membrane and contains the chromosomes whose genes control the structure of proteins within the cell.

Oedema: a swelling of tissues caused by the capillary blood vessels passing out water into the surrounding tissues, and so increasing the intercellular fluid content.

Ontogenetic: development from fertilized egg to full adult.

Organogenesis: the period during embryonic development of an animal when the main body organs are formed.

Parenchyma cells: thin-walled, general purpose plant cells that often have a packing function, *or*, any specific organ cells apart from connective tissues and blood vessels.

Resorption: the taking back into an organism of any structure or secretion produced.

Ribosomes: a small particle found in the cytoplasm of all cells. They bind to the 5' end of mRNA and travel towards the 3' end, with translation and polypeptide synthesis occurring as they go along.

Somite: primitive segment; mesodermal segment; one of the blocklike masses of mesodermal cells arranged along the neural tube.

Translation: the formation of a polypeptide chain in a ribosome during protein synthesis, using the sequence contained in mRNA.

Vegetal hemisphere: is a site on the surface of the blastula where the cells are larger than those on the opposite side.

Appendix B

Decapsulating Artemia Cysts

Decapsulating is the process of chemically stripping away the chorion (shells) from Artemia cysts while maintaining their viability.

The decapsulating mixture is made up of:

333 ml 5.25% liquid chlorine (house bleach)

667 ml tapwater or clean seawater

10 gm sodium hydroxide NaOH (Caustic Soda)

Procedure:

Mix the three ingredients together for about 5 minutes with aeration to dissolve NaOH. Add dry cysts to the mixture and continue aerating vigorously for 10 to 12 minutes. The decapsulation should be completed after 10 to 12 minutes.

Pour off the cysts onto a screen or net and rinse well under tap water to remove excess chlorine. Transfer them to the deactivating solution, then rinse again with deionized water.

Agar preparation:

Ingredients:

6 gm/L $NaCO_3$

29 gm/L non-iodized NaCl

20 mL H_2O

0.47 gm agar.

Procedure:

Mix the above ingredients and boil in a microwave oven for 15 sec. Stir with a stirring rod then put the mixture back in the microwave oven for 15 to 20 sec.

Stir just before boiling then put the agar on a microscope slide.

Collect the brine shrimp with a paint brush and brush them in the agar then place the slice immediately on ice to cool down.

Ensure that there is sufficient water all the time to prevent them from drying.