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A STUDY OF SOME BIOLOGICAL EFFECTS OF NON-IONIZING
ELECTROMAGNETIC RADIATION

DISSERTATION

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Young C. Park, B.EE, MS

Denton, Texas

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The man-made artificial background of electromagnetic fields and radiations (EMFARs) has become a common constituent of civilized society. People living near the sources of EMFARs are exposed to radiation level higher than other people. These different living condition may produce adverse results. Some epidemiological studies have suggested that a link may exist between exposure to non-ionizing electromagnetic radiation (NEMR) and certain types of cancer. Other studies conducted by the researchers in the contra side have found no such link.

The experimental studies of this work were done using a microwave cavity spectrometer, *Escherichia coli* (*E-coli*) bacteria, and other peripheral equipment. The experiment consists of two steps. First, a general survey of frequencies from 8 GHz to 12 GHz was made. Second, a detailed experiment for specific frequencies selected from the first survey were further studied. Interesting frequency dependent results, such as unusually higher growing or killing rates of *E-coli* at some frequencies, were found. It is also concluded that some results are genetic, that is, the 2nd, and 3rd subcultures showed the same growing status as the 1st cultures.

Several factors may affect the biomechanisms of *E-coli*. Some of these are pH and salinity of the environment, the presence of other bacteria in competition with it,

temperature, predatory process, the presence of sunlight, and possibly irradiated electromagnetic radiation and field. However, throughout this experimental study only the frequencies of the electromagnetic radiation were changed and all other factors were kept constant. This work was concentrated on the biological effects of NEMR.

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CHAPTER 1

INTRODUCTION

One argument that is often made in discussion of the biological effects of NEMRs is between “thermal” and “nonthermal” effects. Nonthermal effects are a result of a direct interaction between the radiation and the organism. For example, light sensing of human eyes and photosynthesis of plants and thermal effects are a result of energy loss in the medium. Another example is that of microwave ovens and the microwave radar signals producing hearing phenomena which is a result of voltage generation by electrical stimulation of the cochlea for certain people.¹ There are many reports, basically statistical or epidemiological surveys about nonthermal biological effects of NEMR, and a number of researchers suggest that microwave sources cause biological effects only by thermal heating.

The first survey experimental studies provided information about the nonthermal effects of microwave for certain frequencies. After being exposed to electric and/or magnetic fields, the growing status of *E-coli* bacteria was compared to nonexposed ones for a sufficiently long time (about 8 days) in the incubator. Certain frequencies of electric or magnetic fields produced a higher concentration of bacteria in growing cultures and accelerated the growing rates of bacteria. Through the second more detailed studies near the frequencies selected from the first survey experiment, the reproducibility of biological

effects of nonionizing radiation has been found. By regrowing and investigating the 2nd, 3rd, or even 4th subcultures, it was concluded that some frequencies produced genetic effects. That is, the 2nd, 3rd, or 4th subcultures retained the same growing characteristics as the 1st culture. The fact that *E-coli* bacteria are easy to treat and have a very short division cycle (about 20 ~ 30 minutes under the best conditions), makes it simple to study genetic effects for many generations for a relatively short period of time. It may be possible to extrapolate the results and concepts of this study to human beings.

CHAPTER 2

ENVIRONMENT AND SOME REPORTED BIOLOGICAL EFFECTS OF NEMR

2.1. EMFARs In The Environment

Today, EMFARs have become common constituents of civilized society. The sources of EMFARs, especially nonionizing radiations, are the power-lines, power stations, home appliances, broadcasting antennas, military bases, ships, and airports, etc.. In the United States, there are 9,000 AM and FM radio stations, 1,000 VHF-TV and UHF-TV broadcast stations, and hundreds of military bases.² People living near the above sites are exposed to EMFARs at higher levels than other people. What effects are caused by these signals? The people living near the sources might have higher incidences of diseases, if any. The U.S. EPA measured the broadcast signal intensity at 373 locations throughout 12 large cities in the U.S. and found that approximately 1 % of the population studied or about 380,000 were potentially exposed to levels greater than $1\mu\text{W}/\text{cm}^2$, which is the suggested value in the USSR, and that at any given moment about half the U.S. population is exposed to an intensity of $0.005\ \mu\text{W}/\text{cm}^2$.³ Magnetic fields associated with 60 Hz common electrical appliances are 580 mG, 100 mG, and 44 mG, 13.8 mG for electric razor, separated electric wire, electric irons, and electric blankets, respectively, at distance of 10 cm from their sources.⁴

The Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit and the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values refer to microwave energy in the frequency range of 300 MHz to 300 GHz as follows:

“a. For exposure to continuous wave (CW) sources, the power density level shall not exceed 10 mW/cm^2 for continuous exposure, and the total exposure time shall be limited to an 8 hours workday. This power density is approximately equivalent to a free space electric field strength of 200 V/m rms and a free space magnetic field strength of 0.5 A/m rms.

b. Exposure to CW power density levels greater than 10 mW/cm^2 are permissible up to a maximum of 25 mW/cm^2 based upon an average energy density of 1 mWh/cm^2 averaged over any 0.1 hour period. For example, at 25 mW/cm^2 , the permissible exposure duration is approximately 2.4 minutes in any 0.1 hour period.

c. For repetitively pulsed microwave sources, the average field strength or power density is calculated by multiplying the peak-pulse value by the duty cycle. The duty cycle is equal to the pulse duration in seconds times the pulse repetition rate in Hertz. Exposure during an 8 hours workday shall not exceed the following values which are averaged over any 0.1 hour period:

Power Density: 10 mW/cm^2

Energy Density: 1 mWh/cm^2

Mean Squared Magnetic Field Strength: $0.25 \text{ A}^2/\text{m}^2$

Mean Squared Electric Field Strength: $40,000 \text{ V}^2/\text{m}^2$

d. Exposure is not permissible in CW or repetitively pulsed fields with an average power density in excess of 25 mW/cm^2 or approximately equivalent free space field strengths of 300 V/m or 0.75 A/m .”⁵

2.2 Epidemiologic Survey

2.2.1 Mortality From Leukemia In Workers Exposed To NEMRs

All 438,000 deaths of Washington State resident men of 20 years old or older from 1950 through 1979 were investigated according to occupational classes. Table 1 shows the mortality due to all leukemia and acute leukemia for 11 occupations with presumed exposure to electrical or magnetic fields. In 10 of 11 occupations, the proportionate mortality ratio for leukemia was elevated.⁶

Colman et al.⁷, studied the incidence of leukemia among men in the electrical occupations, basically the same as Milham’s occupational designations, in South-East England. They analyzed 125,887 tumor cases and found 17 % excess of all leukemia in electrical occupations over the standard population.

M. E. McDowall studied all deaths in males aged 15-74 in England and Wales for 1970-1972. He found consistently raised proportionate mortality ratios for the occupations of electrical engineers, telegraph radio operators, and electronic engineers. The results of his second study for 1973 also show increased relative risks for the electrical occupations, the highest risk being for telecommunications engineers.⁸

Pearce, et al.⁹, studied leukemia and agriculture occupation in New Zealand and found an excess of leukemia in the groups of electric equipment assemblers, TV/radio repairmen, and power station operators. In 1971-1983, 1,691 deaths of amateur radio operators in the states of Washington and California were investigated. Twenty-four of them died from leukemia which was about twice the expected number.¹⁰

Forty cases and one hundred sixty controls of underground coal miners, who were working under power distribution lines strung overhead, were investigated and it was found that miners who had worked more than 25 years showed a significantly higher risk for chronic leukemia, chronic lymphocytic leukemia, and myelogenous leukemia.¹¹

Flodin, et al., found that electrical technicians, computer and telephone workers, and electrical welders showed an increased risk of acute myeloid leukemia.¹²

Table 1. Leukemia Mortality in Men Occupationally Exposed to Electrical and Magnetic Fields. (Washington State White Males. 1950-1979)⁶

Occupation	Mortality					
	All Leukemia			Acute Leukemia		
	observed	expected	PMR	observed	expected	PMR
Electronic technicians	6	4	149	3	1.9	162
Radio/telegraph operators	5	4.5	111	3	1.3	239
Electricians	51	37	139	23	12.9	178
Linemen	15	9.4	159	6	3.3	182

Table 1. continued

TV/radio repairmen	5	3.2	157	4	1.4	291
Power station operators	8	3.1	259	3	1.1	282
Aluminum workers	20	10.6	189	11	4.3	258
Welder/flame workers	12	17.9	67	4	7.1	56
Motion picture projectors	4	1.7	234	1	0.9	111
Electrical engineers	7	6.1	114	2	2.1	97
Streetcar/submotormen	3	1.7	175	0	0.4	0
Total	136	99.2	137	60	36.7	163

*PMR (Proportionate mortality ratio): observed/expected $\times 100$

*An PMR of 100 means no increased or decreased risk.

*An PMR of bigger than 100 means increased risk and vice versa.

2.2.2 Electromagnetic Fields and Childhood Leukemia and Cancer

Nancy Wertheimer and Ed Leeper conducted the first study to report a relationship between power lines and cancer.¹³ They found that children who had lived within 40 m of a high-current power lines for their entire lives showed a higher cancer incidence rate than other children.

In 1992, M. Feychting and A. Ahlbom conducted a case-control study to test the magnetic fields produced by high-voltage power line for increases in cancer incidence in children. Cases and controls were selected from the people who had lived on property within 300 m of 220 and 400 kV power lines. The relative risk of childhood leukemia

was 1.5 for magnetic fields of 0.2 μT , and 3.8 for 0.3 μT . There were no elevated risks for other types of childhood cancers.¹⁴

D. A. Savitz, et al.¹⁵, conducted a study of childhood cancer and exposure to 60 Hz magnetic fields for the Denver, Colorado area. The results show that an unusual ratio of total cancer incidence was 1.4. The unusual ratios of leukemia, lymphomas, and soft tissue sarcomas were 1.9, 2.2, and 3.3 respectively.

J. P. Fulton, et al., found no relationship between leukemia and electric power line configurations¹⁶ and a separate study results of S. J. London, et al., support an association between childhood leukemia risk and wiring configuration, but not direct measurements of electric and magnetic fields.¹⁷

2.2.3 Other Electromagnetic Radiation Effects Upon Health

Abraham M. Lilienfeld investigated the Department of State employees and their descendants at the Moscow Embassy who were bombarded by microwaves.¹⁸ He concluded that the Moscow male employees had a three-fold higher risk of acquiring protozoal infections between the time of arrival at the post and the time of last observation, and both men and women in the Moscow group were found to have slightly higher frequencies of most of the common kinds of health conditions.

Robert O. Becker observed that the incidence cases of malignant cancers in the broadcasting and microwave communication relay antenna area in Syracuse, New York where power transmission lines are also concentrated are almost double the expected values.¹⁹ N. Wertheimer and E. Leeper conducted adult cancer studies and found that

high current electrical wiring configurations were located near the patient's residence. But the association was weaker than the childhood cancer cases which were investigated earlier.²⁰

In the United States the counties containing an Air Force base showed a higher cancer mortality compared to the counties without Air Force bases. This mortality is probably due to the microwave radiation from the bases.^{21,22}

Incidence risks of cancer of the working population of the electronic industry in Sweden were investigated. The results showed a higher incidences of cancers at sites of the mesopharynx, larynx, respiratory system, and skin.²³ Another Swedish study of cancer incidence in the electric power industry shows that the relative risk of cancer was higher in the group of linemen. The standardized morbidity ratios (SMR) of both skin and nervous system cancers were 1.5 for linemen compared to the average population.²⁴

A group of 951 adult white male who died from brain tumor in Maryland was studied. The people employed in electricity related occupations showed increased ratios, for example , an unusual ratio of 2.15 of primary brain tumor for men definitely exposed to electromagnetic fields as compared to those not exposed.²⁵

M. R. Spitz , et al., investigated the neuroblastoma cases in children under 15 years old in Texas.²⁶ Children with fathers employed in occupations related to the application of electromagnetic fields showed increased risk of neuroblastoma. When electronic workers' children only were considered, the risk was significantly higher.

In 1991 John Volanti, police psychology and health expert, reported that police radar speed guns, as well as other electromagnetic devices, might be linked to leukemia and lymph node cancer. Early in 1991 the Food and Drug Administration warned police officers not to use the radar gun closer than 6 inches to their bodies.²⁷

2.3 Some Reported Laboratory Experimental Results

In addition to the epidemiologic case-control studies, there are many biological experiments showing some critical effects of nonionizing radiations. M. L. Swicord et al., found that a solution containing DNA showed an increased microwave absorption coefficient compared to the solvent solution alone.²⁸ They extracted DNA from *E-coli* and irradiated it with microwave signals by using a phase fluctuation optical heterodyne spectroscope (PFLOH). They conducted the same experiments for the saline solution, Ringer's solution, and de-ionized water. Figure 1 shows the resulting attenuation coefficients for their work.²⁸

The growing status of yeast cells under millimeter microwave irradiation were observed by Keilmann and Grundler. The narrow width of the resonance, which proves the existence of a nonthermal microwave sensitivity was observed in yeast cells. Figure 2 shows the growth rate versus frequency for their experiment.²⁹

Investigators in New Delhi, India, exposed mice to 2.45 GHz microwave radiation with specific absorption rate of 1.18 W/kg. They found a new DNA sequence in brain and testis tissues.³⁰ When chromosome material is exposed to microwaves, despiralization of chromosome is observed. This aberration is unique to microwave exposure.

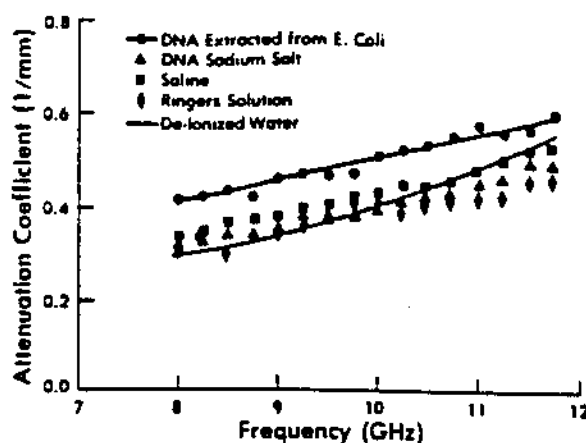


Fig. 1. Variation with frequency of the electric field attenuation coefficient of DNA, saline, Ringers solution and DNA sodium salt in a waveguide of dimensions 5.72×2.86 mm at 29°C (From Swicord and Davis²⁸, An optical method for investigating the microwave absorption characteristics of DNA and other biomolecules in solution)

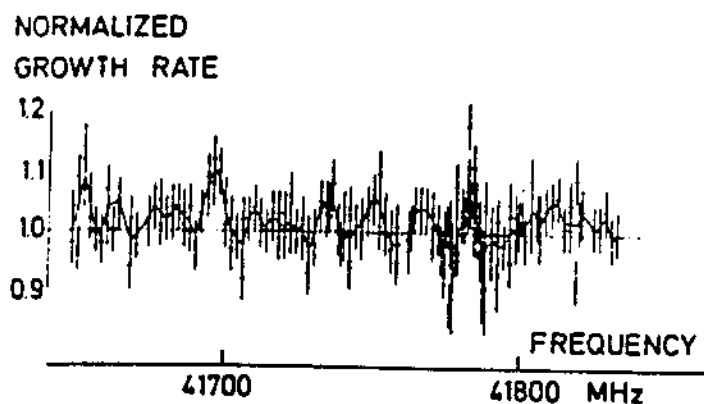


Fig. 2. Nonthermal effect of microwave irradiation on the growth rate of yeast in suspension, versus the microwave frequency. (From Keilmann and Grundler²⁹, Nonthermal resonant action of millimeter microwaves on yeast growth)

CHAPTER 3

THEORIES

Electromagnetism producing biological effects can be divided into ionizing radiation, non-ionizing radiation, and electromagnetic fields. The biological effect of ionizing radiation is relatively well understood, but the effects of non-ionizing radiation and electromagnetic field (EMF) are almost unknown, except for non-selective thermal effect. In the following sections a summary is given of some of the kinds of radiation and energy levels which may produce biological effects.

3.1 Ionizing Radiation

If a radiation has energy higher than 10 eV, then it is referred to as an ionizing radiation. Ionizing radiation can be divided into particle and electromagnetic radiation.

3.1.1 Interaction Between Radiation and Matter

The release of energy by radiation through ionization or excitation is the first physical step in a long sequence of secondary reactions which may finally lead to biological radiation effects. The primary interaction may differ both quantitatively and qualitatively for various types and energies of radiation.

3.1.1.1 Charged Particles

The energy loss formula, the stopping power, is described quantum mechanically for all heavy charged particles by Bethe's formula:

$$\frac{dE}{dx} = 4\pi NZ \frac{z^2 e^4}{mv^2} \left[\ln \left(\frac{2\gamma^2 mv^2}{\hbar \langle \omega \rangle} \right) - \frac{v^2}{c^2} \right], \quad (3.1)$$

where, N: number of bound atoms per unit volume,

Z: number of electrons per atom,

z: number of incident particles with charge e,

m: mass of the electron,

v: speed of incident particles,

e: elementary charge of the particle,

$$\gamma = \frac{1}{\sqrt{1 - \frac{v^2}{c^2}}},$$

$\hbar \langle \omega \rangle$: ionization potential of the absorbing atoms,

c: speed of light.

For electrons, because of their lower mass (and thus stronger deflection) a slightly modified formula holds:

$$\frac{dE}{dx} = 4\pi NZ \frac{z^2 e^4}{mv^2} \left[\ln \left(\frac{\gamma^{3/2} mc^2}{\sqrt{2} \hbar \langle \omega \rangle} \right) - \frac{v^2}{c^2} \right].$$

The linear energy transfer (LET) value substantially corresponds to the mass stopping power, but considers only ionizations and excitations in the vicinity of the primary track, not those of the secondary particles. The secondary particles also interact with the absorbing matter and the same energy loss process must be applied.

In addition to the direct ionization process, the production of bremsstrahlung radiation plays an important role in the energy loss of β particles in matter. A moving electron is slowed down in the field of a heavy nucleus and the energy of deceleration is emitted. The probability for this process increases strongly with particle velocity and the atomic number of the absorbing matter.^{31,32}

3.1.1.2 Ionizing Electromagnetic Radiation

The absorption of these energetic forms of electromagnetic radiation in matter is described by a simple exponential expression:

$$I_x = I_0 e^{-\sigma \rho x}. \quad (3.2)$$

Here I_0 and I_x are the radiation intensities before and after penetration of the matter layer of thickness x , ρ is the number of atoms per unit volume in the matter, and σ is the effective cross-section. The cross-section σ is composed of three components, describing three different interaction processes:

$$\sigma = \sigma_c + \sigma_{PE} + \sigma_{PR}, \quad (3.3)$$

where, σ_c : Compton scattering cross section,

σ_{PE} : photoelectric cross section,

σ_{PR} : pair production cross section.

In the photoelectric effect, a photon delivers all of its energy to a bound electron in the absorbing matter. This process prevails in the region of low energy γ rays. Its probability increases with the fifth power of the material's atomic number:

$$\sigma_{PE} \sim \frac{Z^5}{A}, \quad (3.4)$$

where, Z: atomic number of absorbing matter,

A: atomic weight of absorbing matter.

The energy balance of the photoelectric process is

$$E = h\nu - W, \quad (3.5)$$

where, E: kinetic energy of the ejected electron

W: binding energy of the ejected electron

$h\nu$: energy of the incident radiation

The Compton effect can be considered as a collision process between a γ ray and an electron, during which the photon transfers a part of its energy to the electron. The equation for this process is given by:

$$E + h\nu' = h\nu - W, \quad (3.6)$$

where, E: kinetic energy of electron ejected,

$h\nu'$: energy of the scattered photon,

$h\nu$: energy of the incident photon,

W: binding energy of the ejected electron.

This process predominates for medium energy γ rays. Its occurrence depends on the electron density of the absorber material. This electron density is given by:

$$\sigma_c \sim \frac{Z}{A}. \quad (3.7)$$

During pair formation, a photon materializes in the field of a nucleus and forms an

electron-positron pair. This process, therefore, can occur only if the photon energy corresponds to at least twice the electron rest mass (1.02 MeV). Only at much higher energies does it become the prevailing process. Its probability increases with the square of the atomic number:

$$\sigma_{PR} \sim \frac{Z^2}{A}. \quad (3.8)$$

The energy balance of the pair formation process is

$$h\nu - 1.02 \text{ MeV} = E_+ + E_-. \quad (3.9)$$

The electron and positron lose their kinetic energies through ionization and excitation. Finally, the positron recombines with an electron and disintegrates together with it under emission of two photon of 0.51 MeV each (annihilation radiation).

3.1.1.3 Noncharged Particle (Neutron)

Neutrons passing through matter do not interact with the atomic electrons because the neutrons have no electric charge. Electrons do not “feel” them. The attenuation of a neutron beam inside matter is due to a direct collision with an atomic nuclei. The neutron and matter interaction processes are elastic scattering, inelastic scattering, and neutron capture.

Elastic scattering is a collision process between a neutron and an atomic nucleus, during which total kinetic energy remains unaltered. According to the collision laws of mechanics, the energy transfer is maximum if the two colliding particles have equal masses, which is the case with the hydrogen nucleus and neutrons. The recoil protons

produced dissipate their energy to matter within a short range through ionization and excitation.

In inelastic scattering the total kinetic energy of the system is altered, i.e., some of the kinetic energy goes into the form of internal excitation energy. The nucleus relaxes within a short time by emission of a γ ray. At high neutron energies a second neutron may also be emitted. By such collision processes the neutrons lose kinetic energy until they are in thermal equilibrium with their environment. Their energy is then

$$E = \frac{1}{2} kT \quad (3.10)$$

with $k = 1.38 \times 10^{-23} J / K$ (Boltzmann constant),

T = Absolute temperature (K).

At 293 K temperature, the thermal environmental energy is $E = 0.0025 eV$.

If an atomic nucleus captures neutrons, it gains extra energy and then becomes radioactive. The extra energy will be eliminated by undergoing one or more radioactive transformations. The secondary α , β , or γ rays emitted in this process will then damage the material in the usual way.³³

3.1.2 Ionizing Radiation Effects

Biological effects of ionizing radiation can be divided into the effect on protein, nucleic acid, and membrane. Radiation damages atoms in living cells through ionization. Since biological organs consist of 70 % to 90 % water, most of the primary impact of radiation on molecules will be concentrated on water molecules. If radiation interacts

with water molecules, the collision process results in a positive water ion and a free electron, as given by:



The ejected electron travels some distance and is captured by another water molecule which results in a negative water ion, with a reaction given by:



Both the positive and negative water ions dissociate into radicals and ions, given by:



and



These OH^\bullet and H^\bullet radicals are electrically neutral but they have unsaturated chemical bonds. Therefore, they have a strong tendency to attach themselves to something, i.e., they are very reactive. The reactive radicals disturb the complicated and fundamental building block molecules of living cells such as proteins, enzymes, and nucleic acids.

The possible damages are: breaking chemical bonds, rearrangement of chemical bonds, and formation of extra bonds. Such damages disrupt the normal biochemical processes and may result in the death of living cells. The radiation may also destroy the living molecules directly. DNA breakage produces the loss of reproduction capability or leads to mutations. The physiological effects of radiation are summarized in Table 2.

Table 2. Effects of Acute Radiation Doses (From Hans C. Ohanian, Physics)

Dose	Critical organ	Effect	Mortality
0-100	Blood	Some blood cell destruction	None
100-200	Blood forming tissue	Decrease in white blood cell count	None
200-600	Blood forming tissue	Severe decrease of white blood cell count, internal hemorrhage, infection, loss of hair	0-80 % within 2 months
600-1000	Blood forming tissue	Same as 200-600 case	80-100 % within 2 months
1000-5000	Gastrointestinal effect	Diarrhea, fever, electrolyte imbalance	Nearly 100 % within 2 weeks
Above 5000	Central nervous system	Convulsions, tremor, lack of coordination, lethargy	100 % within 2 days

* Blood-forming tissue: bone marrow, lymphatic tissue

* Dose is for whole body and unit is rem.

3.2 Non-Ionizing Radiation

3.2.1 Thermal Effect

Electromagnetic radiation is completely described by four Maxwell's equations, which in lossy dielectric media are of the form:

$$\nabla \times \vec{E} = -\mu \frac{\partial \vec{H}}{\partial t} \quad (3.15)$$

$$\nabla \times \vec{H} = \sigma \vec{E} + \epsilon \frac{\partial \vec{E}}{\partial t} \quad (3.16)$$

$$\nabla \cdot \vec{E} = 0 \quad (3.17)$$

$$\nabla \cdot \vec{H} = 0. \quad (3.18)$$

\vec{E} and \vec{H} are the electric and magnetic field vectors as usual, t is the time, μ , σ , ϵ are the magnetic permeability, electric conductivity, and electric permittivity of the medium, respectively. Elementary manipulation of the above four equations yields the time-dependent electric and magnetic vector wave equations

$$\nabla(\nabla \cdot \vec{E}) - \nabla^2 \vec{E} + \mu\sigma \left(\frac{\partial \vec{E}}{\partial t} \right) + \mu\epsilon \left(\frac{\partial^2 \vec{E}}{\partial t^2} \right) = 0 \quad (3.19)$$

$$\nabla(\nabla \cdot \vec{H}) - \nabla^2 \vec{H} + \mu\sigma \left(\frac{\partial \vec{H}}{\partial t} \right) + \mu\epsilon \left(\frac{\partial^2 \vec{H}}{\partial t^2} \right) = 0. \quad (3.20)$$

Here, time dependence of \vec{E} and \vec{H} is assumed, i.e., $\vec{E} e^{-i\omega t}$ and $\vec{H} e^{-i\omega t}$. Taking the time derivatives in equation (3.19) yields the vector Helmholtz equation

$$\nabla^2 \vec{E} + (\mu\epsilon\omega^2 + i\omega\mu\sigma) \vec{E} = 0, \quad (3.21)$$

where the quantity inside the parenthesis can be defined by as k^2

$$k^2 = \mu\epsilon\omega^2 + i\omega\mu\sigma. \quad (3.22)$$

Since k is a complex quantity, which is called propagation constant, it can be written as

$$k = \alpha + i\beta, \quad (3.23)$$

where α and β , the real and imaginary parts of the propagation constant, are obtained by combining equations (3.22) and (3.23) as,

$$\alpha = \omega \sqrt{\frac{\mu\epsilon}{2}} \left[\sqrt{1 + \left(\frac{\sigma}{\omega\epsilon} \right)^2} + 1 \right]^{1/2} \quad (3.24)$$

and

$$\beta = \omega \sqrt{\frac{\mu\epsilon}{2}} \left[\sqrt{1 + \left(\frac{\sigma}{\omega\epsilon} \right)^2} - 1 \right]^{1/2}. \quad (3.25)$$

By considering a linearly-polarized plane wave propagating in the z-direction, with amplitude E_0 along the x-direction, and H_0 along the y-direction, the electric and magnetic vectors can be written as,

$$\vec{E} = \hat{i} E_0 e^{i(kz - \omega t)} \quad (3.26)$$

and

$$\vec{H} = \hat{j} H_0 e^{i(kz - \omega t)}. \quad (3.27)$$

Substituting the expression of k into the eq. (3.26) and (3.27) the electric and magnetic field vectors may be written as

$$\vec{E} = \hat{i} E_0 e^{-\beta z} e^{i(\alpha z - \omega t)} \quad (3.28)$$

and

$$\vec{H} = \hat{j} H_0 e^{-\beta z} e^{i(\alpha z - \omega t)}. \quad (3.29)$$

The term $e^{-\beta z}$ indicates an exponential decay of the field energy in the medium. The imaginary part of the propagation constant contributes to this attenuation. Thus, according to eq. (3.25) the attenuation depends on the value of μ , ϵ , and σ of the medium.

Introducing λ as the wavelength, the following simple relation is obtained

$$\lambda = \frac{2\pi}{\alpha}. \quad (3.30)$$

The wavelength depends on the electrical properties μ , ϵ , and σ of the medium. It is apparent then that the wavelength differs in different dielectric media.

The dimensionless dielectric constant (or dielectric coefficient), K_ϵ , of a homogeneous dielectric media is defined as

$$K_\epsilon = \frac{\epsilon}{\epsilon_0}, \quad (3.31)$$

where ϵ_0 is the permittivity of free space. The relative permeability, K_m , of a homogeneous medium is also defined as

$$K_m = \frac{\mu}{\mu_0}. \quad (3.32)$$

For biological tissue throughout the RF range, as well as for air, μ and μ_0 are essentially equal, so that $K_m = 1$. To introduce the loss tangent, $\tan\delta$, equation (3.22) may be rewritten as

$$k^2 = \mu\epsilon\omega^2 \left(1 + i \frac{\sigma}{\omega\epsilon} \right). \quad (3.33)$$

The quantity $(\sigma/\omega\epsilon)$ is termed the loss tangent and is given by

$$\tan \delta = \frac{\sigma}{\omega\epsilon} = \frac{\sigma}{\omega K_e \epsilon_0}. \quad (3.34)$$

The loss tangent is the ratio of the conduction currents to displacement currents. If $\tan\delta=0$ for a medium, then the imaginary part of the propagation constant is zero and the wave passes through the medium unattenuated and this medium is referred to as a perfect dielectric.

The Poynting vector, used to discuss power absorption, is a flow of power through a surface of unit area. It is the energy crossing unit area in unit time. For complex time harmonic field quantities, the Poynting vector is the time average of the product of the real parts of \vec{E} and \vec{H} . Designating the time average Poynting vector as \vec{S} gives:

$$\vec{S} = \left\langle \text{Re}(\vec{E}) \times \text{Re}(\vec{H}) \right\rangle. \quad (3.35)$$

The physical quantity associated with a complex time periodic function is the time average of its real part. An equivalent form of equation (3.35) is

$$\vec{S} = \frac{1}{2} \text{Re} \left(\vec{E} \times \vec{H}^* \right). \quad (3.36)$$

In order to derive an expression for the absorbed power per unit volume in a biological medium, perform first the scalar multiplication of equation (3.15) with \vec{H}^* and

the complex conjugate of equation (3.16) with \vec{E} and subtract the result. Then, use the vector identity for the divergence of the cross product of two vectors results in the expression

$$\nabla \cdot (\vec{E} \times \vec{H}^*) = -\vec{E} \cdot \vec{J}^* - \left(\vec{E} \cdot \frac{\partial \vec{D}^*}{\partial t} + \vec{H}^* \cdot \frac{\partial \vec{B}}{\partial t} \right), \quad (3.37)$$

where \vec{J} is the conduction current, $\sigma \vec{E}$. Using the relationships $\vec{J} = \sigma \vec{E}$, $\vec{D} = \epsilon \vec{E}$, $\vec{B} = \mu \vec{H}$, $\vec{E} = \vec{E}_0 e^{-i\omega t}$ and $\vec{H} = \vec{H}_0 e^{-i\omega t}$ equation (3.37) becomes

$$\nabla \cdot (\vec{E} \times \vec{H}^*) = -\sigma |\vec{E}|^2 + i\omega \left(\mu |\vec{H}|^2 - \epsilon |\vec{E}|^2 \right). \quad (3.38)$$

The mean Poynting vector is given by equation (3.36) and Poynting's theorem states that the divergence of the mean Poynting vector measures the energy transformed per unit volume per second into heat. Applying this theorem to equation (3.38) yields

$$\frac{1}{2} \text{Re} \nabla \cdot \vec{E} \times \vec{H}^* = -\frac{1}{2} \sigma |\vec{E}|^2. \quad (3.39)$$

Thus, the power deposited per unit volume of biological medium by the electromagnetic field is

$$p = \frac{1}{2} \sigma |\vec{E}|^2. \quad (3.40)$$

Another mathematical expression may be derived from equation (3.37) as

$$p = \frac{1}{2} \omega \epsilon_0 \epsilon'' |\vec{E}|^2. \quad (3.41)$$

Here ϵ'' is the imaginary part of a complex permittivity ϵ^* defined by

$$\epsilon^* = \epsilon_0 \left(K_e - i \frac{\sigma}{\omega \epsilon_0} \right) = \epsilon_0 (\epsilon' - i \epsilon''). \quad (3.42)$$

Biological molecules, cells, organs, and tissues may be destroyed by the heat produced by the above power.^{34,35,36}

3.2.2 Nonthermal Effects

In this section the edge effects of the perturbed (severed semi-infinite double helix) atomic lattice vibrations of a dynamical model of DNA homopolymer B poly(dG) · poly(dC) is discussed. This work was primarily done by Putnam et al..^{39,40,41} Also, the theory of lattice vibrations was studied by A. A. Maradudin, E. B. Wilson et al..^{42,43,44,45}

The position of the l^{th} unit cell in the double helix is given by the vector $\mathbf{x}(l)$ and the position of the r^{th} atom in a given unit cell measured from an origin in the cell is given by the vector $\mathbf{x}(\kappa)$, $\kappa = 1, 2, \dots, r$, where r is the number of atoms in a cell.

Thus, the position of the κ^{th} atom in the l^{th} unit cell is given by

$$\mathbf{x}(l\kappa) = \mathbf{x}(l) + \mathbf{x}(\kappa). \quad (3.43)$$

The displacement of the atom ($l\kappa$) from its equilibrium position will be denoted by $\mathbf{u}(l\kappa)$. Here, it is assumed that all coordinates are mass weighted Cartesian (MWC) coordinates. The potential energy in the harmonic approximation in terms of a set of internal coordinates \mathbf{r} is conveniently written as following matrix notation

$$2V = \mathbf{r}\Phi\mathbf{r}, \quad (3.44)$$

where, the components of the atomic force constant matrix Φ are given by

$$\Phi_{ij} = \left(\frac{\partial^2 V}{\partial r_i \partial r_j} \right) \quad (3.45)$$

and the internal coordinates \mathbf{r} are given in terms of the mass weighted Cartesian coordinates \mathbf{u} by the matrix relations $\mathbf{r} = \mathbf{B}\mathbf{u}$, where \mathbf{B} is the transformation matrix.⁴⁶

The potential energy now can be rewritten in terms of MWC coordinates as

$$2V = \mathbf{u}\mathbf{B}\Phi\mathbf{B}\mathbf{u}. \quad (3.46)$$

The kinetic energy also can be written in terms of MWC coordinates as

$$2T = \dot{\mathbf{u}}^2. \quad (3.47)$$

By applying $\frac{d}{dt} \frac{\partial T}{\partial \dot{\mathbf{u}}} + \frac{\partial V}{\partial \mathbf{u}} = 0$, the following equation of motion can be obtained

$$\ddot{\mathbf{u}} + \mathbf{B}\Phi\mathbf{B}\mathbf{u} = \mathbf{0}. \quad (3.48)$$

By assuming a harmonic time dependence of the coordinates, i.e., $u = ue^{i\omega t}$ and setting $\mathbf{B}\Phi\mathbf{B} = \mathbf{F}$, an eigenvalue equation for a perfect long double helical chain, which can be applied to practically any vibration of crystals, can be written as:

$$(\mathbf{F} - \omega^2 \mathbf{I})\mathbf{u} = \mathbf{0}. \quad (3.49)$$

Here, \mathbf{F} is the mass weighted Cartesian potential energy matrix within the harmonic approximation for an infinite perfect helix and can be expressed in terms of the internal force constant Φ as $F_{ij} = \sum_{n'} B_n \Phi_{nn'} B_{r'j}$. The symbol ω is the associated harmonic angular frequency of vibration. The infinite dimensional matrix \mathbf{F} can be factored into smaller

matrices $\mathbf{F}(\theta)$. If the eigenvectors of $\mathbf{F}(\theta)$ are written as $\mathbf{u}_i^j(\theta)$, with the i^{th} coordinate for the j^{th} eigenvector in a given unit cell, then the n^{th} one vibrates, in the same manner, with an amplitude $\mathbf{u}_i^j(\theta)e^{in\theta}$ as given by Bloch's theorem. The associated squared angular frequencies of vibration are denoted by $\omega_j^2(\theta)$.

The orthonormal eigenvectors of eq. (3.49) for the N unit cell can be written as

$$u_{ii}(\theta, j) = \frac{u_i^j(\theta)e^{in\theta}}{N^{1/2}}. \quad (3.50)$$

Since the eigenvectors given by eq. (3.50) satisfy the orthonormality and closure relation, the Green function $\mathbf{g} = (\omega^2 \mathbf{I} - \mathbf{F})^{-1}$ can be expressed in terms of eigenvectors as follows:

$$g_{ii'}(ll', \omega^2) = \frac{1}{\pi} \sum_j \int_0^\pi \frac{\text{Re}[u_i^j(\theta)u_{i'}^{j*}(\theta)e^{i\theta(l-l')}]d\theta}{\omega^2 - \omega_j^2(\theta)}. \quad (3.51)$$

The eigenvalue equation for the perturbed, cut, double helix may be written as

$$(\mathbf{F} - \omega^2 \mathbf{I} + \mathbf{C})\mathbf{u} = 0. \quad (3.52)$$

Here, the perturbation matrix \mathbf{C} has non-vanishing elements only if the lattice sites are directly perturbed by the cut (see Figure 3). That is, the non-zero elements of the perturbation matrix \mathbf{C} are the force constants which connect units (-1) and (0) and are expressed by $C_{ij} = -\sum B_{ii}^* \sigma_{ii'} B_{i'j}$, where the $\sigma_{ii'}$'s are the internal force constants connecting units (-1) and (0). Since \mathbf{F} is the infinitely long perfect helix force constant, the result of $(\mathbf{F} + \mathbf{C})$ is the same as to cut the position between units (-1) and (0).

With the use of the Green's function \mathbf{g} , eq. (3.52) can be expressed as

$$\mathbf{u} = \mathbf{gCu}. \quad (3.53)$$

The matrices \mathbf{u} , \mathbf{g} , and \mathbf{C} can be written in the partitioned form

$$\mathbf{u} = \begin{pmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{pmatrix}, \quad \mathbf{g} = \begin{pmatrix} \mathbf{a} & \mathbf{g}_{12} \\ \mathbf{g}_{21} & \mathbf{g}_{22} \end{pmatrix}, \quad \mathbf{C} = \begin{pmatrix} \mathbf{c} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{pmatrix}. \quad (3.54)$$

Here \mathbf{u}_1 is the coordinates directly affected by the cut, \mathbf{u}_2 is the remaining one, also \mathbf{a} and \mathbf{c} are elements directly affected by the cut. Upon substituting eqs. (3.54) into (3.53), the following two equations are obtained

$$\mathbf{u}_1 = \mathbf{acu}_1 \quad (3.55)$$

$$\mathbf{u}_2 = \mathbf{g}_{21}\mathbf{cu}_2. \quad (3.56)$$

Eq. (3.55) has non-trivial solutions when the determinant of the coefficients vanishes,

$$\Delta(\omega^2) = |\mathbf{I} - \mathbf{ac}| = 0. \quad (3.57)$$

The roots of eq. (3.55) are the frequencies of the perturbed normal modes of the semi-infinite double helix. The atomic lattice displacement vector X may be expressed as a combination of the eigenvectors $u(\theta, j)$ ⁴⁷

$$X = \sum_{\theta, j} u(\theta, j) Q(\theta, j) = \sum_{\theta, j} \frac{1}{N^{1/2}} u_j^j(\theta) e^{i\theta} Q(\theta, j). \quad (3.58)$$

Here $Q(\theta, j)$ are the normal coordinates and can be expressed in terms of annihilation and creation operators within the harmonic approximation as

$$Q(\theta, j) = \left[\frac{\hbar}{2\omega(\theta, j)} \right]^{1/2} [a_j(\theta) + a_j^+(-\theta)]. \quad (3.59)$$

By substitution of the above equation and using the relation $u(-\theta, j) = u^*(\theta, j)$, eq. (3.58) may be expressed

$$X = \sum_{\theta, j} \left[\frac{\hbar}{2\omega(\theta, j)} \right]^{1/2} \left[u(\theta, j)a_j(\theta) + u^*(\theta, j)a_j^*(\theta) \right]. \quad (3.60)$$

The average squared displacement is given by⁴⁸

$$\langle X^2 \rangle = \hbar \sum_{\theta, j} [2\omega(\theta, j)]^{-1} [2n(\theta, j) + 1] u^2(\theta, j), \quad (3.61)$$

where, $n(\theta, j) = \frac{1}{e^{\hbar\omega(\theta, j)/kT} - 1}$.

For a dynamical model of a semi-infinite length of the DNA homopolymer B poly (dG)·poly (dC), Putnam defined a melting coordinate as “an average over the three linking hydrogen bond stretches in a base pair unit cell”. Thus, a resonant melting mode can be obtained when the two conditions are met that the resonant frequencies are the roots of eq. (3.57) and at those frequencies the amplitudes X^2 are large are simultaneously satisfied. In the frequency range from 8 GHz to 12 GHz Putnam calculated two resonance frequencies,⁴⁰ 8.7 GHz and 10.5 GHz for poly (dG)·poly (dC), and Prohofsky calculated 8.4 GHz for poly (dA)·poly (dT).⁴⁹ These postulating of three frequencies stimulated and encouraged us to conduct experimental studies of the nonthermal effects of non-ionizing electromagnetic radiations in these ranges of frequencies.

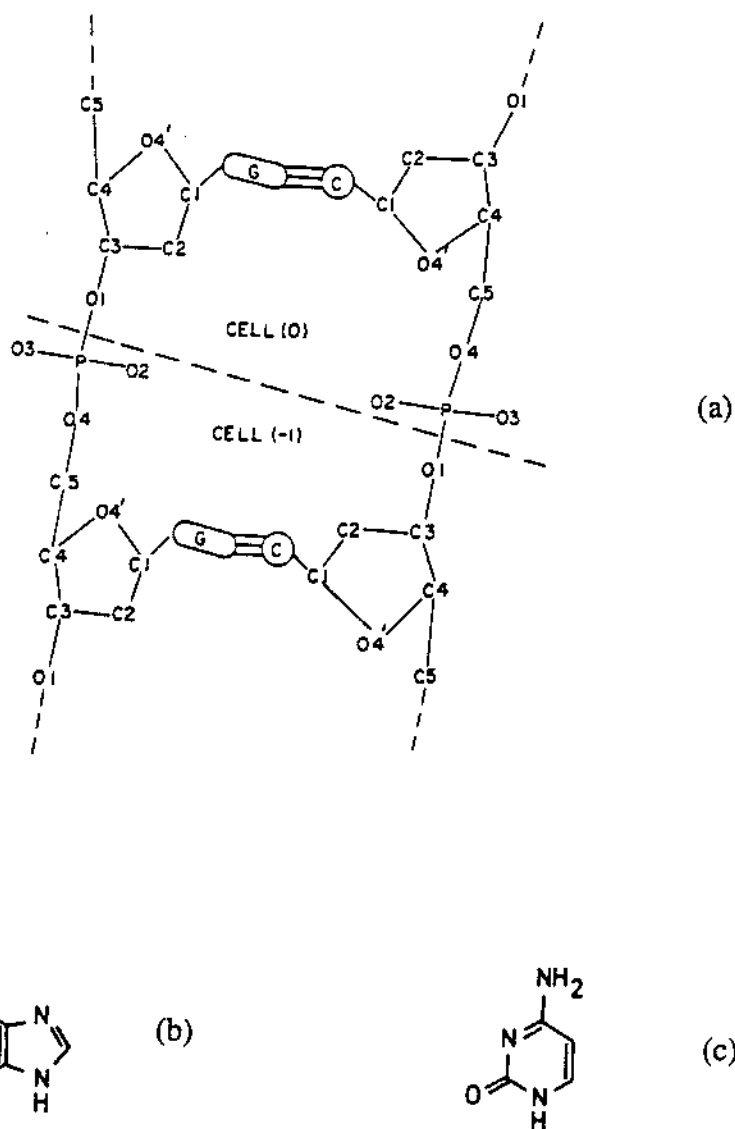


Figure 3. (a). Portion of two unit cells of poly (dG)-poly (dC). The dashed line indicates the position of the cut. The phosphodiester bonds cut are those cleaved by the restriction endonuclease enzymes. (Reproduced by the permission of original author, E. W. Prohofsky) (b). Guanine. (c). Cytosine.

CHAPTER 4

EXPERIMENTAL DETAILS

4.1 First Survey Experiments

During the experiment, *E-coli* DH5 α bacteria were used because the bacterium divides every 20 to 30 minutes in the best growing conditions, every hour in the minimum nutrient solution, and it is one of the safest bacteria to work with. This quick binary “fission” process allows significant data to be recorded over a short period of time. *E-coli* is a prokaryote, which means its cells lack a true nucleus and its cell walls do not contain cellulose.

By using a microwave signal generator and a resonant cavity, *E-coli* bacteria, placed in small pipettes, were irradiated over the range of frequencies 8 GHz to 12 GHz. A signal irradiated the samples for 4 hours each time and the frequency was increased in suitable steps (0.1 GHz) over the desired frequency range. At the same time, one nonirradiated pipette of bacteria was exposed to room temperature for the purpose of comparison. After finishing the radiation treatment, the bacteria were distributed into three culture tubes with minimum nutrient solution. The three tubes were put in incubator which was set at a temperature of 37° C and checked everyday, visually.

The information of the minimum nutrient medium for 1 liter is as follows:

10.5 g of K_2HPO_4 (Dibasic)

4.5 g of KH_2PO_4 (Monobasic)

1.0 g of $(NH_4)_2SO_4$ (Ammonium sulfate)

0.5 g of Na_3 Citrate (Trisodium citrate)

15 g of Agar

1 ml 1M $MgSO_4$

1 ml 1000x B1 (Thiamine)

10 ml 20 % Dextrose of .2 % carbon source

1 liter of distilled deionized water.

A block diagram of the microwave cavity spectrometer used is given in Figure 4. A signal frequency was generated by an Alfred Sweep Oscillator Model 650 and an HP 8555A Spectrum Analyzer was used to monitor the signal. The generated signal was guided by the waveguide to the cylindrical cavity. From the generated signal, the electric field and magnetic field were distributed by adopting TM_{010} cavity mode signal. That is, for a TM_{010} mode in a cylindrical cavity the electric field is the maximum at the center axis of the cylindrical cavity and minimum around the wall. The magnetic field is distributed in an orthogonal way to the electric field.

Due to the simplified setup of the equipment, the frequency accuracy was relatively low in the preliminary experiment. This inaccuracy was removed in the detailed experiment carried out later by using a frequency locking system. The measured Q factor of the resonant cavity is 185 and the output power of the Alfred Sweep Oscillator Model 650 is 50 mW.

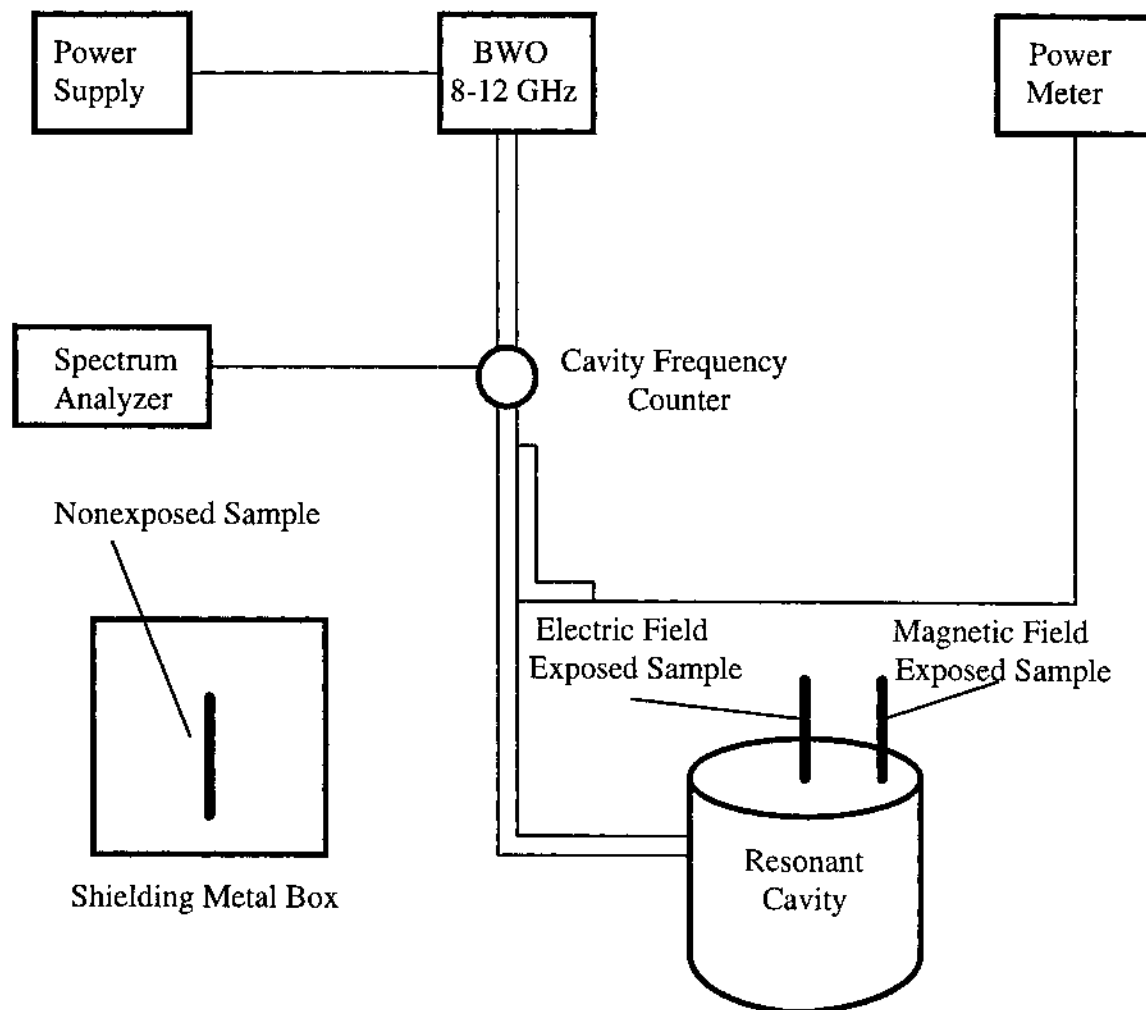


Figure 4. A block diagram of the experimental apparatus used to conduct the first survey research

4.2. Results and Discussion of The First Survey Experiments

The visual observation results of the growing status for electric, magnetic radiation exposed and nonexposed normal *E-coli* bacteria is shown in the Table 2. The colors of the higher concentration cultures were more milky than the lower concentration ones.

Table 3. Visual Observation Results of Growing Status of *E-coli*

Frequency	Result	Frequency	Result	Frequency	Result
8.0 Elec.	No effect	9.4 Elec.	No effect	10.8 Elec.	No effect
8.0 Mag.	No effect	9.4 Mag.	No effect	10.8 Mag.	No effect
8.1 Elec.	No effect	9.5 Elec.	No effect	10.9 Elec.	No effect
8.1 Mag.	No effect	9.5 Mag.	No effect	10.9 Mag.	No effect
8.2 Elec.	No effect	9.6 Elec.	No effect	11.0 Elec.	No effect
8.2 Mag.	No effect	9.6 Mag.	No effect	11.0 Mag.	No effect
8.3 Elec.	Higher	9.7 Elec.	No effect	11.1 Elec.	No effect
8.3 Mag.	Highest	9.7 Mag.	No effect	11.1 Mag.	No effect
8.4 Elec.	No effect	9.8 Elec.	Higher	11.2 Elec.	No effect
8.4 Mag.	No effect	9.8 Mag.	No effect	11.2 Mag.	No effect
8.5 Elec.	No effect	9.9 Elec.	Higher	11.3 Elec.	No effect
8.5 Mag.	No effect	9.9 Mag.	No effect	11.3 Mag.	No effect
8.6 Elec.	No effect	10.0 Elec.	No effect	11.4 Elec.	No effect
8.6 Mag.	No effect	10.0 Mag.	No effect	11.4 Mag.	No effect

Table 3. continued

8.7 Elec.	Faster	10.1 Elec.	No effect	11.5 Elec.	No effect
8.7 Mag.	No effect	10.1 Mag.	No effect	11.5 Mag.	No effect
8.8 Elec.	No effect	10.2 Elec.	Higher	11.6 Elec.	No effect
8.8 Mag.	No effect	10.2 Mag.	No effect	11.6 Mag.	No effect
8.9 Elec.	No effect	10.3 Elec.	No effect	11.7 Elec.	No effect
8.9 Mag.	No effect	10.3 Mag.	No effect	11.7 Mag.	No effect
9.0 Elec.	Higher	10.4 Elec.	No effect	11.8 Elec.	No effect
9.0 Mag.	Higher	10.4 Mag.	No effect	11.8 Mag.	No effect
9.1 Elec.	No effect	10.5 Elec.	No effect	11.9 Elec.	No effect
9.1 Mag.	No effect	10.5 Mag.	No effect	11.9 Mag.	No effect
9.2 Elec.	Higher	10.6 Elec.	No effect	12.0 Elec.	No effect
9.2 Mag.	Highest	10.6 Mag.	No effect	12.0 Mag.	No effect
9.3 Elec.	No effect	10.7 Elec.	No effect	12.1 Elec.	No effect
9.3 Mag.	No effect	10.7 Mag.	No effect	12.1 Mag.	No effect

Higher: Higher culture concentration among the three culture

Highest: Highest culture concentration among the three cultures

Faster: Culture reached the limiting concentration much earlier than others

All frequencies are in GHz.

Clear and significant frequency dependent results were observed at frequencies near 8.3 GHz, 8.7 GHz, 9.0 GHz, 9.2 GHz, 9.8 GHz, 9.9 GHz, and 10.2 GHz. The

electric and magnetic radiation treated bacteria were observed to grow faster or were observed to be at a higher concentration when compared to the nonexposed bacteria.

More specifically, at frequencies 8.3 GHz, and 9.2 GHz, the bacteria exposed to magnetic radiation were grown to the highest concentration and the ones exposed to electric radiation were grown to a higher concentration than the nonexposed ones. At a frequency of 8.7 GHz the three cultures were growing at a concentration which were about the same, but the bacteria exposed to electric radiation reached the limiting concentration much earlier. It usually takes 3 days for *E-coli* bacteria to reach the saturation phase, but the bacteria exposed to 8.7 GHz electric radiation reached this phase in one day. This demonstrated that the electric radiation accelerated the growth. At a frequency of 9.0 GHz, both cultures exposed to electric and magnetic radiations were grown to higher concentration. At frequencies of 9.8 GHz, 9.9 GHz, and 10.2 GHz, the cultures exposed to only the electric radiations grew to a higher concentration.

In this experiment, the nonexposed samples were not heated to the temperatures equivalent to the irradiated samples but were exposed at room temperature condition, so from the thermal point of view, their conditions were not the same as the samples exposed to the radiations. It is still, however, possible to conclude that there are certain nonthermal biological effects, so called window effects. If the thermal effect is the only effect, then every radiation exposed culture should have the same result. Actually, only certain selected frequencies produced some interesting results. Furthermore, the nonexposed cultures didn't reach the concentrations of abnormally grown cultures, which

were exposed to MENR, for sufficiently long time (about 8 days). From this fact, it may be concluded that the MENR acted as an initiator of biological effects instead of acting as a promoter.

There are many known interactions between NEMR and bioorganisms, for example, thermal effects, electromagnetic multipole moments exerted on the molecules, etc. These interactions are nonselective, so they cannot explain the window effect.

To explain the window effect reasonably, a vibrational model of DNA was suggested. E. W. Prohofsky, et al., calculated the resonance frequencies of a semi-infinite DNA chain model and predicted many low frequency microwave regions of interaction. Some of the frequencies, in the 8 GHz - 12 GHz region, are 8.4 GHz, 8.7 GHz, and 10.5 GHz.^{37,38} If the applied frequencies are matched to the resonance frequencies and the atomic vibrational displacement amplitudes are big enough, then some chemical bondings can be broken in the same way that led to the fall of the Tacoma bridge or strong vibration of cars at low critical engine RPM. In the future, the spectral frequency of DNA molecules can be studied by using the molecular spectroscopy technique, which is the major specialization of this laboratory. The molecular spectroscopy technique may be the only method to answer the question of the window effects satisfactorily. The observed biological effects can be either permanent or hold only for short term. To find these effects, samples can be taken from the saturated cultures which were exposed to NEMR, and then these cultured again in a new nutrient solution. These processes can be repeated

several times and will prevent the bacteria from dying, due to their own biochemical waste.

The nonreproducibility of the experimental results reported by some researchers can be easily explained. First, the biological samples used might have different growing background conditions. Samples with different biological history may produce different results. Second, the frequency dependent biological experiments may need to be very accurate and sophisticated equipment used. All experimental processes may not be the same. Thus, the nonreproducibility is somewhat expected. To reduce or eliminate the nonreproducibility, we have used a very accurate frequency locking system which locks the frequency to as low as 10 KHz error.

4.3 Second Detailed Experiments

During this step of the study, the experiment was done for the frequencies selected (8.3, 8.7, 9.0, 9.2, 9.8, 9.9, and 10.2 GHz) from the first survey experiment. The frequencies were swept from 5 MHz below to 5 MHz above in 1 MHz steps around the selected frequencies.

To determine the reproducibility and find out the precise window effect frequencies, a highly stable frequency locking system was used. A block diagram of the frequency locking system is given in Figure 5. A signal frequency is generated by a Varian Backward Oscillator (BWO) which produces an output power of 50 mW and the signal is mixed with the standard frequency generated by a Rold Model 900B Sweep Signal Generator. The Phase Sensitive Detector (PSD) compares the beat signal with

another reference signal generated by a Tektronix Type 190A Constant-Amplitude Signal Generator and produces a dc voltage that is phase and amplitude dependent on the beat frequency between the reference oscillator and the controlled oscillator. The dc voltage is returned to the BWO and controls the output frequency in the standard way.

During the early part of the experiment the culture growing conditions were monitored by using a ESECO Speedmaster Universal Densitometer which measures the transparency (or opacity) of samples. Later a more accurate PERKINS-ELMER Lambda 3A UV/VIS spectrophotometer was used. The growing conditions were quantified and plotted. If some cultures show unusual growing condition, then the secondary samples are taken from the same matured cultures and recultured. The secondary samples, however, were not simply taken from the matured cultures by volume, since the same amount of volume should contain different numbers of bacteria if the culture's growing conditions are not the same. To get the secondary samples containing the same or a very close number of bacteria, different amount of samples were taken and diluted in the nutrient solutions to produce equal transparencies so that the cultures have the same initial bacteria concentrations. The second recultured samples were not the directly irradiated ones, but many generations descendants of the first radiation exposed *E-coli*. Thus, if the recultured samples show the same unusual effects as the first culture, the radiation effects may be concluded as genetic, i.e., the nonionizing radiations interact with DNA instead of (or along with) proteins, membranes, and any other biological molecules. Furthermore, by mapping the nonthermal biological effects to the atoms having the corresponding

resonant frequencies, the genetic roles of DNA constituent atoms or a DNA map may be produced.

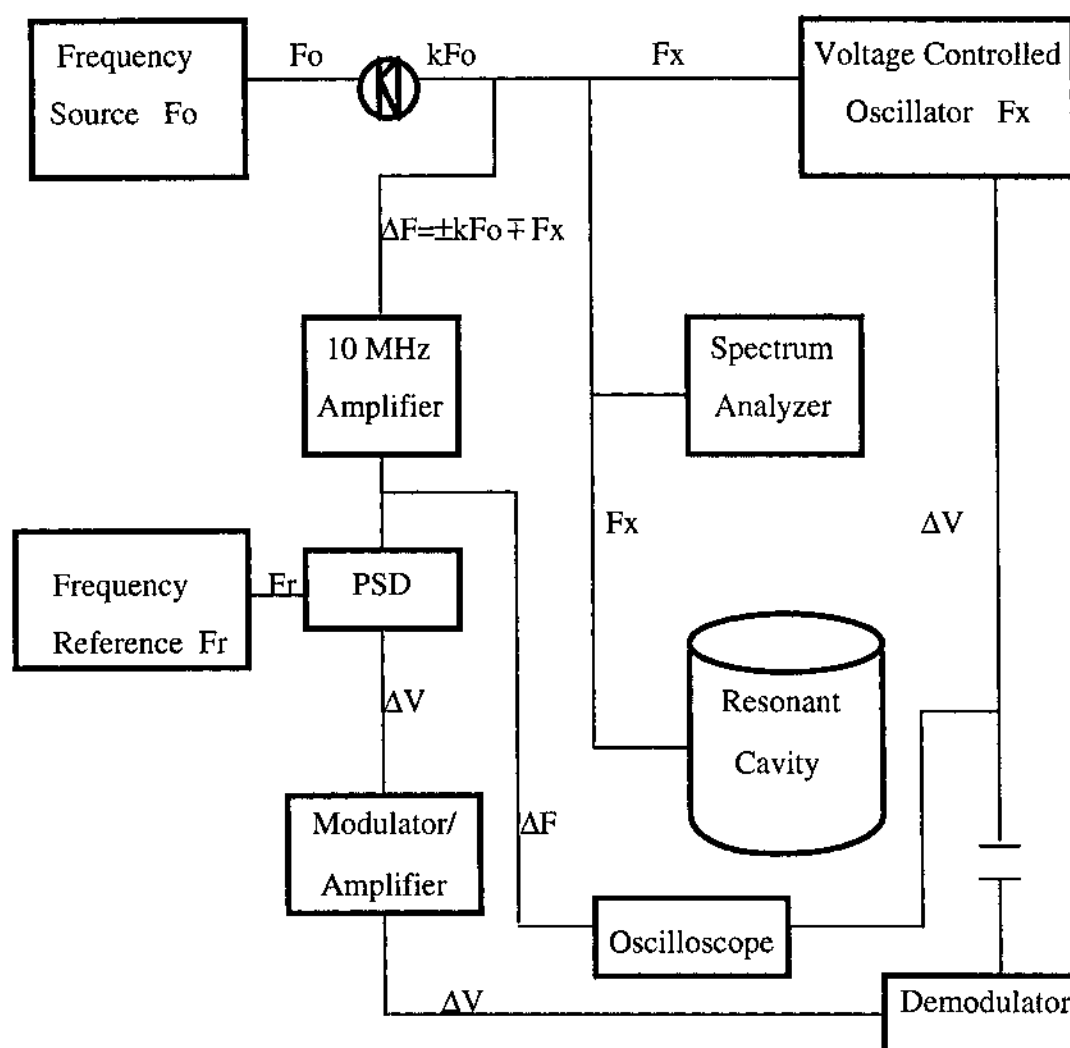


Figure 5. A block diagram of the frequency locking system and microwave source used to perturb the sample

4.4 Results and Discussion of The Detailed Experiments

The frequencies selected from the first survey experiments and neighbors of them have been thoroughly investigated. A total number of 77 frequencies was studied. The results of this detailed experiment generally coincided with the first survey experimental results. For some frequencies, the two results are different. That is, around some frequencies the second experiment showed a result that was not discovered for the first experiment or the second experiment did not show the same result obtained in the first experiment. But, it is too hasty to conclude that the two experimental results produced by the same laboratory show nonreproducibility. It must be remembered that the accuracies of the two experiments are different and the number of frequencies covered by both experiments is at most 117 frequencies in the range of 8 GHz to 12 GHz. So the frequencies covered by the two experiments are similar but may not be the same. The nonreproducibility of such experiment is perhaps due to the nonreproducibility of the experimental conditions such as frequencies and initial bacteria concentration, etc.. Thus, from the two experimental results, it may be concluded that there may still be many theoretically and experimentally undiscovered frequencies. These undiscovered frequencies, if any, can be again confirmed by the techniques of molecular spectroscopy .

The summarization of frequencies and effects are as follows:

- a. 8300 MHz: The electric field irradiated sample reached a higher concentration and the subcultures showed the same effects (genetic).

b. 8700 MHz: The electric field irradiated sample grew faster and the subcultures showed the same effects (genetic).

c. 8997 MHz: The electric field irradiated sample reached a higher concentration and the subcultures showed the same effects (genetic).

The magnetic field irradiated sample reached a higher concentration for the second subculture.

d. 8999 MHz: Both electric and magnetic fields irradiated samples reached a higher concentrations, but the subcultures did not show the same effects (nongenetic, temporary).

e. 9798 MHz: The electric field irradiated samples reached a higher concentration and the subcultures showed the same effects (genetic).

f. 9799 MHz: The magnetic field irradiated sample showed clear transparency, so it is concluded that the magnetic field killed bacteria.

g. 9898 MHz: The magnetic field irradiated culture reached to a higher concentration, but the second subculture showed different results (nongenetic temporary).

h. 9902 MHz: The electric field irradiated sample reached a higher concentration, but the subcultures did not show the same effects (nongenetic temporary).

The growing results of the cultures irradiated by the above frequencies are shown in the following figures.

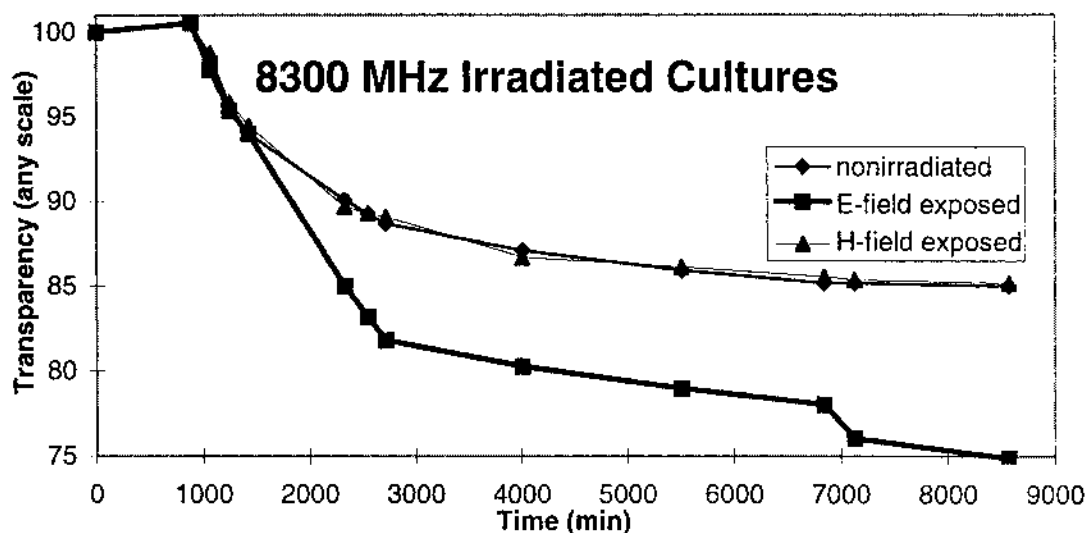


Figure 6. The growing results of 8,300 MHz irradiated cultures. The transparency of the E-field irradiated culture is lower than the nonexposed and H-field irradiated ones.

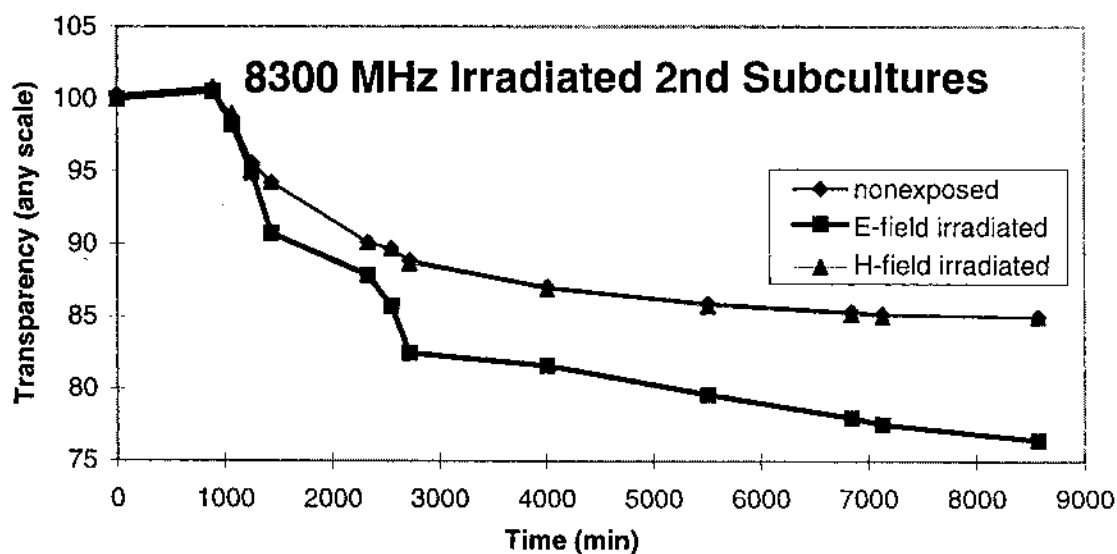


Figure 7. The growing results of 8,300 MHz irradiated second subcultures, which are the descendents of the first irradiated ones. The E-field irradiated culture still shows a lower transparency (possibly a genetic effect of the nonionizing radiation).

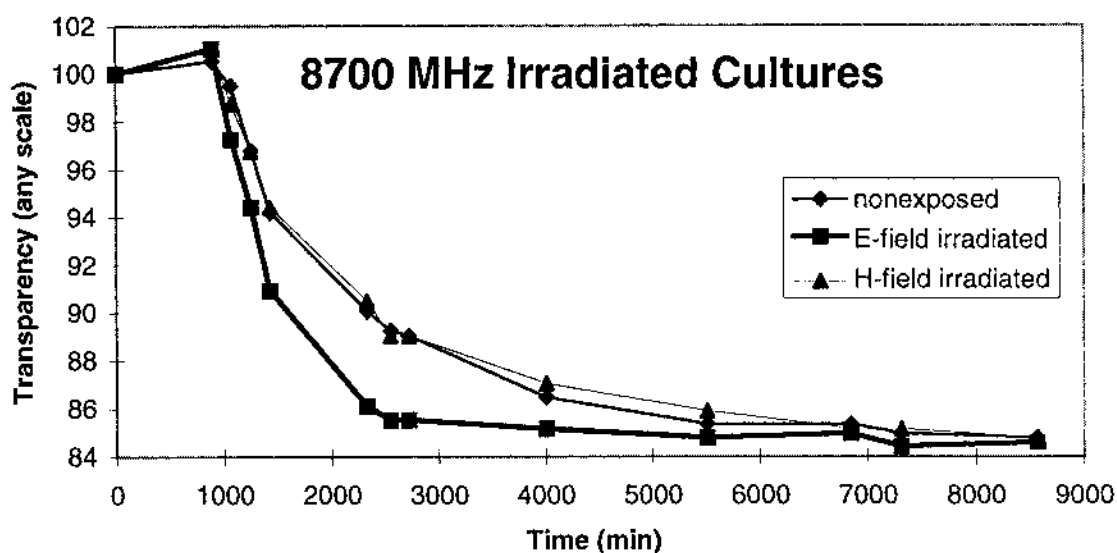


Figure 8. The growing results of 8,700 MHz irradiated cultures. The E-field irradiated culture reached a limiting transparency much earlier than the other ones.

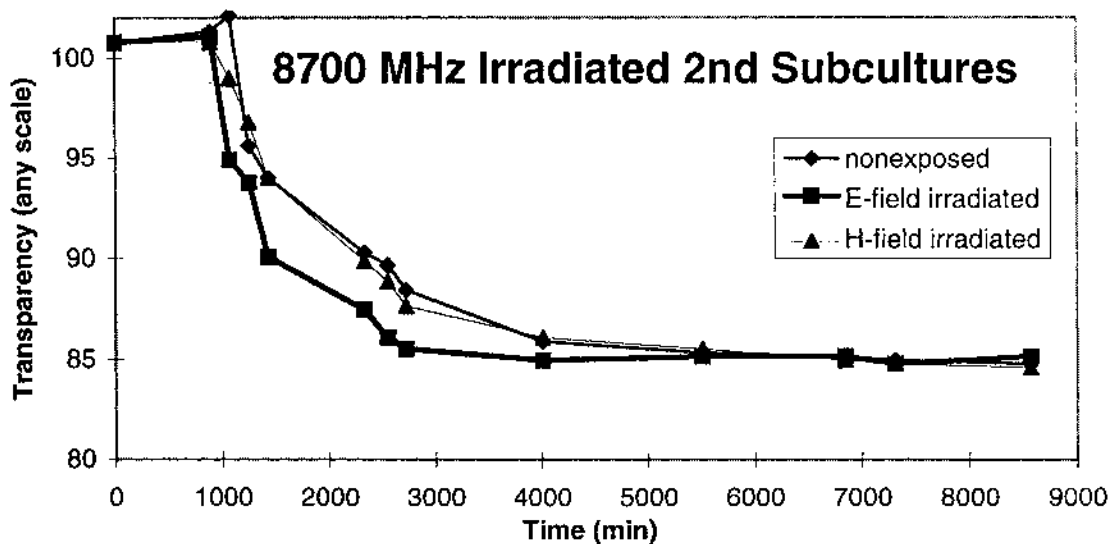


Figure 9. The growing results of 8,700 MHz irradiated second subcultures which are the descendents of the first irradiated ones. The E-field irradiated culture, again, reached a lower limiting transparency earlier (possibly a genetic effect of the nonionizing radiation).

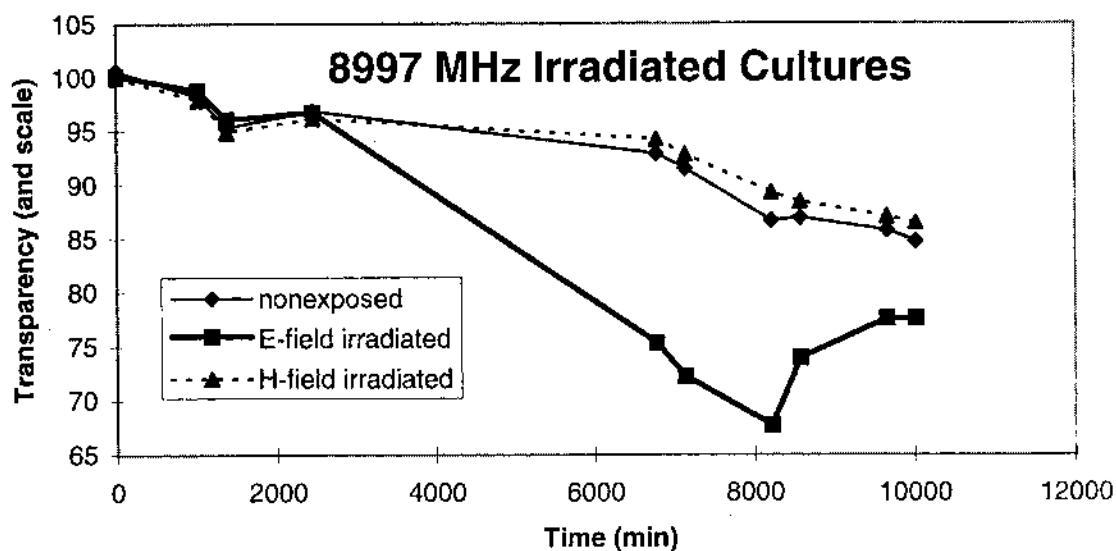


Figure 10. The growing results of 8,997 MHz irradiated cultures. The E-field irradiated culture reached a lower transparency compared to the other ones.

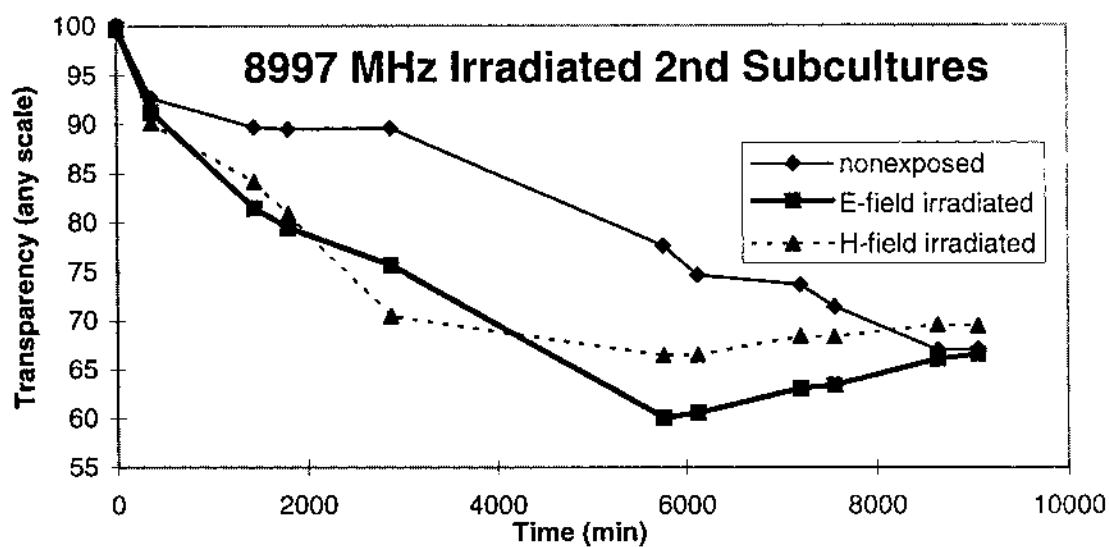


Figure 11. The growing results of 8,997 MHz irradiated second subcultures. The E-field irradiated culture still shows a lower transparency (possibly genetic) and the H-field irradiated culture also shows a lower transparency (delay effect).

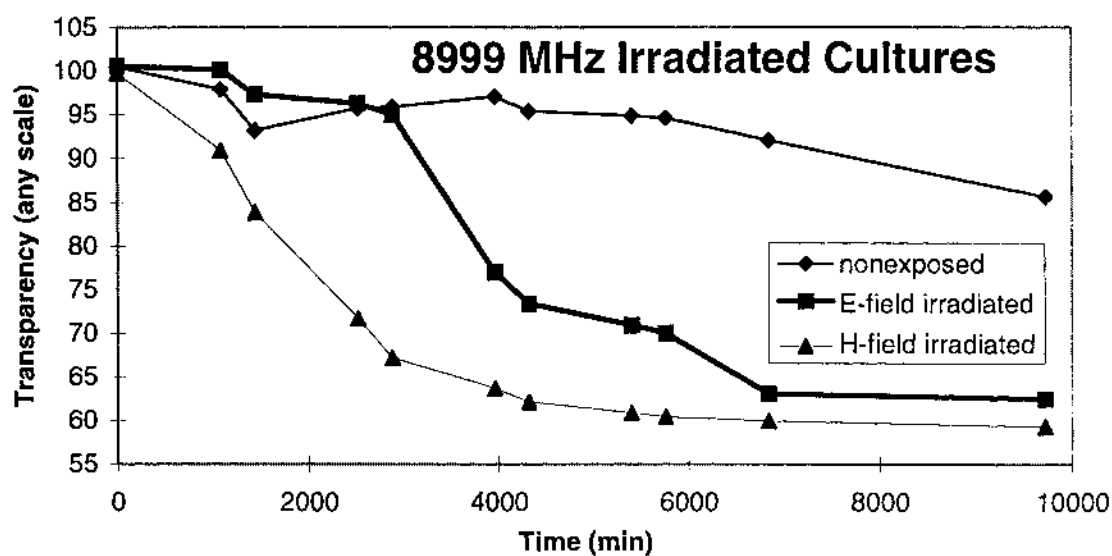


Figure 12. The growing results of 8,999 MHz irradiated cultures. Both E and H field irradiated cultures reached lower transparencies.

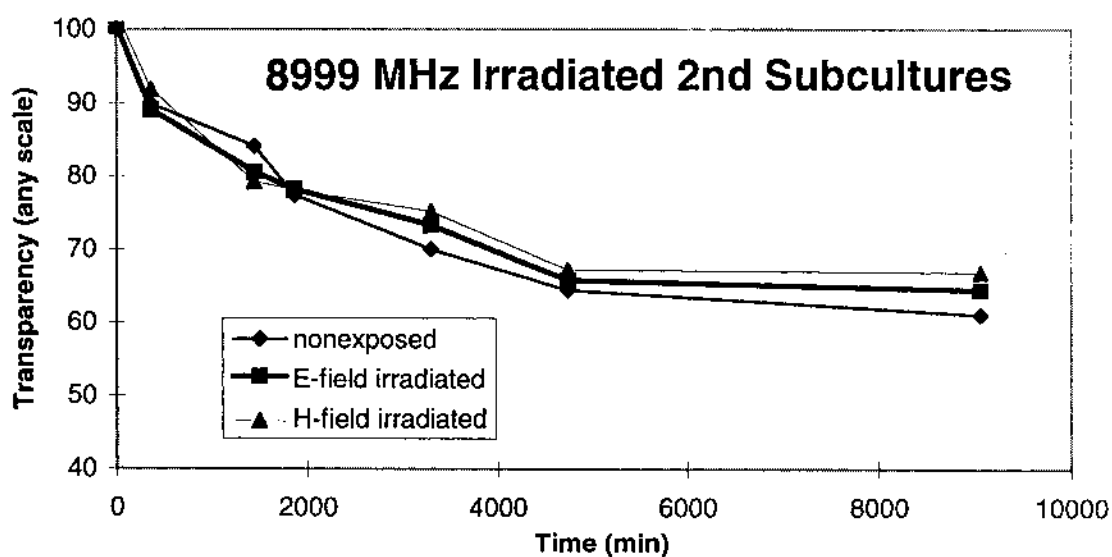


Figure 13. The growing results of 8,999 MHz irradiated second subcultures. The transparencies of the E and H field irradiated cultures are about the same as the nonexposed (nongenetic temporary effects).

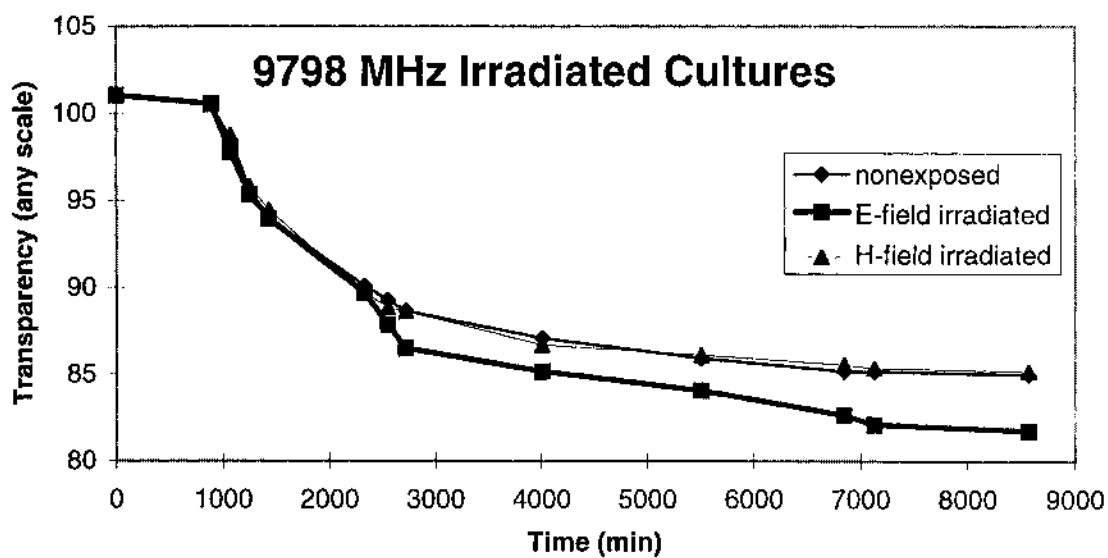


Figure 14. The growing results of 9,798 MHz irradiated cultures. The E-field irradiated culture shows a lower transparency.

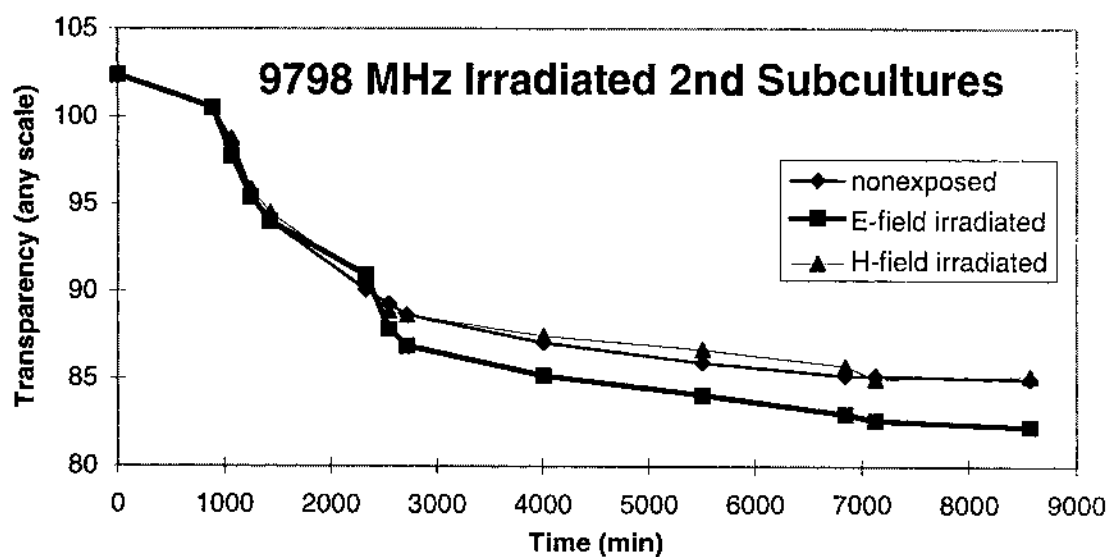


Figure 15. The growing results of 9,798 MHz irradiated second subcultures. The E-field irradiated culture shows a transparency which is the same as the first culture (possibly a genetic effect of nonionizing radiation).

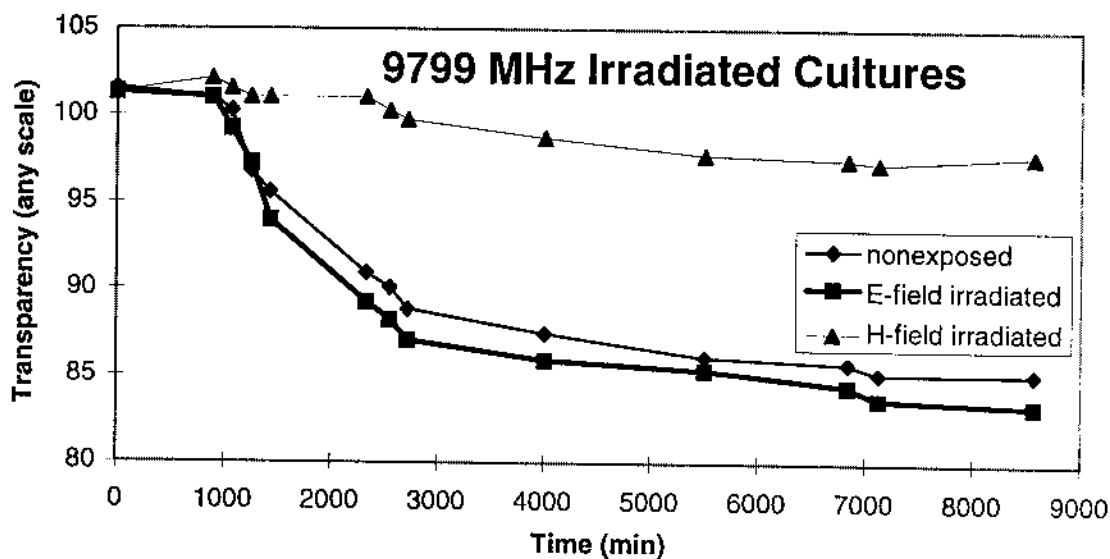


Figure 16. The growing results of 9,799 MHz irradiated cultures. The H-field irradiated culture shows a clear transparency which means the bacteria in the culture were almost completely killed.

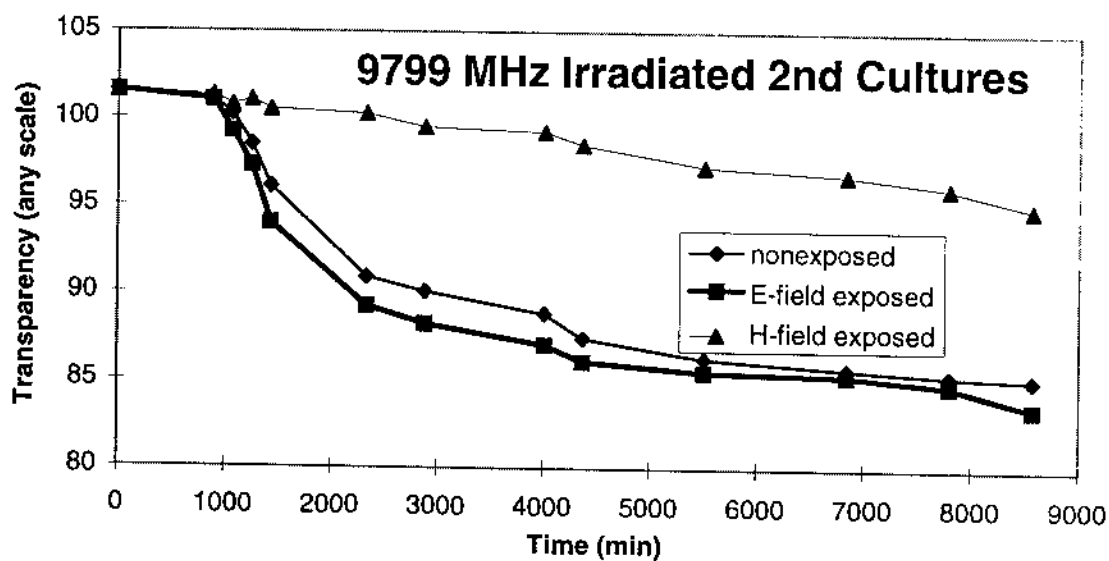


Figure 17. The growing results of 9,799 MHz irradiated second subcultures. The H-field irradiated culture, again, shows a clear transparency (It is not sure whether this effect is genetic or not because the bacteria were killed before showing some effects.).

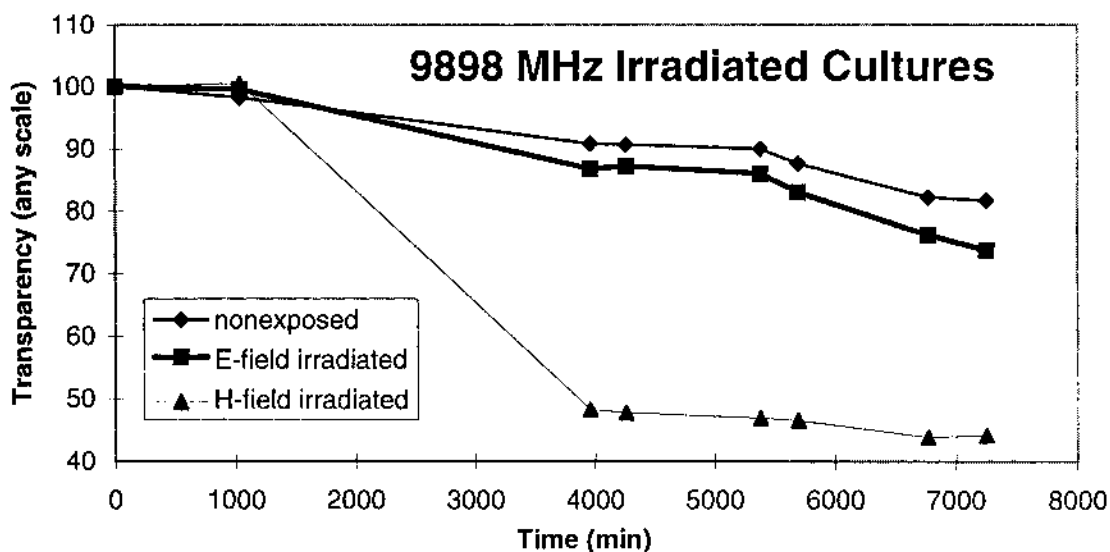


Figure 18. The growing results of 9,898 MHz irradiated cultures. The H-field irradiated culture shows a lower transparency compared to the others.

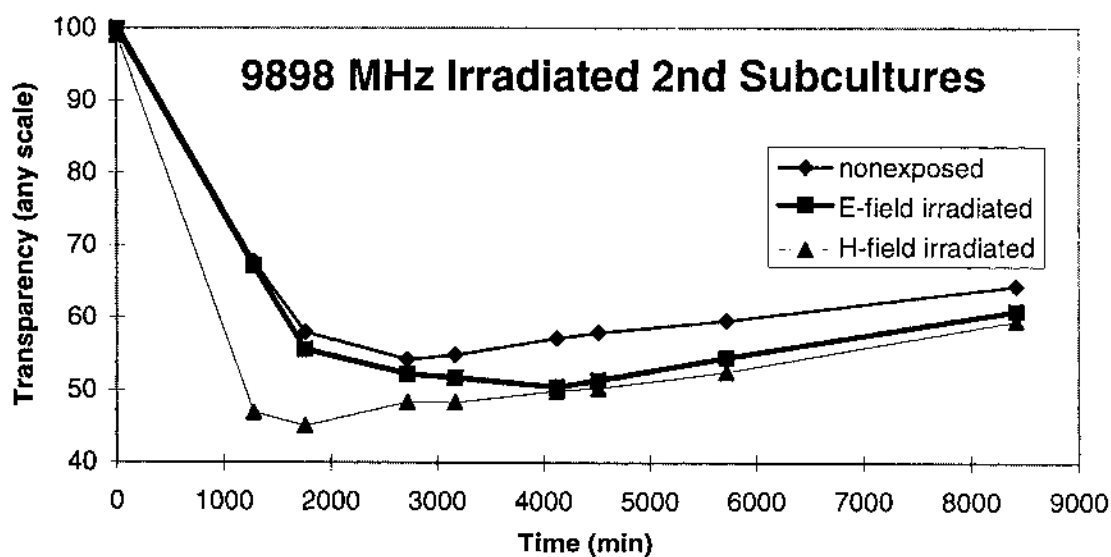


Figure 19. The growing results of 9,898 MHz irradiated second subcultures. All three subcultures show about the same transparency which is different from the first results (nongenetic temporary effects of nonionizing radiation).

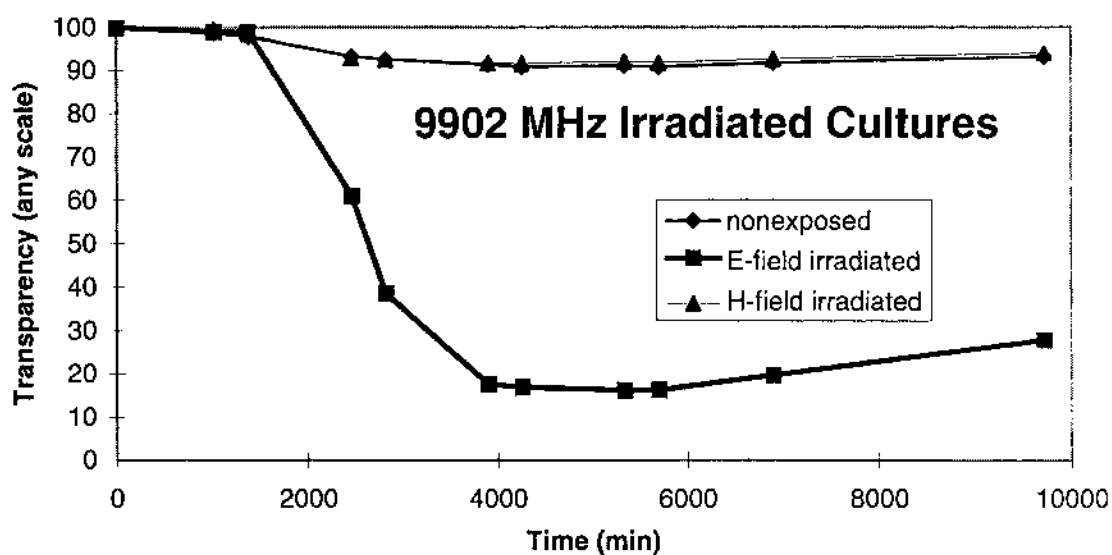


Figure 20. The growing results of 9,902 MHz irradiated cultures. The transparency of the E-field irradiated culture is extremely lower than the others.

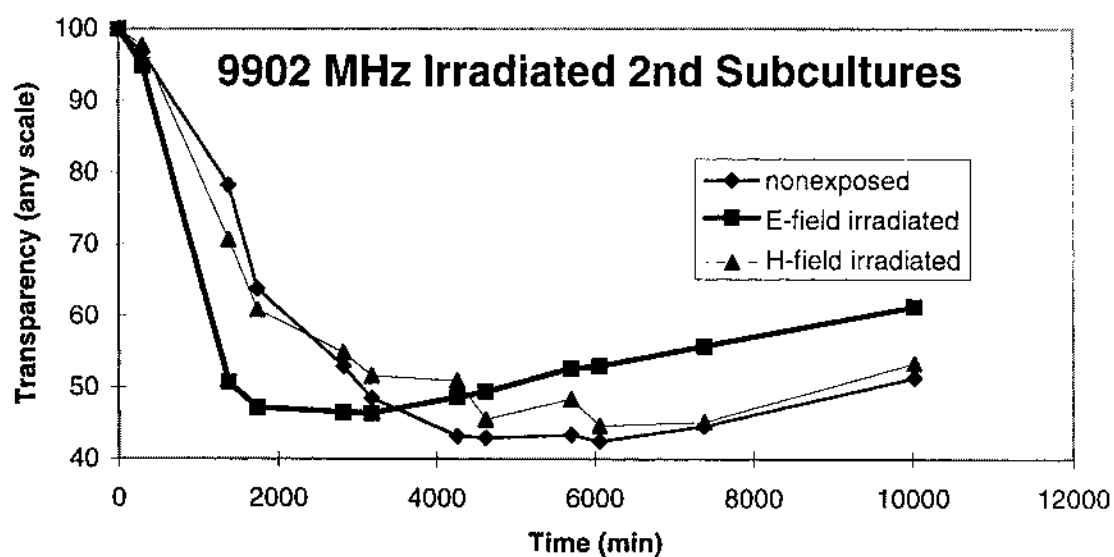


Figure 21. The growing results of 9,902 MHz irradiated second subcultures. The transparencies of all three subcultures are about the same (nongenetic temporary effects of nonionizing radiation).

CHAPTER 5

SUMMARY OF RESULTS

Nonthermal frequency dependent results have been observed and the frequencies were close to or the same as the frequencies of 8.4 GHz, 8.7 GHz, and 10.5 GHz which were theoretically predicted by Prohofsky et al.. The frequencies and their effects are summarized as follows:

- a. At 8,300 MHz: The electric field produced a genetic effect.
- b. At 8,700 MHz: The electric field produced a genetic effect.
- c. At 8,997 MHz: The electric field produced a genetic effect and the magnetic field produced a delayed effect.
- d. At 8,999 MHz: Both the electric and the magnetic fields produced nongenetic, temporary effects.
- e. At 9,798 MHz: The electric field produced a genetic effect.
- f. At 9,799 MHz: The magnetic field appears to have killed all of the bacteria.
- g. At 9,898 MHz: The magnetic field produced a nongenetic temporary effect.
- h. At 9,902 MHz: The electric field produced a nongenetic temporary effect.

The frequencies listed above were selected by conducting the experiments more than three times. That is, they showed the same effects more than three times. Thus, the reliability of the experimental results is very high.

Unfortunately, due to the malfunctioning of the equipment at frequencies higher than 10 GHz and due to limited time, the frequencies around 10.2 GHz which had been selected from the first survey experiment couldn't be studied. The frequency of 10.2 GHz is a very important one because it is located near the theoretically predicted value, which is 10.5 GHz, that should produce an effect.

During the second detailed phase of the experiments, some of the precise frequencies were determined by using a frequency locking system. By sampling from the first irradiated cultures and reculturing those samples, which are not directly irradiated but the descendants of those exposed to the radiations, it is concluded that at least four frequencies (8,300 MHz, 8,700 MHz, 8,997 MHz, and 9,798 MHz) produced genetic biological effects. If the radiation effects are genetic, then the radiations affects the DNA molecule. In future experiments, each part of *E-coli* will be separated and will be studied by using the molecular spectrometer to determine any resonant frequencies which may have strong interaction. The major concern will be concentrated on the macro DNA molecules. Finally, after determining the resonance frequencies of the molecules, it may be possible to investigate what processes are responsible for the interactions between the biological systems and non-ionizing radiations.

CHAPTER 6

FUTURE WORKS

Throughout this experiment the microwave frequencies in the region of 8 to 12 Ghz which produced unusual nonthermal biological effects were determined. The nonthermal effects were determined through the measurement of transparency of the culture medium. The detailed status of growing conditions, for example, whether the size of each bacterium is growing or the number of bacteria is growing or organelle disorder, etc. was not studied. Future efforts should include a detailed study of these properties.

To understand more about the vibrational resonant effects of DNA, the following works should be done:

a). A biological study should be made of the bacteria growing status to determine whether the size of each bacterium is growing or the number of bacteria is growing and whether or not the organ or organelle disorder is initiated, etc..

b). A study of the DNA molecule should be made using molecular spectroscopy techniques to determine the accurate vibrational resonant frequencies. Also, other parts of the bacteria, protein, membrane, bioliquid, etc. should be studied in detail.

d). Construction of a DNA map should be made by mapping the resonant frequencies produced by each atom or atomic chain and any other biological effects.

Another technique to be improved is the bacteria distribution and the field irradiation processes. The growing status of the bacteria cultures may depend on both the effects of radiation and the initial bacteria concentration. Before exposed to the radiations or room temperature, the bacteria were distributed to the micro pipettes and after 4 hours of irradiation the bacteria were diluted with nutrient. Although special care was given to the dilution process to make uniform initial concentrations, the number of bacteria per culture tube might be different, so that an undesired error could be involved. To eliminate or reduce this problem, an improved irradiation technique must be employed in a future study. Before irradiation, the concentrated bacteria should be diluted with nutrient and distributed to the three culture cuvettes instead of the micro pipettes. Thus, each cuvette contains practically the same amount of the bacteria. The big cuvettes, however, cannot be inserted into the resonant cavity through the small holes in which the pipettes can fit. To irradiate the large cuvettes, a larger physical size resonant cavity is required, although it may be impossible, practically, to achieve the conditions needed.

This work has determined select microwave frequencies which deserve more study and a spectrometer has been developed that is capable of making precision frequency studies of *E-coli*. When the procedure suggested above are followed, it will be possible to ascertain the precise mechanism through which the external fields are coupling with *E-coli* specifically and, microorganisms in general.

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