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BIOLOGICAL EFFECTS IN RODENTS EXPOSED TO PULSED ELECTROMAGNETIC RADIATION

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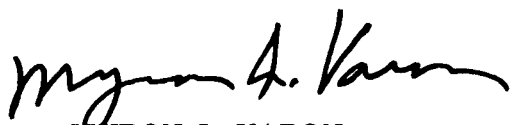

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FOREWORD
(Nontechnical summary)

Electromagnetic pulse (EMP) radiation may be described as a traveling wave consisting of transverse electric and magnetic oscillating fields. The amplitude of the oscillations is directly related to the power density of the field. There could be an effective energy exchange from the electromagnetic field to the medium whenever these forces are sufficient to alter the kinetic or potential energy of the molecules in the medium. However, a 3-cm microwave with a frequency of 10 GHz has an energy only of 4×10^{-5} eV compared with 10^{6} eV for gammas (e.g., ^{60}Co); therefore, an effective energy exchange is not anticipated. Furthermore, a contributing effect of heat is not predicted because of the low average power of the EMP.

Electromagnetic pulse simulators have been used increasingly in recent years by industry and military establishments, and exposure of personnel to EMP radiation has correspondingly increased. There have been a few reports attempting to show that illnesses suffered by some operators are associated with the testing of electronic equipment in EMP facilities. However, results published in the literature do not establish that there is an acute biological hazard. On the other hand, proposed safety standards make the assumption that a biological hazard exists and, if adopted, would restrict healthy workers to 50 kV/m at one pulse per minute. The general public would be restricted to exposures of 200 - 300 V/m peak at a rate of no more than one pulse per minute.

To test the rationale of these safety standards, experiments were designed based on the hypothesis that damage from exposure to EMP should be tested on biological

systems which could respond to rapid changes in electric and magnetic fields. These could be systems with continuously high cellular turnover as seen in the embryo and in the adult bone marrow. The present experiment utilized the AFRRI EMP simulator that provided five pulses per second for 5.1×10^7 pulses and a peak electric field strength of 447 kV/m with a 5 nsec rise time and 5-600 nsec 1/e fall time. This represents a condition in excess of that normally encountered by humans who operate EMP facilities. Exposures of rodents under these conditions indicated no apparent acute injuries based on blood chemistry, blood counts, bone marrow cellular determination, chromosomal aberration, embryology, histology, leukemia, and mammary tumor determinations. Differences between EMP exposed and nonexposed animals were occasionally observed in some blood counts.

It appears that one could safely predict that humans exposed under similar conditions would show no acute injurious biological effects. It is suggested that existing proposed safety standards might be reevaluated, particularly in regard to acute effects. However, since the present experiment has been conducted for only 20 weeks after the onset of EMP exposure, no assessment could be made for the appearance of late somatic effects (e.g., tumors and cancers) possibly induced by early damage at the molecular level. Such injuries usually would be manifested toward the latter part of the life-span in rodents (2nd year).

ABSTRACT

Rodents were exposed to electromagnetic pulse (EMP) radiation to test the hypothesis that rapid changes in electric and magnetic fields would induce injuries in biological systems with high cell turnover rates. The AFRRI EMP simulator provided five pulses per second with a peak electric field intensity of 447 kV/m with a 5 nsec rise time and 5-600 nsec 1/e fall time. Exposures, totaling 5.1×10^7 pulses, were continuous except for approximately 1 hour daily for biological sampling and animal care during 20 weeks. Biological assays were periodically conducted in exposed and nonexposed animals at appropriate intervals.

It was observed that the reticulocyte count in exposed rats was nearly always greater than in nonexposed. However, there were no concomitant differences in peripheral erythrocyte counts between the two groups, nor did radioactive iron incorporation indicate increased cellular production in the irradiated group. Levels or relative counts of circulating leukocytes did not differ between the two groups. Platelet counts in exposed rats occasionally were decreased below those in the nonexposed. Bone marrow cellularity was not different between the two groups. Preliminary analysis of chromosomes showed no detectable increases of defects in EMP exposed rats. Routine chemical analysis of blood demonstrated similar values in the two groups. Observations of fetuses from pregnant rats showed no abnormalities. Other assays (histology, leukemia, and mammary tumor) showed no acute effects from exposure.

The present experiment utilizing the above-described physical parameters represented a condition exceeding by several orders of magnitude that normally encountered by humans who operate EMP facilities. Exposures of rodents under these

conditions indicated no apparent acute injuries. A tentative prediction can be made that humans exposed to EMP in industrial situations would show no acute biological injuries.

I. INTRODUCTION

The utilization of electromagnetic pulse (EMP) simulators by industry and military establishments and the exposure of personnel during routine operation have greatly increased in recent years.[2,5] A potential hazard to man has been a matter of concern, and safety standards have been proposed.[3] However, there are not enough biological data to support these standards.

Basically, EMP consists of a pulse of radio-frequency waves with a nearly instantaneous rise in the electric and magnetic fields and a subsequent decline in the fields. EMP radiation may be represented as a traveling wave consisting of transverse electric and magnetic oscillating fields; the amplitude of the oscillations is directly related to the power density of the field. There could be an effective energy exchange from the electromagnetic field to the medium whenever these forces are sufficient to alter the kinetic or potential energy of the molecules in the medium. A contributing effect of heat is not predicted because of the low average power of the EMP. It may therefore be questioned whether exposure to EMP could present a hazard to man.

There is a lack of data dealing with field exposures or simulated laboratory experiments to support a definitive answer. Exposure of the enzyme alcohol dehydrogenase and deoxyribonucleic acid to a radio-frequency electric field resulted in no biological or chemical changes.[12] On the other hand, radio-frequency exposures have caused genetic damage in the fruit fly[7,8] and chromosome aberrations in plant and mammalian cells.[4,9] These reported effects occurred at approximately 100 MHz with high peak powers which were not great enough to produce heating. The field strength and frequency range are similar to that of an EMP.

Exposures of a few rats, dogs and monkeys to EMP have shown no apparent acute biological effects.[5,6] A small reversible effect on learned behavior was seen in rats exposed to pulses of 600 kV/m peak fields.[6]

The reported biological changes do not answer the question of what possible physical interaction with biological systems could have occurred. Many biological processes depend on electrical interaction and orientation. The rapid rise and fall of the electric and magnetic fields during EMP could disrupt some of those processes. If this results in biological damage at the molecular level, manifestations of acute injury would depend on the frequency of induced molecular and ultrastructural changes, whereas late effects (e.g., tumors) could be initiated by a few events. The hypothesis that exposure to EMP results in biological damage should be tested on biological systems which might respond to rapid changes in electric and magnetic fields. These could be systems with continuously high cellular turnover rates as seen in the embryo and in the adult bone marrow.

In the present experiment, rodents were exposed to the AFRRI EMP simulator utilizing a frequency of pulses and peak electric field strengths several magnitudes higher (worst type) than existing operating conditions involving exposures of humans. Biological tests involved parameters from systems of high cellular turnover and from tumor inducers. It appears that exposure to 5.1×10^7 electromagnetic pulses during 20 weeks does not result in an acute biological hazard.

II. METHODS

EMP exposure. The AFRRI EMP generator employed in this study provided five pulses per second with a peak electric field intensity of 447 kV/m with a 5 nsec

rise time and 5-600 nsec 1/e fall time. The pulse generator fed a parallel-plate transmission line structure. Provisions were made for the placement of 200 nonmetallic cages between the transmission plates.

Biological parameters were periodically assayed in exposed and nonexposed animals at appropriate intervals during the nearly continuous EMP irradiation. Exposures were interrupted daily for only about 1 hour for biological sampling and animal care. Food and water were supplied ad libitum.

Biological tests.

A. Bone marrow. This study was designed to include 700 male Sprague-Dawley rats. Of this number, 300 were utilized for the determination of bone marrow cellularity and of possible chromosome aberrations every 2 weeks after exposure to EMP. An equal number of animals served as the nonirradiated group. To determine the proliferative capacity of rat bone marrow, the concentration of mitotic cells was determined 6 hours after the injection intraperitoneally of colchicine (1 mg/kg).[10,11] Bone marrow differential counts and estimates of cellularity of one femur were obtained semimonthly from each animal from groups of six irradiated and six nonirradiated male rats.

Bone marrow cells in mitoses were analyzed for possible chromosomal aberrations semimonthly after the onset of EMP irradiation. Mitotic cells were arrested in metaphase using colchicine in vitro.[8,9]

B. Blood. Blood samples (0.2 ml) were obtained via the jugular vein from two groups of 10 continuously irradiated male Sprague-Dawley rats and from their controls so that one group of 10 animals was utilized per week. The concentrations

per mm^3 of erythrocytes, leukocytes, neutrophils, lymphocytes, reticulocytes and platelets were determined from these blood samples. In addition, hematocrit values were obtained. A group of 30 male Sprague-Dawley irradiated rats and an equal number of nonirradiated controls were used for the measurement of ^{59}Fe incorporation into newly formed erythrocytes. The radioiron (1 μCi of ^{59}Fe in 0.01 μg of total iron) was injected via the tail vein into five groups of six rats each at 6 hours, 1 day, 7, 14, and 21 days after the onset of the EMP irradiation. Blood sampling and testing for radioactivity was identical to that described before and was conducted 7 days after ^{59}Fe injection.[1] In addition to the procedures described above, blood obtained from five irradiated and five control rats sacrificed for bone marrow was utilized for standard blood chemistry assays, as follows: protein, albumin, calcium, phosphorus, cholesterol, urea nitrogen, uric acid, creatinine, bilirubin and alkaline phosphatase.

C. Histology. Histological studies as well as postmortem examinations were performed on the animals sacrificed for bone marrow assays.

D. Embryology. Five pregnant rats were placed in the EMP irradiation facility and exposed to 7×10^6 pulses during 17 days of gestation. Five other pregnant animals were utilized as nonirradiated controls. At the end of the exposure time, the fetuses were removed and fixed in Bouin's solution. They were examined grossly for abnormalities and were saved for histological studies.

E. Mammary tumors. Twenty female rats were continuously exposed and observed for possible development of mammary tumors and were compared with an equal number of nonexposed animals.

F. Leukemia. To determine whether EMP exposure would induce early onset of leukemia, 50 irradiated male leukemia-prone mice (AKR/J) and an equal number of nonirradiated animals were observed.

Statistics. The t-test was used to determine the significance of differences between groups.

III. RESULTS

As may be seen in Figure 1, the number of reticulocytes of the EMP exposed rats is nearly always significantly greater than that of the nonirradiated animals. However, Figure 2 indicates no differences in the concentration of circulating

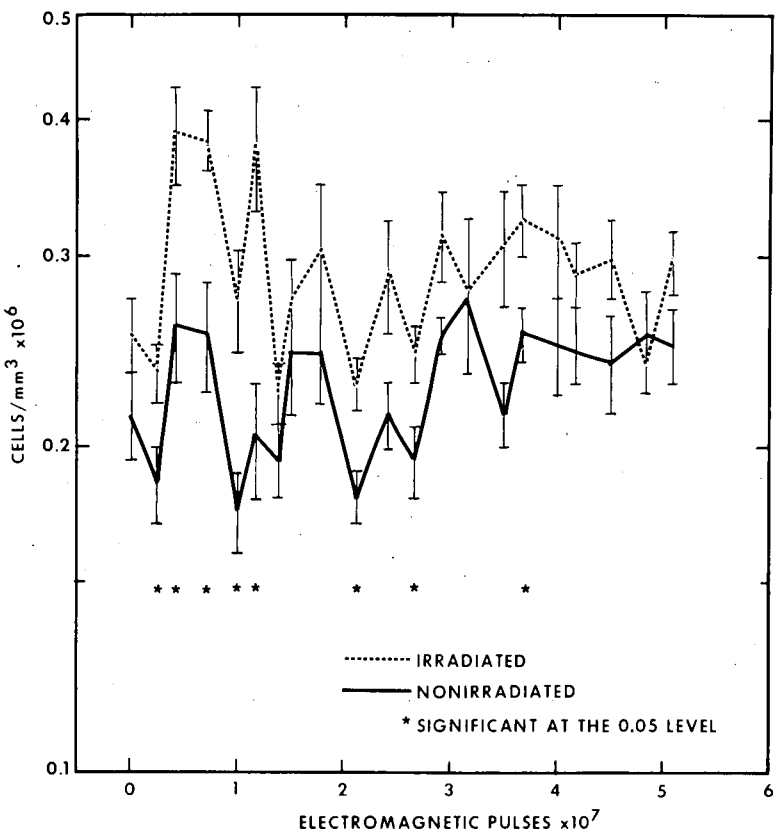

Figure 1. Reticulocytes in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

erythrocytes between the two groups. Furthermore, radioactive iron incorporation was similar in both the nonirradiated and the irradiated groups subjected from 0.1 to 7.7 x 10^6 pulses (Table I). With the exception of five isolated instances, it

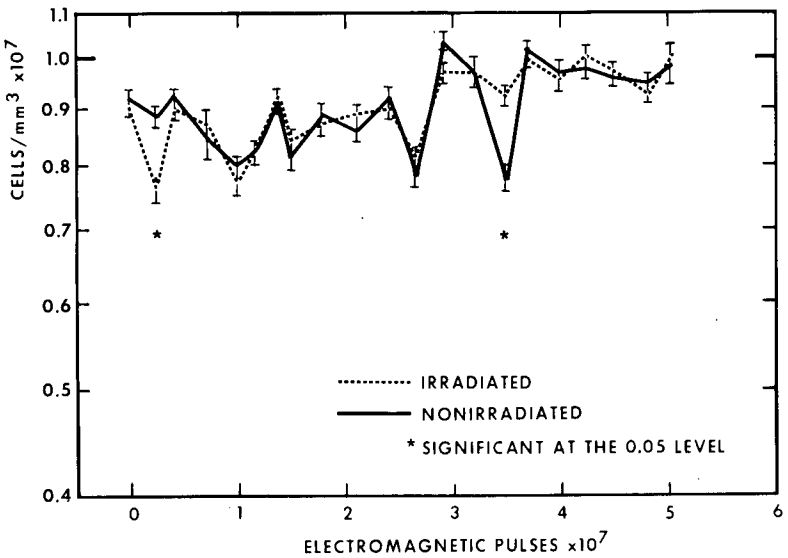

Figure 2. Red cells in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

Time group	Pulses (x 10^6)	^{59}Fe uptake* (percent)
6 hours	0	82.7 ± 2.1
	0.1	86.4 ± 4.0
1 day	0	81.7 ± 4.6
	0.4	77.9 ± 6.3
7 days	0	80.1 ± 5.3
	2.8	79.7 ± 2.3
14 days	0	72.4 ± 2.9
	5.0	64.8 ± 3.6
21 days	0	85.3 ± 5.2
	7.7	82.9 ± 3.3

* Mean ± standard error (number = 6)

Table I. ^{59}Fe Incorporation into Erythrocytic Precursors of Rats Exposed to EMP Radiation

does not appear that the number of circulating leukocytes differs significantly between the two groups (Figure 3). A significant elevation in circulating neutrophils was measured in animals exposed to 10^7 pulses (4 weeks). However, beyond these numbers of pulses and to 5.1×10^7 pulses, no further difference was noted (Figure 4). Again, except for several isolated sampling periods, the number of circulating lymphocytes does not differ between the two animal groups (Figure 5). There appear to be two periods, from 2.7 to 3.2×10^7 pulses and from 4.2 to 5.1×10^7 pulses, when platelets from irradiated animals were significantly decreased (Figure 6).

Bone marrow cellularity, determined up to 1.4×10^7 pulses, showed no differences between the two animal groups.

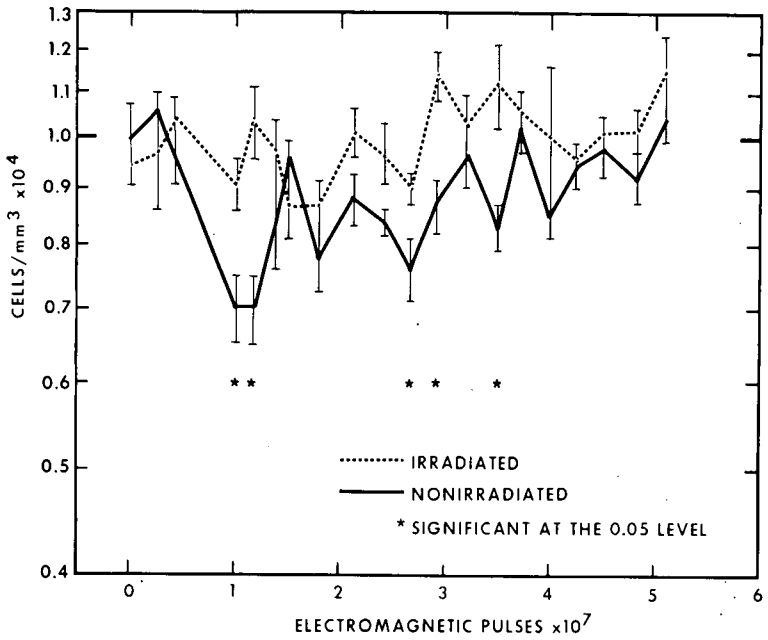

Figure 3. White cells in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

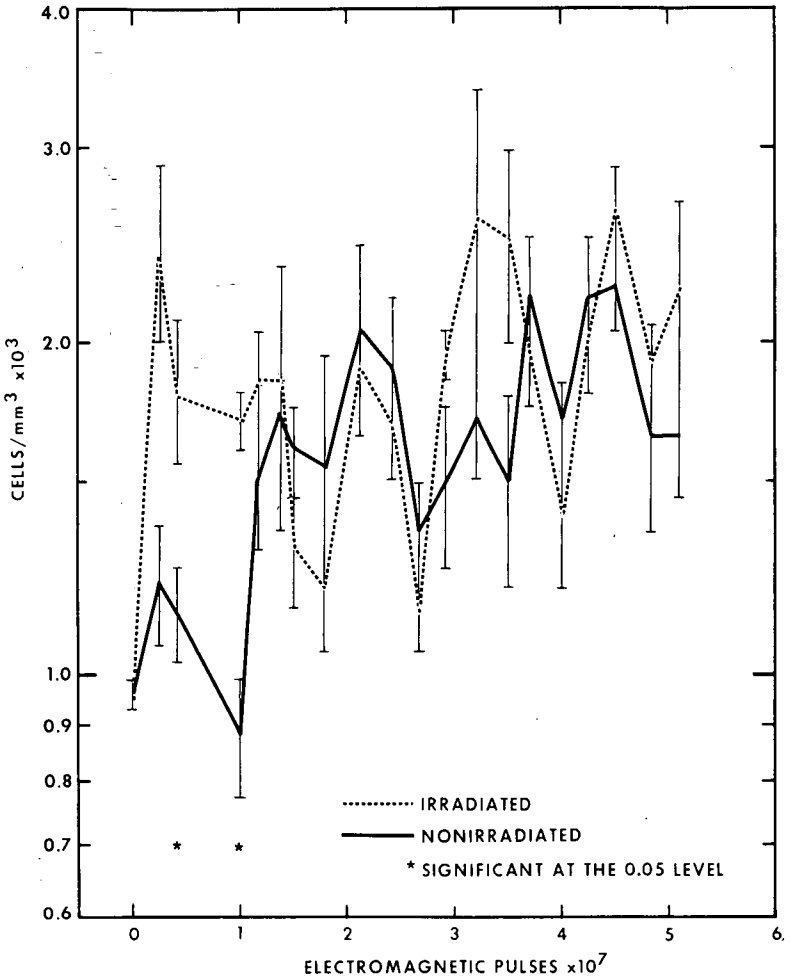

Figure 4.
Segmented neutrophils in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

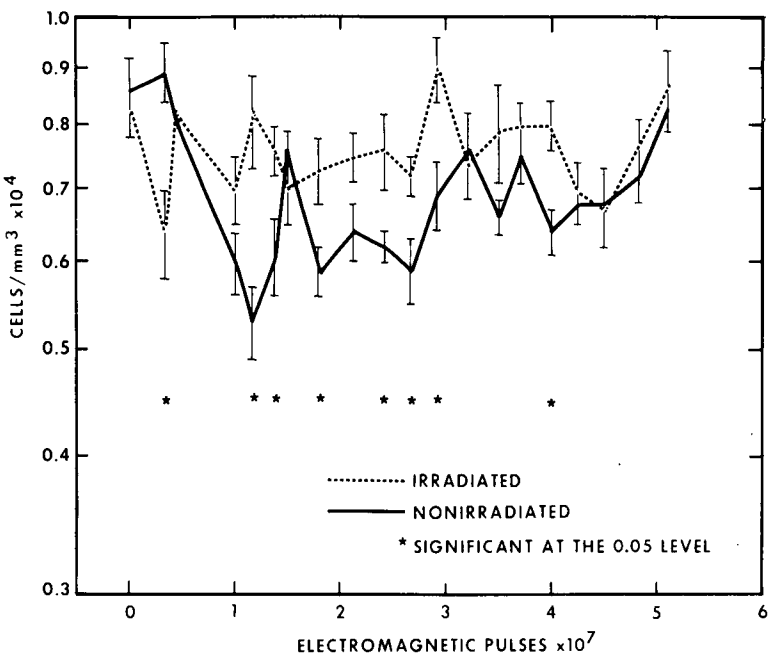

Figure 5.
Lymphocytes in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

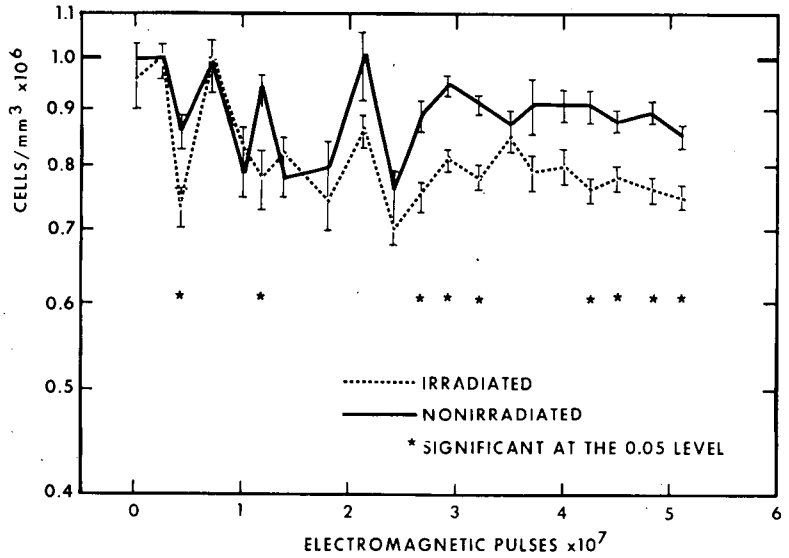

Figure 6. Platelets in peripheral blood from rats during 20 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

Preliminary analysis of chromosomes showed no detectable increases in defects in EMP exposed rats. Routine chemical analysis of blood constituents indicated no difference between the two groups (Table II). Preliminary observations of fetuses from five pregnant rats in each group showed no abnormalities (Table III).

Serum assay	Nonirradiated*	Irradiated* (5×10^7 pulses)
Protein (g/100 ml)	6.2 ± 0.15	6.2 ± 0.12
Albumin (g/100 ml)	2.8 ± 0.11	2.6 ± 0.11
Calcium (mg/100 ml)	9.3 ± 0.25	9.3 ± 0.23
Phosphorus (mg/100 ml)	5.0 ± 0.15	5.2 ± 0.10
Cholesterol (mg/100 ml)	105 ± 10.0	93 ± 7.2
Urea nitrogen (mg/100 ml)	21 ± 1.0	22 ± 1.0
Uric acid (mg/100 ml)	1.0 ± 0.31	1.3 ± 0.27
Creatinine (mg/100 ml)	0.5 ± 0.02	0.6 ± 0.05
Bilirubin (mg/100 ml)	0.3 ± 0.02	0.3 ± 0.03
Alkaline phosphatase (units/100 ml)	177 ± 30	178 ± 39

* Mean ± standard error (number = 5)

Table II. Blood Chemistry

As may be seen in Table IV representing a summary of the biological parameters tested, all other assays (histology, leukemia and mammary tumors) show no demonstrable effects from exposure to 5.1×10^7 pulses during 20 weeks of irradiation.

Table III. Embryology

Group	Normal fetuses*	Resorptions*
Nonirradiated	13.0 ± 1.3	0 ± 0
Irradiated (7×10^6 pulses, 17 days)	13.4 ± 0.6	0.6 ± 0.4

* Mean ± standard error (number = 5)

Table IV. Summary after 5.1×10^7 Pulses during 20 Weeks of Chronic EMP Irradiation

Experiment	Biological effect
Blood chemistry	none
Blood count	variable
Bone marrow	none
Chromosomal aberration	none
Embryology	none
^{59}Fe uptake	none
Histology	none
Leukemia	none
Mammary tumor	none

IV. DISCUSSION

Evaluation of the experimental results in the present experiment dealing with rodents exposed to 5.1×10^7 pulses of electromagnetic radiation clearly indicates that they did not experience acute biological injuries. Occasionally, significant differences

between the irradiated and nonirradiated groups for hematological parameters were observed which never approached physiological abnormality. It is of interest to note that in a preliminary experiment conducted at the Lovelace Foundation, utilizing dogs, some hematological differences were observed.[5]

There appears to be no definite physiological explanation for the observed hematological changes which might propose the possibility that they represent extremes of normal biological fluctuations. For example, increases in the number of reticulocytes normally indicate either a temporary sudden release of cells from bone marrow compartments or a true increase in red cell production. The latter usually persists over a long period of time and eventually shows a corresponding increase in the circulating number of erythrocytes. The data in the present experiment show elevation of reticulocytes in the irradiated animals for several weeks without concurrent increases in peripheral erythrocytes. Furthermore, no increases in ^{59}Fe incorporation of erythrocytic precursors in the EMP exposed rats were measured, clearly indicating no increased cellular production.

The early increase in peripheral neutrophils of irradiated animals might indicate possible minor infections or exposure to a new general stressful condition. However, no distinct adverse effects could be noticed and indeed these changes could also have no pathophysiological basis. The occasional apparent lymphocytic increases may be similarly explained.

As had been emphasized earlier, the present experiment utilizing five pulses per second for 5.1×10^7 pulses and a peak electric field strength of 447 kV/m represents

a condition in excess of that normally encountered by humans who operate EMP facilities.[5] Exposures of rodents under these conditions indicated no apparent acute injuries based on the biological assays employed. It appears then that one could safely predict that humans exposed under similar conditions would show no acute biological effects. It is suggested that existing safety standards might be reevaluated, particularly in regard to acute effects.[3] However, since the present experiment has been conducted for only 20 weeks after the onset of EMP radiation exposure, no assessment could be made for the appearance of late somatic effects (e.g., tumors and cancers) possibly induced early by damage at the molecular level. Such injuries usually would be manifested toward the latter part of the life-span in rodents (2nd year).

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13. ABSTRACT

Rodents were exposed to electromagnetic pulse (EMP) radiation to test the hypothesis that rapid changes in electric and magnetic fields would induce injuries in biological systems with high cell turnover rates. The AFRRI EMP simulator provided five pulses per second with a peak electric field intensity of 447 kV/m with a 5 nsec rise time and 5-600 nsec 1/e fall time. Exposures, totaling 5.1×10^7 pulses, were continuous except for approximately 1 hour daily for biological sampling and animal care during 20 weeks. Biological assays were periodically conducted in exposed and nonexposed animals at appropriate intervals.

It was observed that the reticulocyte count in exposed rats was nearly always greater than in nonexposed. However, there were no concomitant differences in peripheral erythrocyte counts between the two groups, nor did radioactive iron incorporation indicate increased cellular production in the irradiated group. Levels or relative counts of circulating leukocytes did not differ between the two groups. Platelet counts in exposed rats occasionally were decreased below those in the nonexposed. Bone marrow cellularity was not different between the two groups. Preliminary analysis of chromosomes showed no detectable increases of defects in EMP exposed rats. Routine chemical analysis of blood demonstrated similar values in the two groups. Observations of fetuses from pregnant rats showed no abnormalities. Other assays (histology, leukemia, and mammary tumor) showed no acute effects from exposure.

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