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**BIOLOGICAL MEASUREMENTS IN
RODENTS EXPOSED CONTINUOUSLY
THROUGHOUT THEIR ADULT LIFE TO
PULSED ELECTROMAGNETIC RADIATION**

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Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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FOREWORD
(Nontechnical summary)

Earlier work at the Armed Forces Radiobiology Research Institute (AFRRI) clearly demonstrated that exposure to the AFRRI electromagnetic pulse (EMP) simulator which provided five pulses per second for 10^8 pulses during 38 weeks and a peak electric field strength of 447 kV/m did not produce acute injuries to vital blood cell producing and reproductive organs of rodents. However, the possibility existed that the rapid rise and fall of electric and magnetic fields could induce minute injuries within cells of vital organs which would later manifest themselves in life shortening diseases.

The present report deals with rodents which were subjected continuously to EMP radiation for nearly all their adult life, receiving a total of 2.5×10^8 pulses. None of the biomedical parameters measured indicated a significant difference between irradiated and nonirradiated rats. It was found that EMP exposure did not aggravate an existing respiratory infection and it caused no changes in the blood forming and reproductive organs. Female rats exposed throughout their gestation period to approximately 3.4×10^7 electromagnetic pulses gave birth to normal progeny. Finally, exposure to electromagnetic radiation did not induce either earlier appearance or late increased numbers of tumors or any other cause for life shortening. Results obtained in the present study clearly indicate that EMP radiation presented no biological hazard to rodents.

The radiation exposure employed in the present experiment represents a condition in excess of that normally encountered by workers who operate EMP facilities by

factors of tens of millions. If the negative effects observed in rodents hold true for larger mammals and man, they should have a great influence on future proposed safety standards.

ABSTRACT

Rodents were exposed continuously for 94 weeks of their adult life to a total of 2.5×10^8 pulses from the AFRRRI electromagnetic pulse (EMP) simulator which provides five pulses per second with a peak electric field intensity of 447 kV/m, a 5-nsec rise time and 550-nsec 1/e fall time. The following biological parameters were measured: blood chemistry, blood and bone marrow cellular concentration, chromosomal aberrations, erythrocyte production, effects on fertility and reproductive capability and appearance of tumors and other late effects. At no time before and particularly as the rodents approached the end of their life-span did any of the biological measurements indicate an effect of the EMP radiation. While it is extremely difficult to prove the absence of any injury, it can be unequivocally stated that EMP exposure presented no biological hazard to the rodents of the present study.

I INTRODUCTION

In previous reports⁸ it was hypothesized that electromagnetic pulse (EMP) radiation presents no biological hazard from current flow or thermal heating for a body insulated from the ground. However, it was not clear if the rapid rise and fall of electric and magnetic fields could induce changes at the molecular level which would result in either acute injuries or which would manifest themselves in later life due to earlier somatic changes.

Extensive studies in rodents⁸ which were continuously exposed up to 10^8 electromagnetic pulses during 38 weeks did not indicate acute injuries of the vital hematopoietic and embryological systems based on organ, histopathologic and cellular examinations. This, of course, did not preclude the occurrence of minute effects at the molecular level which could later facilitate the activation of latent viruses or result in vasculoconnective tissue changes.⁹ Such late effects were observed in mice exposed to 0.2 R per day of ¹³⁷Cs gamma rays throughout their adult life, an exposure which did not cause acute biological injury.⁶

The present report deals with a study designed to discover possible late effects in rats exposed to 2.5×10^8 electromagnetic pulses during 94 weeks of their adult life. Careful examination of the biological data revealed no biological hazards for the rodents.

II. METHODS

A detailed description of the AFRRRI EMP generator may be found in previous publications.^{2,8} Essentially, the system provides five pulses per second with a peak electric field intensity of 447 kV/m. The rats were exposed continuously for 94 weeks to 2.5×10^8 pulses of EMP radiation beginning at 4 months after birth. Exposures were

interrupted daily, from Monday through Friday, for approximately 1 hour for biological sampling and animal care. Food and water were available ad libitum to 640 male and 40 female Sprague-Dawley rats.

A total of 300 irradiated male Sprague-Dawley rats and an equal number of non-irradiated male rats were studied over a period of 60 weeks to determine the effect of EMP irradiation on bone marrow cellularity and the induction of chromosomal aberrations. The techniques used were described in detail in an earlier publication.⁸ Blood samples were obtained via jugular venipuncture for 94 weeks from 20 continuously irradiated male Sprague-Dawley rats and from an equal number of nonirradiated controls. The concentrations per mm³ of erythrocytes, leukocytes, neutrophils, lymphocytes, reticulocytes and platelets were routinely determined once every week.

Rats utilized for bone marrow studies were also examined for possible pathologic changes in other organs and tissues. The animals were euthanatized by exposure to ether in a closed chamber. At time of death, all tissues were examined for macroscopic changes.

Tissues saved for histologic examination were fixed in 10 percent neutral buffered Formalin, embedded in paraffin, sectioned at 6 μ m and stained with hematoxylin and eosin. Representative specimens of the following organs were collected: lung, kidney, urinary bladder, spleen, thymus, thyroid gland, adrenal gland, heart, skeletal muscle, bone, skin, brain, eye, testicle, preputial gland, salivary gland, stomach, small intestine, large intestine, liver and pancreas. Tissues were selectively screened for microscopic lesions. At necropsy gross lesions which were suspected

of having a bacterial or mycoplasma component were cultured by routine microbiologic technique.

Twenty female Sprague-Dawley rats which were 4 months old at the beginning of the study were continuously subjected to EMP radiation for 94 weeks and compared with an equal number of nonexposed female animals for the incidence and frequency of mammary tumor development. Rats bearing visible tumors and animals which appeared to be clinically ill were euthanatized with CO₂ in a closed chamber. Necropsies were immediately accomplished. The location, size and general appearance of tumors and other lesions were recorded.

In order to assess possible effects of EMP exposure on fertility, five pairs of 4-month-old rats were exposed to 3.4×10^7 electromagnetic pulses and compared with an equal number of pairs of nonirradiated animals. Male rats were separated from their female partners for the first 8 days (approximately 3×10^6 electromagnetic pulses). Thereafter, pairs were housed together for the remainder of the experiment. At the termination of pregnancy, the number of progeny was recorded and individual neonates were examined for possible anatomical abnormalities.

The effects of long-term chronic exposures on fertility were measured in five 2-year-old male rats subjected to 2.5×10^8 pulses which were paired with 4-month-old female rats exposed to 2.5×10^7 pulses. An equal number of nonexposed animals of the same age served as controls.

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

III. RESULTS

Rats exposed chronically to EMP radiation had the same number of nucleated bone marrow cells as the nonexposed animals. The final combined mean number of nucleated cells per mm³ of bone marrow was $8.1 \times 10^5 \pm 10^5$ *. Similarly, no differences were observed for the mitotic rubricytes (combined mean value per mm³ of bone marrow, $9.9 \times 10^4 \pm 9.3 \times 10^3$) or the mitotic myelocytes ($4.5 \times 10^4 \pm 4.3 \times 10^3$). One may observe in Table I that there were no differences in the final number of the various blood cells. It is indicated in Figure 1 that earlier increases in the numbers of

Table I. Final Values of Cellular Constituents Per mm³ of Blood in Rats Exposed to 2.5×10^8 Pulses during 94 Weeks

	EMP irradiated rats (mean value \pm standard error)	Nonirradiated rats (mean value \pm standard error)
RBC's	$5.2 \pm 0.1 (x 10^6)$	$5.2 \pm 0.2 (x 10^6)$
WBC's	$10.6 \pm 1.0 (x 10^3)$	$10.3 \pm 0.9 (x 10^3)$
Neutrophils	$2.0 \pm 0.5 (x 10^3)$	$2.1 \pm 0.4 (x 10^3)$
Lymphocytes	$8.2 \pm 0.6 (x 10^3)$	$7.7 \pm 0.6 (x 10^3)$
Reticulocytes	$.86 \pm 0.14 (x 10^5)$	$.82 \pm 0.17 (x 10^5)$
Platelets	$.68 \pm .02 (x 10^6)$	$.72 \pm .01 (x 10^6)$

reticulocytes of irradiated rats were no longer observed during the last 25 weeks of exposure. Similarly, decreases in the concentration of platelets were no longer evident during the same period (Figure 2).

Necropsies were conducted on half of the EMP exposed and nonexposed rats employed for the bone marrow cellularity studies. Macroscopic and microscopic

* Standard error

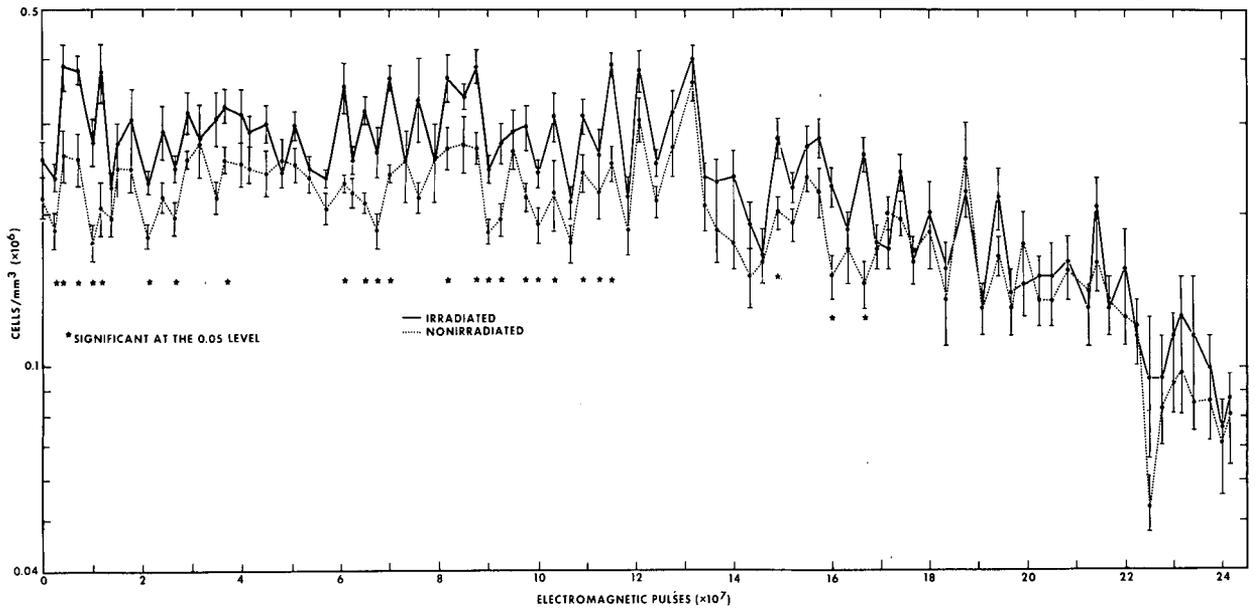


Figure 1. Reticulocytes in peripheral blood from rats during 94 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

examinations revealed that the irradiated animals did not deviate from controls in any perceptible manner. Sprague-Dawley rats in our colony as well as those from other laboratories show evidence of chronic respiratory disease, particularly as they get older. The important point for the present research effort was that the degree of

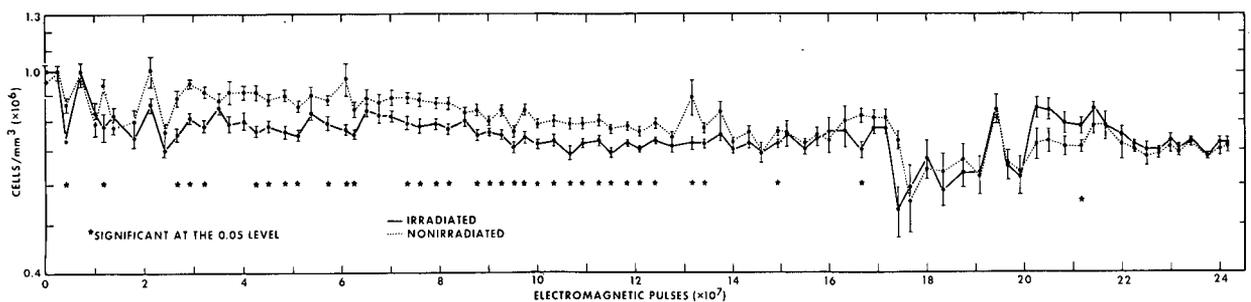


Figure 2. Platelets in peripheral blood from rats during 94 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

involvement was quite similar for exposed and nonexposed animals. Furthermore, EMP radiation did not represent an activating or stress factor for this endemic disease.

Table II shows that the number of male rats afflicted with tumors was similar for both groups and the incidence and type of tumors seen are those usually observed in older rats.⁷

Table II. Summary of Tumor Findings in Male Rats

Rat #	Date necropsied	Control	No.	EMP exposed	No.
79	12-5-72	Adenocarcinoma (kidney)	1	Undifferentiated sarcoma (subcutis)	1
16	3-13-73				
275	6-19-73	Fibrosarcoma (subcutis)	1		
467	10-23-73	Undifferentiated sarcoma (abdominal cavity)	1		
25	7-24-74	Fibroma (abdominal cavity wall)	1		
40	10-11-74			Pituitary tumor	1
28	6-13-74			Adenocarcinoma, low grade malignancy, mammary	1
Total			4	Total	3

Table III clearly indicates that EMP exposure did not increase the occurrence of mammary tumors in the female Sprague-Dawley rats.

Pairs of rats exposed to 3.4×10^7 electromagnetic pulses show no effect on fertility (Table IV). No anatomical abnormalities were observed in the progeny. Male rats which had been exposed to 2.5×10^8 electromagnetic pulses and were 2 years old were mated with 4-month-old female rats subjected to 2.5×10^7 electromagnetic pulses.

Table III. Summary of Tumor Findings in Female Rats

Rat #	Date necropsied	Control	No.	EMP exposed	No.
356	7-31-73			Adenocarcinoma, * low grade malignancy (mammary tumor)	1
358	7-31-73			Fibroadenoma (mammary tumor)	1
367	11-6-73	Fibroadenoma (mammary tumor)	1		
343	1-31-74	Fibroadenoma (mammary gland tumor)	1		
352	1-31-74			Fibroadenoma (mammary tumor)	1
371	1-31-74	Adenofibroma (mammary gland)	1		
380	1-31-74			Adenofibroma (mammary gland)	1
370	1-31-74			Adenocarcinoma, * low grade malignancy (mammary gland)	1
360	4-18-74			Adenofibroma (mammary gland)	1
369	4-18-74	Adenofibroma (mammary gland)	1		
378	4-18-74			Adenofibroma (mammary gland)	1
379	7-19-74	Fibroadenoma (mammary gland)	1		
349	10-11-74	Adenocarcinoma, * low grade malignancy (mammary gland)	1		
Total			6	Total	7

* Tumors were adenocarcinomas based on histological criteria

As may be seen in Table V, there was no significant difference in the number of progeny produced by these pairs as compared with that of nonirradiated animals of the same age. Again, no neonatal abnormalities were noted.

Table IV. Fertility Tests with 4-Month-Old Rats Exposed to 3.4×10^7 Electromagnetic Pulses during 14 Weeks

	EMP irradiated	Nonirradiated
Number of pairs	5	5
Number of days irradiated	98	0
Number of days housed in pairs	90	90
Mean number of pregnancies per pair	$2.2 \pm .2^*$	$1.8 \pm .1^*$
Mean number of progeny per pair	$14.6 \pm 3.2^*$	$10.6 \pm 2.5^*$
Number of neonatal abnormalities observed	0	0

* Standard error

Table V. Fertility Tests with 24-Month-Old Male Rats Exposed to 2.5×10^8 Electromagnetic Pulses during 94 Weeks and 4-Month-Old Female Rats Exposed to 2.5×10^7 Electromagnetic Pulses during 10 Weeks

	EMP irradiated	Nonirradiated
Number of pairs	5	5
Number of days irradiated		
Males	658	0
Females	70	0
Number of days housed in pairs	70	70
Mean number of pregnancies per pair	$1.0 \pm .2^*$	$1.6 \pm .1^*$
Mean number of progeny per pair	$6.0 \pm 1.1^*$	$10.4 \pm 2.1^*$
Number of neonatal abnormalities observed	0	0

* Standard error

It is of interest to note that a spontaneous disease condition was found equally distributed in exposed animals and controls beyond 40 weeks of age. This was a purulent preputial gland infection caused by organisms from the Enterobacteriaceae group.

A summary of all the tests employed in this study is presented in Table VI. As may be seen, similar to the results obtained during the early phase of this project, no injurious late effects were detected.

Table VI. Summary after 2.5×10^8 Pulses during 94 Weeks of Chronic EMP Irradiation

Experiment	Animal species	Biological effects	
		Early*	Late†
Blood chemistry	Rats	None	--
Blood count	Rats	Variable	None
Bone marrow	Rats	None	None
Chromosomal aberrations	Rats	None	None
^{59}Fe uptake	Rats	None	--
Embryology	Rats	None	None
Fertility	Rats	--	None
Histology	Rats	None	None
Leukemia	Mice	None	--
Mammary tumors	Rats	None	None
Other late effects	Rats	--	None

* 10^8 pulses during 38 weeks of exposure

† 2.5×10^8 pulses during 94 weeks of exposure

IV. DISCUSSION

In the absence of ionizing and thermal events, it is not likely that EMP radiation would initiate acute injurious effects, particularly in cell renewal systems. Indeed, in a previous report⁸ no evidence was found of an acute biological hazard in rodents even after exposure to 10^8 electromagnetic pulses. However, minute intracellular molecular changes while not causing immediate overt effects might initiate chains of reactions which would be manifested in injury at a much later time.¹ This has been

clearly demonstrated for small doses of ionizing radiation.⁶ Late effects such as cataracts, tumors, or general life shortening might have been induced by earlier injury causing later somatic changes. Such injuries would include arteriolocapillary fibrosis, glomerulosclerosis and intracellular molecular changes which would decrease the capability of cellular and organ systems to perform their biological function.⁴ The hypothesis underlying the present research design proposed that the rapid rise and fall of electric and magnetic fields would initiate minute somatic changes which would result in late biological injury.

However, none of the biological parameters measured when the rats approached the end of their life-span indicated a significant difference between irradiated and non-irradiated rats. As indicated earlier even though most animals presented evidence of chronic respiratory disease, EMP radiation did not further aggravate this condition. Female rats exposed throughout their gestation period to approximately 3.4×10^7 electromagnetic pulses gave birth to normal progeny. Similar results were obtained from a subsequent second pregnancy under EMP exposure. No deleterious effects were measured for the hematopoietic and reproductive systems. Finally, exposure to EMP did not induce either earlier appearance or late increased numbers of tumors or any other cause for life shortening.

While it is extremely difficult to prove the absence of minute biological injury, it might be unequivocally stated that even if EMP radiation caused such effects it presented no biological hazard to the rodents of the present study.

The present experiment used an EMP simulator with five pulses per second for 2.5×10^8 pulses and a peak electric field strength of 447 kV/m. This represents a

condition considerably in excess of that normally encountered by workers who operate EMP facilities.⁵ If the negative effects observed in rodents hold true for larger mammals and man, they should have a great influence on future proposed safety standards.³

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