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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION
EXPOSURE ON RATS (U) UNIVERSITY HOSPITAL SEATTLE WA
BIOELECTROMAGNETICS RESEARCH L A W GUY ET AL AUG 85
SR-29 USAFSAM-TR-85-64 F33615-80-C-0612 F/G 6/18

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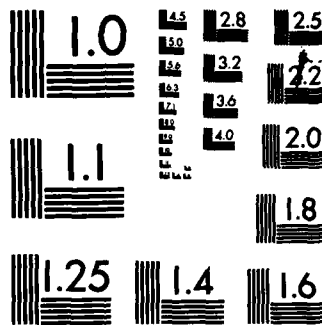
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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 9. SUMMARY

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August 1985

Final Report for Period June 1980 - July 1984

Approved for public release; distribution is unlimited.

Prepared for
USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
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NOTICES

This final report was submitted by the Bioelectromagnetics Research Laboratory, Department of Rehabilitation Medicine, School of Medicine, University of Washington, Seattle, Washington 98195, under contract F33615-80-C-0612, job order 7757-01-71, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks AFB, Texas. Dr. Jerome H. Krupp (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility nor any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

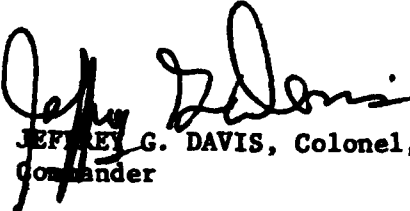
The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.


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REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			
4. PERFORMING ORGANIZATION REPORT NUMBER(S) SR 29		5. MONITORING ORGANIZATION REPORT NUMBER(S) USAFSAM-TR-85-64	
6a. NAME OF PERFORMING ORGANIZATION Bioelectromagnetics Res Lab Dept of Rehabilitation Med	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION USAF School of Aerospace Medicine (RZP)	
6c. ADDRESS (City, State and ZIP Code) School of Medicine, University of Washington Seattle, WA 98195		7b. ADDRESS (City, State and ZIP Code) Aerospace Medical Division (AFSC) Brooks Air Force Base, TX 78235-5301	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER F33615-80-C-0612	
8c. ADDRESS (City, State and ZIP Code)		10. SOURCE OF FUNDING NOS.	
		PROGRAM ELEMENT NO. 62202F	TASK NO. 7757
		WORK UNIT NO. 01	71
11. TITLE (Include Security Classification) EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS. VOLUME 9. SUMMARY			
12. PERSONAL AUTHOR(S) Guy, Arthur W.; Chou, Chung-Kwang; Kunz, Lawrence L.; Crowley, John; Krupp, Jerome			
13a. TYPE OF REPORT Final Report	13b. TIME COVERED FROM 1 Jun 80 TO 31 Jul 84	14. DATE OF REPORT (Yr., Mo., Day) 1985 August	15. PAGE COUNT 25
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD 06	GROUP 18	SUB. GR.	RFR Health; Immune response; Neoplasm; 2450 MHz Behavior; Pathology; Longevity; Rats Blood; Metabolism;
19. ABSTRACT (Continue on reverse if necessary and identify by block number) <i>ag cm</i> For 25 months 100 male SPF rats were exposed to pulsed 2450-MHz circularly polarized micro-waves at an average power density of 0.48 mW/cm ² . Another 100 rats served as sham-exposed controls. This report summarizes the results of the eight previous volumes which reported on measurements of 155 parameters. For most of the parameters no statistical difference was found between the exposed and sham-exposed groups. This report discusses a few end-points that were statistically different. KEYWORDS:			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT. <input type="checkbox"/> OTIC USERS <input type="checkbox"/>		21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Jerome H. Krupp, D.V.M.		22b. TELEPHONE NUMBER (Include Area Code) (512) 536-2576	22c. OFFICE SYMBOL USAFSAM/RZP

CONTENTS

	<u>Page</u>
INTRODUCTION	1
EXPERIMENTAL DESIGN	4
Objectives	4
Exposure Criteria	4
Rationale of Biological Assessment	5
Statistical Considerations	6
Schedule of Final Protocol	7
RESULTS OF BIOLOGICAL ASSESSEMENT	8
Behavioral Evaluations	8
Plasma Corticosterone	9
Evaluation of Immune Competence	10
General Health Profile	11
Metabolism	13
Body Mass and Consumption of Food and Water	14
Total-Body Analysis	15
Oxygen Consumption and Carbon Dioxide Production	15
Gross Pathological and Histopathological Evaluation	16
REFERENCES	20

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY
RADIATION EXPOSURE ON RATS

VOLUME 9. SUMMARY

INTRODUCTION

More than 6000 articles have been published about the biological effects of radiofrequency radiation (RFR), but the question of whether long-term low-level exposure to such fields presents a human health hazard remains unanswered (Elder and Cahill, 1984). Most exposure protocols completed to date have been of relatively short duration and restricted sample size, thus providing little insight into cumulative effects.

To provide data derived from long-term experiments, the Bioelectromagnetics Research Laboratory at the University of Washington has conducted, under Air Force sponsorship, the largest single evaluation study of the bioeffects of microwaves yet undertaken. Beginning in September 1978, we devoted two years of effort to facility and equipment design, exposure device construction, protocol development and refinement, and pilot operation.

During September 1980 to October 1982, we conducted a 25-month lifetime exposure of 200 rats. The goal of our project was to investigate purported adverse effects of long-term exposure to pulsed microwave radiation on health. The major emphasis was to expose a large population of experimental animals to microwave radiation throughout their lifetimes and monitor them for cumulative effects on general health and longevity.

As part of this project, we developed a unique exposure facility that enabled 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed individually in circularly polarized waveguides. The exposure facility consisted of two rooms, each containing 50 waveguides

for the active exposure of experimental rats and 50 waveguides for the sham exposure of control subjects. Each room contained two 2450-MHz pulsed microwave generators, all capable of delivering a maximum of 10-W average power at 800 pps with a 10- μ s pulse width. This carrier was square-wave modulated at an 8-Hz rate. The power distribution system delivered 0.144 W to each exposure waveguide, for an average power density of 0.48 mW/cm². Whole-body calorimetry, thermographic analysis, and power meter analysis indicated that these exposure conditions resulted in average specific absorption rates (SARs) ranging from approximately 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

We randomly assigned 200 male rats, obtained at 3 weeks of age from a commercial barrier-reared colony, to exposed and sham-exposed treatment conditions. Exposure began at 8 weeks of age and continued for 25 months. Throughout this period all surviving animals were bled at regular intervals and blood samples were analyzed for a panel of serum chemistries, hematological values, protein electrophoretic patterns, and thyroxine and plasma corticosterone levels. In addition to daily measures of body mass and food and water consumption, oxygen consumption and carbon dioxide production were periodically measured in a subpopulation of the exposed and sham-exposed groups. Activity was assessed in an open-field apparatus at regular intervals throughout the study. After 13 months, 10 rats from each treatment condition were killed for immunological competence testing, whole-body analysis, and gross and histopathological examinations. The surviving 11 rats in the sham-exposed group and 12 rats in the exposed group were killed at the end of 25 months for similar analyses.

The design and results of this study have been published in a series of eight technical reports covering major subtopics (Guy et al., 1983a,b; Chou et al., 1983; Johnson et al., 1983, 1984; Kunz et al., 1983, 1984, 1985). The first volume describes the design, facilities, and procedures. The second volume reports the dosimetric data on humans exposed to 450-MHz RFR, which is chosen to represent a typical midrange Air Force system. Dosimetric data on rats exposed in 2450-MHz circularly polarized waveguides are published in volume 3. Volumes 4-8 report the biological results: volume 4--open-field behavior and corticosterone; volume 5--immune system response; volume 6--hematological, serum chemistry, thyroxine, and protein electrophoresis evaluation; volume 7--metabolism, growth, and

development; and volume 8--longevity, cause of death, and histopathological findings. This volume presents a summary of the previous eight volumes; readers should refer to those volumes for details.

EXPERIMENTAL DESIGN

Objectives

The broad goal of this study was, in a population of experimental animals throughout their natural lifetimes, to simulate the chronic exposure of humans to 450-MHz RFR at an incident power density of 1 mW/cm^2 . One hundred rats were used for the exposed treatment condition, and 100 were sham-exposed as controls. Our primary interest was to investigate possible cumulative effects on general health and longevity.

All possible RFR exposure parameters of interest could never be incorporated in a single study, nor could all desired biological endpoints be assessed. We therefore extensively considered both the exposure parameters and the biological indices of general health.

Exposure Criteria

Our first criterion was to select a test animal and exposure condition to model the human situation for a well-documented experiment on dosimetry. Much of the past work on chronic exposure of large numbers of test animals used anechoic chambers, metal capacitor plates, or resonant cavities. With these methods, the energy coupled to each animal is a function of the group size, group orientation, and the orientation of each animal within the group, as well as of the presence and orientation of water and food dispensers. Also, estimates of exposure absorption rate are uncertain, and extrapolation of biological results from animals to humans is virtually impossible. In addition, the cost in time and resources for even simple experiments involving chronic exposures of animal populations in large anechoic chambers is prohibitive.

For this study we chose a system of cylindrical wiremesh waveguides for exposing a population of animals to a common source while independently maintaining relatively constant and quantifiable EM power coupling to each animal regardless of position, posture, or movement. The system,

consisting of a number of independent waveguides, allows each animal to be continuously exposed, while unrestrained and living under normal laboratory conditions with access to food and water.

So that the rat would have approximately the same size-to-wavelength ratio as a human exposed to 450 MHz, we selected an exposure frequency of 2450 MHz. Our initial consideration was to produce the same average SAR in test animals as predicted for man exposed to 1-mW/cm² 450-MHz RF fields. Based on our previous experience with the 2450-MHz circular waveguide exposure system, we estimated that an average power density level of approximately 0.50 mW/cm² for the rat exposure would result in an average SAR equivalent to that for the human exposure at the lower frequency.

Our secondary criterion for the exposure parameters was the modulation frequency and its effects. In addition to using pulse modulation (10- μ s pulse, 800 pps), we decided to square-wave modulate the microwave power. The inclusion of this square-wave modulation was prompted by the Ca⁺⁺ efflux increase observed in chick and cat brains reported in the literature. Since the demonstrated effects are most pronounced when the modulation frequencies correspond to the dominant EEG frequency, we selected a modulation frequency of 8 Hz because it is near the dominant EEG frequency of rats.

More detailed discussions of the exposure units, power generation, and distribution network are presented in volume 1 of this series of reports. Dosimetry data on humans and rats exposed to microwaves are in volumes 2 and 3.

Rationale of Biological Assessment

We not only selected reported biological effects from low-level microwaves as endpoints (e.g., alterations of hematopoietic, immunologic, and specific blood chemistry indices), but also included possible cumulative effects on general health, metabolism, and lifespan. We also considered which endpoints could be assessed without seriously compromising the health of the animal, the value of concurrent measurements, or the power of the statistical evaluations of the chosen endpoints. Consultation with researchers within the community concerned with the bioeffects of

microwaves and the scientific community at large tempered the final protocol. We finally selected the following endpoints: 1) behavior--open field activity, 4 parameters; 2) corticosterone level, 1 parameter; 3) immunology--4 assays, 10 parameters; 4) hematology, 11 parameters; 5) blood chemistry, 21 parameters; 6) protein electrophoresis, 4 parameters; 7) thyroxine, 1 parameter; 8) urinalysis, 1 parameter; 9) metabolism, 7 parameters; 10) total-body analysis, 39 parameters; 11) organ weight, 9 parameters; 12) histopathology, 46 tissues and organs; 13) longevity, 1 parameter. A total of 155 parameters was studied. Details of the rationale of the biological assessment are described in volume 1.

Statistical Considerations

For any failure-time endpoint, such as time to death, time to cancer diagnosis, or time to some specified change in animal weight or blood chemistries, we calculated an initial sample size of 100 in each group to be sufficient for detection, at the .05 significance level, of a 50% increase (or 33% reduction) in instantaneous failure rate with a probability (power) of 90%. For any normally distributed endpoint (including transformations on failure-time variables), a sample size of 100 per group permits the detection, at the .05 level of significance, of a difference between groups of 40% of one standard deviation, with power 90%. Adjustment for a differential effect due to the altered experimental procedure for the 36 rats subjected to metabolic rate measurements has very little effect on the power calculations made, nor does adjustment for an interim kill of 20 animals.

Differences between groups on single measurements were assessed by t-tests, in some cases after transformation to improve the approximate normality of the data. Logical groupings of variables were compared across groups using the multivariate Hotelling's T^2 statistic. Differences in tumor prevalence or incidence were assessed using time-adjusted analysis, as described more fully later. Reported p-values must be considered in the light of the multiple endpoints analyzed.

Schedule of Final Protocol

During the first year of the chronic exposure period, the rats were bled every 6 weeks, with the first bleeding during the seventh week of exposure. In addition to the hematological and serum chemistry evaluation of the blood collected during the first bleeding, corticosterone levels were determined on all samples having adequate amounts of serum. In subsequent bleedings, corticosterone and thyroxine levels were determined only quarterly, whereas the hematology and serum chemistry were evaluated for each sample (every 6 weeks). We considered this frequency of bleeding sufficient to detect the onset of most degenerative or disease states that would occur during the lives of the individual rats without stressing the animals unduly. Every 3 months a urinalysis was done on all rats, the first during the fourth week of exposure. This frequency of biochemical evaluation increased the opportunity to detect subclinical abnormalities and follow their pathophysiological course. Open-field assessment was conducted every 6 weeks.

During the second year of the study, we reduced the frequency of bleeding to once every 12 weeks, eliminated corticosterone analysis except just prior to the final sacrifice at the end of the 2 years, did a partial urinalysis every 2 weeks, and conducted open-field analysis quarterly.

RESULTS OF BIOLOGICAL ASSESSMENT

Behavioral Evaluations

Behavioral testing is a valuable tool for assessing the possible bioeffects of microwaves. Many constraints of both design and logistics, however, made selection of appropriate tests for this project a difficult task. Tests should not jeopardize the health of the animals or the reliability of data obtained from other measures. A test protocol must not entail differential treatment of an animal based on its performance (e.g., shock density or reward magnitude) and thereby produce secondary effects as artifacts that must be distinguished from any primary (microwave) bioeffects. In addition, all testing must be performed within the SPF environment and in such a manner as not to interfere with the normal daily maintenance procedures or exposure protocols.

The risk of physical harm to the animals eliminated many standard behavioral tests, so we chose to use a simple behavioral test based on quantification of naturally occurring behavior. Open-field or exploratory behavior has long been used as a sensitive endpoint in pharmacology and teratology and is accepted as a measure of general arousal or anxiety. In addition, East European researchers have used the open-field test extensively in studies of the bioeffects of microwaves.

The open-field test is not the most impressive of the behavioral test procedures considered; but it is simple in nature, does not rely on elaborate or time-consuming training procedures or shock-motivated performance, and can be routinely administered by laboratory personnel under the rigid SPF protocol.

We selected an open-field apparatus with an infrared-light-emitting sensing schema. This apparatus provided a readout of both activity/motion within the field and of position along the x-y axis of the field. The latter information was used to indicate an animal's field position in one of the four possible quadrants. The data collected for each animal included a general activity count; i.e., beam interruptions, the number of quadrants entered, their spatial distribution, and the number of moves

between quadrants. In addition, at the end of each test session the field was inspected for signs of urination and defecation.

Analysis of the data from the 14 open-field assessments supports the conclusion that, except for the first test session, 2 years of exposure to low-level pulsed-microwave radiation did not lead to significant alterations in behavior as measured by activity, defecation, or urination. During the first test session, the general activity level of the exposed animals was significantly lower, by approximately 9%, than that of the sham-exposed animals.

The open-field activity pattern during the course of this study resembles that normally observed as a function of age/experience and apparently was not affected by a lifetime of low-level pulsed-microwave exposure.

Plasma Corticosterone

Pituitary-adrenal axis activity as indexed by plasma corticosterone levels has long been interpreted as an indicator of general arousal, i.e., anxiety, fear, or stress. If cumulative biological effects of long-term exposure to pulsed RFR disrupt normal physiological functions or are psychologically disturbing to the animal, we may expect to see such effects mirrored in an increased basal level of corticosterone. The functioning of the endocrine system could provide for summation of multiple, otherwise subthreshold, effects. Individual corticosterone data are of value for correlation with results from individual animals or subpopulations exhibiting possibly abnormal blood chemistry indices or high rates of tumor incidence, and also as a measure of some possible nonspecific microwave bioeffect.

Analysis of the data obtained during the five sampling periods indicates that serum corticosterone levels were not dramatically altered in either the exposed or sham-exposed groups. The multivariate statistical analyses of the data indicate that no overall cumulative effects of microwave radiation were measurable by levels of serum corticosterone.

When the serum corticosterone values of the exposed and sham-exposed groups were compared for each session, the t-test analysis indicated that

the exposed animals may have had elevated serum corticosterone values at the time of the first sampling session and the sham-exposed animals may have had elevated levels at the time of the third session. The exposed and sham-exposed groups had comparable levels of corticosterone on all other regular sampling sessions.

Evaluation of Immune Competence

Alterations in the immune system due to microwave exposure have been reported in the literature. The conflicting nature of the work to date compelled including an assay of immunocompetence in our study of long-term low-level RFR effects.

The immune-system evaluation consisted of several basic tests designed to detect profound immunological effects resulting from exposure to RFR:

- 1) Blood lymphocyte evaluation with respect to numbers of B- and T-cell antigen-positive lymphocytes and complement receptor-bearing lymphocytes.
- 2) Spleen lymphocyte evaluation for response to the following mitogens: phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and purified protein derivative of tuberculin (PPD).
- 3) Direct plaque-forming cell assay (with spleen cells) and serum antibody titration of exposed rats immunized with the T-dependent antigen sheep red blood cells (SRBC).

The following immunological tests were performed coincidentally with the interim killing of 10 animals from each treatment group and, after 25 months of exposure, with the terminal kill of 10 animals from each group: response of splenic lymphocytes to various mitogens, plaque-forming ability, complement-receptor formation, and enumeration of B- and T-cells.

When compared with the sham-exposed group, after 13 months of RFR exposure the exposed animals had a significant increase in both splenic B- and T-cells. This apparent general stimulation of the lymphoid system in exposed animals was not detected in the animals evaluated after 25 months of RFR exposure: Comparison of the exposed and sham-exposed rats in the terminal kills did not reveal any significant differences in the percentage

or total numbers of B- and T-cells per spleen. The lack of a significant difference in the terminal-kill animals may be the result of age and the onset of immunosenescence.

No significant differences were seen between the exposed and sham-exposed rats in the percentage of complement-receptor-positive cells in the spleen at either the interim or terminal kills (after 13 or 25 months of exposure). This finding indicates no difference between the treatment groups in the maturation of lymphocytes as indicated by this procedure.

The plaque assay performed on animals immunized with SRBC in the 13-month exposure group revealed a slight but statistically insignificant increase in plaques per spleen for the exposed animals relative to the sham exposed. This trend appeared reversed in the 25-month exposure group where the exposed animals, compared with the sham exposed, showed a slight but statistically insignificant decrease in plaques per spleen. This assay indicated no statistically significant alteration of the reticuloendothelial system, which first processes the SRBC antigen, and no deficiency in the B-cells' ability to produce antibodies in the presence of T-cells, as the SRBC antigen is T-cell dependent.

The mitogen-stimulation studies following 13 months of exposure revealed a significant difference between groups in their responses to various B- and T-cell specific mitogens. The RFR-exposed animals had a nonsignificant increase in response to PHA and a significant increase in response to LPS and PWM. As compared to the sham-exposed animals, the exposed animals also had a significantly increased response to ConA and a decreased response to PPD ($p = .01$). These results suggest a selective effect of RFR on the response of the lymphoreticular system to mitogen stimulation. Mitogen response data was not available from the 25-month exposure studies because the lymphocyte cultures failed to grow.

General Health Profile

In this study we investigated purported adverse effects on health after long-term exposure to pulsed microwave radiation. In an attempt to detect and document any cumulative effects on general health and longevity of the exposed animal population, we monitored several biochemical and

hematological parameters: serum chemistry components, hematological constituents, protein electrophoretic patterns and fractions, and thyroxine levels. Two hundred Sprague-Dawley rats, divided equally into exposed and sham-exposed groups, were sampled for blood every 6 weeks for the first year and then every 12 weeks until the project was terminated after 25 months of exposure.

Eleven hematological parameters, indices, and absolute cell counts for the leukocytes were analyzed statistically. Multivariate analyses with Hotelling's T^2 statistic on a truncated data set indicated no overall difference between the exposed and sham-exposed populations. Individual t-test comparisons for all parameters for each of the 15 sampling sessions indicated a significant reduction in the absolute eosinophil count for the exposed population during session 2 and marginally significant reductions in absolute neutrophil count during sessions 2 and 3. None of the other individual comparisons was significant. These findings indicate that, despite the 25-month duration of exposure, no detectable effects were produced in the bone marrow erythropoietic cells or in the juxtaglomerular apparatus of the kidney and its production of erythropoietin.

Twenty-one serum chemical constituents were measured in serum samples collected from all 15 sampling sessions. The serum chemistry tests were sensitive enough to detect population changes due to aging. Statistical analysis of the data by Student's t-test did not indicate any differences between the exposed and sham-exposed groups.

Electrophoresis of the serum proteins revealed no significant changes in the electrophoretic patterns and absolute protein fractions between the population groups. Both groups showed a gradual decrease in the albumin/globulin ratio with increasing age, and the overall level of globulin fractions observed in these barrier-sustained animals was lower than reported from conventional-colony animals. The RFR exposure had no apparent effect on the functioning of various organ systems contributing to serum protein concentrations.

Thyroxine levels did not differ significantly between the exposed and sham-exposed animals. Thus, RFR exposure had no effect on the entire hypothalamic-pituitary-thyroid feedback mechanism. The absolute level of serum thyroxine developed to a maximum in young animals and decreased gradually as they aged. The correlation of this age-related decrease in

thyroxine levels with increasing cholesterol and triglyceride levels shows it to be a reliable indicator of metabolic activity in the rat.

The major conclusion that can be reached from the evaluations of the hematology, serum chemistry, protein electrophoretic patterns and fractions, and thyroxine levels is that any significant alterations of these parameters seen during the lifetime of the exposed animals were to be expected with age and were not due to exposure to pulsed microwave radiation.

Metabolism

The actual ground for possible concern for the long-term exposure of rats was that the nominal 0.4-W/kg average SAR value used throughout the chronic exposure period is about 5% of the average metabolic rate of any active, young 200-g rat and about 10% of its resting rate. This SAR may be as high as 15% of the average rate of a lethargic, old 600-g rat and 25% of its resting rate.

We felt that exposure to microwave radiation for long periods at the levels used for this project could have different consequences for longevity, either life shortening or life lengthening, depending on the energy-budgeting option chosen. Therefore, given the importance of the metabolic- vs extrinsic-energy-budget question, the protocol provided for taking the following measurements of the animals:

- 1) Daily/lifetime body mass measures, i.e., growth
- 2) Daily/lifetime food and water consumption
- 3) 24-h cycles of oxygen consumption and carbon dioxide production, measured at regular intervals throughout the lifespan
- 4) Periodic assessment of thyroxine level
- 5) Periodic assessment of urine production and semiquantitative analysis
- 6) Total-body analysis upon spontaneous death or termination

Despite the importance of direct metabolic measurements through respiratory gas exchange analysis, two factors precluded their application to all 200 animals in the study: (1) physical as well as financial restraints made it impossible to instrument all 200 waveguides, and (2) to

rotate all animals through a few instrumented waveguides would have an associated risk of mismanagement of animal transfers and a subsequent loss of data. In addition, were such a mass rotation attempted, the need to allow each animal a minimum of 2 days in the instrumented waveguide to adapt to the new environment would have led to a rotation schedule allowing data to be obtained from an animal twice a year at most, which would have been too infrequent.

Therefore, we selected only a subgroup of the exposed and control populations for rotation through waveguides specially adapted for the measurement of oxygen consumption and carbon dioxide production. This procedure did not result in any loss of overall statistical power and produced more frequent measures on the specific animals involved. Given the modular arrangement of the rooms, 36 animals (18 exposed and 18 sham exposed) were measured for respiratory gas exchange.

Body Mass and Consumption of Food and Water

Growth curves for microwave and sham-exposed animals through this study are in general agreement with those reported for the Sprague-Dawley rat. The asymptotic body mass approach may have been somewhat lower than expected, possibly owing to the periodic "stunting" effect coincident with the start of the regular blood-sampling sessions.

The average daily food intake of approximately 25-26 g is higher than usually reported for the rat and indicated by the feed manufacturer (12-15 g/day). These "normal" values, however, are for animals housed in a standard animal facility maintained at a higher ambient temperature (25°C). The amount of food eaten by the animals in our facility is in consonance with that reported for animals housed at lower ambient temperatures and in our previous studies that used the waveguide apparatus.

The similarity in overall patterns of growth, food and water consumption, and body-mass loss and recovery for the exposed and sham-exposed populations indicates that no cumulative effects of microwave energy absorption were apparent at this level of exposure and with these measures of long-term energy balance.

Total-Body Analysis

With one exception, the combined analyses of organ mass, general carcass composition, fatty acid profile, and mineral content provided no evidence that metabolic processes were irreversibly altered in the animals exposed for 13 or 25 months to microwave radiation. A highly significant elevation of adrenal mass was indicated by the 75% increase observed for the exposed rats compared to the sham exposed. However, when the animals with benign tumors in the adrenal gland were separated from those without tumors, the difference became insignificant. For animals with tumors, the adrenal weight was significantly higher in the exposed group than in the sham group. This analysis indicated that the increased adrenal weight was related to the tumors and irrelevant to the metabolic processes in the rats. The mean adrenal mass in the exposed animals without tumors was slightly heavier, but statistically insignificant, as compared with those of the sham group. This increase in weight was attributed to one animal with a hyperplastic adrenal cortex that was secondary to a pituitary tumor.

Oxygen Consumption and Carbon Dioxide Production

We attempted to measure the metabolism of the rat maintained under standard waveguide cage conditions, spanning the circadian cycle of light/dark photoperiod, food consumption, and physical activity. Our measures of oxygen consumption and carbon dioxide production at times approximate "basal," or resting, levels. At other times during the day/night cycle, however, our measures are considerably higher than those often reported and are in the range consistent with values for active or exercising animals or for animals maintained at low ambient temperatures.

Differences between the exposed and sham-exposed treatment conditions occurred in oxygen consumption, carbon dioxide production, and metabolic quotient for the young but not for the mature animals.

The effects observed in the young animals were less pronounced during the second round of measurements. On an hour-to-hour basis, the mature animals' metabolic measures appear less variable than those of the young. The young animals demonstrate more marked responses to the lights-off

condition and generally higher levels on each measure during the nighttime hours, i.e., the active portion of the rats' circadian cycle. This apparent synchronization of metabolic activity with the light/dark cycle has been noted by others investigating the variation of activity, food/water consumption, and energy balance patterns as a function of photoperiod.

Gross Pathological and Histopathological Evaluation

The nature of this experiment suggested that an extensive histopathological examination of the animals be completed for detection and classification of all possible morphological lesions and as a help in providing a definitive diagnosis for any organ system abnormalities found. Evaluation of sporadically occurring pathologic lesions in aging rats may help explain abnormal results of biochemical tests. We deemed that documenting the onset of neoplastic and age-associated lesions was important for detecting any differences between the age of onset and frequency of occurrence of the lesions between the control and exposed animals. We organized and analyzed the pathological data to compare survival curves, age-associated neoplastic and nonneoplastic lesions, incidence of tumor metastases, and multiple lesions for each rat in the exposed and control groups.

As part of a general health screen at the time of animal procurement, 10 rats, 21 days old, received gross and histopathological examination. After 13 months, 10 exposed and 10 sham-exposed controls were randomly selected and killed for examination; at 25 months, the surviving 12 exposed rats and 11 sham-exposed rats were killed and examined. In addition to these 43 animals, 157 animals were examined when they died spontaneously or were terminated in extremis during the study.

The pathology consultant provided animal-evaluation data to the Bioelectromagnetics Research Laboratory, which was responsible for computer entry and quality control. The statisticians then evaluated the data, and the final results were reviewed by the pathologist for appropriate interpretative comments.

The occurrence of nonneoplastic and neoplastic lesions was recorded along with the age of the animal and whether the animal was sacrificed

or had died spontaneously, also the cause of death for each animal. The pathological data were collected to compare exposed and sham-exposed groups' survival curves, age-associated lesions, incidence of tumor metastasis, and the occurrence of multiple lesions per rat.

Product-limit estimates and the log-rank statistic were used, respectively, to estimate and compare cumulative survival curves for the exposed and sham-exposed animals. The histopathology data were grouped with respect to the age, at 6-month intervals, and the data were divided into neoplastic and nonneoplastic diagnoses. The incidence of neoplastic or nonneoplastic lesions is given as the proportion of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals examined pathologically (denominator). For tissues that required gross observation for detection of lesions (i.e., skin or subcutaneous tumors), for lesions that appeared at several sites (i.e., multiple lymphomas), or for tissues that were examined histologically only when lesions were detected grossly, the denominator consisted of the number of animals necropsied in that experimental group.

The analysis of the lesions involved a 4-way table with factors of age at death, treatment condition, mode of death (terminated or spontaneous), and organ. The tables were then collapsed with respect to individual organs. From these tables the Mantel-Haenszel estimate of the odds ratio was computed, and the Chi-square statistic was used to test whether or not the odds ratio was significantly different from 1. This statistic reflects the difference in prevalence of lesions, over time, between the exposed and sham-exposed animals. For malignant lesions, time-to-tumor was also analyzed using time-to-death-with-tumor as a surrogate. If no malignant lesions were present, the time-to-tumor was considered censored. The log-rank statistic was used to compare these times-to-tumor of the exposed animals with those of the sham-exposed animals.

Evaluation of the cumulative survival curves for both the exposed and sham-exposed animals revealed that the median survival time was 688 days for the exposed animals and 663 days for the sham exposed. Despite subtle differences in the survival curves in the early and late stages of the study, statistical analysis indicated that no significant effect existed during any phase of the lifespan of the animals.

Statistical evaluation indicated no association between a specific cause of death and the treatment condition; however, for cause of death due to urinary tract blockage, there is some indication that the survival times are longer in the exposed animals.

The documentation of morphological lesions showed 2184 pathological changes in the 200 animals examined. The nonneoplastic lesions comprised 1992 of the observed changes, with 217 unique combinations of organs and lesions. The neoplastic lesions accounted for 192 of the observations, with 83 unique combinations of organs and type of neoplasms.

Chronic glomerulonephropathy was the most frequent cause of death and one of the most consistently encountered lesions. Statistical analysis indicated that glomerulonephropathy was less frequently observed in the exposed than in the sham-exposed animals. Analysis of the other non-neoplastic lesions did not indicate that the specific lesions were more likely in either treatment condition.

To detect a progressive development of the chronic glomerulonephropathy, the severity of the lesions also was evaluated. This analysis revealed no significant differences between the treatment condition and the severity of the lesions.

A total of 85 animals had neoplastic lesions; 45 were exposed and 40 were sham-exposed. Statistical evaluation revealed no significant difference in the incidence of neoplasia between the exposed and sham-exposed groups (Chi-square = 0.32).

The neoplastic lesions were identified as benign or malignant, with the malignant lesions classified as primary or metastatic. The incidence of neoplastic lesions corresponds to that reported for this strain of rat; only two tumors were present in rats younger than 12 months, and the incidence rapidly increased after 18 months of age. The endocrine system had the highest incidence of neoplasia in the aging rats, as is to be expected in this experimental animal.

Only 8 rats had primary malignant tumors without any benign tumors present: 6 were from the exposed group and 2 from the sham-exposed group.

The low incidence of neoplasia with no increase in any specific organ or tissue required the data to be collapsed and evaluated with respect only to occurrence of the neoplasm, with no attention given to the area of occurrence. This analysis indicated that neither group had an excess of

benign lesions, also no statistical difference in total neoplastic lesions. There is statistical evidence that the number of primary malignancies was higher in the exposed animals than in the sham exposed, but the biological significance of this difference is reduced by several factors. First, detection of this difference required the collapsing of sparse data without regard for the specific type of malignancy or tissue of origin. Also, when the incidence of the specific primary malignancies in the exposed animals is compared with specific tumor incidence reported in the literature, our exposed animals had an incidence similar to that of untreated control rats of the same strain and maintained under similar SPF conditions. It is important to note that no single type of primary malignancy was enhanced in the exposed animals. From the standpoint of carcinogenesis and under the assumption that the initiation process is similar for both benign and malignant tumors, benign neoplasms have considerable significance. The fact that treatment groups showed no difference in benign tumor incidence is an important element in defining the promotion and induction potential of microwave radiation for carcinogenesis. The collapsing of sparse data without regard for tissue origin is useful in detecting possible statistical trends, and the finding here of a relative increase in primary malignancies in the exposed animals is provocative; however, when this single finding is considered in the light of other parameters evaluated, it is questionable if the statistical difference reflects a true biological activity. No meaningful statistical analysis could be made of metastatic neoplasms because of their low incidence.

To standardize the experimental animals as much as possible, we housed the exposed and sham-exposed animals under identical conditions and subjected them to identical diet, handling, husbandry, lighting, air change, and sample-collection procedures. We also monitored the animals for any parasitic, bacterial, mycoplasmal, or viral agents during the 25-month experimental period. No significant infections occurred that would complicate or produce erroneous results in the gross or histopathological evaluation of the experimental animals.

In summary, no defensible trends in altered longevity, cause of death, or spontaneous aging lesions and neoplasia can be identified in the rats exposed to this long-term low-level radiofrequency radiation exposure.

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