Long-Term, Low-Level Microwave Irradiation of Rats

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Our goal was to investigate effects of long-term exposure to pulsed microwave radiation. The major emphasis was to expose a large sample of experimental animals throughout their lifetimes and to monitor them for effects on general health and longevity.

An exposure facility was developed 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 active waveguides and 50 waveguides for sham (control) exposures. The experimental rats were exposed to 2,450-MHz pulsed microwaves at 800 pps with a 10-µs pulse width. The pulsed microwaves were square-wave modulated at 8-Hz. Whole body calorimetry, thermographic analysis, and power-meter analysis indicated that microwaves delivered at 0.144 W to each exposure waveguide resulted in an average specific absorption rate (SAR) that ranged from 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

Two hundred male, Sprague-Dawley rats were assigned in equal numbers to radiation-exposure and sham-exposure conditions. Exposure began at 8 weeks of age and continued daily, 21.5 h/day, for 25 months. Animals were bled at regular intervals and blood samples were analyzed for serum chemistries, hematological values, protein electrophoretic patterns, thyroxine, and plasma corticosterone levels. In addition to daily measures of body mass, food and water consumption by all animals, O_2 consumption and CO_2 production were periodically measured in a sub-sample (N=18) of each group. Activity was assessed in an open-field apparatus at regular intervals throughout the study. After 13 months, 10 rats from each group were euthanatized to test for immunological competence and to permit whole-body analysis, as well as gross and histopathological examinations. At the end of 25 months, the survivors (11 sham-exposed and 12 radiation-exposed rats) were euthanatized for similar analyses. The other 157 animals were examined histopathologically when they died spontaneously or were terminated *in extremis*.

Statistical analyses by parametric and non-parametric tests of 155 parameters were negative overall for effects on general health, longevity, cause of death, or lesions associated with aging and benign neoplasia. Positive findings of effects on corticosterone level and immune system at 13 months exposure were not confirmed in a follow-up study of 20 exposed and 20 control rats. Differences in O_2 consumption and CO_2 production were found in young rats. A statistically significant increase of primary malignancies in exposed rats vs. incidence in controls is a provocative finding, but the biological significance of this effect in the absence of truncated longevity is conjectural. The positive findings need independent experimental evaluation. Overall, the results indicate that there were no definitive biological effects in rats chronically exposed to RF radiation at 2,450 MHz.
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INTRODUCTION

Advances in dosimetry, and a better understanding of energy absorption by biological tissues, have eliminated many concerns regarding effects of radio-frequency electromagnetic radiation [Tyler, 1975; Elder and Cahill, 1984; NCRP, 1986; Polk and Postow, 1986; Lin, 1989; Gandhi, 1990]. Despite this lessening of concern for low-level, acute exposures, lack of data on long-term, low-level radiation has fueled public and scientific concerns. In this context, officials of the United States Air Force sought to support research in this area to provide data for use in the development of environmental impact studies for present and planned Air Force systems.

The goal of the project was to investigate effects on health of long-term exposure to low-level, pulsed microwave radiation. The approach was to expose a large population of experimental animals to microwave radiation throughout most of their lifetimes and to monitor them for effects on general health and longevity.

Although the initial impetus for the study was the question of environmental impact of the Air Force PAVE PAWS system, early on it was decided not to study a replica of the PAVE PAWS emissions, but to create a generalized level of radiation that would provide whole-body exposure based on the maximum of permissible absorption [ANSI C95.1-1982, 1983; IEEE C95.1–1991,1992] at the resonant frequency in human beings (0.4 W/kg), as scaled to the proportions of the experimental animal of choice.

Following a period of pilot studies and training of technicians, exposures to microwaves commenced on September 1, 1980, and concluded September 27, 1982. The 100 experimental and 100 sham-exposed animals underwent the longest near-continuous exposure ever completed. The findings were reported in a series of 9 Air Force technical reports, which are available through the National Technical Information Service (Springfield, Virginia). Interested readers should refer to the technical reports for details [Guy et al., 1983a, b, 1985; Chou et al, 1983; Johnson et al., 1983, 1984; Kunz et al., 1983, 1984, 1985].

METHODOLOGY

Experimental Design

Exposure criteria. Much of the past work on chronic exposure of large numbers of text animals has been based on 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 location of water and food dispensers. Because estimates of energy absorption are uncertain, quantitative extrapolation of biological results from laboratory animals to human beings is virtually impossible. In addition, the cost in time and resources of even simple experiments involving chronic exposures of animal populations in large anechoic chambers is prohibitive.

For this study, we chose a system of cylindrical, wire-mesh waveguides to expose a large number of animals to a common source while independently maintaining relatively constant and quantifiable coupling of electromagnetic energy to each animal regardless of position, posture, or movement [Guy and Chou, 1977; Guy et al., 1979]. The system, which consists of a number of independent waveguides, allows individual animals to be continuously exposed under normal laboratory conditions while living unrestrained and with continuous access to food and water.

A frequency of 2450 MHz was selected so each rat would have approximately the same size-to-wavelength ratio as a human being exposed at 450 MHz, the frequency near which PAVE PAWS operates. The initial consideration was to produce the same average SAR in test animals as predicted for man exposed to a 1-mW/cm^2, 450-MHz RF field. To simulate radar exposure, pulse modulation was used (10 μs pulse, 800 pps). In addition to the pulse modulation, we decided to square-wave modulate the microwaves. The inclusion of square-wave modulation was prompted by the evidence of altered movement of Ca^{++} ions in chicken and cat brains exposed to ELF-modulated RF fields [Adey, 1981]. Because 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 at the peak of the rat's hippocampal theta rhythm [Coenen, 1975].

Rationale of biological assessment. Not only were reported biological effects from low-level microwaves selected as end points (e.g., alterations of hematopoietic, immunologic, and specific blood chemistry indices), but assays for effects on general health, metabolism, and life span were also included (references listed in later sections). In addition, end points were considered that could be assessed without seriously compromising the health of the animal, the value of concurrent measurements, or the power of the statistical evaluations on the chosen end points. Only male rats were used to minimize statistical variation, i.e., to avoid the hormonal variations characteristic of female rats. Use of female rats would have required a substantial increase in the number of animals. A total of 155 parameters was studied. The end points selected are shown in Table 1. Due to space limitations, details of rationale and methods of biological assessments cannot be provided here. The original NTIS reports should be consulted.

Statistical considerations. For any failure-time end point, such as time to death, time to cancer diagnosis, or time to some specified change in animal mass or blood chemistry, an initial sample size of 100 in each group was calculated 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 end point (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 a power of 90%. Adjustment for a differential effect due to the altered experimental procedure for

TABLE 1. Endpoints Selected to Study the Effects of Long-Term, Low-Level RF Exposure on Rats

Category	Parameters	No.
Behavior	Open field behavior (activity, quadrant change, urination, defecation)	4
Corticosterone	Serum corticosterone	1
Immunology	Mitogen stimulation (PHA,LPS,ConA,PWM,PPD), B-cell, T-cell, %CRPC, total CRPC, plaque	10
Hematology	WBC, RBC, HCT, Hgb, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophil, monocytes	11
Blood chemistry	Glucose, BUN, creatinine, Na, K, Cl, CO_2, uric acid, total bilirubin, direct bilirubin, Ca, phosphorus, alkaline phosphatase, LDH, SGOT, SGPT, cholesterol, triglycerides, total protein, albumin, globulin	21
Protein electrophoresis	Albumin fraction, alpha-1 and 2 fractions, beta fraction, gamma fraction	4
Thyroxine	Thyroxine	1
Urinalysis	Urine observation	1
Metabolism	Body mass, food consumption, water consumption, O_2 consumption, CO_2 production, respiratory quotient, metabolism quotient	7
Total body analysis	Body mass, moisture, protein nitrogen, crude fat, nonprotein nitrogen, total ash, mineral contents (aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, phosphate, potassium, selenium, silver, sodium, strontium, tin, titanium, vanadium), fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic)	39
Organ mass	Heart, brain, liver, kidneys, testicles, adrenals	9
Histopathology	All tissues and organs	46
Longevity	Survival days	1
Total		155

the subset of 36 rats subjected to metabolic rate measurements had very little effect on the power calculations made, nor did adjustment for an interim euthanasia of 20 animals.

Differences between the two groups on single measurements were assessed by Student's t tests, in some cases after transformation to improve the normality of the data. Reported P values must be considered in the light of the multiple end points analyzed. Logical groupings of variables were compared across groups of the multivariate Hotelling's T^2 statistic.

Differences in tumor prevalence or incidence were assessed with time-adjusted analyses. The occurrence of neoplastic and non-neoplastic lesions was recorded along with the age of the animal and whether the animal had died spontaneously or was euthanatized. Survival curves of the exposed and sham-exposed animals were estimated by product-limit estimates [Kaplan and Meier, 1958] and compared by the log-rank statistic [Mantel, 1966]. The histopathological data were grouped with respect to age, at 6-month intervals, and the data were divided into neoplastic and non-neoplastic diagnoses. The incidence of neoplastic or non-neoplastic lesions was 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 the odds ratio was significantly different from unity [Mantel and Haenszel, 1959]. This statistic reflects the difference in prevalence of lesions, over time, between the exposed and sham-exposed animals, and is appropriate if the lesions are "incidental" (do not affect the animal's survival). The time to a malignant lesion was also analyzed with survival-analysis techniques, as would be appropriate if lesions were fatal. If an animal had malignant lesions, its time-to-tumor was taken as its survival time. If there were no malignant lesions present, the time-to-tumor was considered censored (i.e., the time to appearance of a tumor is assumed to be longer than the time to death). The log-rank statistic was used to compare the times to tumor of the exposed animals with those of the sham-exposed animals [McKnight and Crowley, 1984].

Final protocol. During the first year of the study, the rats were bled from the orbital artery every 6 weeks, with the first bleeding during the 7th week of exposure. In addition to the hematological and serum-chemistry evaluation of blood collected during the first bleeding, corticosterone levels were determined in 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). This frequency of bleeding was considered sufficient to detect the onset of most degenerative or disease states that would occur during the lives of the individual rats without unduly stressing the animals. Every 3 months a urinalysis was done on all rats, the first during the fourth week of exposure. This frequency of biochemical evaluations increased the opportunity to detect subclinical abnormalities and to follow their pathophysiological course. Open-field assessment was conducted every 6 weeks.

During the second year of the study, the frequency of bleeding was reduced to 12-week intervals, and the corticosterone analysis was eliminated except just prior to euthanasia of remaining animals at the end of the 2 years; urinalysis was done every 2 weeks, and open-field analysis was conducted quarterly.

Facilities

Animal facility. To maintain the colony of rats used in this study in the healthiest possible state, free of chronic disease and other problems common to rats, two specific-pathogen-free (SPF) rooms in the Division of Animal Medicine were acquired (Fig. 1). Access to the clean hall is via a shower room, through which all personnel must pass to shower and don autoclaved garments. A walk-in autoclave connected the cage-washing facility with the clean hallway so that, once washed, all materials entering the clean hall must have passed through autoclaving before being returned to the animal rooms. All soiled cages and waste collectors left the clean rooms via the dirty hallway and were then taken to the cage-washing facility.

Each alcove housed 20 waveguides mounted on four horizontal shelves, five waveguides per shelf. The exposure and sham-exposure waveguides were randomly
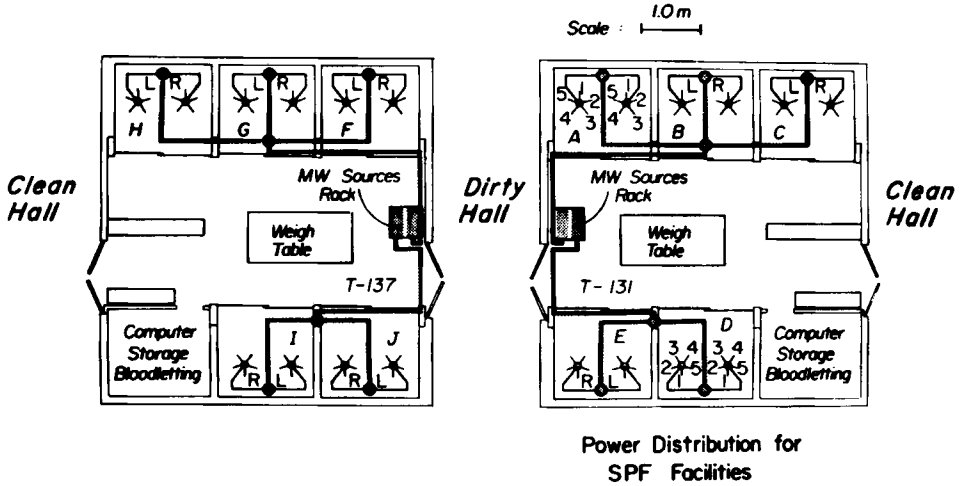
Fig 1. Top view of exposure rooms (T-131 and T-137) with alcove designations and associated exposure-cell identification system.

arranged, except that only sham-exposure waveguides could be in the center position because of a sliding-glass-door operation. One of the alcoves in each room was equipped as a metabolism alcove, in which O_2 consumption and CO_2 production were measured. The sixth alcove in each room was partitioned off as a procedures area that was used for bleeding and as housing for the main data-collection computer and miscellaneous supplies.

In the SPF rooms the airflow rate was programmed for 22 exchanges each hour, to maintain positive-pressure flow. Over the course of the project, ambient temperatures were balanced between the workspaces and alcoves to maintain a fairly constant $21\pm1°C$ environment in the facility. Humidity was in the range of 30–70%. Sound-pressure measurements indicated an average level in the central workspace of approximately 60 dBA (relative to 20 $\mu N/cm^2$) and alcove levels that were 6 to 10 dBA lower, depending on position within the alcove. Light-intensity measurements during the light cycle (0700–1900) indicated a 13-lux average workspace level and 6-lux average alcove level.

Microwave exposure system. As shown in Figure 2, when an animal housed in a plastic cage was exposed in the circular waveguide to microwaves fed into the terminal (P_{IN}), some energy (P_A) was absorbed by the animal, some (P_W) was absorbed by the walls of the chamber, some was reflected in the form of both right-hand (P_{RR}) and left-hand (P_{RL}) circularly polarized waves that couple back to the probes on the feed section of the waveguide, and some (P_{TA} and P_{TB}) were absorbed at the termination terminals. The reflected component P_{RR} was measured as CP_{RR} at the reflecting arm of the bidirectional coupler, which was placed between the source and the input probe (C is the coupling coefficient of the bidirectional coupler). The reflected component P_{RL} was measured directly at the other terminal of the transmitting transducer. The power level of the incident energy launching the right-hand circularly polarized waves was measured (as CP_{IN}) at the incident-wave terminal of the coupler. The power level of energy transmitted beyond the animal was measured at the terminals (P_{TA} and P_{TB}) of the termination transducer. The sum

WATER
BOTTLE

P_{TA}

P_{TB}

P_W

P_A

P_{RL}

CP_{RR}

CP_{IN}

P_{IN}

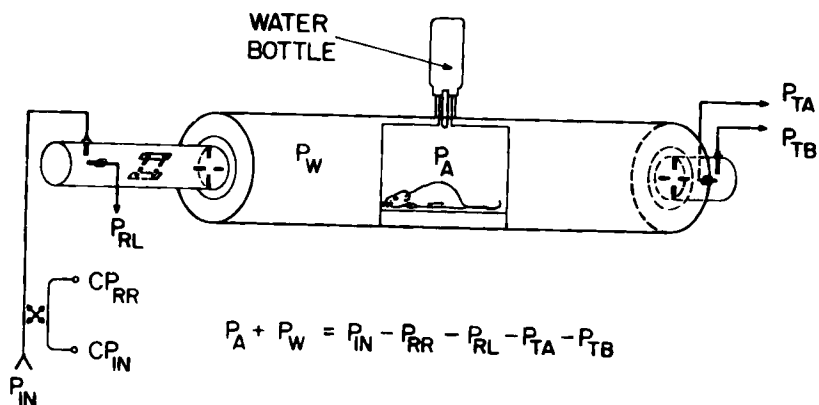$$P_A + P_W = P_{IN} - P_{RR} - P_{RL} - P_{TA} - P_{TB}$$

Fig. 2. The exposure chamber was a circularly polarized waveguide operating at 2,450 MHz. A rat, housed inside a plastic cage, was exposed in the 20.3-cm diameter wire-mesh tube. A circular polarizer, at the left end, converted the linearly-polarized TE_{11} mode to the circularly-polarized TE_{11} mode. Tuning stubs inside the polarizer matched the impedance of the propagating modes. Transmitted microwaves were terminated in the transducer at the right side of the tube.

of power levels of energy absorbed by the animal and the chamber walls can be obtained from the equation in Figure 2.

The water bottle in each waveguide was electrically decoupled from the animal by two concentric 1/4-wavelength choke sections so that the tip of the water nozzle had an extremely high impedance, virtually preventing conduction currents between it and any contacting object. The theory of the waveguide operation has been described elsewhere [Guy et al., 1979].

Microwave generation and distribution. Each exposure room was equipped with two 2,450-MHz pulsed microwave sources (Epsco, model PG5KB, Trenton, NJ), each source capable of providing an average output power of 20 W and a peak power of 5 kW. These generators were controlled by a microprocessor to deliver repetitive pulse trains as shown in Figure 3.
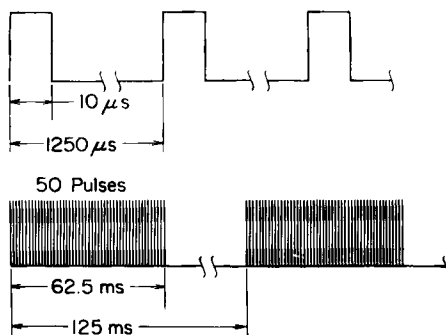

10 μs

1250 μs

50 Pulses

62.5 ms

125 ms

Fig. 3. Modulation characteristics of the microwave pulses: 8 groups per second, 50 10-μs-wide pulses per group, with a repetition rate of 800 pps. The period was 125 ms; with pulse onsets separated by 1.25-ms intervals. This is the equivalent of an 800-pps source square-wave modulated at 8 Hz.

MICROWAVE CIRCUITRY for LOW DUTY CYCLE HIGH PEAK POWER
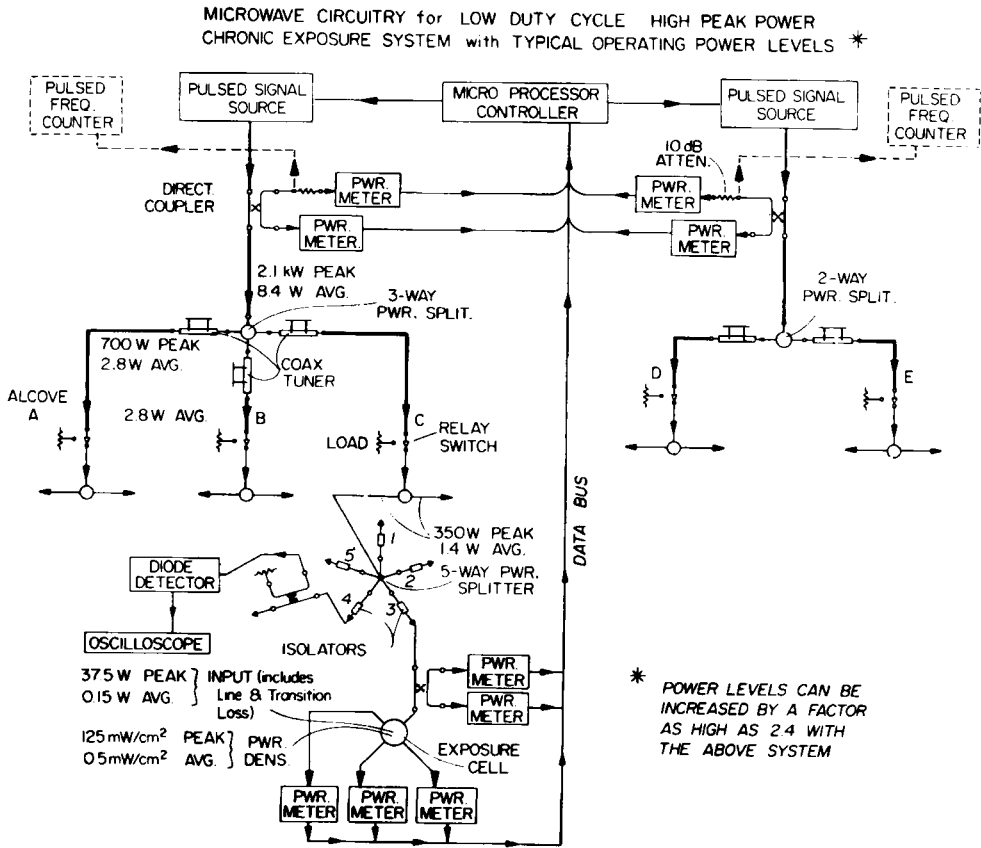CHRONIC EXPOSURE SYSTEM with TYPICAL OPERATING POWER LEVELS *

Fig. 4. Schematic of the microwave-distribution system in room T-131. The microwave energy was divided by means of low-loss coaxial cable via a 3-way splitter. Then the microwaves were fed through a single-pole double-throw (SPDT) coaxial relay to a 2-way splitter. Microwaves from each arm of the 2-way splitter were fed to a 5-way splitter; thus, the power-level of microwave radiation was again equally divided and transmitted through isolators to the two groups of five active exposure waveguides in each alcove. The distribution system of the second generator in each room was similar except that the microwaves were initially split in two ways to energize two alcoves. Power levels of forward and reflected energy at each generator output terminal was measured and recorded through a directional coupler and digital power meters interfaced with a microprocessor.

The microwaves from one generator in each equipment rack were transmitted to three alcoves (Fig. 4). The power levels of input, reflected, and transmitted energy associated with one exposure waveguide per room were monitored to obtain a recording of the average absorption loss of the waveguide-rat assembly; the average SAR could be calculated from the known waveguide loss and the mass of the rat. Each room contained a total of nine power meters, two each for the incident and reflected energy at each generator and five for the incident, reflected, and transmitted energy at the multiple terminals of the respective waveguides. The average SAR in the experimental animal was determined from the power meters. Throughout the chronic study, the monitoring system was connected each day to a different exposure waveguide so that every waveguide was monitored 1 day every 50

days over the course of the experiment. There was insignificant down time due to microwave power failure. Spare generators were available for this rare occurrence.

Dosimetry

Dosimetry studies conducted in preparation for this experiment were directed toward determining the power level for each waveguide that would best simulate with rats the exposure of man to an RF field. To determine the conditions necessary for simulating such exposure, the relation between the input power and the average and distributed SAR in the body of an exposed rat living in the exposure waveguide had to be quantified.

A microprocessor-controlled, twin-well calorimetry system was developed to measure the average SAR in rat carcasses. The average SARs for live exposed rats over the first year of exposure are shown in Figure 5. The results show that the SARs calculated from the data on the live animals are very close to but slightly less than the values calculated from measurements on rat carcasses. The results of this study have been published by Chou et al. [1984]

To best simulate the exposure of human beings, from child to adult, to radiation at the maximal levels allowed by ANSI C95.1-1982 [1982], the input power level for each alcove cluster was set so that the average input power was 0.144 W, which resulted in an initial average SAR of 0.4 W/kg in young rats of 200-g body mass.
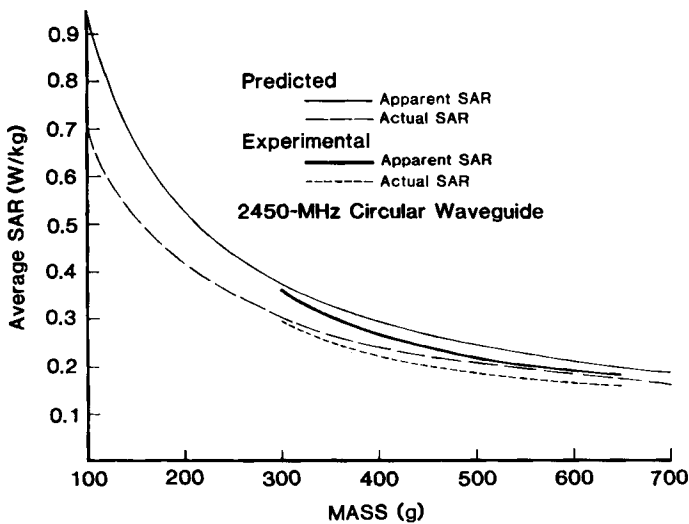

Fig. 5. Average SAR values measured for rat carcasses (Predicted) compared with average SAR values measured for free-roaming exposed rats (Experimental); SARs were averaged over weekly periods during first-year chronic exposure (input power 0.144 W to the waveguide). The predicted actual SAR was measured calorimetrically on rat carcasses of various body masses; bodies were exposed in five orientations in the waveguide: center, far corner, side, transverse, and diagonal positions. Predicted apparent SAR was measured by power meters on rat carcasses. Experimental apparent SAR was measured by power meters in live rats. Assuming that a live rat would spend equal time in each of the five orientations, the experimental actual SAR data were calculated from the apparent SAR and correction factors. The correction factors were the ratios between actual SAR and apparent SAR measured on rat carcasses.

Experimental Animals and Exposure Regimen

Two hundred male, Cesarean-derived, barrier-reared, Sprague-Dawley rats were obtained at 3 weeks of age from Camm Laboratory (Wayne, New Jersey): the rats were randomly assigned to exposure and control groups. Exposure began at 8 weeks of age, 21.5 h/day, 7 days a week, for 25 months. Maintenance procedures were done between 8 A.M. and 12 A.M. to minimize circadian-rhythm effects. The two and one-half hours off-time was used for cage cleaning, measurements of body mass, food and water consumption, blood letting, and other biological procedures.

Biological Assessment

Behavior testing. Behavior is a valuable end point for assessing neurological effects of exposure to microwaves [Lovely et al., 1977; cf. Shandala et al., 1979]. 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 intensity or reward magnitude) and thereby produce secondary effects as artifacts that must be distinguished from any primary (microwave) effect. In addition, all testing must be performed within the SPF environment and in such a manner so 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 a simple behavioral test based on quantification of a naturally-occurring behavior. Open-field or exploratory behavior has long been used as a sensitive endpoint in pharmacology and teratology, and it is accepted as a measure of general arousal or anxiety [Walsh and Cummins, 1976]. In addition, East European researchers have used the open-field test extensively in biological studies of microwaves [Shandala et al., 1979].

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

An open-field apparatus with infrared-light-emitting sensors was used. This apparatus provided a readout of both motion activity and the coordinates in the field. The latter information was used to indicate an animal's field position in one of the possible quadrants. In addition, at the end of each test session the apparatus was inspected for urination and defecation.

Evaluation of the immune system. Alterations in the immune system due to microwave exposure have been reported and disputed in the literature [cf., e.g., Mayers and Habeshaw, 1973; Czerski et al., 1974; Huang et al., 1977; and Wiktor-Jedrzejczak et al., 1977]. The conflicting results justified an assay of immunocompetence in this study. The immune-system evaluation consisted of several basic tests that were designed to detect immunological effects that might result from exposure to RF fields:

a. Blood lymphocyte evaluation of the numbers of B- and T-cell, antigen-positive lymphocytes, and complement-receptor-bearing lymphocytes.

b. Spleen lymphocyte evaluation for response to the following mitogens: phytohemagglutinin (PHA), concanavalin A (ConA), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and purified protein derivative of tuberculin (PPD).

c. 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 at the 13-month interim euthanasia of 10 animals from each treatment group, and after 25 months of exposure with the final euthanasia 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.

Blood sampling for corticosterone and health profile. Pituitary-adrenal axis activity as indexed by plasma corticosterone levels has long been interpreted as an indicator of general arousal, i.e., alerting borne of anxiety, fear, or stress. If long-term exposure to pulsed RF fields disrupts normal physiological functions or is psychologically disturbing to the animal, an increased basal level of corticosterone can be expected [Lotz and Michaelson, 1978]. The endocrine system can provide evidence of summation of multiple, otherwise subthreshold, effects. Individual corticosterone data are of value for correlation with results from individual animals or subpopulations that might exhibit abnormal indices of blood chemistry or a high incidence of tumors, and also as a measure of a possible nonspecific microwave effect.

The research protocol required the rapid collection of blood from all test animals in a 2-h period per day over 4 days for each blood sampling. The collection procedure was designed to be as rapid and atraumatic as possible. To prevent artifactual elevation of corticosterone, blood samples for serum corticosterone were drawn within 2 min after a rat was removed from its cage [Zimmermann and Crutchlow, 1967; Davidson et al., 1968]. The animals were rapidly anesthetized by a mixture of halothane, nitrous oxide, and oxygen; blood samples were drawn by the relatively atraumatic retro-orbital technique. Alternate eyes were sampled for blood in successive samplings so as to minimize ocular damage. A single blood sample, 1.8 to 2.0 ml, was taken at each session for all determinations.

Metabolism. An important consideration in performing the long-term exposure of rats is that the nominal 0.4-W/kg average SAR, initially is about 5% of the average metabolic rate of an active, young 200-g rat and about 10% of its resting rate. This SAR may be as high as 15% of the average metabolic rate of a lethargic, old, 600-g rat and 25% of its resting rate. The decision was, therefore, made to use a constant power density, which resulted in a declining SAR as the animals matured.

Exposure to microwave radiation for long periods could have different consequences for longevity, either life-shortening or life-lengthening, depending on the energy-budgeting option [Sacher and Duffy, 1978]. Therefore, given the importance of the metabolic versus extrinsic-budget question, the protocol provided the following animal measurements:

a. Daily-lifetime body mass measures, i.e., growth.
b. Daily-lifetime food and water consumption.
c. 24-h cycles of oxygen consumption and carbon dioxide production, measured at regular intervals throughout the life span.
d. Periodic assessment of thyroxine level.
e. Periodic assessment of urine production.
f. Total-body analysis at 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:

(1) physical as well as financial constraints made it impossible to instrument all 200 waveguides, and (2) rotating all animals through a few instrumented waveguides would have an associated animal-transfer-management risk 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, at most, twice a year from an animal, which would have been too infrequent. Therefore, we selected a subset of the exposed and control samples for rotation through waveguides adapted for the measurement of oxygen consumption and carbon dioxide production. This procedure did not result in loss of overall statistical power, and it 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.

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

A pathologist (L.L.K.), without knowing the identity of the rats, provided evaluative data to the technical personnel of the Bioelectromagnetics Research Laboratory, who were responsible for computer entry and quality control. Statisticians then evaluated the data, and the final results were reviewed by the pathologist for appropriate interpretative comments.

The occurrence of neoplastic and non-neoplastic lesions was recorded along with the age and the cause of death of each animal, whether the animal was euthanatized or had died spontaneously. The data on pathology were collected to permit comparison of survival curves of exposed and sham-exposed animals, age-associated lesions, and incidence of tumor metastases, as well as the number of lesions per rat.

RESULTS

Behavioral Evaluations

Figure 6 shows data from the 14 sessions of open-field assessment; except for the first test session, 2 years of exposure to the low-level, pulsed-microwave radiation did not lead to significant behavior alterations as measured by activity, defecation, or urination. During the first test session, the general activity level of the exposed animals was significantly lower (t = -2.24, P = .026, df = 195), 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 and experience, and it apparently was not affected by a lifetime of exposure to the low-level pulsed microwaves (Hotelling's T^2 statistic F = 8.73, P = .40, df = 8,168).

Plasma Corticosterone

Analysis of the data obtained during the five sampling periods (Fig. 7) indicates that serum corticosterone levels were not dramatically altered in either the
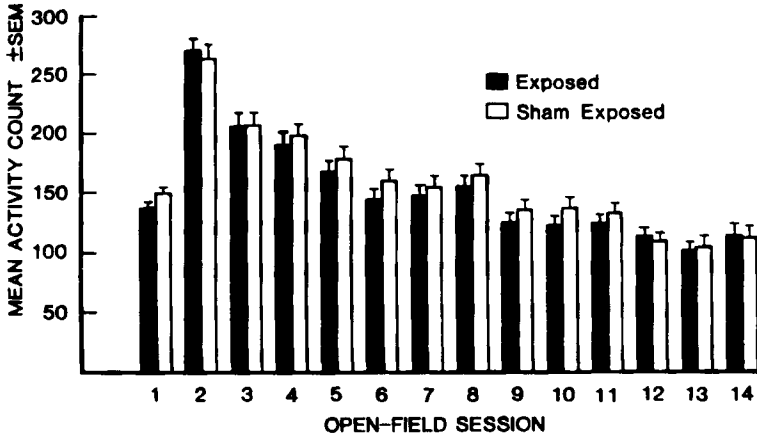
Fig. 6. Comparison by treatment group of mean levels of activity throughout the 14 open-field assessment sessions.

exposed or sham-exposed rats. The multivariate statistical analyses of the data ($\underline{F}$ = 1.38, $\underline{P}$ = .24, df = 5,133) indicate that no overall effects of microwave radiation were measurable by levels of serum corticosterone.

When the serum corticosterone values of exposed and sham-exposed animals were compared for each session, a t test indicated that exposed animals had relatively elevated serum corticosterone levels at the time of the first sampling session ($\underline{t}$ = 2.06, $\underline{P}$ = .04, df = 154), and that sham-exposed animals had elevated levels at the time of the third session ($\underline{t}$ = -2.25, $\underline{P}$ = .026, df = 161). Exposed and sham-exposed animals had comparable levels of corticosterone on all other regular sampling sessions.

The finding of elevated corticosterone was tested in a follow-up study [Chou et al., 1986]. Two groups of 20 animals each were exposed for 6 and 12 months, respectively, under the same exposure parameters as in the original study. An equal
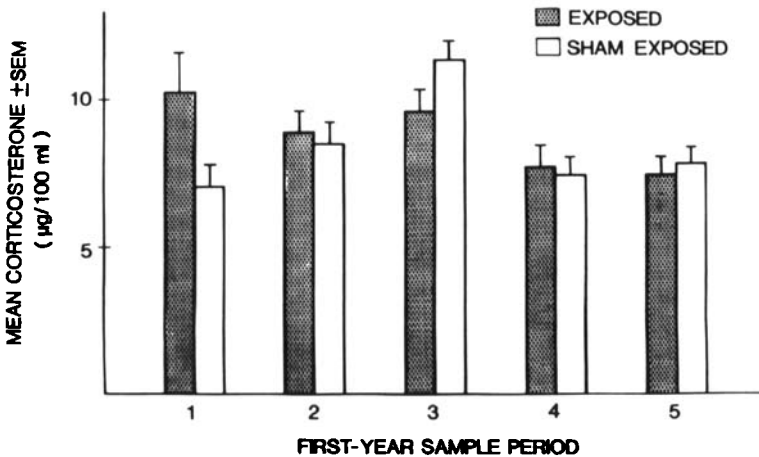

Fig. 7. Comparison of mean corticosterone levels from five quarterly determinations during the first year of the project.

number of sham-exposed rats served as controls. Corticosterone measured at 6 weeks, 6 months and 12 months did not show any statistically significant differences ($\underline{P} >$ .05) between 20 control and 20 exposed rats.

Immunological Competence

When compared with sham-exposed rats after 13 months of exposure (Fig. 8), exposed animals had a significant increase in both splenic B-cells ($\underline{t} = 3.76$, $\underline{P} = .002$, df = 16) and T-cells ($\underline{t} = 3.48$, $\underline{P} = .003$, df = 16). This apparent general stimulation of the lymphoid system in exposed animals was not detected in the animals after 25 months of exposure: Comparison of exposed and sham-exposed rats at euthanasia of survivors did not reveal any significant differences in the percentage or total numbers of B and T cells per spleen.

No significant differences were seen between exposed and sham-exposed rats in the percentage of complement-receptor-positive cells in the spleen at either the interim or final euthanasia. These findings indicate no difference between the treatment groups for lymphocyte maturation.

The plaque assay performed on exposed animals immunized with SRBC in the 13-month exposure rats exhibited a slight but statistically insignificant increase in plaques per spleen relative to the sham-exposed. This difference reversed after 25 months when exposed animals showed a slightly lower and statistically insignificant number of plaques per spleen. This assay indicated no statistically significant alteration of the reticuloendothelial system, which first processes antibodies in the presence of T-cells, because the SRBC antigen is T-cell dependent.

The mitogen-stimulation studies following 13 months of exposure revealed significant differences between groups in their responses to various B- and T-cell specific mitogens. The radiated animals had a nonsignificant increase in response to PHA but a significant increase in response to LPS (mean of 6.06 vs. 3.67, $\underline{t} = 2.35$, $\underline{P} = .032$) and PWM (mean of 6.41 vs. 4.61, $\underline{t} = 2.43$, $\underline{P} = .027$). As compared with sham-exposed animals, exposed animals also had a significantly increased response to ConA (mean of 17.0 vs. 10.7, $\underline{t} = 2.65$, $\underline{P} = .018$) and a decreased response to PPD (mean of 2.74 vs. 6.98, $\underline{t} = -2.65$, $\underline{P} = .018$). These results indicate a selective effect of exposure on the lymphoreticular system's response to mitoge-
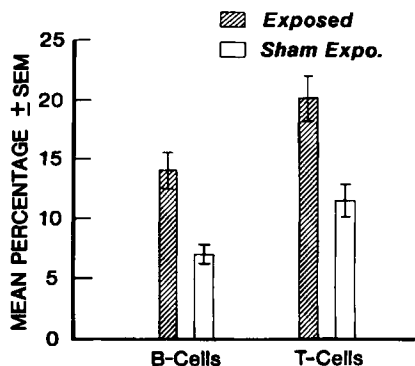

Fig. 8. Mean percentages of B-cells and T-cells within culture population of splenic lymphocytes for exposed and sham-exposed groups.

nic stimulation. Mitogen-response data were not available from the 25-month exposure studies because the lymphocyte cultures failed to grow.

In a follow-up study [Chou et al., 1986], no significant differences between 20 exposed and 20 sham-exposed rats were observed in the proliferation of thymocytes to ConA, PHA, and PWM after 6- and 12-months of RF exposure. The same lack of differences was found for splenocytes stimulated by LPS, PHA, PPD, ConA, and PWM. Flow cytometry revealed no group alterations in the number and frequency of B- and T-cells. However, after 12 months of exposure, a reduction in cell surface expression of Thy 1.1 (T-cell related) surface antigen, and a reduction in the mean cell-surface density of s-Ig (B-cell related) on small lymphocytes in spleen were observed. The stimulatory effect observed in the original study was not confirmed.

General Health Profile

In an attempt to detect and document any effects on the general health of the exposed animals, the following biochemical and hematological parameters were monitored: serum chemistry components, hematological constituents, protein electrophoretic patterns and fractions, and thyroxine levels. Multivariate analyses with Hotelling's T^2 statistic on a truncated data set (outliers removed) indicated no overall

Fig. 9. Comparison of serum glucose for exposed and sham-exposed animals for 15 sampling sessions.
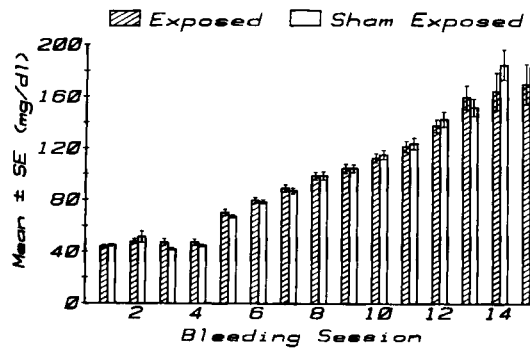

Fig. 10. Comparison of serum cholesterol levels of exposed and sham-exposed animals from 15 sampling sessions.

differences among all parameters between exposed and sham-exposed samples. Figures 9 and 10 present two representative examples of glucose and cholesterol levels from 36 sets of data. Individual t tests of all parameters across all 15 sampling sessions indicated a significant reduction in the absolute eosinophil counts of exposed rats during session 2, and marginally significant reductions in absolute neutrophil count during sessions 2 and 3. None of the other comparisons was significant. Therefore, these findings indicate that after the 25-month exposure no consistent effects were produced in bone-marrow erythropoietic cells or in the juxtaglomerular apparatus of the kidney and its production of erythropoietins.

Twenty-one serum chemical constituents were measured in serum samples collected during 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 tests did not indicate any differences between exposed and sham-exposed animals.

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 that reported in conventional-colony animals. The microwave exposure had no apparent effect on the functioning of various organ systems that contributed to serum-protein concentrations.

Thyroxine levels did not differ significantly between exposed and sham-exposed animals (Fig. 11). Thus, exposure had no effect on the 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 (Fig. 10) and triglyceride levels in both test and sham groups shows it to be a reliable indicator of metabolic activity in the rat.

The major conclusion that can be reached from the evaluations of hematology, serum chemistry, protein electrophoretic patterns and fractions, and thyroxine levels is that any significant variations of the parameters observed during the lifetime of the exposed animals were to be expected as a function of aging.
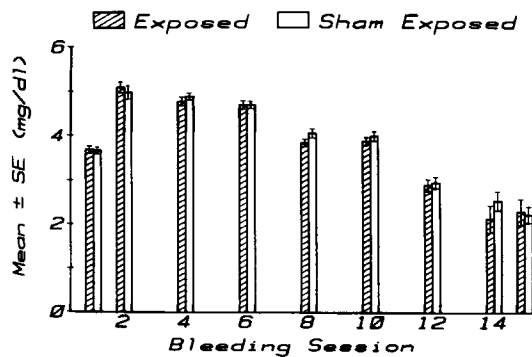

Fig. 11. Comparison of thyroxine data for exposed and sham-exposed animals for blood sampling sessions for which analysis was made.
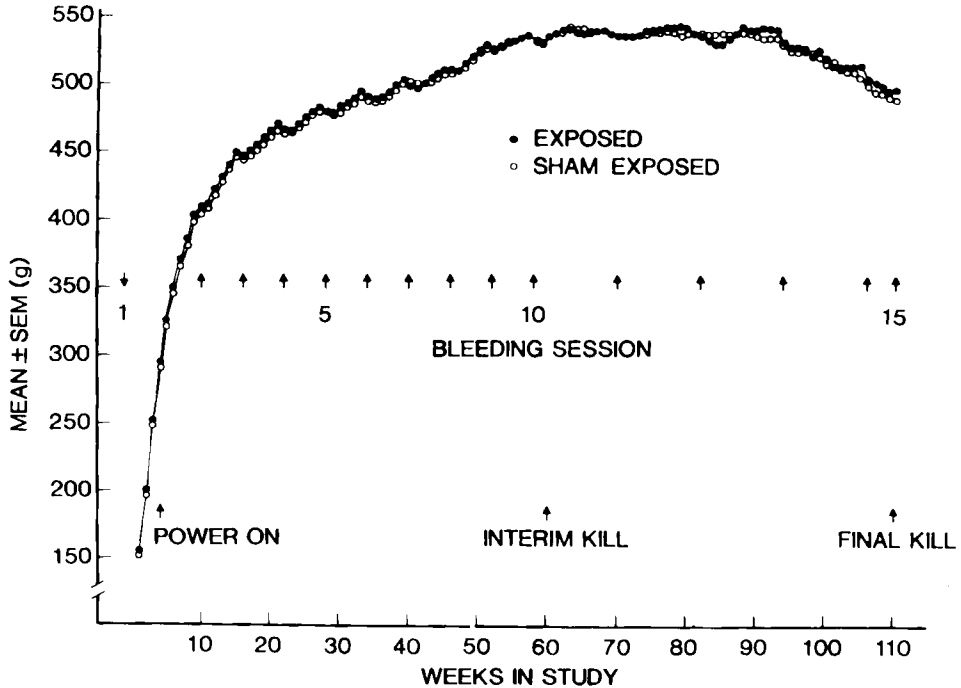
Fig. 12. Mean weekly body mass throughout 25-month study. Arrows indicate periodic bleeding sessions as well as other significant events during the course of the study.

Metabolism

Body mass and consumption of food and water. Growth curves for microwave-exposed and sham-exposed animals throughout this study (Fig. 12) are in general agreement with those reported for the Sprague-Dawley rat [Berg, 1960; Masoro, 1980]. The asymptotic body mass was somewhat lower than expected, possibly because of a periodic "stunting" effect coincident with the start of the regular blood-sampling sessions.

The average daily food intake of approximately 25 to 26 g is higher than that usually reported for the rat [Brobeck, 1948; Hamilton, 1967; Jakubczak, 1976] and indicated by the feed manufacturer (12 to 15 g/day). These food-intake norms, 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, which was maintained at 21 ± 1 °C, is in agreement with that reported for animals housed at lower ambient temperatures [Brobeck, 1948; Hamilton, 1967; Jakubczak, 1976] and in other studies in our laboratory that had used the waveguide apparatus [Lovely et al., 1977]. Throughout the 25 months, no overall differences were observed between treatment groups in either food or water consumption.

The similarity in overall patterns of growth, food and water consumption, and body-mass loss and recovery in exposed and sham-exposed samples indicates that no effects of microwave irradiation were apparent in 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 adversely affected 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 exposed rats as compared with sham-exposed animals. 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 mass was significantly higher in the exposed group than in the sham group. This analysis indicated that the increase in adrenal mass was related to the tumors and was independent of the metabolic processes in the rats. The mean adrenal mass in exposed animals without tumors was slightly larger, but statistically insignificant, as compared with that of the sham-exposed rats. This increase in mass was attributed to one animal with a hyperplastic adrenal cortex, which was secondary to a pituitary tumor.

O_2 consumption and CO_2 production. Differences between exposed and sham-exposed rats occurred in O_2 consumption and CO_2 production in younger rats (body mass 300–400 g) but not in the more mature animals (17–24 months old, body mass 550–600 g). The average hourly O_2 consumption for the young rats during the nocturnal period (1900–0600 lights off) was significantly different between the treatment conditions (Hotelling T^2 statistic, $F = 2.29$, $P = 0.025$, df = 11,44). Although individual t tests of hourly CO_2 productions of the young animals did not show consistent significant difference between treatment groups, the Hotelling T^2 statistic was significant during the diurnal (1300–1900 lights on) period ($\underline{F} = 2.73$, $\underline{P} = .023$, df = 6,49) and even more significant during the night time hours ($\underline{F} = 2.91$, $\underline{P} = .006$, df = 11,44). The effects observed in the young animals were less pronounced during the second round (36 days later) of measurements. On an hour-to-hour basis, the mature animals' metabolic measures appeared less variable than those of the young. The young animals demonstrated more marked responses to the lights-off condition and generally higher levels on each measure during the night time 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 and water consumption, and energy balance patterns as a function of photoperiod [Zucker, 1971; Besch and Woods, 1977].

Gross Pathological and Histopathological Evaluation

Longevity. Product-limit estimates and log-rank statistics were used to estimate and compare survival curves of exposed and sham-exposed animals (Fig. 13). Evaluation of the curves revealed that the median survival time was 688 days for 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 no significant differences during any phase of the life span of the animals. Statistical evaluation indicated no association between a specific cause of death and treatment condition; however, for cause of death due to urinary tract blockage (9 in exposed group and 19 in sham group), there is some indication that survival times were longer in the exposed animals.

Histopathology. Parasitic, bacterial, mycoplasmal, and viral agents were monitored during the 25-month period. A low-level (15%) infestation of the colony with pinworms, *Syphacia muris*, occurred but no histological lesions were attributed to these nematodes. The microflora of the animals was altered over the course of the experiment by the sporadic occurrence of *Proteus* sp. (*mirabilis, rettgeri,* and
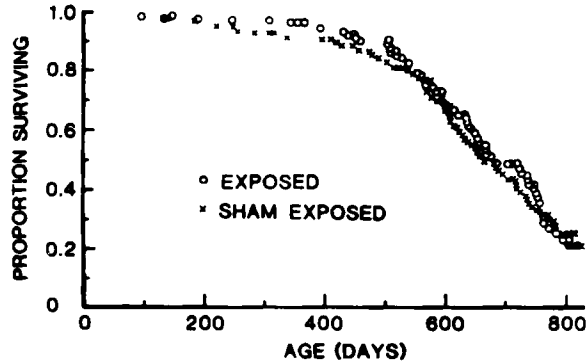
Fig. 13. Survival data for microwave-exposed and sham-exposed animals throughout the 25-month study.

vulgaris), *Staphylococcus epidermidis, Neisseria* sp., *Escherichia coli,* and *Klebsiella* sp. These intestinal flora became opportunistic organisms in the few cases of preputial adenitis and wound infections that occurred. *Mycoplasma* sp. was not isolated, either by culture or serology, and serological monitoring failed to reveal any significant elevations in titers of any of the common rodent viruses. There were no underlying diseases that complicated or produced erroneous results in the gross or histopathological evaluations of the experimental animals.

The histopathology data were grouped with respect to the animal's age, at 6-month intervals, and the data were divided into neoplastic and non-neoplastic diagnoses. The documentation of morphological lesions showed 2,184 pathological changes in the 200 animals examined. The non-neoplastic lesions comprised 1,992 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 types of neoplasms.

Chronic glomerulonephropathy was the most frequent cause of death and one of the most consistently encountered non-neoplastic lesions. Statistical analysis (Mantel-Haenszel estimate and chi-square statistics) indicated that glomerulonephropathy was less frequently observed in the exposed than in the sham-exposed animals ($\underline{P}$ = .04, df = 1). 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 was also evaluated. This analysis revealed no significant differences between the treatment condition and the severity of non-neoplastic lesions.

The neoplastic lesions were identified as benign or malignant, with the malignant lesions classified as primary or metastatic. A summary of these combinations is presented in Table 2, which indicates the total number of primary and metastatic malignancies and benign lesions observed in both exposed and sham-exposed animals. The incidence of neoplastic lesions corresponds with that normally reported for the Sprague-Dawley rats: Only two tumors were present in rats younger than 12 months, and tumor incidence rapidly increased after 18 months of age [MacKenzie and Garner, 1973; Altman et al., 1985]. The endocrine system had the highest incidence of neoplasia in the aging rats, as is expected in this animal. The incidence of benign pheochromocytoma of the adrenal medulla was much higher in the exposed group than in the controls (7 out of 100 vs. 1 out of 100). However, Fisher's exact test did not show a statistically significant effect ($\underline{P}$ = .065).

TABLE 2. Neoplastic Lesions Per Organ System

Organ	Lesions	Exposed			Sham-exposed		
		B	P	M	B	P	M
Adrenal	Adenoma	0	0	0	1	0	0
	Carcinoma	0	0	0	0	1	0
	Cortical adenoma	10	0	0	10	0	0
	Cortical carcinoma	0	3	0	0	0	0
	Myelomonocytic leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Pheochromocytoma	7	0	0	1	0	0
Blood vessel	Hemangiosarcoma	0	1	0	0	0	0
Bone marrow	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	1	0	0	0	0
Brain	Myelomonocytic leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	2	0	0	0
Cervical	Myelomonocytic leukemia	0	0	0	0	0	1
Lymph node	Lymphocytic lymphoma	0	0	0	0	1	0
	Malignant lymphoma	0	0	0	0	0	1
Colon	Malignant lymphoma	0	0	1	0	0	0
Duodenum	Myelomonocytic leukemia	0	0	1	0	0	0
	Malignant lymphoma	0	0	1	0	0	0
	Squamous cell carcinoma	0	0	1	0	0	0
Edipidymis	Squamous cell carcinoma	0	0	1	0	0	0
Eye	Leukemia	0	0	0	0	0	1
Heart	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Neurinoma	1	0	0	2	0	0
Kidney	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Nephroblastoma	1	0	0	1	0	0
Liver	Adenoma	2	0	0	0	0	0
	Carcinoma	0	0	0	0	1	0
	Hepatocellular adenoma	1	0	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	2	0	0	1
	Malignant lymphoma	0	0	1	0	0	1
	Squamous cell carcinoma	0	0	1	0	0	0
Lung	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	0
	Malignant lymphoma	0	0	1	0	0	0
Lymph node	Myelomonocytic leukemia	0	1	2	0	1	0
	Malignant lymphoma	0	0	1	0	0	0
	Transitional cell carcinoma	0	0	1	0	0	0
Mesentery	Transitional cell carcinoma	0	0	1	0	0	0
Nasal cavity	Leukemia	0	0	0	0	0	1
Pancreas	Adenoma	0	0	0	1	0	0
	Islet-cell adenoma	1	0	0	1	0	0

Continued

TABLE 2. Continued.

Organ	Lesions	Exposed B	P	M	Sham-exposed B	P	M
Pancreas	Squamous cell carcinoma	0	0	1	0	0	0
Parathyroid	Malignant lymphoma	0	0	1	0	0	0
Parotid SG	Myelomonocytic leukemia	0	0	1	0	0	0
Peritoneum	Liposarcoma	0	1	0	0	0	0
Pituitary	Adenoma	17	0	0	21	0	0
	Carcinoma	0	2	0	0	0	0
Preputial gland	Malignant lymphoma	0	0	1	0	0	0
Skeletal muscle	Myelomonocytic leukemia	0	0	1	0	0	0
Skin	Auditory sebaceous sq carcinoma	0	1	0	0	0	0
	Basal cell carcinoma	0	1	0	0	0	0
	Basal cell tumor	1	0	0	0	0	0
	Keratoacanthoma	1	0	0	1	0	0
	Malignant lymphoma	0	0	1	0	0	0
	Pilomatricoma	1	0	0	0	0	0
	Sebaceous adenoma	2	0	0	0	0	0
Spleen	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
Stomach	Malignant lymphoma	0	0	1	0	0	0
	Squamous cell carcinoma	0	1	0	0	0	0
	Squamous cell papilloma	3	0	0	4	0	0
SubQ tissue	Fibroma	1	0	0	0	0	0
	Fibrosarcoma	0	1	0	0	0	0
	Lipoma	1	0	0	0	0	0
	Neurinoma	0	0	0	1	0	0
Testes	Benign interstistial cell tumor	1	0	0	0	0	0
	Squamous cell carcinoma	0	0	1	0	0	0
Thymus	Myelomonocytic leukemia	0	1	0	0	0	0
	Lymphocytic lymphoma	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	0	1	0
Thyroid	Adenoma C-cell	10	0	0	9	0	0
	Carcinoma C-cell	0	2	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
Ureter	Malignant lymphoma	0	0	1	0	0	0
Urin/bladder	Transitional cell carcinoma	0	1	0	0	0	0
	Transitional cell papilloma	1	0	0	0	0	0
Zymbal's gland	Leukemia	0	0	0	0	0	1
Total		62	18	36	53	5	18

This table lists neoplastic lesions found per organ system. These lesions may be benign (B), a primary malignancy (P), or a metastatic malignancy (M) arising from a primary malignancy in another organ system (i.e., a malignant neoplasm may occur as a metastatic malignancy in many organs of a single animal, but as a primary malignancy in only one organ system of an animal).

The low incidence of neoplasia with no significant increase in any specific organ or tissue required the data to be collapsed and evaluated with respect to occurrence per se of neoplasms, with no attention given to the site or organ of occurrence. For benign lesions, as shown in Table 3, the Mantel-Haenszel (M-H) estimate of the odds ratio was 1.04. The chi-square statistic, which tests whether the relative risk is 1, was .001 ($\underline{P}$ = ,97, df = 1); therefore, we found no evidence that either group had an excess of benign lesions. For total neoplastic incidence including benign and malignant lesions, statistical evaluation revealed no significant difference between the exposed and sham-exposed groups (χ^2 = 0.32, $\underline{P}$ > .05).

A similar set of tables was prepared for primary malignant neoplastic lesions and is presented in Table 4. When all age categories for the primary malignant lesions were considered, the M-H estimate of the odds ratio was 4.27 and the chi-square statistic was 7.66 ($\underline{P}$ = .006, df = 1). With the first three age categories combined and the analysis repeated, the M-H statistic was 4.38 and the chi-square statistic was 7.9 ($\underline{P}$ = .005, df = 1). When the first four age categories were collapsed (leaving two categories: 1–24 and 25–30 mo), the M-H statistic was 4.47 and the chi-square was 6.97 ($\underline{P}$ = .008, df = 1). When age at death was ignored completely, the M-H estimate of the relative risk was 4.46 and the chi-square was 8.00 ($\underline{P}$ = .005, df = 1). It is interesting that the estimate of the odds ratio and the chi-square statistic are both insensitive to the way the data were grouped with respect to age at death.

A survival-type analysis also was done with time of death as a surrogate for time to tumor development if a primary malignant lesion were present. If no primary malignant lesions were found, time to tumor was considered censored at the time to death. From that analysis, the log-rank statistic is 7.63 with a $\underline{P}$ value of .006. This analysis indicates that the primary tumors occurred earlier in exposed rats than in sham-exposed animals.

DISCUSSION

We investigated the effects on health of long-term exposure to low-level, pulsed, microwave radiation. Among the 155 parameters studied, most of them showed no

TABLE 3. Incidence of Benign Neoplasms at Death

Age	Benign neoplasms	No. of animals	
		Exposed	Sham
Age considered (mo)			
1–6	Yes	0	0
	No	3	3
7–12	Yes	0	3
	No	5	5
13–18	Yes	1	5
	No	24	18
19–24	Yes	16	11
	No	19	24
25–30	Yes	22	19
	No	10	12
Age not considered			
	Yes	39	38
	No	61	62

TABLE 4. Incidence of Primary Malignant Lesions at Death

Age	Primary malignant lesions	No. of animals	
		Exposed	Sham
Age considered (mo)			
1–6	Yes	0	0
	No	3	3
7–12	Yes	0	0
	No	5	8
13–18	Yes	2	2
	No	23	21
19–24	Yes	9	1
	No	26	34
25–30	Yes	7	2
	No	25	29
Age not considered			
	Yes	18	5
	No	82	95

significant differences associated with exposure during the 25-month period. However, a few parameters showed positive effects. There was a statistically significant increase in the mean of the serum corticosterone level in exposed rats at the time of the first blood sampling, and there was a significantly lower level at the third session of measurement as compared with sham-exposed animals. The other significant effects involved the immune response of the rats at 13 months of exposure and the O_2/CO_2 metabolism in young rats.

The early finding of elevated corticosterone levels was not found in the later sessions of the 25-months study. The failure to repeat may be due to maturational differences, to the decreasing SAR as animals grew, or to a combination of the two. The lack of a significant difference in the total number of B and T cells in the terminal-euthanasia animals of the original study may also be the result of aging, the onset of immunosenescence, or the declining SARs. The role of a decreased SAR in animals across time should be considered if a similar study is conducted. One could avoid this problem by increasing the power level to keep the SAR constant.

A follow-up study was conducted to confirm both corticosterone and immune system effects [Chou et al., 1986]. Neither effect was confirmed in two groups of 20 animals each exposed for 6 and 12 months, respectively, under the same exposure condition as the original study. An equal number of sham-exposed animals served as controls. The sample size of 20 animals per group was chosen to have good statistical power (80%) to detect the same magnitude of differences observed in the original study. The failure to confirm indicates that the original findings are not robust.

The lack of discernible differences in O_2 consumption and CO_2 production in the mature animals at this level of microwave exposure is in agreement with the results of Phillips et al. [1975]. They exposed male adult rats to various intensities of 2,450-MHz microwaves. Animals receiving 27 cal/min (~ 2W) showed no difference from controls. The microwave exposure in our study resulted in an energy deposition of 1.5–2.0 cal/min (144 mW) throughout the lifetime of the animal,

well below levels employed by Phillips et al. Under the ambient environmental conditions of temperature, humidity, and airflow, the rate of energy deposition used in our study was not sufficient to produce robust changes in the metabolism of the mature rat exposed to microwave irradiation. Changes in the O_2 consumption and CO_2 production were observed in young, exposed animals—and these changes were more pronounced during the first round of the measurements—are consistent with the fact that the rate of energy absorption in our waveguide apparatus decreases with increasing body mass. Due to the fast growth rate of the rats (Fig. 12), the animals were subjected to higher SARs only during the first month.

The incidence of benign pheochromocytome of the adrenal medulla was higher but not statistically significantly so in the exposed group. However, we note that the incidence of this tumor in the exposed group does not exceed the incidence of tumors reported in the literature for this strain of rat housed under specific pathogen-free conditions [Anver et al., 1982]. Strict comparisons of these data with those from other laboratories cannot be made, however, because the animals were not subjected to parallel conditions. A reference control—large numbers of untreated rats except for observation of longevity and post-mortem analysis—would be desirable in future studies.

The finding of a near fourfold increase of primary malignancies in the exposed animals is provocative. These data cannot be considered as an artifact because different statistical analyses led to similar results. Although the overall difference in numbers of primary malignancies is statistically significant, the biological significance of this difference is open to question. 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 exposed animals was compared with specific tumor incidence reported in the literature, the exposed animals had an incidence similar to that of untreated control rats of the same strain 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. That treatment groups showed no difference in incidence of benign tumors is an important element in defining the promotion and induction potential of microwave radiation for carcinogenesis.

Morphologically, carcinogenesis proceeds through transitory or progressive states of growth, including hyperplasia and/or dysplasia, benign neoplasia, and finally overt malignant neoplasia. This morphological continuum, which often, but not always occurs, is the basis for grading systems and staging systems in common usage in medical pathology. Although the exact cause of cancer remains illusive, there is considerable morphological and biochemical evidence that neoplasms in humans and animals progress through a series of stages and ultimately become completely autonomous, invade surrounding tissue, and metastasize widely. Although there are readily recognizable histopathologic differences between the cancer cell and the normal cell, the biochemical differences, especially relating to the molecular biology of DNA and RNA synthesis, protein and polypeptide synthesis, enzyme activity, and membrane receptions to ultrastructural and cellular components is far from being completely understood [Busch, 1974, 1979].

The incidence of benign pheochromocytomas of the adrenal medulla was higher in the exposed group than in the controls; however, no other single type of tumor was significantly increased by the treatment, even though the primary malignancies of all types is significantly elevated in the exposed group. In considering this issue, one perspective to keep in mind is that, with the induction of cancer by a carcinogen, tissue-specific effects are usually induced, so that an agent is not usually considered carcinogenic unless it induces a significant response in any one tissue. The U.S. Environmental Protection Agency Guidelines for carcinogenicity risk assessment states, "A statistically significant excess of tumors of all types in the aggregate, in the absence of a statistically significant increase of any individual tumor type, should be regarded as minimal evidence of carcinogenic action unless there are persuasive reasons to the contrary" [U.S. EPA, 1986].

The combining of malignant tumors from all sites for statistical comparison of incidence in the exposed and control groups is questionable as to its biological relevance. A major factor that one must consider is the different response found in this study from what is expected in a chemical carcinogenesis study. There was no discernible induction of benign tumors in the organs that were apparently developing malignant neoplasms. Considering that the majority of the 155 parameters evaluated showed no differences, and especially that longevity was not affected, the biological significance of the increased primary malignancies is unknown. Chance variations may be the reason for difference in numbers of malignancies [Ward, 1983].

Scientists of the Georgia Institute of Technology have performed a complementary study, also supported by the Air Force; 200 rats were exposed to 435-MHz fields in circular, parallel-plate waveguides, 22 hr/day for 6 months. No significant differences in blood-borne end points were found [Toler et al., 1988]. To explore the possibility of RF-induced tumor initiation or promotion, the Georgia Tech group exposed a large population (200 exposed and 200 sham-exposed) of mammary-tumor-prone mice to 435 MHz fields for 21 months. This study was specifically designed to examine the effects of low-level, pulsed RF fields on cell growth and differentiation, unlike our project which was designed to study effects on general health and longevity. Their experiment is completed and the data are being analyzed. It will be interesting to compare their results with ours.

CONCLUSIONS

Microwave exposure of 100 male rats (and 100 sham-exposed controls) at SARs of 0.4 to 0.2 W/kg (pulsed, 2,450-MHz circularly-polarized microwaves at 21.5 h/day, for 25 months) showed no biologically significant effects on general health, serum chemistry, hematological profiles, longevity, cause of death, and lesions associated with aging and benign neoplasia. Statistically significant effects were found in corticosterone levels and immunological parameters at 13 months exposure, but these findings were not confirmed in a follow-up study. O_2 consumption and CO_2 production were lower in exposed young rats. These effects were not observed in mature rats. The findings of an excess of primary malignancies in exposed animals is provocative. However, when this single finding is considered in light of other parameters, it is conjectural whether the statistical difference reflects a true biological influence. The overall results indicate that there are no defintive, biologi-

cally significant effects on rats chronically exposed to this form of microwave irradiation. Positive findings need further independent experimental evaluation.

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