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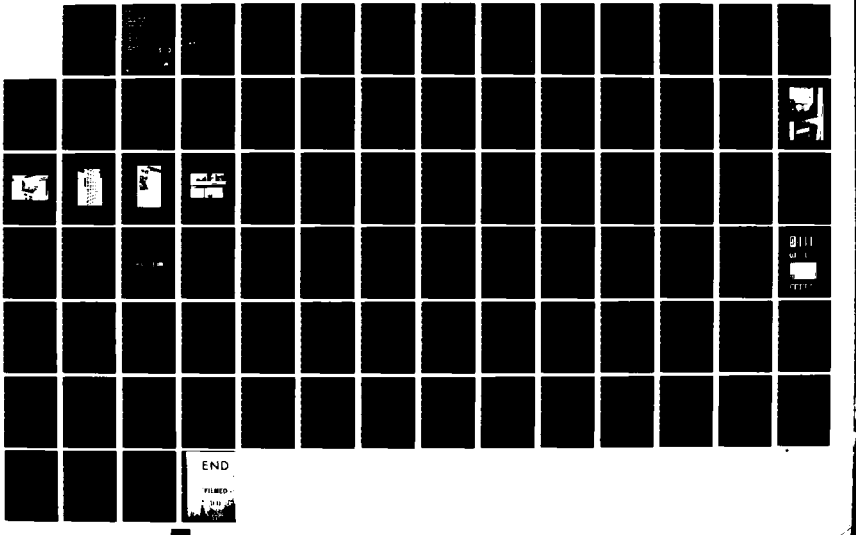

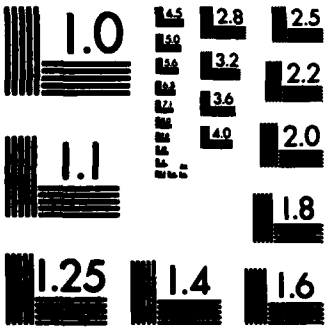

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EFFECTS OF LONG-TERM LOW-LEVEL RADIOFREQUENCY RADIATION EXPOSURE ON RATS

VOLUME 1. DESIGN, FACILITIES, AND PROCEDURES

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Interim Report for Period June 1980 - December 1982

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Prepared for

USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

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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 A. Brown (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 or 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 licensing the holder, or any other person or corporation; or 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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This is the first of a series of reports on the effects of long-term low-level RFR exposure on rats. The design, facilities, and procedures for exposing 100 experimental and 100 sham-exposed rats to 2450-MHz pulsed electromagnetic fields are described. The rats were exposed to 10-μs pulsed microwaves at 800 pps and 8-Hz modulation for 21 h per day for 25 months at an average power density of 480 μW/cm^2. The maximum specific absorption rate in rats was 0.4 W/kg. Reports on individual endpoints are presented in subsequent reports.

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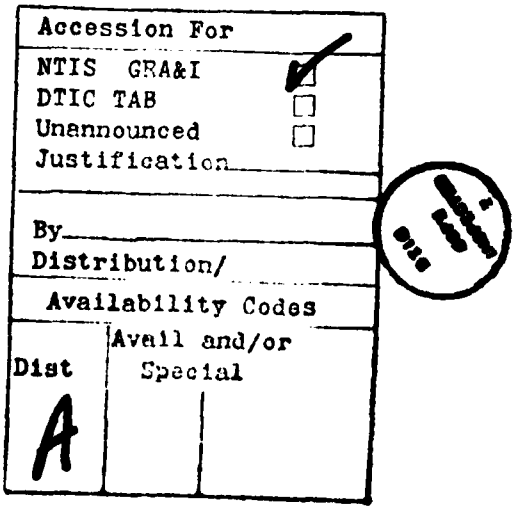
TABLE OF CONTENTS

LIST OF ILLUSTRATIONS AND TABLE



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

VOLUME 1. DESIGN, FACILITIES, AND PROCEDURES

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 unclear (Czerski et al., 1974; Glaser and Dodge, 1977; Tyler, 1975; Justesen and Guy, 1977; Justesen and Baird, 1979; Gandhi, 1980). Most exposure protocols completed to date have been of relatively short duration and restricted sample size, thus providing little insight into questions about cumulative effects.

During the past three years, 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. The goal of the 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 on longevity.

As part of this project, a unique 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 waveguides for use in active exposure of experimental rats together with 50 waveguides for use in 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-μsec 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 480 $\mu W/cm^2$. 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.

Two hundred male rats obtained at 3 weeks of age from a commercial barrier-reared colony were randomly assigned 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 at regular intervals throughout the study in an open-field apparatus. 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 10 rats from each group were killed at the end of 25 months for similar analyses.

This technical report is the first of a series covering the major subtopics of interest concerning the conduct and results of the chronic exposure study. It presents an overview of the organization and rationale of the project and describes the basic facility, equipment, and procedures used, including the animal exposure facility, waveguide construction, power distribution, data collection system, animal selection and procurement procedures, and daily animal maintenance procedures. Each subsequent report will concentrate on the specific equipment, methodologies, and experimental results of one subtopic: human dosimetry, animal dosimetry, analysis of open-field behavior, immunological competence, hematology and blood chemistry, metabolic profile, and gross pathology and histopathology. A final summary volume will complete the series.

EXPERIMENTAL DESIGN

Objectives

The broad goal of this study was to simulate in a population of experimental animals, throughout their natural lifetimes, 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. The 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. Therefore, both the exposure parameters and the biological indices of general health to be used were extensively considered.

Exposure Criteria

The first exposure criterion was to select a test animal and exposure situation to best model the human exposure situation in a well-documented experiment in terms of dosimetry. Much of the past work on chronic exposure of large numbers of test animals involved using 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. The problem of absorbed energy variation with orientation of the animals can be partially eliminated by restricting the size of the exposed groups to a single restrained animal. However, an

animal cannot be exposed for an extended period of time in a restrained position without dramatic physiological side effects (Perhach and Barry, 1970; Keim and Sigg, 1976).

For this study, we chose to use the system of cylindrical wiremesh waveguides described by Guy and Chou (1977) and Guy et al. (1979) 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. Systems such as this, operating at 918 and 2450 MHz, have been used successfully in our laboratory since 1975 for physiological and behavioral studies (Moe et al., 1977; Johnson et al., 1977; Lovely et al., 1977, 1978).

We selected an exposure frequency of 2450 MHz so that the rat would have approximately the same size-to-wavelength ratio as a human exposed to 450 MHz. The initial consideration was to produce the same average SAR in test animals as predicted for man exposed to 1-mW/cm^2 450-MHz RF fields. We estimated, on the basis of previous experience with the 2450-MHz circular waveguide exposure system, that an average power density level of approximately 500 μW/cm^2 for the rat exposure would result in an average SAR equivalent to that for the human exposure at the lower frequency.

The secondary criterion for the exposure parameters was the modulation frequency and its effects. In addition to using pulse modulation (10-μsec 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 by Bawin et al. (1975) and replicated later by Blackman et al. (1979). 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 (Coenen, 1975).

More detailed discussions of the exposure units, power generation, and distribution network are presented later in this report.

Rationale of Biological Assessment

Considerable effort was made not only to select reported biological effects from low-level microwaves as endpoints (e.g., alterations of hematopoetic, immunologic, and specific blood chemistry indices), but also to include 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. Throughout the following sections some, but not necessarily all, of the rationale is given for inclusion or exclusion of an endpoint or its frequency of monitoring. The rationale for each endpoint is discussed more fully in the technical reports covering each major division of the research effort.

We tried to choose endpoints that were both sensitive and generally replicable in providing information about the health of the animals. Although some endpoints can at best detect only major alterations of a biological system, they have been included as negative indicators.

Metabolism

In principle two distinct actions of microwave radiation on mammals can be distinguished: (1) the hypothesized direct effects on biomolecules and cell structures through as yet undemonstrated mechanisms and (2) the well-known consequences produced by heat during exposure of biological material. The exposure levels selected for this project are well below those known to produce thermal effects and related cell damage.

Given the uncertainty regarding cumulative effects of a lifetime exposure, however, increased longevity and reduced cancer incidence in the exposed population were possibilities to be considered. The probability of either occurring would depend upon how the average adult male rat "metabolizes" small amounts of energy such as those received in the form of

heat in this study. This heat energy might be utilized as part of the rat's energy budget in maintaining thermal equilibrium with the environment, as an alternative to using internal chemical stores of energy. With an exposure environment maintained at an ambient temperature below the thermoneutral zone of the rat, this lowered requirement for chemical energy could result in reduced food intake, or in increased utilization of chemical energy for growth, or in simple deposition of this energy in fat stores.

A strong negative correlation between caloric intake and lifespan has been repeatedly demonstrated (McCay et al., 1939; Berg and Simms, 1960). In rats, at least, caloric restriction prolongs life, partly by reducing kidney disease and decreasing cancer incidence. Similarly, increased energy expenditure due to being housed in cold environments reduces life expectancy of rats. An inverse relation also exists between the resting metabolism of a species and the maximum (or mean) species lifespan. Sacher and Duffy (1978) showed a similar relationship between metabolic rate and lifespan for different genotypes of mice; the same negative relationship between metabolic rate and lifespan may hold true for rat and man. The amplitude of the diel cycle of metabolism also correlates positively with the lifespans of individual animals, independently of the correlation of resting metabolism and lifespan.

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 an 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

These measurements provided a set of mutually supportive data that could make possible a conclusion about the effects of microwave exposure on energy metabolism, thermoregulation, growth, and the influence of these variables on the lifespan of the animals in the study. Should the exposed population have a longer lifespan than the control population, the detailed study of metabolic and thermoregulatory variables would be especially useful, providing a rational explanation of an otherwise inexplicable outcome.

General Health Profile

Many research projects for assessing the biological effects and health hazards of microwave radiation have been concerned with single or limited biological endpoint evaluations. During the last few years, a health profile has proven more useful to researchers in other fields for diagnosing and understanding abnormalities in their experimental animals. A profile is an aid in uncovering unsuspected organ system malfunctions. In animals with subclinical or undiagnosed abnormalities, the emphasis is placed on the correct interpretation of the profile results and the interrelation of different test results rather than on individual test selection. The health profile permits a better understanding of the pathophysiology of abnormal or disease states and can demonstrate multisystemic organ involvement which can often be missed if only individual tests are selected and measured. A health profile consisting of serum chemistry tests, hematology measures, protein electrophoresis,

thyroxine levels, and a urinalysis provides a data base from which presumptive and definitive diagnoses can be made.

The following test parameters were included in the blood chemistry analysis:

1) Glucose
2) BUN
3) Creatinine
4) Sodium
5) Potassium
6) Chloride
7) Carbon dioxide
8) Uric acid
9) Total bilirubin
10) Direct bilirubin
11) Calcium
12) Phosphorus
13) Alkaline phosphatase
14) Lactate dehydrogenase (LDH)
15) Serum glutamic-oxaloacetic transaminase (SGOT)
16) Serum glutamic-pyruvic transaminase (SGPT)
17) Cholesterol
18) Triglycerides
19) Total protein
20) Albumin
21) Globulin

Although serum protein electrophoresis (SPE) is not a method used for determination of specific proteins, it is a valuable determinant in organ panels or health profiles and was therefore included in our study. SPE is the single most sensitive procedure for detecting monoclonal gammopathies and is used for determination of certain proteins such as albumin and immunoglobulins. With a well-resolved system, even small monoclonal or oligoclonal bands can be easily identified, giving evidence of intense immunologic stimulation such as may accompany serious viral infections or tissue necrosis.

Elevation of serum cholestrol and triglycerides and a mild normochromic, normocytic anemia are suggestive of hypothyroidism, although they do not occur in all hypothyroid animals. The thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), are active in controlling the growth, development, and proper function of organ systems and in the regulation and maintenance of normal metabolism throughout the body. The synthesis of T_3 and T_4 in the thyroid gland and the release of these hormones into the circulatory system are regulated by a complex feedback mechanism involving the hypothalamus and the anterior pituitary hypophysis.

These hormones are therefore an index of the intact functioning of these neural structures; thus, T_4 levels were evaluated regularly.

Urine abnormalities almost always occur in urologic disorders and frequently occur in extraurinary disorders such as hemolytic anemia, diabetes mellitus, or liver disease. Therefore, urinalysis was performed on every animal suspected of having urologic disease and on those with undiagnosed abnormalities. The complete urinalysis consisted of

1) observation of physical properties
2) estimation of solute concentration
3) chemical analysis
4) sediment examination

The following hematological parameters were monitored by means of standard techniques:

1) Hemoglobin
2) WBC
3) RBC
4) PCV
5) Indices
6) Differential count

When a pathophysiological approach is used for evaluation of hematological data, their interpretations are seldom diagnostic as individual tests but are invaluable as an adjunct to the health profile. Our major interpretations of the hematological tests pertained to the presence or absence of systemic stress, inflammatory disease, bone marrow disease, and neoplasia.

As part of the data base for the metabolic energy studies, carcass analysis was included as an endpoint that might reflect shifts in body composition due to the cumulative influence of absorbed energy on metabolic processes. Carcass analysis included:

1) Total moisture
2) Total protein
3) Crude fat
4) Nonprotein
5) Total ash

6) Spectrographic analysis (27 element readout)
7) Selenium
8) Fatty acid profile

The total moisture, crude fat, nonprotein nitrogen, protein, and ash reflect differences in lean body mass, fat, and skeletal components of the animals. The levels of calcium, magnesium, zinc, iron, selenium, and phosphorus show possible differences in mineral deposition. The fatty acid profile gives the amounts of the different fatty acids present and aids in detecting subtle metabolic changes that can affect the type of fatty acids produced in the body.

Evaluation of Immune Competence

Alterations in the immune system due to microwave exposure have been reported in the literature. Czerski et al. (1974) and Huang et al. (1977) reported increased numbers of peripheral lymphoblasts in exposed animals. Wiktor-Jedrzejczak et al. (1977) reported a microwave-induced increase in the frequency of complement receptor lymphocytes in the spleen. Mayers and Habeshaw (1973) showed that human macrophages have decreased phagocytic ability when exposed to microwaves in vitro. 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).

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. Exposure to microwave radiation alters pituitary-adrenal activity under various exposure frequencies and power levels (Lu et al., 1980; Johnson et al., 1978), albeit at levels substantially higher than used in this study.

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 possible abnormal blood chemistry indices or high rates of tumor incidence, also as a measure of some possible nonspecific microwave bioeffect. Because of the nature and cost of this assay, it was done on a quarterly basis.

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. Documenting the onset of neoplastic and age-associated lesions was deemed important in detection of any differences between the age of onset and frequency of occurrence of the lesions between the control and exposed animals. The pathological data were organized and analyzed for comparison of survival curves, age-associated neoplastic and nonneoplastic lesions, incidence of tumor

metastases, and multiple lesions for each rat in the exposed and control groups.

For adequate evaluation of aging lesions in long-term studies, rats that have survived until at least the 50% survival age are required. The 50% survival age of Sprague-Dawley rats is about 24 months. To obtain the best survival and lesion-age incidence curves for comparison of exposed and control animals, ideally we should allow all animals to live until spontaneous death occurs. This means that the oldest rats may reach lifespans longer than 40 months. A practical method to limit the duration of the experiment with minimal compromising of the data is to expose the animals until 90% survival age is reached and then kill the remaining animals as a terminal group for comparison.

Behavioral Evaluations

Behavioral testing is a valuable tool for the assessment of 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 used 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. However, quantification of the data is generally a product of a human observer visually scoring the behavior of the animal; and if more than one observer is used, as would have been required in this project, interrater reliability must be continually evaluated and controlled. Also, the

subjective expectations of the observer can, even under "blind" conditions, unintentionally influence the rating of an animal's behavioral output. Therefore, for this project we acquired commercial automated open-field apparatus, thus eliminating the human factor in the quantification of the data and ensuring the reproducibility of the results.

Physical Limitations

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 be too infrequent.

Therefore, only a subgroup of the exposed and control populations was selected for rotation through waveguides specially adapted for the measurement of oxygen consumption and carbon dioxide production. This would not result in any loss of overall statistical power and would produce more frequent measures on the specific animals involved.

Given the modular arrangement of the rooms, as described in the Animal Facility section, 36 animals were included in measurements of respiratory gas exchange, 18 exposed and 18 sham-exposed.

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, an initial sample size of 100 in each group was calculated to

be sufficient for detection, at the 0.05 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 0.05 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.

Outline and 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 7th 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 hematological and serum chemistry evaluations were done on 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 but yet not to stress the animals unduly. A urinalysis was conducted on all rats every 3 months, with the first being done during the 4th 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, the frequency of bleeding was reduced to once every 12 weeks, corticosterone analysis was eliminated except just prior to the final sacrifice at the end of 2 years, and a partial urinalysis was conducted every 2 weeks. Open-field analysis was conducted quarterly during the second year. Detailed schedule is shown in the following calendar of events.

Date	CALENDAR OF EVENTS
1 Oct 1978- 28 May 1980	Construction of 200 waveguides and preparation of animal exposure facility; initial animal vendor screening; training of animal maintenance personnel
29 May 1980	Animals shipped/arrive from CAMM Labs (230 rats, 90 days old)--Used for 4 weeks training period in a) SPF techniques and equipment operation b) Anesthesia and bleeding c) Urine collection d) Necropsy procedures
2 June 1980	Health screen (vendor screen) 10 rats
8 July 1980	Animals arrive from CAMM Labs (60 rats, 21 days old) -- Used for training in anesthesia and bleeding
July 1980	Kill all rats and collect serum for pooled control serum Clean and disinfect room (25 June-3 July) Health screen 10 rats for mycoplasma
29 July 1980	Animals shipped/arrive from CAMM Labs (400 rats, 21 days old) for initial physical exam
30 July- 1 Aug 1980	Toe clip ID numbers Health screen (10 animals) 50 rats assigned for immunological tests
1 Aug 1980	Start quarantine
5 Aug 1980	Kill 20 rats for total body analysis
4-7 Aug 1980	Bleed 250 rats for individual baseline values
8 Aug 1980	Bleed 50 rats for group baseline values Start waveguide adaptation (196 rats)
1 Sept 1980	Power on
29 Sept 1980	Urinalysis (every 6 weeks thereafter)
13 Oct 1980	Bleeding (every 6 weeks thereafter)
4 Nov 1980	Open-field assessment (every 6 weeks thereafter)
31 Sept 1981	Interim necropsy and immune system evaluation
Oct 1981	Frequency of bleedings and open-field assessments changed to every 12 weeks, urinalysis every 2 weeks
March 1982	50% mortality reached
27 Sept 1982	Power off, final necropsy, and immune evaluation

ANIMAL FACILITY

To maintain the colony of rats used in this study in the highest state of health possible, free of chronic respiratory disease and other common rat problems, we acquired two rooms in the T-wing vivarium in the Division of Animal Medicine at the University of Washington. These rooms are part of a facility designed to be used under barrier conditions to maintain SPF animals. They are located within a system of "clean" and "dirty" hallways designed to isolate them from adjoining rooms in the facility (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 connects the cage-washing facility with the clean hallway so that once washed, all materials entering the clean hall must pass through autoclaving before being returned to the animal rooms. All soiled cages and waste collectors leave the clean rooms via the dirty hallway and then are taken to the cage-washing facility.

The original configuration of each clean room was a central workspace surrounded by six smaller alcoves, five of which could be closed off from the main workspace by vertical sliding glass doors. Each room measured approximately 5.5 x 6.0 m, with center workspace dimensions of 5.5 x 3.0 m and each alcove approximately 1.57 x 1.44 m deep.

The rooms had to be modified since the length of the waveguide apparatus with associated cabling exceeded the depth of the original alcoves. Each alcove was extended into the central space approximately 0.5 m and a new set of horizontal sliding doors was installed, with the original doors intact in the open position. Racks were built so that each alcove could house 20 waveguides mounted on four horizontal shelves, five waveguides per shelf. The exposed and sham-exposed waveguides were randomly arranged on these shelves, except that only sham waveguides could be in the center position (Fig. 2) because of the operation of the sliding glass door. One of the alcoves in each room was equipped as a metabolism alcove, where oxygen consumption and carbon-dioxide production were measured.

*Throughout this report, figures will be placed at the end of the main section in which they are first mentioned.

The sixth alcove in each room was partitioned off completely for use as a procedures area. This area was used during the bleeding and to house the main data-collection computer and miscellaneous supplies (Fig. 3).

An island was constructed in the center of each room to serve as a workspace during the daily maintenance procedures. Each island had two independent data-entry stations for weighing animals and food and water containers (Fig. 4). A water-delivery system was constructed within the island to deliver water to faucets on the island top for filling water bottles. This system had stainless-steel tanks filled with autoclaved water, pressurized by air.

Two large storage shelves were constructed to house the animals, in filter-bonneted plastic holding cages, during the 2 h each day that the regular waveguide cage was being cleaned (Fig. 5).

We housed the microwave-generation equipment in a rack near the dirty-hall doorway to facilitate inspection without entering the clean room (Fig. 6). Equipment needed to determine oxygen consumption and carbon-dioxide production was located above the door of the metabolism alcove used for these measurements (Fig. 7).

Each room and each alcove had its own source of ventilation and temperature control. The clean hall was kept at positive pressure with respect to each room; the rooms, in turn, were kept at positive pressure with respect to the dirty hallway. The alcoves were kept at positive pressure with respect to the central workspace. Thus, an air pressure gradient was maintained throughout the system so that any airborne contaminants would be carried from the cleanest to the dirtiest portions of the facility and away from the animals.

In the SPF rooms we kept the airflow rate to approximately 22 exchanges each hour to maintain the positive pressure flow. We carefully balanced temperatures between the workspaces and alcoves to maintain a fairly constant 21 $\pm$ 1 ^{o}C environment throughout the facility. Fig. 8 shows the temperature fluctuations of the alcoves and workspaces throughout the course of the project.

Sound pressure measurements indicated an average level in the central workspace of approximately 60 dBA (re 20 $\mu N/cm^2$) and alcove levels that were 6-10 dBA lower depending upon position within the alcove. Light intensity

measurements indicated an average workspace level of 13 lux and average alcove levels of 6.

All surfaces were scrubbed and the entire facility was filled with a fog of germicidal detergent for 24 h before the animals were brought in.

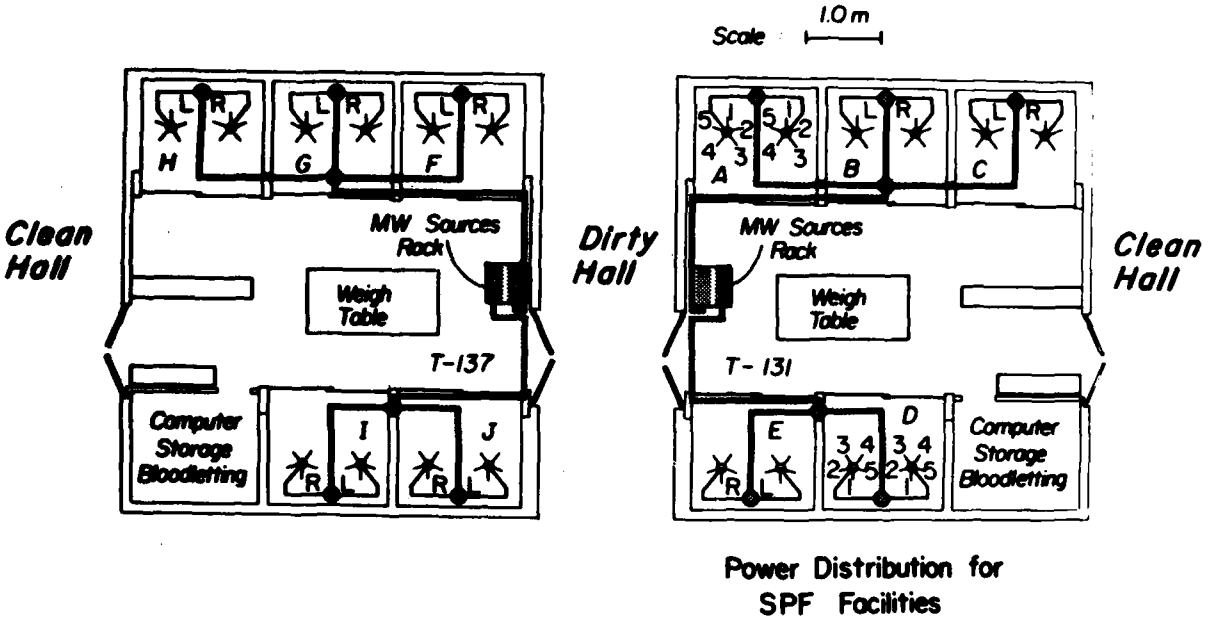


Figure 1. Top view of exposure rooms with alcove designations and associated exposure-cell identification system.

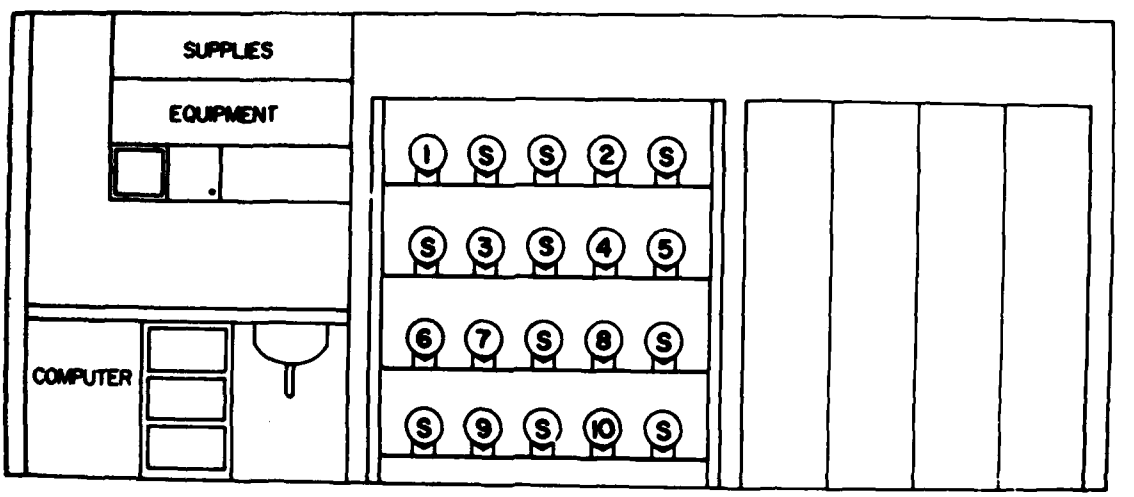


Figure 2. A procedures room and an alcove; arrangement of exposure and control screen waveguides.

Figure 3. One alcove in each lab is partitioned off for use as procedures room.

Figure 4. Two weigh stations are located centrally in each clean room for easy access by technicians servicing alcoves on either side.

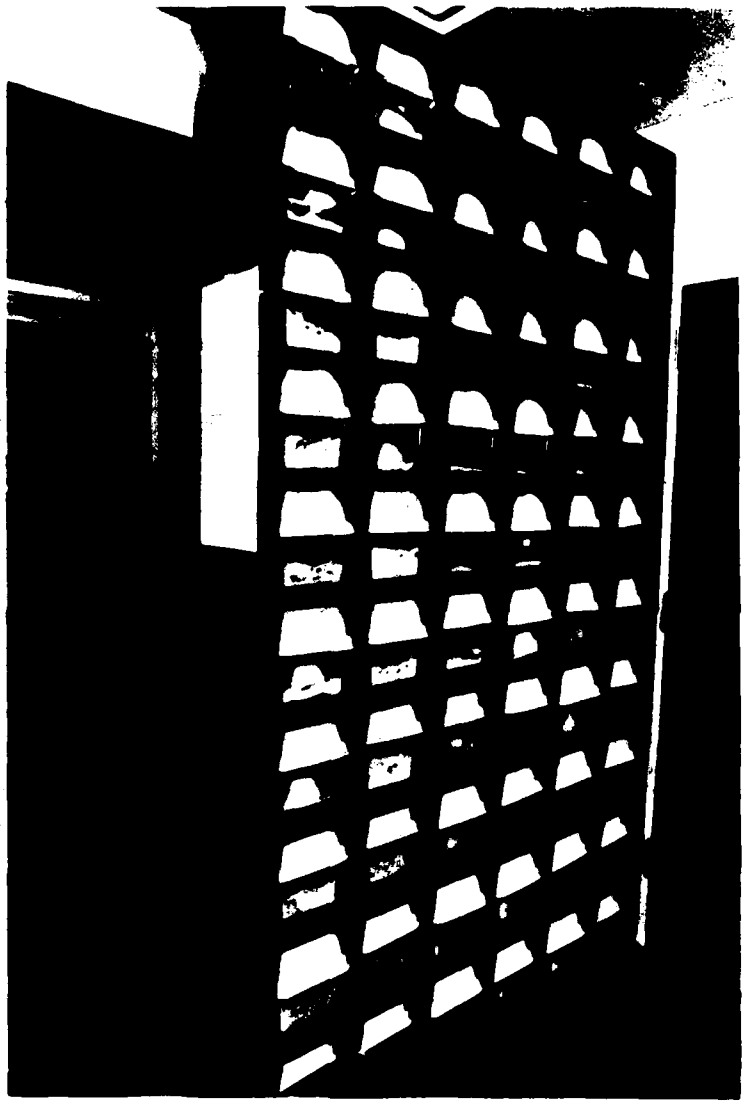


Figure 5. Filter-bonneted plastic cages, housed in compartmentalized shelves, used to hold rats during the 2-h daily maintenance period.

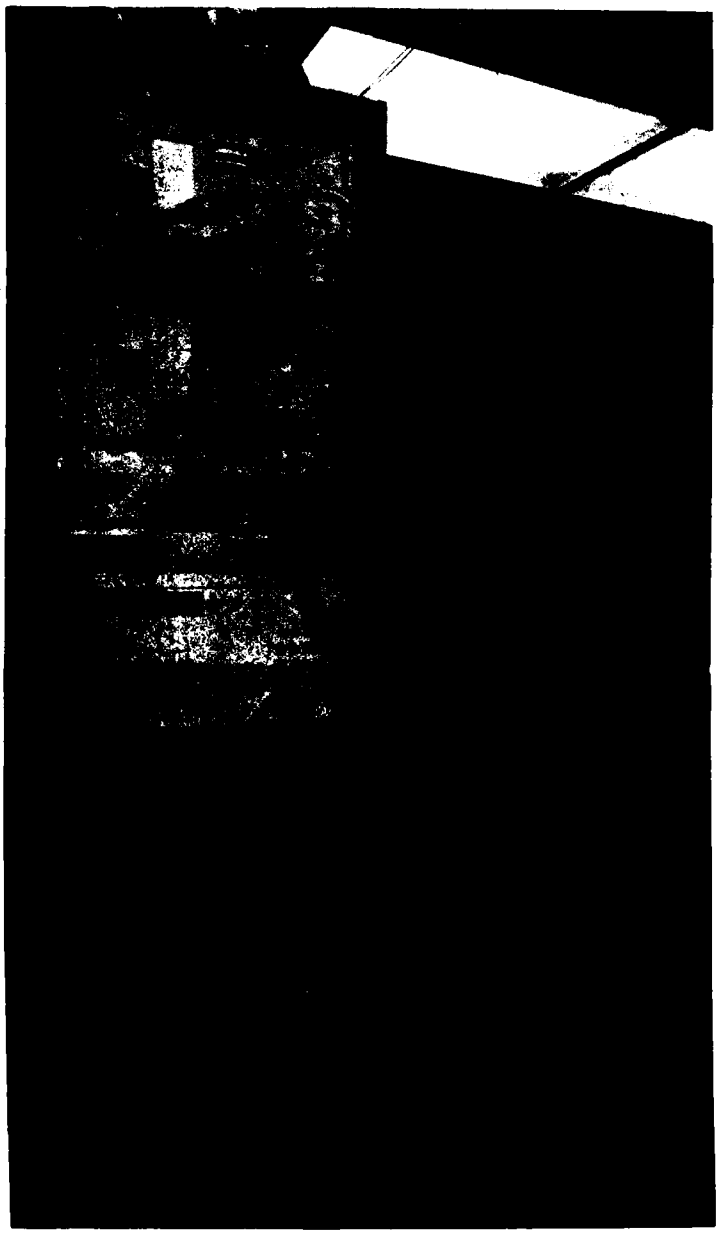


Figure 6. Equipment rack showing (from top to bottom) two power generators, oscilloscope, temperature humidity meter, microcomputer power meters and emergency power supply.

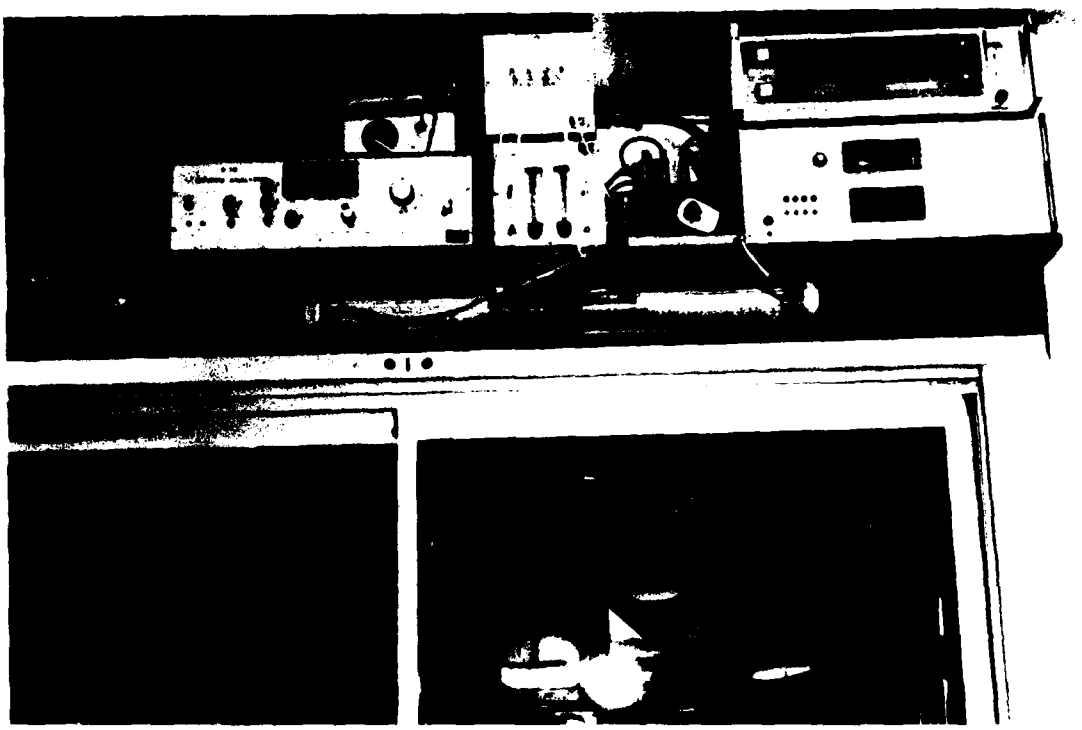


Figure 7. Oxygen and carbon dioxide analyzers, pumps, and control unit (located on a shelf above the metabolism alcove) used in determining respiratory gas exchange.

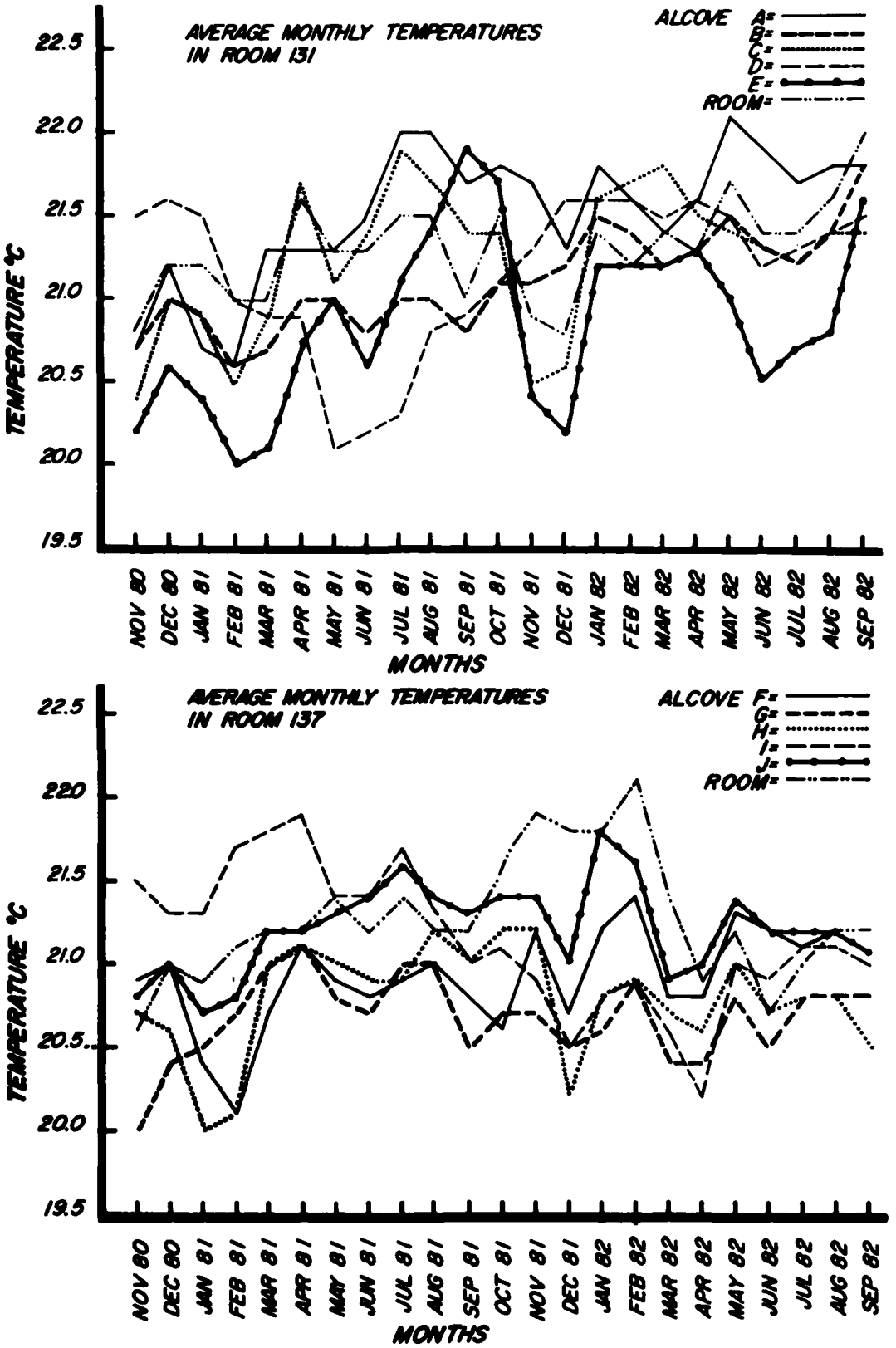


Figure 8. Temperature fluctuations within the alcoves and rooms throughout the chronic exposure study.

MICROWAVE EXPOSURE SYSTEM

For this study, we chose to use the cylindrical-waveguide exposure system described by Guy and Chou (1977) and Guy et al. (1979). This system, consisting of many independent waveguides, allowed each animal to be continuously exposed while unrestrained and living under normal laboratory conditions with access to food and water.

Theory of Operation

The operation of an exposure waveguide is shown in Fig. 9. The main body of the 20-cm-diameter circular exposure waveguide is constructed of wire screen for ventilation and visibility. At one end of the wire-mesh tube, a transducer is attached consisting of a section of smaller-diameter waveguide fabricated from a standard 3 1/8" (approximately 8 cm) diameter EIA coaxial transmission line. Two orthogonal excitation probes are at the input end of this transducer, of proper length and distance from its connecting end so that each is matched to a 50-ohm coaxial feedline when the input end of the transducer is terminated in the characteristic impedance of the TE_{11} mode excited by the probe. When energy is fed to the vertical probe, it excites a propagating TE_{11} mode in the transducer. A circular polarizer, located near the excitation probe, consists of four stubs that convert the linearly polarized TE_{11} mode to the circularly polarized TE_{11} mode. The circularly polarized mode in the transducer is, in turn, matched by four tuning stubs to the circular exposure waveguide which is terminated in the characteristic impedance of the propagating modes.

Three major propagating modes (TE_{11}, TM_{11}, and TE_{31}) can be excited at the aperture of the transmitting transducer. The electrical-field configuration of the three modes is shown at the top of Fig. 9(a). Since the first two of these modes have electrical-field distributions that most closely match those of the exciting aperture, they represent most of the propagating energy in the waveguide. Thus, the length of the exposure

waveguide is chosen so that these two propagating modes will reach the termination in the circular waveguide in the same phase relation that exists at the feed end. This phase relationship requires that the length (L) of the guide be equal to an integer (n) multiplied by the reciprocal of the difference between the reciprocals of the wavelengths λ (TE_{11}) and λ (TM_{11}) of the two important modes.

The termination end of the waveguide has a transducer similar to the transmitting transducer but without the circular polarizing stubs. Energy of the propagating modes in the circular waveguide is matched to the termination transducer through four impedence-matching stubs where it finally couples to probes that are connected to 50-ohm loads.

The fields in the circular waveguide vary as a function of both transverse and longitudinal position owing to interference between the different propagating modes. Tests have shown, however, that the average SAR in the tissues of an exposed subject will remain relatively constant regardless of the position or movement of the animal. When the exposure waveguide is empty, energy coupled to the transmitting transducer is transmitted through the circular waveguide in the form of circularly polarized TE and TM modes with very little insertion loss, approximately 10%, and very little reflection to the source (voltage standing wave ratio [VSWR] less than 1.5).

When an animal is placed in the chamber as shown in Fig. 9(b) and is exposed to the traveling waves by energy fed into the terminal (P_{IN}), some energy (P_A) will be absorbed by the animal, some (P_W) will be absorbed by the walls of the chamber, some will be 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 2450-MHz circular waveguide, and some (P_{TA} and P_{TB}) will be absorbed at the termination terminals. The reflected component P_{RR} can be measured (as CP_{RR}) at the reflecting arm of the bidirectional coupler placed between the source and the input probe, where C is the coupling coefficient of the bidirectional coupler. The reflected component P_{RL} can be measured directly at the other terminal of the transmitting transducer. The power level of the incident energy launching the right-hand circularly polarized waves can be measured (as CP_{IN}) at the incident wave terminal of the bidirectional coupler. The power level of energy transmitted beyond the animal can be measured at the

terminals (P_{TA} and P_{TB}) of the termination transducer. The sum of power levels of energy absorbed by the animal and the chamber walls can be obtained from the equation in Fig. 9(b) in terms of the known components of energy transmitted to the various terminals of the transducers and the directional couplers.

Since the wavelengths of the TM_{11} and TE_{11} modes are 18.06 and 13.09 cm, respectively, a large-diameter (20.3 cm) circular waveguide approximately 95 cm long satisfies the equation for a good match to the termination transducer for a value of n = 2. The transverse components of the electric fields for the two important propagating modes is in phase on the axis in the center of the circular waveguide. The maximal deviation from this phase relationship for a distance of 12 cm on each side of the midpoint is 90^{o}. A distance of 24 cm centered at the midpoint corresponds to the region where the dominant propagating modes are reasonably in phase, ensuring that a subject is well exposed to the electric and magnetic fields of both modes.

Experimental measurements have shown that reflections from the animal placed at the midpoint position undergo little change from the original direction of rotation or polarization with respect to propagation. Thus, the reflections arrive at the transmitting transducer chiefly as circularly polarized waves of the opposite direction of rotation and with a phase relationship that couples quite well with the P_{RL} terminal of this feed transducer. Energy transmitted beyond the animal remains chiefly in the same sense, as circular polarization coupling to terminals P_{TA} and P_{TB} at the termination transducer. Laboratory measurements indicate that the mean value of input VSWR is below 1.7 at the input terminal of the cell when it is loaded with a freely moving rat, and the net mean power coupling to the animal varies from 25% to 90%, depending on its position.

Fig. 10 illustrates the construction and dimensions of the exposure cell. The cell consists of a section of circular waveguide constructed of galvanized wire screen of 6.3-mm mesh. Short sections of brass tubing are attached to the mesh to provide stability for maintaining a proper circular shape and to form a base for anchoring the phosphor bronze fingers that provide electrical contact between the sections of the chamber. Details of the transmitting and termination transducers attached to each end of the

exposure waveguide are shown in Fig. 11, and details of the plastic cage for holding the animal within the exposure waveguide are shown in Fig. 12.

The water supply shown in Fig. 13 is electrically decoupled from the animal by two concentric 1/4-wavelength choke sections so that the tip of the water nozzle is extremely high in impedance, virtually preventing conduction currents between it and any contacting object.

Power Generation and Distribution

Each exposure room was equipped with two 2450-MHz pulsed microwave sources (Epsco, model PG5KB, Trenton, NJ), each capable of providing an average output power of 20 W and a peak power of 5 kW. These generators are controlled by a microprocessor to deliver repetitive pulse trains, consisting of 50 10-μs-wide pulses, with onsets separated by 1.25-ms intervals. This is the equivalent of an 800-pps source being square-wave modulated at the rate of eight times per second with equal on/off durations of 62.5 ms. These modulation characteristics, shown in Fig. 14, provide an 8-Hz modulation component corresponding to the dominant EEG frequency in the rat (Coenen, 1975).

The power from one generator in each equipment rack was transmitted to three alcoves by means of a 7/8"-diameter foam-filled coaxial cable. In room T131, the power was equally distributed to alcoves A, B, and C (and by one generator in room T137 to alcoves F, G, and H) via a 3-way power splitter and was transmitted to the interior of each alcove by means of another section of 7/8" cable. At the back wall of each alcove, the power was fed through a single-pole double-throw (SPDT) coaxial relay to a 2-way power splitter. Power from each arm of the 2-way splitter was fed through sections of 1/2"-diameter foam-filled coaxial cable to a 5-way power splitter, thus the power was again equally divided (left and right) and transmitted through isolators to the two groups of five active exposure waveguides in each alcove (Fig. 15). The distribution system of the second generator in each room was similar to that just described except that the power was initially split in two ways, rather than three, to power alcoves D and E in room T131 and I and J in room T137.

We could remove the power to any particular alcove by shunting it to a 20-W load located at the SPDT coaxial relay activated by switches located

on the control panel at the equipment rack containing the signal sources and the microprocessor control unit. A red light above the door to an alcove indicated when the waveguides within were activated.

The average attenuation for RF power transmitted through the transmission lines, power splitters, coaxial relay, and isolator to the waveguide exposure cell was approximately 2.7 dB. Thus, for typical operating conditions with an output power of 8.4 W from the source, the power delivered to each of the 10 exposure cells in an alcove was 0.15 W, producing an average power density of 500 $\mu W/cm^2$ and a peak power density of 125 mW/cm^2 in each exposure cell. The actual incident average power density, however, may be set at any level from 0 to 1.2 mW/cm^2 as determined by the output of the power generator. The maximum average power density of 1.2 mW/cm^2 allows peak power densities as high as 300 mW/cm^2 to be produced in each exposure cell with the selected modulation parameters.

The forward and reflected power at each generator output terminal was measured through a directional coupler and digital power meters interfaced to the microprocessor. Also, the input, the reflected, and the transmitted power associated with one exposure cell per room was monitored to obtain a recording of the average absorption loss of the waveguide/rat assembly from which the average SAR could be calculated from the known waveguide power loss and weight of the rat.

Calibration of Power Distribution System

We made several measurements to determine the relationship between the output power from the signal generator and the power delivered to each exposure cell, and the total transmission loss in dB to each exposure cell. The mean and standard deviation of the power delivered to each cell was also calculated for each alcove cluster (groups of cells fed by a particular signal generator). The output power of a calibrated CW source of known frequency was measured with a calibrated directional coupler and power meters and compared with the power delivered to each exposure cell as measured with a calibrated directional coupler and power meter.

During the course of the measurements, we found that power delivered to groups of cells in different alcoves fed by the same generator varied

considerably. The problem was traced to the power splitters which were extremely sensitive to any slight impedance differences, as seen at the output cables to the different alcoves. This caused significant variations of power delivered to the different output arms of the power splitter. The problem was eliminated by the use of coaxial tuners connected to each output arm of the power splitters delivering power to each alcove. The tuners were adjusted to compensate for the imbalances in impedance. During calibration a conventional electronic counter was used to set a CW signal source to the proper frequency. During actual pulse operation, however, the pulse generator frequencies were set to the correct frequency periodically by means of an HP 5345A frequency counter designed for measuring pulsed signals.

Each room contained a total of nine power meters, two each for the incident and reflected power at each generator and five for the incident, reflected, and transmitted power at the multiple terminals of the respective exposure cells designated for determining the average SAR in the experimental animal. Throughout the chronic study, the monitoring system was connected each day to a different exposure cell so that every cell was monitored for 1 day every 50 days over the course of the experiment.

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 450-MHz 1-mW/cm^2 RFR. To determine the conditions necessary for simulating such exposure, we first had to quantify the relationship between the input power and the average and distributed SAR in the body of an exposed rat living in the exposure waveguide. We also had to determine the relationship between 450-MHz 1-mW/cm^2 radiation to the average and distributed SAR in an exposed man.

We developed a microprocessor-controlled twin-well calorimetry system to measure the average SAR in the bodies of the exposed rats. Rats of various body weights were exposed in five orientations in the waveguide. Both power meter and calorimetric measurements were made. The final SAR data were averaged by weight range, on the assumption that a live rat would

spend equal time in each of the five orientations. The ratio between the actual SAR (measured calorimetrically) and apparent SAR (measured by the power meters) was the final correction factor. The SAR in live animals was then predicted by the power meter measurement and the correction factor. Complete description can be found in Volume 3 of this report.

A 10-kW 2450-MHz klystron high-power microwave amplifier mainframe was used to provide sufficient power for simulating the exposure of man to 450-MHz radiation through the use of 1/5 scale models of man. The models were then exposed to 2450-MHz radiation in a large anechoic chamber. Average SAR was measured by exposure of scale man-models filled with a liquid synthetic tissue, and SAR distribution patterns were measured thermographically by exposure of similar models filled with synthetic gel, as described by Guy et al. (1976, 1978). Details are described in Volume 2 of this Final Report.

Selection of Exposure Levels

During the planning stages of the experiment, three options were formulated regarding the criteria for simulating chronic human exposure to RFR. These options are outlined in Table 1. The first option is consistent with the original intent to simulate exposure of man to 450-MHz 1-mW/cm^2 RFR. The second option would be to simulate an exposure regime in which the worst-case conditions corresponding to the maximum allowed average SAR (0.4 W/kg) specified in the 1982 ANSI C95.1 RFR Standard would exist at some period in time, but would not be exceeded. The third option would be to set an exposure level for an average SAR, as averaged over the lifetime of the animal, equal to the maximum 0.4 W/kg allowed by the new standard.

After considering all three options, we decided to use option 2 because it best simulated the exposure of humans, from childhood to adulthood, to the maximum exposure levels allowed by ANSI C95.1. Therefore, an input power level to each exposure alcove cluster was set so that the average input power, averaged over time and all exposure waveguides for the entire group, was 0.144 W, which resulted in an initial average SAR of 0.4 W/kg in young rats.

TABLE 1. OPTIONS FOR CIRCULAR WAVEGUIDE EXPOSURE PARAMETERS FOR SIMULATING CHRONIC EXPOSURE OF A HUMAN TO RFR

Option 1	Option 2	Option 3
450-MHz 1-mW/cm^2 RFR From child to adult (based on 0.68 W/kg for adult)	Equivalent to no more than allowed by ANSI C95.1 at any time during lifetime (max. 0.4 W/kg during childhood)	Equivalent to maximum allowed by ANSI C95.1 (0.4 W/kg averaged over entire life-time)
Waveguide input = 0.097 W	Waveguide input = 0.144 W	Waveguide input = 0.390 W
Average power density = 0.324 mW/cm^2	Average power density = 0.480 mW/cm^2	Average power density = 1.30 mW/cm^2
Average SAR 200-g rat = 0.27 W/kg	Average SAR 200-g rat = 0.40 W/kg	Average SAR 200-g rat = 1.08 W/kg
Average SAR 800-g rat = 0.68 W/kg	Average SAR 800-g rat = 0.10 W/kg	Average SAR 800-g rat = 0.27 W/kg
Predicted range of hot-spot magnitude in 330-g rat 0.52-1.09 W/kg	Predicted range of hot-spot magnitude in 330-g rat 0.63-1.33 W/kg	Predicted range of hot-spot magnitude in 330-g rat 1.71-3.60 W/kg

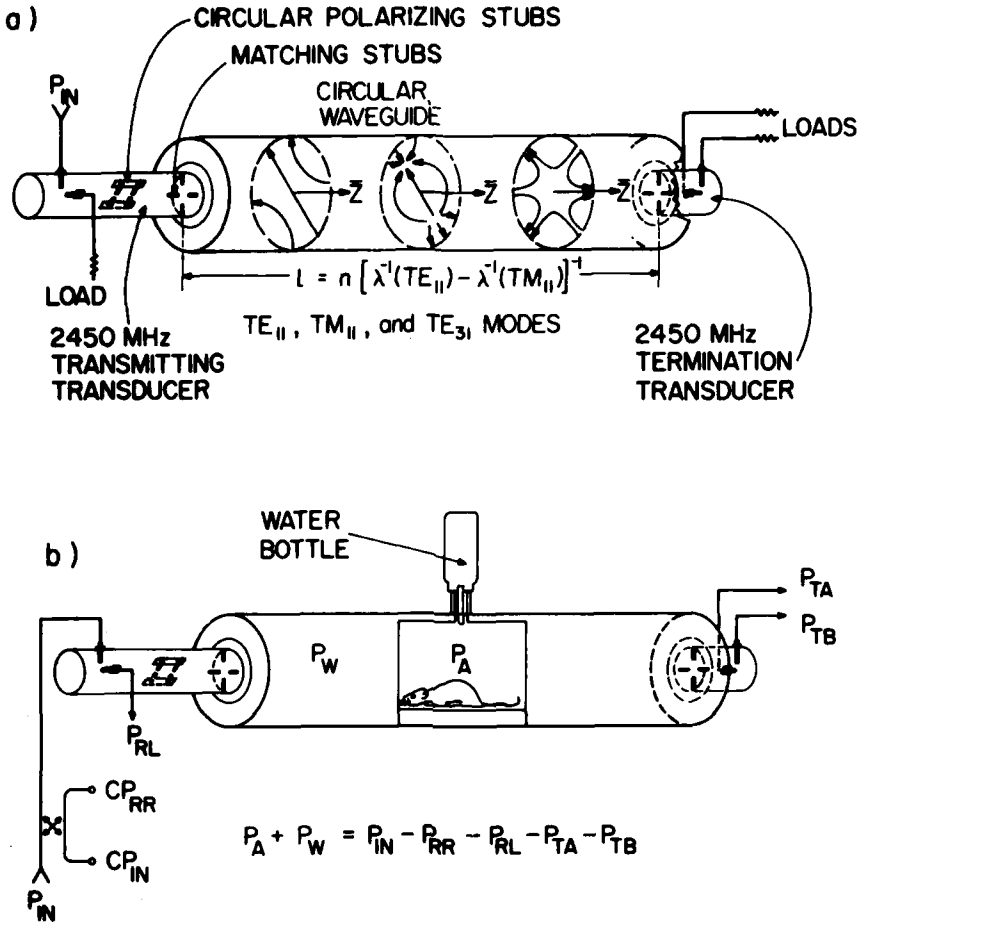


Figure 9. Operation of 2450-MHz exposure chamber: a) empty, b) with animal.

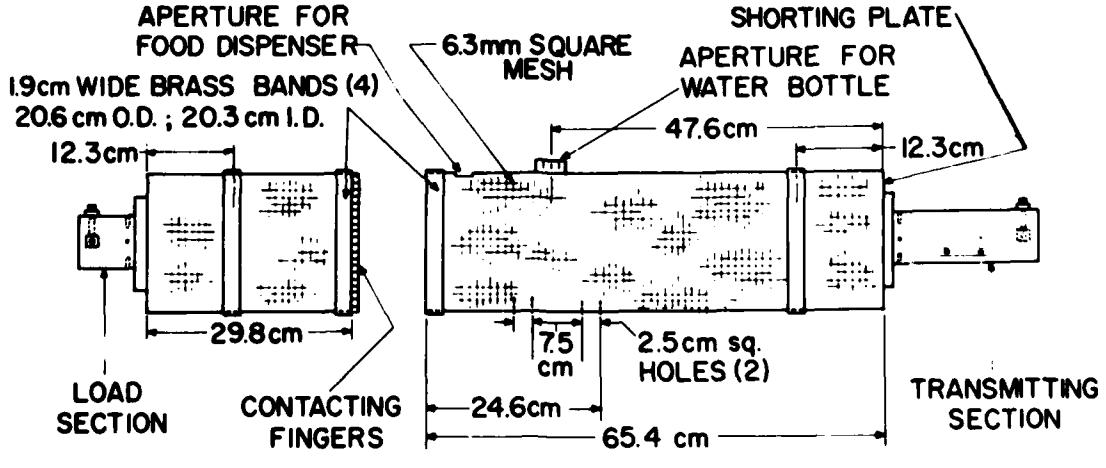


Figure 10. Schematic of exposure chamber.

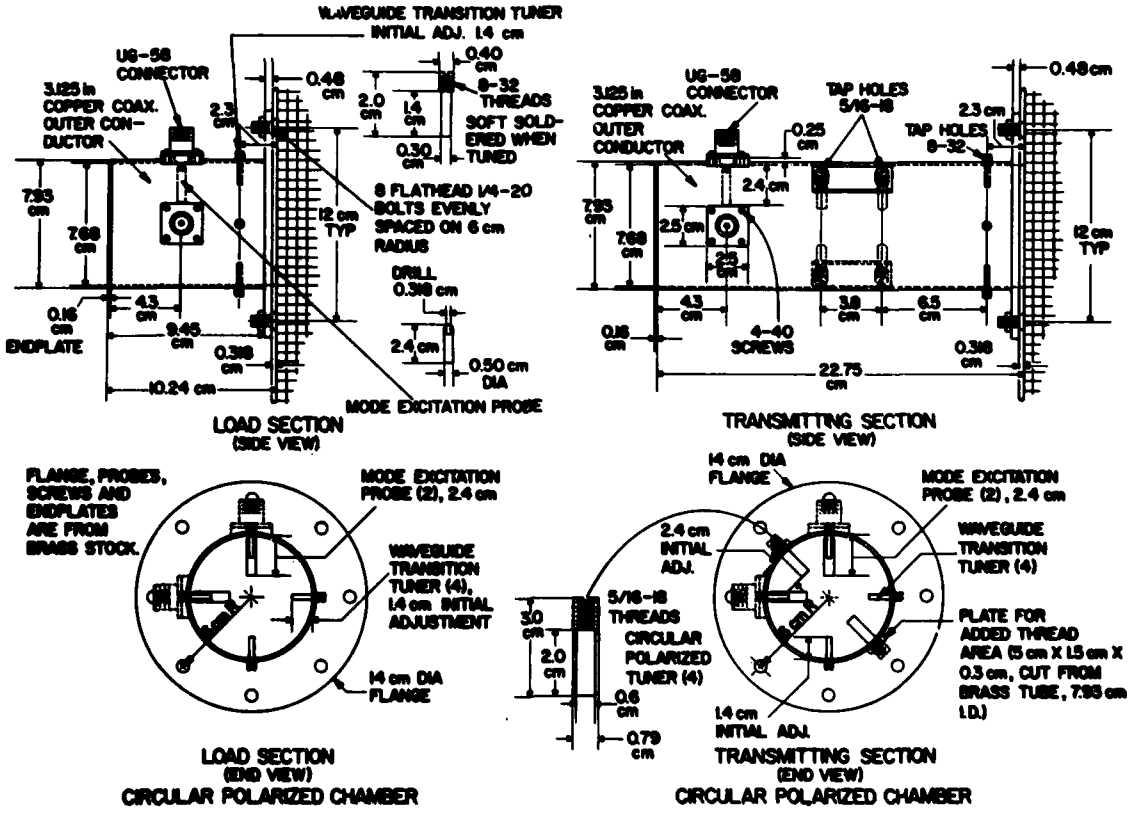


Figure 11. Schematic of transmitting and load (termination) transducers.

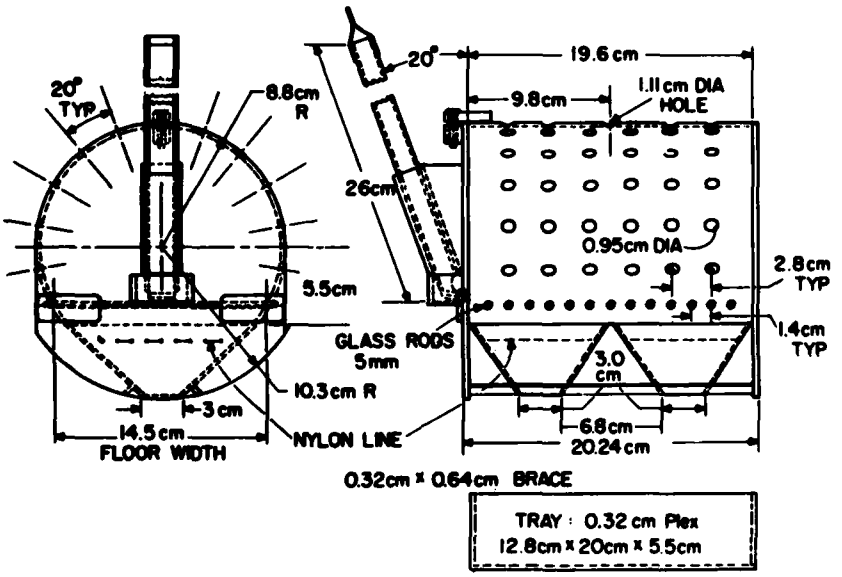


Figure 12. Schematic of plastic cage for holding animal in exposure chamber.

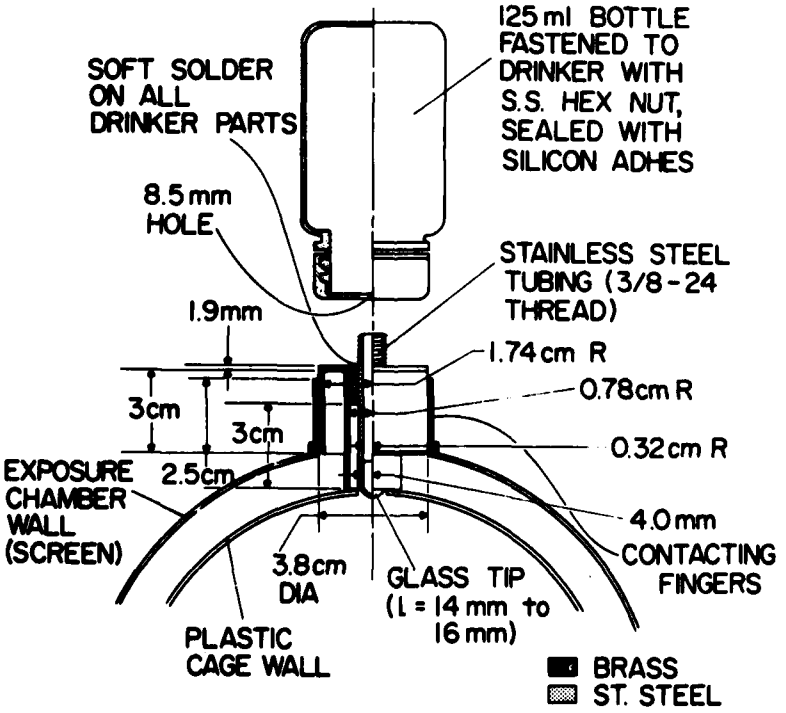


Figure 13. Schematic of water supply for exposure system.

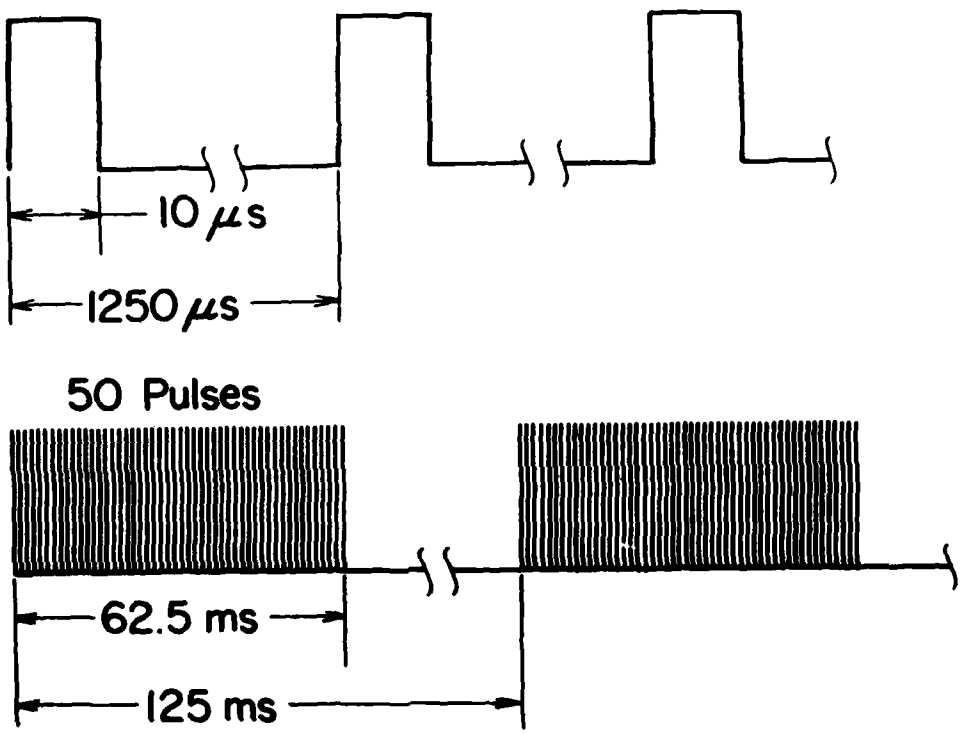


Figure 14. Modulation characteristics of the microwave pulse groups per second: 50 10-μs-wide pulses per group, with a repetition rate of 800 pps.

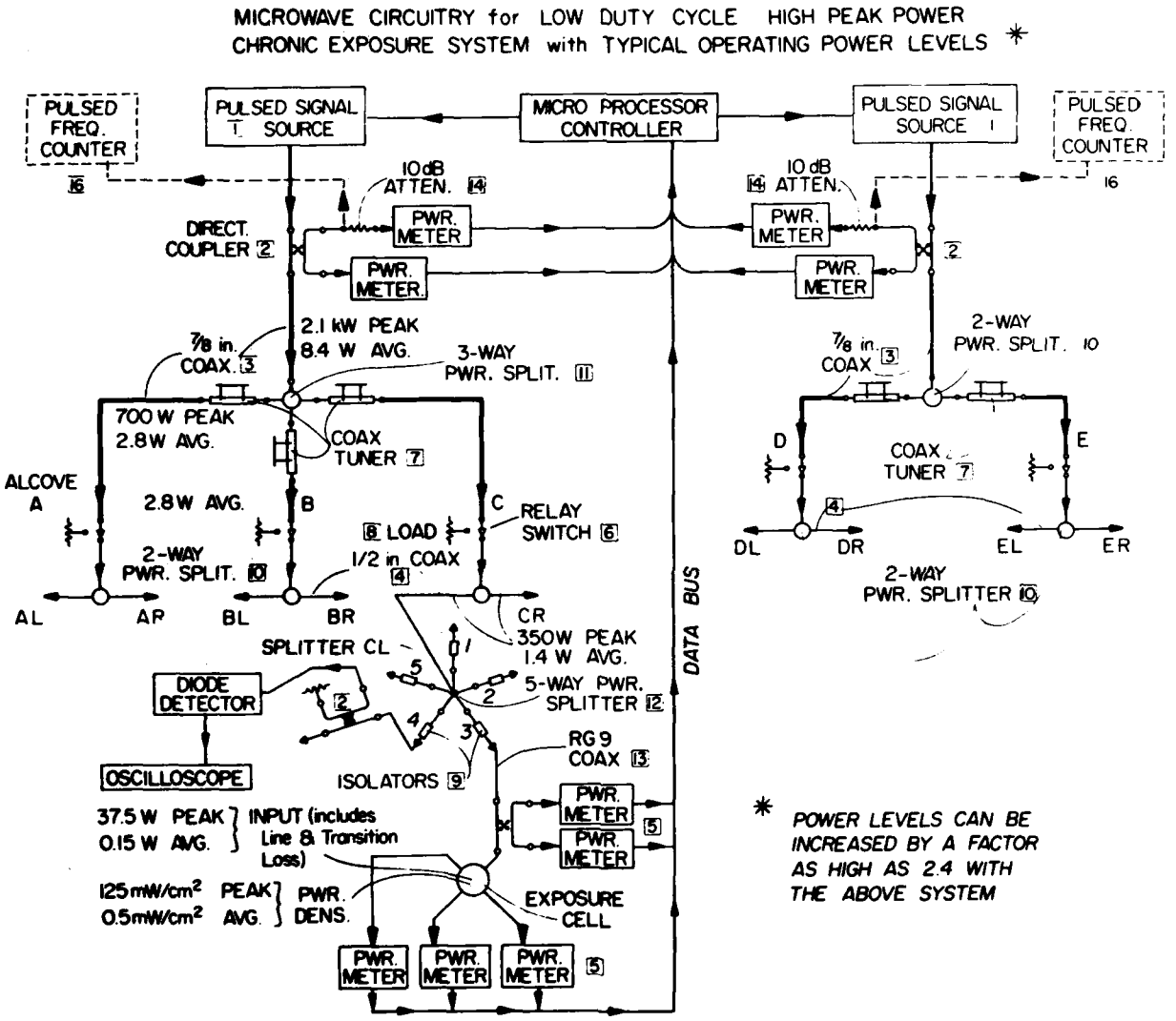


Figure 15. Schematic of the power-distribution system in room T-131.

DATA ACQUISITION SYSTEM

The system used for collection of daily data was designed specifically to meet the needs of this project and to be low in cost compared with commercially available systems. The system design includes the following functions within each experimental room:

Modulating of the microwave signal generators
Monitoring of total power output of each generator
Insertion loss of one waveguide daily for SAR calculations
Monitoring of temperature and humidity in each alcove
Collection of weight measurements, e.g., body weight, food, water
Real-time calculation and display of weight gains or losses
Real-time calculation and display of food and water consumption
Maintenance of two independent data-collection stations
Measurement of O_2 consumption/CO_2 production rates
Maintenance of autoanswer telephone link for remote access
Storage of data on floppy diskettes for off-line analysis
Immediate printout of data as hard-copy backup

To implement these functions, we used a distributed processing system that combined the features of both a multitasking system and a networking system (see Fig. 16). The tasks were logically divided into three groups: those related to (1) the weighing of animals, food, and water; (2) the control and monitoring of the microwave distribution network; and (3) the measurement of O_2 and CO_2. Three separate microcomputers were constructed to perform the specific tasks required by each of these task groups. The individual microcomputers were respectively designated the Master, the Microwave Slave, and the Metabolism Slave. The Master ran a multitasking operating system executive that supported ten concurrent tasks. Two of these tasks were the network communication links with the Slave computers (the Master served as the hub of the network). More details of the three-microcomputer system located in each room follow.

Master Microcomputer

The functions of the Master system were related to the acquisition of the data collected during daily maintenance procedures, including the prompting of the animal technicians, the collection of data from the electronic balances, the calculation of weight gains and losses and food and water consumption, and the display and archiving of this information for later analysis. In addition, as the hub of the network, it maintained communication links with the other microcomputers as well as a remote telephone link.

The basic hardware chosen for the Master was an S-100 bus compatible mainframe with slots for 22 individual function cards. The system was configured with the following 12 function cards:

Z-80 based processor	Time-of-day/interrupt clock
16K ROM (read-only memory)	Video interface #1
16K RAM (random-access memory)	Video interface #2
4-port parallel interface	Video interface #3
2-port serial interface	Disk drive controller
8K RAM	Autoanswer/dial modem

To protect this system and its associated electronic balances and video monitors from loss of data during abrupt power failures, we used a 1000-W uninterruptible power supply. This system was housed in a standard relay rack in each procedures alcove.

The software developed for this system included the following custom assembly language packages:

-- multitasking system executive driven by 60-Hz interrupts. At each interrupt the processor stored the relevant information concerning the currently executing task and resumed execution with the next task in the queue. This queue supported 10 tasks and was circular in nature, i.e., after the tenth-task information was stored, the processor resumed where it had previously exited the first task. If a task was inactive, or simply in a wait loop (as when waiting for data from a balance), the 1/60-s time slice currently allotted the task was forfeited and the processor proceeded to the next task.

This feature allowed the executive to service each task more rapidly than the expected 6/s rate suggested by the interrupt frequency.

-- modified drive handlers for the commercially obtained disk operating system to provide compatibility with the interrupt-driven multitasking executive. Disk accesses could be interrupted except during the actual transfer of a data sector.

-- a reentrant and shared-access BCD format floating-point arithmetic package, including formatting and conversion subroutines.

-- data link protocols for remote access to the system via the autoanswer modem interface. The remote user appeared to the system as the main console device.

-- communications protocols for data exchange between the Master and Slave microcomputers.

The above listed software, as well as most of the application programs, resided in 16K of erasable/programmable read-only memory (EPROM) so that powerline disturbances or other electromagnetic interference would not affect the integrity of program execution. Scratchpad areas, transient programs such as BASIC, and data buffers were maintained in 24K of RAM. A detailed memory-map for the Master system is shown in Fig. 17.

Two tasks of the Master system were identical, comprising the routines for collecting daily weight data. Each of these tasks supported the hardware of a separate weigh station consisting of a Mettler P3000 electronic balance with a range of 0-3500 g and resolution of 0.1 g, a custom-built data entry keyboard (Fig. 18), and a CRT display unit. The balance and the keyboard were controlled through a multiplexed parallel data channel controlled by a parallel port from the Master. The CRT display was interfaced via a 1-Kbyte memory-mapped buffer in the address space of the Master. On the central work island in each experimental room, two weigh stations were located; each had access to the data base for all alcoves, so either could be used to perform the daily routine for any alcove.

The daily routine included weighing the animals and the food and water containers in the order specified by the system. As the animals, food chutes, and water bottles were weighed, internal checks were made to ensure that inputs outside the possible range of legitimate weights were rejected by the system.

Each morning a copy of the previous day's data was loaded into memory for use in calculating body weight gains and losses and food and water consumption. These values were calculated after the acceptance of valid data from the balance and were displayed concurrently with the balance data on the CRT display for inspection by the technician. Before any information was entered into the data base, it was inspected on the screen by the technician for acceptance or rejection. Data could be rejected if an obvious error were detected, e.g., mistared balance. Once a data value for an animal on a particular variable (e.g., body weight) was entered, it could be overwritten only if a technician took intentional action to correct a value. For example, when a food container was spilled after weighing, a new weight would have to be taken.

Microwave Slave Microcomputer

This microcomputer was mounted in the same rack as the microwave signal generators and power meters. Its functions included generating the modulation signal for the microwave generators and monitoring the microwave power meters, temperature and humidity, and the switching network that turned the alcoves on and off (i.e., diverting the energy to the load or to the waveguides).

This microcomputer was a custom S-100-compatible unit with room for eight function cards. The heart of the system was a single-card computer that contained a Z-80 processor, 8K ROM, 1K RAM, one serial interface, and three parallel interfaces. An analog-digital converter interface digitized the analog output from each of nine power meters upon command. The serial port was used for data communication with the Master processor at 19.2K baud. This baud rate, as well as other features of this unit's operating system, could be reprogrammed by the Master. One line of one parallel

output port was used to synthesize a TTL timing pulse as an external sync trigger to the microwave generators. A parallel input port monitored the state of the switches/coaxial relays that controlled delivery of radiation to the various alcoves. A four-digit panel meter was used to display the averaged forward power from either generator. A block diagram of this system is presented in Fig. 19.

The temperature- and humidity-monitoring device was a custom-built unit capable of monitoring 16 channels of analog dry-bulb and wet-bulb temperature sensor input. The Analog Devices A/D590 sensor with a 1 μA/^{o}K output was used. Each sensor was prepared for use by attachment of the leads to the end of RG174 miniature coax and encapsulation of all but the tip. Since it was a current device, it could be used remotely on cable lengths up to 50 ft long. A current-to-voltage conversion was made for each sensor, and this signal was then multiplexed to the input of a commercial panel meter with 0-1.999 V input range. The decimal point display was adjusted to display 0-300.0^{o}K. The panel contained an integral A/D convertor and BCD output channel. This BCD could be read by the microcomputer. An analysis of the wet-bulb/dry-bulb calculation of humidity revealed that, for the narrow range of temperatures used in this study, a small table containing the limit values could be retained in memory; and by interpolation, the correct humidity could be obtained with 1% accuracy.

The operating system for this unit consisted of a foreground/background executive. The foreground task continually monitored the communication link with the Master and performed any requested data transfers and reprogramming requests. The foreground program also performed calculations at 1-s intervals on data collected from the power meters and the temperature/humidity monitor. These calculations were available to the Master as instantaneous readings or as 1-min averages. The background task was interrupt-driven and contained routines to synthesize the sync signal for the microwave sources, select channels on the A/D convertor for collection of power meter data, and select channels for accepting data from the temperature sensors. Data from each meter were collected at the rate of 36 Hz and averaged once each second by the foreground routines before use by the Master. Support routines included floating-point arithmetic

routines and a monitor that allowed for calibration of the A/D converter and the various sensors of the temperature/humidity monitor.

We used the data for the five power meters associated with one waveguide per room per day to calculate the insertion loss for that waveguide system. These calculations were based on the raw data obtained from the power meters and the attenuation factors associated with the directional couplers used. For easy alteration, these attenuation factors were stored in a separate ROM in the system.

Metabolism Slave Microcomputer

This unit performed the functions associated with collecting data from oxygen-consumption and carbon-dioxide-production measuring devices attached to the two waveguide assemblies in each room. The unit was a Standard-bus-compatible unit containing a Z-80 processor, 32K RAM, 12-channel A/D convertor, and serial-and parallel-port interfaces. Like the microwave Slave, this unit ran a foreground/background system. Again the foreground program was primarily responsible for performing internal data manipulation and communications with the Master. The background routines were interrupt-driven and performed the tasks of switching a solenoid that selected the air lines to be monitored and converting of analog information from the three flow meters, two oxygen analyzer cells, and the carbon dioxide analyzer. A block diagram of this system is presented in Fig. 20.

Central Data Analysis System

Data collation and various levels of reduction were performed at multiple sites within our research facility. The first level of this process was performed by research technicians on the small microcomputer dedicated for that use. This system provided tight-loop feedback to the SPF laboratory personnel via specific statistical indices indicating how uniformly they were fulfilling their responsibilities as primary animal care personnel.

Intermediate-level reduction and simple daily, weekly, and monthly analyses were performed (with a commercial package similar to the statistics package for the social sciences) on a microcomputer system. This system had 2 Mbytes of on-line floppy disk storage.

Large multivariate analyses were conducted with a DEC 10 installed at a campus facility or on the DEC PDP 11/34 located in the main lab. The PDP 11/34 computer had 256K memory and 150 Mbytes of hard-disk storage.

Data Auditing Procedures

We established procedures and continually refined them to ensure data integrity at various points during collection, reduction, and analysis. This "audit trail" involved the data collected automatically by the computer monitoring systems in each room (e.g., temperature, oxygen consumption, and insertion losses), the daily computer-assisted data collection by the technicians during the waveguide maintenance procedures (e.g., body weights), and the data that must be entered by hand (e.g., findings with regard to blood chemistry, corticosterone, and pathology).

To validate the computer-assisted collection of data during daily maintenance, we wrote programs to scan the data after placement on the disk. If data were poorly transferred from memory to disk, repeated attempts to obtain a proper transfer could be made. Hard-copy outputs, obtained immediately after completion of each alcove function, were kept in case disk data for off-line entry into the data base were lost. This allowed as much time as required for the data collected daily on diskette to be transferred to the final archiving disk packs on the PDP 11/34 and for multiple printed summaries to be obtained and filed.

The data from blood chemistry sheets were entered into the computer system by hand; e.g., a program prompted the keypunch operator for each entry and performed immediate range checking specific to the parameter being requested. All data were entered for a complete blood chemistry run (e.g., 200 animals each with 26 chemistry variables) within approximately 1 week after the bleeding session.

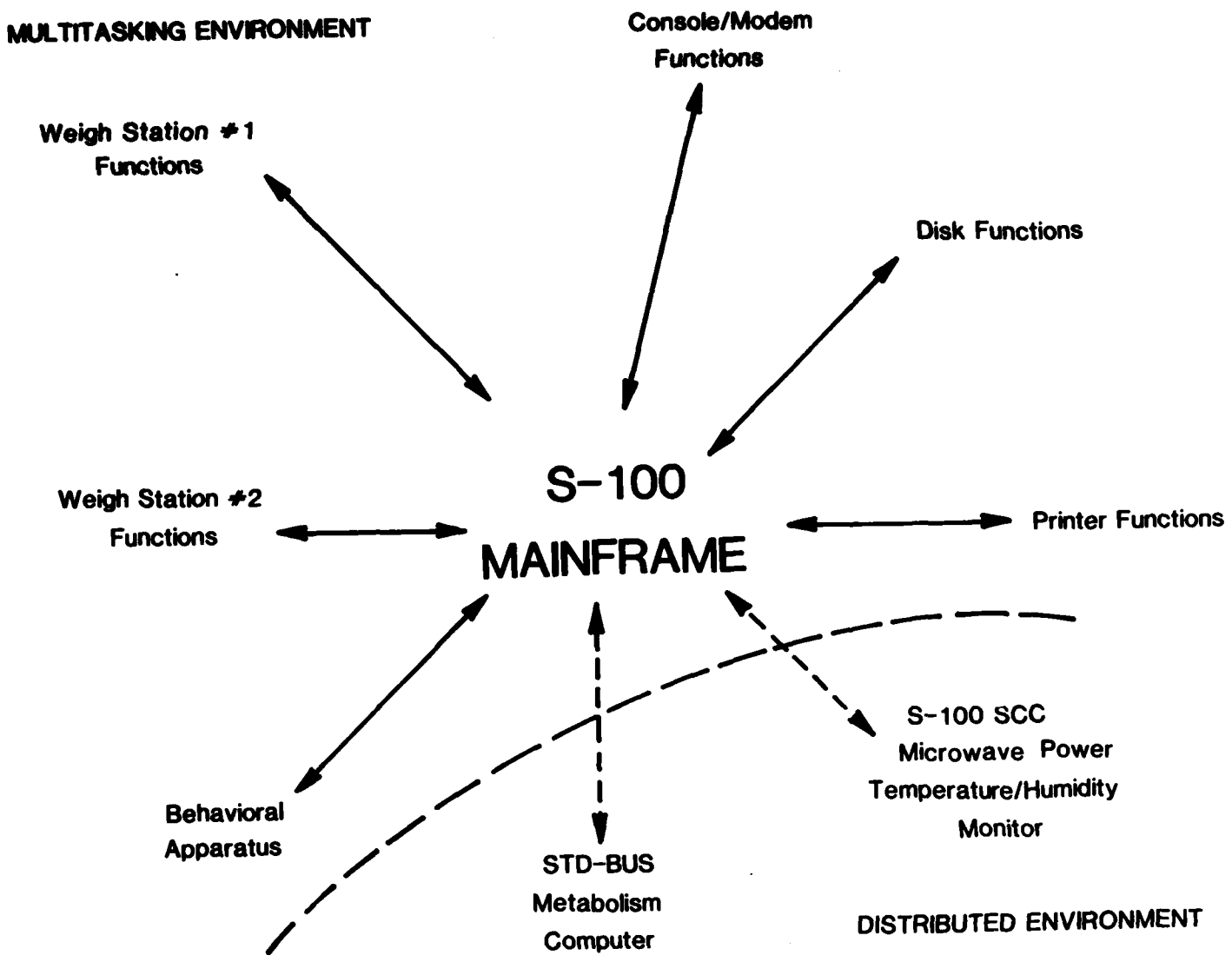


Figure 16. Schematic of data-collection system used in each animal room: multitasking and network environments.

MEMORY MAP

Start Address	Memory Type	Function	Ram Use
0000	RAM	Interrupt Vectors	
0050	RAM	Data Buffer	
1800	RAM	Buffer For Monitor	
2000	RAM	DOS Routines	2000-27FF
2A00	RAM	Transient Programs	2A00-6000
B000	VRAM	Console Output	B000-B3FF
B400	VRAM	Weigh Station #1	B400-B7FF
B800	VRAM	Weigh Station #2	B800-BBFF
C000	EPROM	Multi-tasking Routines	1C00-1C80
C400	EPROM	Messages	
C800	EPROM	Re-entrant Routines	
CC00	EPROM	Messages	
D000	EPROM	Temperature Summary	
D400	EPROM	Behavioral Analysis	
D800	EPROM	Printer Routines	
D000	EPROM	Modem Driver	2930-2940
E000	EPROM	Monitor Routines	2940-29FF
E400	EPROM	Monitor Extensions	1C70-1CFF
E800	ROM	N* Disk Controller	
EC00	EPROM	Station #1 Program	1D00-1DFF
E000	EPROM	Station #2 Program	1E00-1EFF
F400	EPROM	Floating Point Routines	1F80-1FFF
FC00	EPROM	Interruptible DOS Driver	

Figure 17. Memory map showing distribution of control programs within the Master computer.

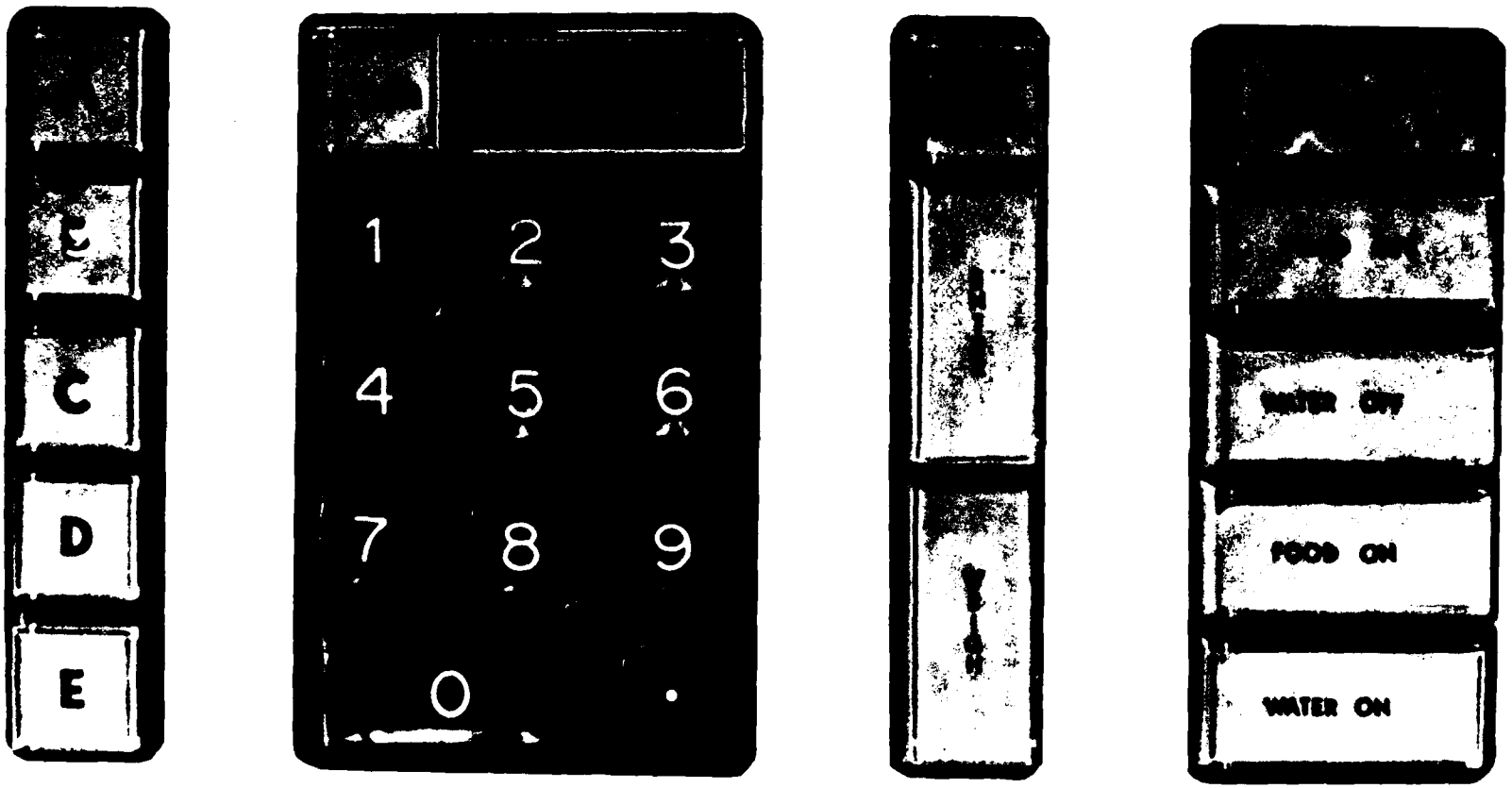


Figure 18. Photograph of custom data-entry keyboard showing the various collection options.

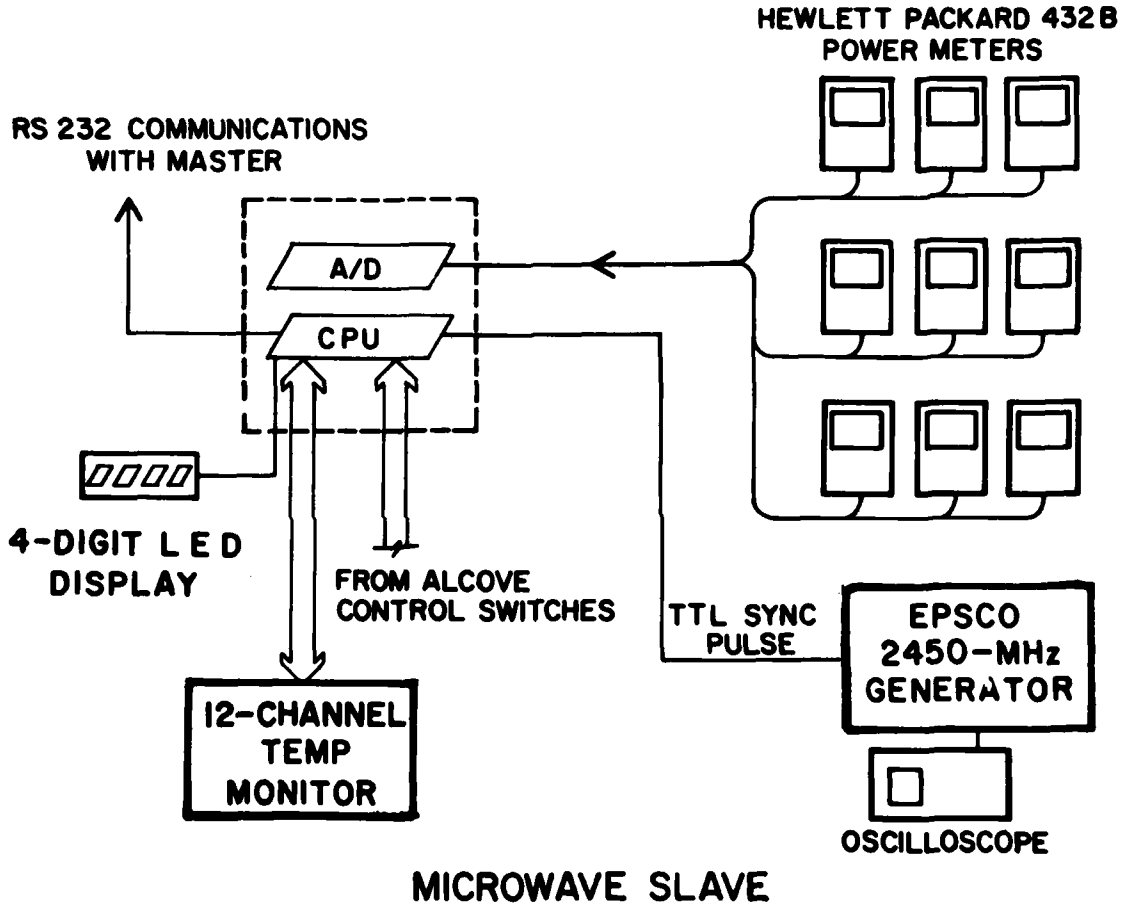


Figure 19. Block diagram of interconnections of microwave Slave microcomputer, power meters, microwave generator, and temperature monitor.

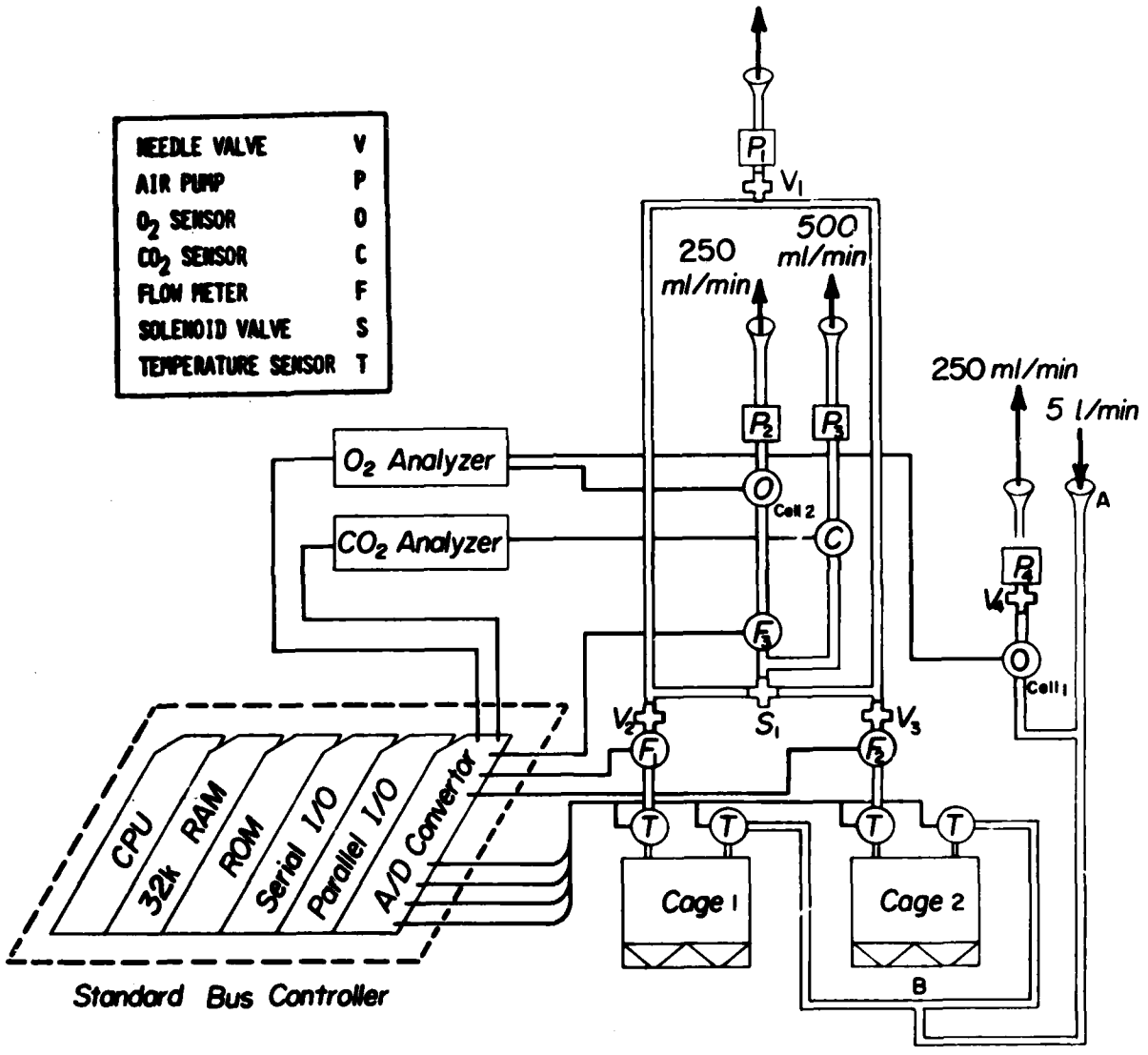


Figure 20. Schematic of metabolism Slave microcomputer, oxygen and carbon dioxide analyzers, and airflow monitoring hardware.

ANIMAL SELECTION AND PROCUREMENT

During early planning of the study, we concluded that using conventionally bred and reared rats would present a problem during interpretation of the data because of the possible interaction of the general health status of the animals and the exposure situation. We therefore decided to acquire a colony of cesarean-derived barrier-reared animals and maintain them under SPF conditions throughout the study.

The Sprague-Dawley (SD) rat was suggested as an appropriate animal since it has a rapid growth rate and reaches an average weight of 360 g (male) by 84 days of age. This large size was important because of the frequency and volume of the blood samples drawn. The gentle disposition of the animal was also important. Information on spontaneous tumor incidence, growth curves, genetics, incidence of congenital malformations, reproductive data, and base line hematological and blood chemistry values for the SD rat was obtained from various vendors and the Laboratory Animal Data Bank.

Experimental Animals and Health Screen

We made initial arrangments during the first phase of this project to use rats obtained from Charles River Breeding Laboratories. Although a few months prior to the start of the study they confirmed that Proteus sp. was present in the colony of SD rats from which we were scheduled to obtain our test animals, we decided to continue to use the Charles River rats. However, 6 months prior to the start of the exposure period, the Charles River SD rat colony became serologically positive for Kilham rat virus. This outbreak precluded continued use of this colony as a source of animals for the project.

Serological testing by Yale University indicated that the Camm Research Institute, Inc., (414 Black Oak Ridge Road, Wayne, NJ 07470) maintained a colony of SD rats free of pathogens. The Camm (SD) BR rats were cesarean-derived and had the following defined microflora:

Lactobacillus casei ssp. rhamnosus
Lactobacillus acidophilus
Bacteroides fragilis ssp. ovatus
Streptococcus faecalis ssp. liquefaciens
Streptococcus lactis ssp. diacetilactis

A group of ten 90-day-old male rats from Camm were subjected to a vendor health screen 3 months prior to the start of the exposures. The results indicated that these animals were high quality SD rats consistent with the requirements of this contract.

Animal Identification Procedures

We made every attempt during the design and conduct of the experiment to use blind procedures; this included a nondescriptive waveguide identification system and a complete double-blind protocol for testing animals during open-field behavioral assessments. However, ultimately some permanent positive means of identifying the animals was required. Various methods of tagging and tattooing were ruled out in favor of a simple toe-clipping procedure (Kiemar, 1979) that enabled positive identification of each animal throughout the project.

In this system various terminal phalanges are removed (never more than two from any one appendage) in a specified pattern (Fig. 21). A pair of iris scissors dipped in 95% alcohol is used. The first digit is never clipped so that the animal's ability to grasp is not destroyed. The toes on each foot are read from left to right when the animal is viewed from the dorsal aspect. Units identification is accomplished by first reading the left hind foot (hundreds), then the left forefoot (tens), and finally the right forefoot (units).

Arrival Data

On 29 July 1980, four hundred 21-day-old male rats arrived from Camm Laboratory and were admitted to the clean hall of the vivarium complex for

a short quarantine period. During this period each animal was physically examined and toe-clipped for individual ID. Ten animals were randomly selected from the colony for the general health screen procedures, including serological testing. Fifty other animals were randomly assigned to be used for initial establishment of the immunology test procedures, and twenty others were removed to provide baseline values for a whole-body carcass analysis.

One week after arrival, 250 animals were bled to obtain individual baseline values. The next day, 200 animals were randomly assigned to treatment groups and began waveguide adaptation, in which they experienced the same daily procedures used throughout the study but without actual microwave exposure.

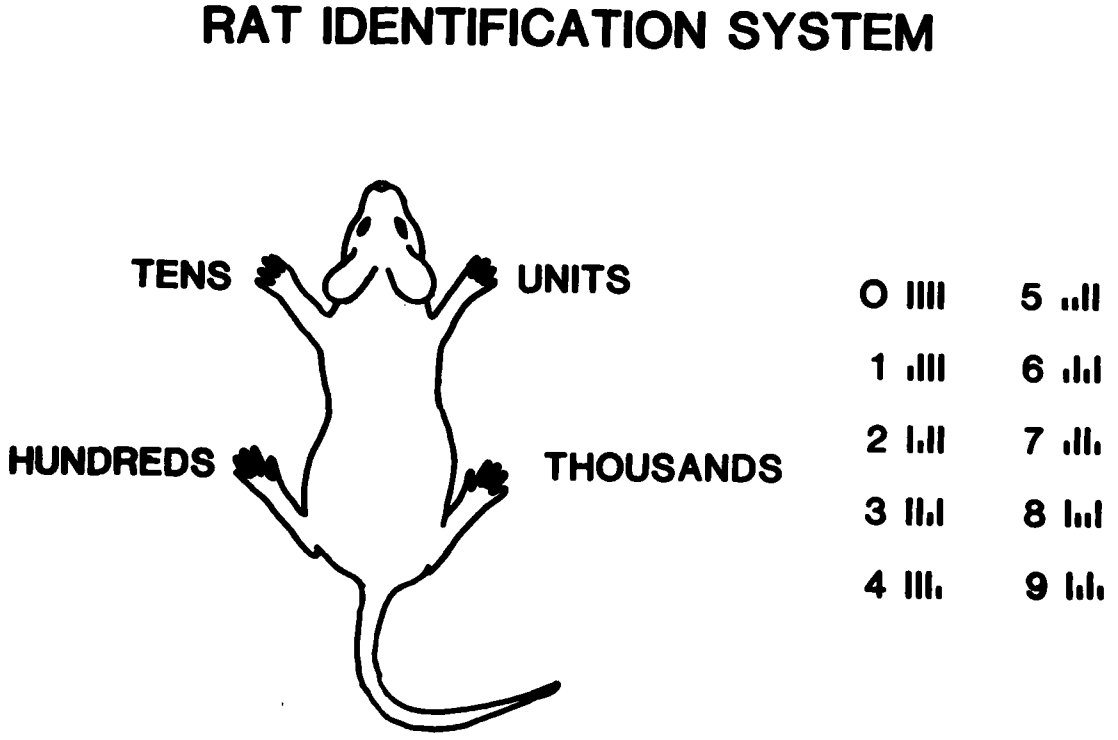


Figure 21. Illustration of the toe-clipping system used for individual animal identification.

MAINTENANCE PROCEDURES

We conducted two pilot studies during the first phase of this research project: 1) a one-room, 3-month, 100-animal pilot study to develop daily operating procedures and gain familiarity with animal care under SPF conditions, and 2) a 1-month 100% simulation of the final protocol using 200 animals divided between the two rooms. During these pilot studies many alterations were made in the daily protocol and in the data-collection programs. The finalized protocol and the criteria that shaped its development are presented in the following sections.

Procedure Criterion

The first criterion was that the total daily exposure time be maximized for all animals. The modular arrangement of the rooms, where each alcove could be independently removed from the microwave distribution network, made exposure time easy to manage. The maintenance for one alcove could be completed in about 2 h; therefore, three full-time technicians using a daisy-chain operation could conceivably have performed the maintenance of all 10 alcoves during an 8-h period. This would have cut personnel costs, but spreading maintenance out over the entire work day would have presented investigative problems. Foremost, the data collected from animals of different alcoves would have reflected as much as a 6-h difference into the rats' circadian cycles of body weight, food intake, and blood chemistry values; thereby the variability of the data collected would have been increased. We therefore scheduled the daily alcove maintenance during the 2-h window between 0800 and 1000 to obtain the most homogeneous values for the parameters of interest.

Procedure Outline

We designed the original daily procedure to minimize the time required for waveguide and cage maintenance and provide a 22 h/day exposure period. This schedule staggered the maintenance of the five alcoves in one room during the period of 0800 to 1200 such that the down time for any one alcove did not exceed 2 h and only required three technicians per room to complete. The schedule was as follows: two alcoves started at 0800, one at 0900, and two at 1000, with completion times of 1000, 1100, and 1200 respectively. Once working on an alcove, a technician was concerned only with the maintenance of that alcove until it was completed. Unhousing 20 animals from their waveguides took approximately 30 min; rehousing them once the cages had been cleaned, an additional 30 min. This allowed 60 min for the transport of cages to and from the washroom and for the washroom procedures to be completed. Additional technicians did ancillary procedures, such as bloodletting, during the hour the animals were outside their waveguides.

This maintenance schedule proved to be unworkable because the length of time required for the cages to be transported from the rooms, washed, and returned was not consistent owing to scheduling problems with other washroom activities. Also, the food and water maintenance procedures for an alcove took only 30 min, leaving the technician with nothing to do until the clean cages were returned. The cages were often returned late, so the technician was unable to rehouse the animals within the 2-h limit and the remaining schedule for that day was disrupted.

With the time for maintenance of each alcove expanded to 2.5 h, a technician could begin the process of unhousing the next alcove while waiting for the cages from the first alcove to be returned. This procedure was implemented, starting with two alcoves at 0800, two at 0830, and the last at 0900. Vivarium personnel were allowed 1.5 h to complete the washroom procedures. After unhousing two alcoves, a technician would provide the food and water maintenance for an alcove just before the cages were returned. This schedule permitted a 21.5-h/day exposure period. The procedure worked well; it kept the technicians more uniformly occupied and reduced the number of entry errors resulting from pressure to meet an unrealistic timetable.

During the two-room pilot study this schedule was further modified to interleave the schedules of two rooms so that in one room the first two alcoves were started at 0800, two at 0845, and the last at 0930; in the second room the alcoves were started at 0815, 0900, and 0945 respectively.

The magnitude of this project required that rather stringent protocols be established in great detail. Twenty persons came in contact with the SPF environment on a regular basis: the primary animal-care personnel (research technicians, lab technicians, lab helpers, and washroom personnel) as well as additional engineering support and supervisory personnel. Operating instructions for all daily and periodic activity were written and are included in the Appendix.

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APPENDIX A. SPF BARRIER PROCEDURES

LABORATORY ENTRY

I. Personnel

A. Shower

1. Shower (all personnel) with a bacteriostatic soap immediately before entering the changing room.

NOTE: MORNING SHOWERS AT HOME DO NOT SUBSTITUTE FOR ADMITTANCE SHOWERS.

2. Dry off (towels are supplied) and enter the changing room.

NOTE: ALL STREET CLOTHES AND SHOES MUST REMAIN IN THE OUTER RESTROOM AREA.

B. Don uniforms. (Coveralls are packed and autoclaved with bonnet, mask, gloves, and booties.)

1. Don coveralls first, then the bonnet, then the mask, and last, the gloves.

2. Don booties. Don the first bootie, step into clean hall with the bootied foot, then proceed in the same manner with the remaining foot.

NOTE: BARE FEET SHOULD NEVER COME IN CONTACT WITH THE CLEAN HALL FLOOR; BOOTIED FEET SHOULD NEVER COME IN CONTACT WITH THE CHANGING-ROOM FLOOR.

3. Proceed to the laboratory (clean room) via the clean hall.

C. Enter laboratory and exchange booties for tennis shoes.

a. Set booties aside. Tennis shoes are exchanged for booties when leaving at the end of the day.

b. Always keep tennis shoes in lab except for monthly washing and autoclaving.

II. Materials and Equipment

A. All materials/equipment should enter via the clean-hall side of the autoclave.

1. Autoclave items, if possible.

2. Wash nonautoclavable items thoroughly with a disinfectant solution.

B. Nothing may enter the laboratory without first passing through the clean hall.

C. Entry into clean hall is restricted to doors from changing rooms (for personnel) or autoclave (for inanimate objects).

D. Nothing may enter the clean hall from laboratories. Exit from labs is restricted to dirty hall.

RAT ADMITTANCE

I. On the day preceding arrival of the SPF rats, the following preparations should be made.

A. Prepare Data Sheets.

1. Rat's date of arrival
2. Date of birth
3. Box number (that rat was in)
4. Rat number
5. Tail clipper's ID number
6. Rat's weight
7. Strain of rat
8. Breeder
9. Comments
10. Data keeper's ID number

B. Set up necessary toe-clipping equipment.

1. Alcohol
2. Surgery tray
3. Cotton
4. Scissors (two pairs)
5. Lidocaine
6. Topical antibiotic

C. Set up acclimation-period equipment and supplies.

1. Label holding bins, metal lids, and filter bonnets.
2. Wash holding bins, metal lids, and filter bonnets.
3. Fill holding bins with bedding, and autoclave along with metal lids and filter bonnets.
4. Wash and autoclave water bottles.

5. Autoclave distilled drinking water and food pellets.
6. In clean hall

    a. divide food among holding bins.
    b. replace metal lids.
    c. fill water bottles and set aside.
    d. set filter bonnet aside (in numerical order).

II. On day the rats arrive, the following procedures apply:

A. Laboratory helper #1

1. Dons sterile mask, bonnet, and gloves.
2. Remains in dirty hall outside clean-room door.
3. Retrieves boxes of rats and holds box lid to enable clean-room personnel to remove SPF rats with a minimal amount of contamination.
4. Avoids coming in contact with anything in the clean room (including rats and inside of clean-room doors).

B. Laboratory helpers #2 and #3

1. Don sterile uniforms (refer to Laboratory Entry I.A.-I.C.)
2. Remain in laboratory.
3. Lab helper #2

    a. removes rats by tail from box; and places, individually, in holding bin, closing with metal lid.
    b. avoids coming in contact with anything not clean (e.g., the outside of the box or the dirty-hall floor).
    c. hands rat (in holding bin) to research tech for ID clipping.

4. Lab helper #3

    a. gets rats from research tech (post toe clipping).

b. fits holding bin with water bottle and respective filter bonnet.
c. sets bin in assigned pigeonhole.

C. Four research technicians

1. Research techs #1 and #2 clip toes of rats as they are handed to them by lab helper #2; they

a. secure rat in one hand.
b. wash toes with cotton swab dipped in alcohol.
c. avoid leaving cotton fibers on toes.
d. numb toes to be clipped with Lidocaine.
e. clip toes (ID number determined by holding-bin number).
f. apply antibiotic to clipped toes.
g. return to holding-bin area and hand to research techs #3 and #4.
h. disinfect gloves.
i. proceed with next rat.

2. Research techs #3 and #4

a. take rats after toe clipping and compare toe-clipped number with holding-bin number.
b. write rat ID number in data records.
c. obtain rat body weight and record data.
d. hand to lab helper #3.
e. disinfect gloves.
f. proceed with next rat.

III. After a 2-week acclimation period, rats will be reweighed, paired on an equivalent body weight basis, and randomly assigned to an alcove and waveguide number.

LABORATORY CLEANLINESS

I. Personnel

A. Upon entering the lab set booties aside in specified areas and exchange for lab shoes (tennis shoes).

B. Minimize contamination from gloves.

1. Disinfect gloves

a. immediately after donning lab shoes, to minimize contamination to the lab equipment and rats.
b. after every five rats when removing animals from or returning them to their respective hutches.
c. after removing waste-collection trays, vacuuming, and/or disinfecting bases--prior to returning to direct work with rats, food, or water.
d. after vacuuming and/or mopping the floor (of lab/alcove).

2. Replace gloves

a. when they get a hole in them.
b. after handling a rat suspected of carrying disease or parasites/fungi.

II. Lab Room

A. Animal facilities

1. Vacuum and disinfect bases daily.
2. Vacuum and disinfect floor daily.

3. Wash animal housing (hutches) and waste collectors daily.
4. Wash holding bins in which animals are housed for 2.75 h/day, fill with fresh bedding, and autoclave weekly.
5. Wash water bottles and food tubes weekly.
6. Sort food pellets (Purina Rodent Chow--certified autoclavable) according to size, autoclave, re-sort, and store in covered polypropylene containers (to prevent vermin infestation) in the clean hall until needed in a lab.

    a. keep autoclaved food no longer than 2 weeks (to guard against oxidative rancidity).
    b. keep nonautoclaved food no longer than 4 weeks.
    c. analyze preautoclaved and postautoclaved food to insure availability of heat labile nutrients.

7. Distill and autoclave drinking water before putting it into clean hall. Store autoclaved drinking water in sealed polypropylene containers in the clean hall until needed in a lab, at which time transfer it to the water tank reservoir (located under the workbench in the central workspace in each lab).

B. Lab area

1. Spray labs with a pesticide (Entacide = pyrethrins, n-octyl bicycloheptene dicarboximide) on a routine basis. Spray pesticides around both doorways of our two labs as well as the doorways of the other labs (on the clean-hall side) on a routine basis.
2. Clean up urine and feces immediately to prevent their being spread and ground into surfaces.
3. Discard food dropped on the floor.
4. Send equipment (i.e., hutch doors, filter bonnets, food tubes, water bottles) dropped on the floor to the washroom for rewashing and, if possible, autoclaving. A broken item must be reported to the research tech and the item replaced.

5. Clean counter tops and stools daily with disinfecting solution.
6. Never stand on the counters, sink, bases, or chair. When in need of something to stand on, use the step stools.

DAILY LABORATORY PROTOCOL

I. Calibrate balances.

II. Unhouse animals by alcove.

A. Call for runner to pass empty rack into lab.

B. Turn alcove power off at scheduled time (two go off at 0800, two at 0845, and one at 0930).

C. Open right side (Nos. 13-20).

1. Remove exposure and sham caps.

a. Store top-row caps on shelf above the alcove, standing each cap on its open end.

b. Store remaining caps on alcove floor.

1) Gently slide exposure caps in lengthwise, open end first, and lay on side.

2) Slide sham caps in so that open end slips over loads on exposure caps.

3) Store caps two deep only.

2. Place food separator box on counter next to balance, and a paper towel next to the food separator box.

3. Remove water bottles and food tubes.

a. Remove both the water bottle and the food tube from a given cage before proceeding to the next; be careful to minimize juggling of cage or waveguide, to avoid excess stress to the rat.

1) Water bottle

a) Gently but firmly lift the water bottle out of the waveguide, taking care to minimize spillage. As much as possible, handle the water bottle only around the plastic lid, avoiding the brass fitting and the bottle itself.

b) Top row. To remove the water bottle, gently push it against the ceiling. (This is necessary because of insufficient space between the top-row waveguides and the ceiling of the alcove.)

c) Lower rows. Push bottom of the bottle up through the hole cut in the base above, displacing the waste tray enough to get the bottle cap out of the waveguide.

d) Place bottle in the "V" of the base in front of the cage.

2) Food tube

a) Top row

1 Lift the food tube up through the food-tube cutout as far as possible.

2 Using food-tube support in cage door as a handle, gently slide cage backwards.

3 Pull down on food tube and slide it out of the waveguide.

b) Bottom row

1 Slide the tube up through the opening in the base above until the bottom of the tube clears the waveguide.

2 Tilt the tube to permit its being pulled back down and out in front of the waveguide. This may require slight rotation of the cage and/or waveguide. Minimize this as much as possible; however, when unavoidable, do so gently to minimize stress to the rat.

4. Place water bottles in appropriate slot in water-bottle carrier.
5. Place food tubes in food-tube carrier with lip facing upwards.

D. Open left side of alcove (Nos. 1-12) and repeat Step C.

III. Determine body weight by alcove (1-20 in order).

A. Obtain appropriate holding bin from pigeonhole and place on balance.

B. Tare balance and set filter-bonneted lid aside.

C. Gently pull cage out of the waveguide, using the food-tube support on the door as a handle.

D. Remove peg securing cage door to cage and place in appropriate hole (located in side of base "V").

E. Remove door from cage and set aside in base "V"; or if that door requires washing, place it inside of the respective empty cage on the rack.

F. Take the cage to the bin (use both hands as much as possible when handling cages) and place it in the bin such that the back edge of the cage is flush with the back of the bin; allow the rat to climb into the bin.

1. This may become unnecessary as time progresses and the animal becomes accustomed to the routine. Most animals learn readily to climb into the bin if the cage is held just above it.
2. In all processes, disturb the animal as little as possible.

G. After the rat is in the holding bin, remove the cage and replace the filter-bonneted lid.

H. Hit "Weigh" on data-entry keyboard (DEK).

I. Remove any food pellets remaining in cage: use the copper rod to gently force pellets between the nylon strings and out through the feces chute, taking care not to break the strings. Put the food into the appropriate compartment in the food separator box.

J. Place the dirty cage onto the rack.

K. Check data-entry monitor (DEM) for weight and, barring an error, hit "Enter" on DEK to enter the data.

L. Return holding bin to appropriate pigeonhole.

M. Bring next bin to balance and repeat as above for all animals (1-20), disinfecting hands after every fifth rat.

IV. Remove feces trays.

A. Remove trays one at a time.

B. Check contents for food, remove dry pieces and match wet ones, and place into appropriate compartment in food separator box.

C. Place the dirty tray onto rack shelf.

D. When all 20 trays are on rack (in addition to previously placed cages), roll the rack into the dirty hall.

E. Phone the washroom, instructing the personnel to pick up rack for cleaning (washroom will phone back when rack is ready for removal from the autoclave).

V. Vacuum alcove.

A. Clean inside waveguide, front end of base, and in middle where feces tray stands.

B. Remove any remaining pieces of food and place into food separator box.

VI. Disinfect alcove.

Each alcove has its own individual disinfectant kit (located on floor of respective alcove), which consists of disinfectant bucket, disinfecting rag, and scrub brush.

A. Fill bucket with tap water and specified amount of disinfectant.

B. Wipe down each waveguide at feces chute cutouts with rag. If dried urine has accumulated around cutouts, use scrub brush to remove.

C. Disinfect front half of base.

D. Disinfect midportion of base (where feces tray sits).

1. If urine is present on base, mark that feces tray "LEAKS" and set it on floor in dirty hall, next to door (after it has been returned from washroom).
2. Replace with new feces tray.

E. Maintain cleanliness of glass doors and runners (if necessary, on a daily basis).

VII. Weigh food and water (off-cage weight).

A. Call "Menu."

B. Select "A" for "Auto Series."

C. Select appropriate function ("Food Off" or "Water Off").

D. Enter alcove designation and operator ID.

E. Disinfect gloves.

F. Tare balance.

G. Weigh food tubes.

1. Place first food tube and any food in separator box or water bottle on balance and press "Weigh."

a. If weight and consumption appear correct, depress "Enter" on DEK, then proceed to next food tube.

b. If screen data appear suspicious depress "Clear," then "Weigh." Suspicious readings:

1) negative consumption
2) low consumption
3) excessively high consumption

These could indicate

1) error in the system
2) faulty water bottle or food tube
3) sick animal

2. Remove food tube from balance and proceed to next food tube, repeating Step G.1 each time.

NOTE: CHECK BALANCE PERIODICALLY FOR PROPER ZEROING.

3. Having completed the "Food Off" series, check on "Display Status" and, barring error in food weigh-off, discard all food wastage from food separator tray.

H. Weigh Water Bottles.

1. Place first water bottle on balance and depress "Weigh" on DEK.

a. If weight and consumption appear correct, depress "Enter" on DEK, then proceed to next bottle.
b. If screen data appear suspicious, depress "Clear," then "Weigh," on DEK (See G.1.b).

2. Remove water bottle from balance and proceed to next water bottle, repeating Step H.1 each time.

NOTE: CHECK BALANCE PERIODICALLY FOR PROPER ZEROING.

VIII. Weigh food and water (on-cage weight).

A. Disinfect gloves.

B. Refill food tubes.

C. Refill water bottles.

1. If water level in the bottle is greater than one-half, this step is unnecessary.
2. When refilling bottles, leave an air space in the bottle (otherwise, the rat will be unable to get water from it).

D. Call "Auto Series".

E. Select appropriate function ("Food On" or "Water On")

F. Enter alcove designation and operator ID.

G. Place first food tube/water bottle on balance and press "Weigh."

1. If data appears correct, depress "Enter" on DEK and proceed to next tube/bottle.
2. If data appears suspicious (weight is either too high or too low), depress "Clear," then "Weigh."
3. Remove tube/bottle from balance and proceed to next, repeating Step G for each.

NOTE: It is not imperative to carry out every action in Steps V through VIII in the above order. Ability to do so will depend upon amount of time available before beginning the next alcove and/or availability of a balance. However, the alcove must be vacuumed before the food is weighed off so that any food accidentally left on the bases when the hutches and feces trays are removed can be included in the off weight, and the food and water must be weighed off before being weighed on.

IX. Return cages and feces trays to respective alcove.

A. Disinfect gloves.

B. Ask runner for your rack.

C. Set cages into the waveguide so that the front of the cage lip remains just outside the end of the waveguide. Avoid putting hands into cage.

D. Place door in "V" of base.

E. Place feces trays in midsection of base.

F. Pass empty rack into dirty hall for rewashing.

G. Put water bottles 1-3 upright on "V" of bases 4-6 directly in front of the cage; water bottles 4-6 in "V" of bases 7-9; and so on.

1. Bottom-row bottles go on floor, far enough back to prevent their being accidently kicked over.
2. Place food tubes in "V" of respective bases.

X. Rehouse animals by alcove.

A. Call "Display Status" to check that all data has been collected for alcove to be rehoused.

B. Remove first bin from pigeonhole and remove lid.

C. Bring the bin close to hutch #1 and lower one corner down next to the opening of the cage so that the rat can step into it.

D. Place empty bin aside on another base or on table counter.

E. Place door on cage and secure it with peg (located in side board in "V" of base).

F. Gently move the cage back into the waveguide so that the feces chutes line up with the matched cutouts in the waveguide.

G. Insert food tube and water bottle.

1. Insert both food tube and water bottle in a given cage before proceeding to the next.
2. Top row

a. Food tube

1) Gently slide cage back as far as it will go.
2) Slide food tube approximately three-quarters the way up through food tube cutout, tilting top part of tube toward you.
3) Pull cage toward you until food tube can be slid into place.

b. Water bottle. Gently push up on ceiling of alcove with bottom of water bottle, then insert into middle hole of hutch. Take care to minimize spillage.

3. Bottom row

a. Food tube. Insert in same manner as for top rows with the exception that, since the holes in the base of the above alcove provide adequate room in which to manipulate the tube, the hutch needn't be slid backwards.
b. Water bottle. Insert in same manner as for top rows, taking advantage of hole in base above.

4. Slight adjustment of the waveguide and/or cage may prove helpful.

H. Insure that the food tube is not in backwards.

I. Check to see that cage floor is level.

J. Replace lid on holding bin and return it to its respective pigeonhole.

K. Proceed to next rat, repeating steps B through K for each. Disinfect gloves after every fifth rat.

L. Replace waveguide caps. Caps should be securely coupled with the waveguide, and the waveguides pushed all the way back to the stop.

1. Do this gently to minimize disturbance to the animal or water bottle (which may result in water leakage).

2. Situate exposure caps such that the loads point up and outward at equal angles.

3. Situate sham caps such that the indentation faces upwards.

M. Check feces trays for proper alignment (they occasionally get knocked around during insertion of the water bottles).

N. Vacuum alcove floor and runners and damp mop with disinfectant solution.

O. Close alcove doors, making sure to interlock them securely.

P. Turn power on at scheduled time. Two come on at 1045, two at 1130, and one at 1215.

XI. Clean up.

A. Prepare disinfectant solutions for next day.

B. Check distilled water supply.

C. Vacuum around balances and computer terminals on work table.

D. Disinfect table counter, equipment, and splashguards.

E. Vacuum and damp mop entire floor with disinfectant solution.

F. Disinfect counter in control center.

G. Check the numbers on the food and water containers to insure that they belong to the appropriate rat.

H. Check the feces trays to ensure that they are in place.

I. Check the cages to ensure that they are straight.

XII. Exit from lab.

A. Remove lab shoes and put on booties.

B. Gather up trash and exit into dirty hall.

C. Discard gloves, mask, bonnet, and booties in shower room because they are not to be reused.

D. Fold coveralls and place in appropriate box for washing.

NOTE: Runner's duties are to remove clean cages, distilled water, bedding, etc., from autoclave and bring to lab. Mop clean-hall floor on a daily basis. Vacuum weekly. Prepare uniform packs. The runner must never enter the lab except at the end of the day in order to exit to the dirty hall.

be reported to the research tech and the item replaced.