PNL--3258

INTERIM REPORT
March 1, 1978 - September 30, 1979

Biological Effects of High-Strength Electric Fields on Small Laboratory Animals

R. D. Phillips
L. E. Anderson
W. T. Kaune

December 1979

DO NOT MICROFILM
COVER

Prepared for
Electric Energy Systems Division
Department of Energy
Washington, D.C.

Pacific Northwest Laboratory
Richland, Washington 99352
Operated by Battelle Memorial Institute

Battelle

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

The views, opinions and conclusions contained in this report are those of the contractor and do not necessarily represent those of the United States Government or the United States Department of Energy.

DO NOT MICROFILM
COVER

PACIFIC NORTHWEST LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
Under Contract EY-76-C-06-1830

Printed in the United States of America
Available from
National Technical Information Service
United States Department of Commerce
5285 Port Royal Road
Springfield, Virginia 22151

Price: Printed Copy $____*; Microfiche $3.00

*Pages	NTIS Selling Price
001-025	$4.00
026-050	$4.50
051-075	$5.25
076-100	$6.00
101-125	$6.50
126-150	$7.25
151-175	$8.00
176-200	$9.00
201-225	$9.25
226-250	$9.50
251-275	$10.75
276-300	$11.00

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

PNL--3258

BIOLOGICAL EFFECTS OF HIGH-STRENGTH ELECTRIC FIELDS ON SMALL LABORATORY ANIMALS

INTERIM REPORT

March 1, 1978 - September 30, 1979

PNL--3258

DE83 000006

December 1979

Project Manager: R. D. Phillips

Associate Project Manager: L. E. Anderson

Project Engineer: W. T. Kaune

Pacific Northwest Laboratory
Richland, Washington 99352
Operated by Battelle Memorial Institute

NOTICE

for **PORTIONS OF THIS REPORT ARE ILLEGIBLE. It has been reproduced from the best available copy to permit the broadest possible availability.**

Electric Energy Systems Division
Department of Energy
Washington, D.C.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

EXECUTIVE SUMMARY

This interim report describes progress made on the project from March 1, 1978 to September 30, 1979 in assessing the biological effects of exposing small laboratory animals (rats and mice) to 60-Hz electric fields.

Exposure Systems

Previous interim reports provided detailed information on the systems that are being used to expose rats and mice to uniform, vertical 60-Hz electric fields. The earlier reports include documentation that the exposure systems are free of corona discharge and ozone formation, and that the animals do not receive spark discharges or other shocks during exposure to electric fields. This report describes additional measurements that have been made to characterize the exposure systems. Total harmonic distortions of the electric fields outside and inside the body of an exposed animal are 0.3 and 0.7%, respectively. Electric-field-induced vibration of the floors of the exposure cages is small relative to ambient levels of vibration; average rms vibrations at 60 and 120 Hz are 1.4×10^{-3} and 0.5×10^{-3} gravities, respectively. The electric field inside cages of sham-exposed (control) rats is less than 2 V/m. Current that flows through the mouth of a rat when it drinks during electric-field exposure is about 0.6 of the rat's short-circuit current; for a 400-gram male rat exposed at an unperturbed electric field of 100 kV/m, the upper limit for current flow through the mouth is about 5 μA.

Dosimetry

Measurements were made using a hemi-ellipsoidal rat model and dead rats to quantify the magnitude and distribution of electric fields inside the rat exposure cages and to determine the distribution of electric-field-induced currents that originate in various parts of the body. Based on measurements made with the rat model, the electric field is attenuated about 5% by the presence of the cage in the field. There is an additional 35% attenuation in field strength due to the interaction (mutual shielding) by animals in adjacent cages. Hence, at an "unperturbed" field strength of 100 kV/m (i.e., the measured field strength without cages or animals in the field), the "effective" field strength (the field to which the animals are exposed) is about 60 kV/m.

The short-circuit current of a 400-gram rat exposed to an unperturbed field of 100 kV/m (an effective field strength of $\sim$60 kV/m) is about 8.6 μA when the animal is in its normal resting posture. About 15% of this short-circuit current originates in the head, 16% in the thorax, 23% in the abdomen and 43% posterior to the abdomen. When the rat rears, these values change to 41%, 30%, 18%, and 11%, respectively, and the short-circuit current is doubled.

Biological Studies

Experiments in ten major areas were conducted during this reporting period to screen for biological effects over a wide range of parameters in rats and mice exposed to a 60-Hz electric field at an unperturbed field strength of 100 kV/m for up to 120 days. In addition, studies were conducted to assess the mutagenic potential of dc electric fields on bacteria and fruit flies.

Several features of experimental design were common to all experiments with rodents. All animals received from the supplier (Hilltop Laboratories) were examined before being used in experiments to insure that they were healthy. A sample population was sacrificed from each group of animals at delivery for virologic screening and histopathologic examination. Both rats and mice were ear-coded, then acclimated for 14 days in cages identical to the ones used for exposure to electric fields. Sham-exposed rats and mice were maintained in the exposure systems along with their exposed counterparts to serve as controls in each experiment. Environments and conditions for these controls were identical to those of exposed animals except that the electrodes of their racks were not energized. Ambient temperature and humidity were 21°C (range 20-23°C) and 30% (range 20-50%), respectively. Except for several behavioral experiments, the unperturbed field strength used for all exposures was 100 kV/m. The animals were in electrical contact with the reference ground electrode in all experiments. Neither animal caretakers nor investigators were informed which animals were being or had been exposed or sham-exposed until all measurements were completed. The exposure period was about 20.5 hr/day; this allowed about 3.5 hr/day for cage cleaning, feeding and watering while electrodes were not energized. Animals had free access to water and food in all experiments where exposure was >8 hr/day.

The following is a brief summary of the biological studies and the results:

- Hematology and Serum Chemistry: This study measured 14 different parameters concerning blood cells and 14 parameters of serum chemistry in female rats and mice exposed for 120 days. In addition, ferrokinetic studies were conducted in female rats and mice exposed for 60 days. Also, hematologic and serum chemistry parameters were evaluated in three successive generations of mice (F_1, F_2 and F_3) after exposure in utero and for 77 days after birth. All experiments were conducted in replicate in order to differentiate effects induced by exposure to electric fields from spurious false positive findings, intrinsic in multiparameter evaluations.

There appears to be no unequivocal hematologic or serum chemistry effects of exposing young adult female rats or mice to an unperturbed, 100 kV/m, 60-Hz electric field for 120 days (as well as 15, 30 or 60 days; Third Interim Report, 1978). Although there were sporadic statistical differences in some measures between exposed and sham-exposed groups, the apparent effects were not consistent across exposure replicates. No significant differences were seen in plasma clearance of iron of exposed and sham-exposed animals. In the 3-generation mouse study, consistent differences between exposed and sham-exposed mice were observed in the first and third generations. In the first generation, white-blood-cell counts of exposed mice were higher than those of sham-exposed in both sexes and in both experiments. In the third generation, red-blood-cell parameters were elevated in male animals in both experiments. In general, more effects were seen in exposed male mice than in female mice in the three-generation study.

- Immunology: Studies were conducted to evaluate the effects of exposure of mice to 60-Hz electric fields on both the humoral and cellular

functions of the immune system. In humoral studies, no effect of 30- or 60-day exposure on the primary antibody response was observed. In cellular studies, in vivo assays of delayed and contact hypersensitivity showed instances of statistically significant decreases in skin thickness in animals exposed for 30 days. No statistically significant changes were observed in mice exposed for 120 days. Cell-mediated immunity was also evaluated by assessing the response of spleen cells in vitro to mitogen stimulation. Exposure of mice for 90 or 150 days had no significant effect on the response of spleen cells to four different mitogens (concanavalin-A, lipopolysaccharide, pokeweed mitogen and phytohemagglutinin).

- Pathology: No significant differences were observed between exposed and sham-exposed rats and mice (120-day exposure) in an extensive histopathological survey of 23 tissues.

- Metabolic Status and Growth: Exposure of male and female rats and mice to electric fields for 30 or 120 days had no significant effect on their body weights, organ weights, or food and water consumptions. There were no effects of exposure on oxygen consumption, carbon dioxide production or growth and metabolic parameters.

- Bone Growth: Bone growth and structure in juvenile male and female rats was not altered by exposure to a 60-Hz electric field at 100 kV/m for 30 days.

• Endocrinology: The effects of exposure to 100 kV/m electric fields on the endocrine system were examined in adult and in developing (juvenile) rats. Exposure of young adults for 30 days had no effect on serum levels of growth hormone or thyroid stimulating hormone. No significant change in serum corticosterone level was observed; however, variance in the levels of this hormone in serum was lower in exposed animals than in sham-exposed controls. Following 120 days of exposure of young adult male rats, no differences from controls were seen in serum concentrations of follicle-stimulating hormone, leuteinizing hormone, prolactin, or thyroid-stimulating hormone. Serum levels of testosterone and corticosterone in exposed animals were significantly lower than levels in sham-exposed animals. Inconsistently lower levels of testosterone and corticosterone also were observed in the 36-day exposure of weanling rats (i.e., rats exposed from 20-56 days of age). Growth rate of reproductive organs in these weanling rats, as well as levels of follicle-stimulating hromone, luteinizing hormone and prolactin were not affected by exposure.

• Cardiovascular Function: Exposure of rats for 1 or 4 months (100 kV/m) had no detectable effect on ECG's, heart rate, blood pressure, or vascular reactivity. No cardiovascular changes were observed during a 1-hr exposure period. To test for physiological reserve capabilities, rats were exposed to an electric field (100 kV/m) for 1 month and subsequently subject to cold stress. Heart rate, skin and deep-clonic temperatures were recorded before, during and after cold exposure. Again, no significant differences were observed between electric-field exposed and sham-exposed animals. Plasma corticosterone and thyrotropin levels before and after cold exposure were not affected by electric-field exposure.

• Neurophysiology: Transmission of impulses between nerves (in synapses) was investigated using the superior cervical sympathetic ganglia from rats that had been exposed (100 kV/m) or sham-exposed for 30 days. Characteristics of the postsynaptic compound action potential, conduction velocity and high-frequency-induced fatigue were not altered by exposure of the rat to the electric field. However, the conditioning-test response curve of exposed rats was significantly higher than that of sham-exposed rats, supporting previous observations (Third Interim Report, 1978). It appears that exposure to a 60-Hz electric field may promote increased excitability of nerve synapses.

Detailed neurophysiologic tests of myelinated (sciatic) and unmyelinated (vagus) nerves from exposed and sham-exposed rats did not show any consistent changes as the result of electric-field exposure.

• Growth and Development: Experiments have been completed on the assessment of electric-field exposure on reproduction and on fetal and post-natal growth and development in rats and mice. In one experiment, exposure of the pregnant rat was begun on day 0 of gestation and continued until the offspring reached 8 days of age. In another experiment, exposure started at 17 days of gestation and continued through 25 days of postnatal development. Few statistically significant effects were observed on growth, survival, size or morphology of fetuses, or on neuromuscular development of the offspring. Tests of reproductive integrity of the offspring did not disclose any deficits.

In another experiment, mice were conceived, born and raised for 3 successive generations in a 60-Hz, 100-kV/m electric field. There were no apparent effects on conception, litter size, mortality, body weight, or neuromuscular development.

- Animal Behavior: Rats were tested for side preference in a shuttlebox, one end of which was shielded and the other end unshielded from a 60-Hz electric field. In short-duration tests (45 min), animals exhibited a statistically significant preference for the shielded end of the shuttlebox at field strengths of 90 kV/m or higher. Chronic exposure of rats (20 hr/day for 30 days at 100 kV/m) prior to this short-duration (45 min) test significantly attenuated the preference behavior.

In longer duration tests (24 hr), rats showed a preference for the shielded end of the shuttlebox at field strengths of 75 kV/m and above. At 50 kV/m or lower, rats spent more time in the exposed end of the shuttlebox than in the shielded end, but only during the light period of a 12-hr light:12-hr dark cycle. In both the short and longer duration tests, traverses by the rat between exposed and shielded ends of the shuttlebox appeared to be transiently increased among exposed animals relative to sham-exposed controls.

Central nervous system excitability was assessed by examining the sensitivity of seizure-prone mice to high-intensity sound after electric field exposure. Exposure of mice to 100 kV/m for 14 or 30 days had no detectable effect on sound-induced seizure sensitivity of mice.

In summary, exposure of rats and mice to 60-Hz electric fields at 100 kV/m for up to 120 days showed few statistically significant, reproducible effects. Consistent, replicated effects were observed in experiments designed to provide a measure of synaptic excitability. Serum levels of corticosterone and testosterone appear to be reduced in exposed animals, and significant reproducible effects were documented in several behavioral tests. The results of several experiments showed trends which suggest the possibility of effects; decreased in vivo cell-mediated immunity, alteration in several hematologic parameters, e.g., higher white cell levels in mice exposed from conception to 77 days of age and higher red blood cell parameters in the 3rd generation of exposed mice. Experiments designed to screen for biological effects are essentially completed. Studies are now in progress to: 1) examine, in greater detail, the observed effects outlined in this report; 2) determine if the observed effects are transitory or permanent; and 3) establish the relationship between the observed effects to the intensity/duration of exposure.

CONTENTS

EXECUTIVE SUMMARY iii

I. INTRODUCTION 1

II. EXPOSURE SYSTEMS 9

III. BIOLOGICAL INVESTIGATIONS 43

IV. HEMATOLOGY AND SERUM CHEMISTRY 47

V. IMMUNOLOGY 95

VI. PATHOLOGY 111

VII. METABOLIC STATUS AND GROWTH 119

VIII. BONE GROWTH 139

IX. ENDOCRINOLOGY 147

X. CARDIOVASCULAR FUNCTION 173

XI. NEUROPHYSIOLOGY 195

XII. REPRODUCTION AND DEVELOPMENT 227

XIII. ANIMAL BEHAVIOR 247

XIV. MUTAGENESIS 285

XV. DOSIMETRY 295

XVI. SUMMARY OF BIOLOGICAL INVESTIGATIONS 323

I. INTRODUCTION

Research was initiated on this project on March 9, 1976. The first three interim reports (Phillips et al., 1976; Phillips and Kaune, 1977; Phillips et al., 1978) described progress made during the first 24 months, through February 28, 1978. This interim progress report describes research completed during the period March 1, 1978 to September 30, 1979.

NEED, SCOPE AND OBJECTIVES

A comprehensive research study is in progress to assess possible biological effects of exposure to 60-Hz electric fields and to develop a reliable data base for establishing safe exposure limits. The major objective is to determine the biological effects on small laboratory animals of exposure to 60-Hz electric fields. Specific aims are:

1. To conduct a broad series of screening experiments encompassing major biological organ systems in order to identify which system(s) may be affected by exposure to 60-Hz electric fields
2. To establish threshold field strengths and durations of exposure for producing effects observed in the screening experiments
3. To determine whether effects produced by exposure are transitory or longer lasting, i.e. to ascertain the time course of any biological effect
4. To investigate observed effects in depth in order to obtain a fundamental understanding of the interaction of electric fields

with biological systems. Such data will provide the biological basis for extrapolating effects on experimental animals to possible effects on humans

5. To measure and characterize the surface electric fields and current distributions in bodies of rats and models of rats. Data obtained from these "dosimetry" studies will be compared to data derived from similar studies on models of humans to provide the physical basis for extrapolating effects on experimental animals to possible effects on humans.

SCHEDULE AND MILESTONES

The schedule and major achievements of this project that were accomplished prior to this report are included in previous interim reports. Briefly, major milestones from the start of the project in March 1976 through February 1978 were:

1. Design, fabrication and characterization of three systems for exposing small laboratory animals to uniform, vertical, 60-Hz electric fields: a rat exposure system, capable of simultaneously exposing 144 rats individually housed in plastic cages; a mouse exposure system in which 288 mice, three per cage, can be exposed simultaneously; a special test system for dosimetry studies and for experiments requiring direct data acquisition from individuals or small numbers of animals during or immediately after exposure
2. Extensive measurements to characterize the exposure systems and document that they are free of corona, audible noise, ozone,

vibration or other possible secondary field factors that could produce artifactual biological effects, such as spark discharge between the animal and the caging/watering/feeding systems

3. Measurements of the short-circuit current and surface electric fields on models of rats as a first approximation of the interaction of rats with a uniform, 60-Hz electric field
4. Completion of experiments in 9 of 11 major areas (hematology, serum chemistry, pathology, immunology, endocrinology, male reproduction, neurophysiology, cardiovascular function, bone growth, reproduction and development, and central nervous system morphology) to screen for biological effects in mice and rats exposed $\sim$ 20 hr/day for 30 days to a 60-Hz electric field.

Major accomplishments since the last interim report (March, 1978) are:

1. Completion of experiments evaluating hematology and serum chemistry of mice and rats exposed to 100 kV/m for 30, 60 or 120 days
2. Completion of experiments assessing cardiovascular function of rats exposed to 100 kV/m for 30 to 120 days
3. Completion of experiments evaluating the cellular and humoral components of the immune system of mice exposed to 100 kV/m for 30 to 150 days
4. Completion of experiments evaluating growth and metabolic status of rats and mice exposed to 100 kV/m for 30-120 days

5. Completion of experiments examining bone growth of juvenile rats exposed to 100 kV/m for 30 days
6. Completion of experiments evaluating both the possible effects of exposure (100 kV/m) on the development of the endocrine system of rats and possible effects of chronic exposure for 30 to 120 days on endocrine function
7. Completion of histological examination of tissues from rats and mice exposed to 100 kV/m for 30 to 120 days
8. Completion of experiments examining the functional aspects of synaptic transmission and conduction in peripheral nerves of rats exposed to 100 kV/m for 30 days
9. Completion of experiments assessing both the perception and avoidance of electric fields (25 to 100 kV/m) by rats, and excitability of the central nervous system of mice
10. Completion of experiments evaluating possible effects of exposure to 100 kV/m on neonatal and postnatal development and growth of rats
11. Completion of experiments to assess possible effects of exposure to 100 kV/m on reproduction, growth and development of three generations of mice raised under continuous exposure to the electric field
12. Quantitative measurements of electrode vibration induced by the electric field in the rat and special studies exposure systems
13. Quantitative measurements of the currents induced by the electric field in various parts of the rat; a comparison of these data to values obtained in models of humans exposed to 60-Hz electric fields.

Future milestones for the project are:

1. To complete experiments now in progress in neuromuscular function, behavior, cell-mediated immunity, bone-fracture repair, hormone levels, hematology and serum chemistry of rats exposed in utero, Ames mutagenesis assays, cell-membrane function, and growth and development of F_4 generation of mice
2. To initiate experiments on possible effects of exposure on neurotransmitters and brain chemistry
3. To investigate threshold field strengths and durations of exposure for producing effects observed in cell-mediated immunity, synaptic transmission and behavior
4. To investigate whether effects observed to date are transitory or of long duration, i.e. determine time course of effects
5. To determine the consequences of spark discharge on growth, serum corticosterone levels and selected hematologic and serum chemistry parameters
6. To conduct in-depth studies in neurophysiology, immunology and behavior to obtain a fundamental understanding of the importance of observed effects relative to humans exposed to 60-Hz electric fields
7. To continue dosimetry experiments to quantify electric fields at the surface of and inside rats, and compare these data to those from models of humans exposed to 60-Hz electric fields.

C. PROJECT ORGANIZATION

Dr. Richard D. Phillips will continue as Project Manager on this project, as well as on the EPRI-funded companion project using miniature swine. Dr. Larry E. Anderson will serve as Associate Project Manager and as Task Leader for the Biological Studies. Dr. William T. Kaune will serve as Task Leader for the Dosimetry and Exposure Systems.

The following list gives the organizational structure of the project in terms of Tasks and Subtasks, indicating key personnel responsible for each.

TASK A Administration - R. D. Phillips

TASK B Exposure System - W. T. Kaune

- Subtask: Operations - W. T. Kaune
- Subtask: Repair and Maintenance - W. T. Kaune

TASK C Dosimetry - W. T. Kaune

TASK D Spark Discharge Study - W. T. Kaune

TASK E Biological Studies - L. E. Anderson

- Subtask: Hematology and Serum Chemistry - H. A. Ragan
- Subtask: Immunology - J. E. Morris
- Subtask: Neurophysiology - R. A. Jaffe
- Subtask: Endocrinology - M. J. Free
- Subtask: Behavior - R. H. Lovely
- Subtask: Bone Growth - B. J. McClanahan
- Subtask: Cell and Membrane Physiology - R. P. Schneider
- Subtask: Genetics - F. P. Hungate

Subtask: Neurochemistry - L. E. Anderson

Subtask: Analytical Chemistry - B. Wilson

Subtask: Growth and Development - M. R. Sikov

Subtask: Biometrics - R. L. Buschbom

Subtask: Animal Care - V. G. Horstman

REFERENCES

Phillips, R. D., W. T. Kaune, J. R. Decker and D. L. Hjeresen. 1976. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Interim Progess Report. CONS/1830-1, Conservation, Energy Research and Development Administration, Washington, DC.

Phillips, R. D. and W. T. Kaune. 1977. Biological Effects of High Strength Electric Fields. Second Interim Progress Report, CONS/1830-2, Conservation, Energy Research and Development Administration, Washington, DC.

Phillips, R. D., et al. 1978. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Annual Report. HCP/T1830-03, Division of Electrical Energy Systems, U.S. Department of Energy, Washington, DC.

II. EXPOSURE SYSTEMS

Principal Investigator: W. T. Kaune

ABSTRACT

Accurate measurements of harmonic distortion in the Special Studies Exposure System have been made. With no corrective steps, the electric field induced within the body of an exposed animal has 17% fifth harmonic and 18% seventh harmonic admixtures. With the addition of damping resistance and line filtering, these harmonic amplitudes are very small and the total harmonic distortions in the electric fields outside and inside the body of an exposed animal are 0.2 and 0.6%, respectively.

The measured total harmonic distortions in the electric fields inside and outside the body of a rat exposed in the Rat Exposure System are 0.3% and 0.7%, respectively.

Measurements with piezoelectric accelerometers have detected 60- and 120-Hz electric-field-induced vibrations in empty and full rat cage modules. In cages occupied by rats for more than 1 hr, the average rms levels of 60- and 120-Hz vibration are 1.4×10^{-3} and 0.5×10^{-3} gravities, respectively.

Measurements of the leakage of 60-Hz electric fields from the exposure to sham-exposure facilities are reported; the upper limit on the 60-Hz electric fields to which sham-exposed animals are exposed is 0.2 V/m.

Currents passing through the mouth of a drinking rat have been studied. Measurements with water-deprived male rats have shown that the upper limit on the fraction of short-circuit current passing through the mouth is about 0.6. Data are presented which suggest that this fraction is higher for female rats.

A. HARMONIC DISTORTION MEASUREMENTS

1. Introduction. Previous progress reports (Phillips et al., 1976; Phillips and Kaune, 1977; Phillips et al., 1978) have described the three exposure systems used in this project: the Special Studies

System, used for experiments involving dosimetry, or for small numbers of animals; the Rat Exposure System (accommodates 144 exposed, 144 sham-exposed); and the Mouse Exposure System (accommodates 288 exposed, 288 sham-exposed). Initial measurements of the harmonic distortion in the Special Studies Exposure System were reported earlier (Phillips et al., 1978). Additional measurements were made during this reporting period, resulting in: (1) the development of improved measurement techniques, (2) more accurate measurements of the Special Studies Exposure System harmonic amplitudes, and (3) measurement of the Rat Exposure System harmonic amplitudes.

2. Methods. Higher frequency electric fields will be coupled more strongly in the body of an exposed animal because of the capacitance between the body and the exposure electrodes. Thus, harmonic analyses of the electric fields outside and inside the body need to be separately performed.

Electric fields outside the body will have the same harmonic spectra as the voltage producing the field, since the capacitive reactances between the body and the exposure electrode are large in comparison to the impedances inside the body (Geddes and Baker, 1967). Voltage waveforms were obtained using a frequency-compensated voltage divider (Ross Engineering Corp. Model VD-60-6.2-B-IC-A, Campbell, CA), and were digitized (85 points over one cycle) with a digital oscilloscope (Nicolet Instruments, Inc. Model 1090A, Madison, WI). Fourier-

analyses of the data were performed with a desk calculator (Hewlett-Packard, Model 9825A, Loveland, CO).

The harmonic spectra of the induced currents and electric fields inside the body will be approximately the same, since the conductivities of living tissues are relatively frequency-independent below 1000 Hz (Schwan and Kay, 1956; Geddes and Baker, 1967). To measure the harmonic spectra of induced current, a field-probe was used which was capacitively coupled to the exposure electrode. (Phillips, et al., 1976; Kaune, 1979). The resulting waveforms were digitized and analyzed in the same way as the voltage waveforms described above.

3. Results. Photographs of typical high-voltage and induced-current waveforms for the Special Studies Exposure System were given in a previous progress report (Phillips et al., 1978). As noted in that report, substantial levels of harmonic distortion were observed, resulting from resonant interaction between the output inductance of the high-voltage transformer and the load capacitance of the exposure system. This was corrected by: (1) inserting a resistance of 10 Ω in series with the primary of the high-voltage transformer to "damp" the resonance; and (2) energizing the high-voltage supply with undistorted 60-Hz power from AC line conditioners (California Instruments Model LC1201B, San Diego, CA). Measurements are given in Table II-1 of high-voltage and induced-current harmonic amplitudes in the Special Studies Exposure System operating with an electrode voltage of 25 kV in the following three configurations: no damping resistor or filtering; damping resistor; damping resistor and filtering.

TABLE II-1. Measured Harmonic Amplitudes for the Special Studies Exposure System Operating at 25 kV. Data for high-voltage (HV) and induced-current (I) waveforms are given. Harmonic amplitudes are expressed as fractions of the fundamental (60-Hz) amplitudes. Total harmonic distortions are presented at the bottom of each column.

	No Damping Resistor; No Filtering		10-Ω Damping Resistor; No Filtering		10-Ω Damping Resistor; Input Power Filtered	
Harmonic	HV	I	HV	I	HV	I
2	0.000	0.000	0.001	0.001	0.000	0.001
3	0.006	0.016	0.004	0.010	0.002	0.005
4	0.001	0.001	0.000	0.001	0.000	0.001
5	0.034	0.169	0.010	0.050	0.001	0.002
6	0.001	0.002	0.000	0.000	0.000	0.000
7	0.026	0.184	0.004	0.029	0.000	0.001
8	0.000	0.001	0.000	0.000	0.000	0.000
9	0.002	0.018	0.001	0.007	0.000	0.001
10	0.000	0.003	0.000	0.002	0.000	0.001
11	0.000	0.004	0.000	0.004	0.000	0.000
Total	0.043	0.251	0.012	0.059	0.002	0.006

Harmonic amplitude measurements were also made in the Rat Exposure System, with 10-Ω damping resistors installed and using line conditioned power (Phillips and Kaune, 1977; Phillips et al., 1978). In this system, adjacent electrodes are energized by equal-magnitude, oppositely phased voltages produced by a transformer with two secondary windings and a common primary winding. Harmonic analyses of both high-voltage waveforms and of the induced-current waveform from the field probe, capacitively coupled only to the lowest electrode, are presented in Table II-2 for an electrode-to-ground voltage of 20 kV (corresponding to an unperturbed electric field strength of 100 kV).

4. Discussion and Conclusions. The harmonic amplitude data for the Special Studies Exposure System (Table II-1) show clearly that the major impact of the addition of a damping resistor (which lowers the Q to about 0.75) is on the fifth and seventh harmonics. This indicates that the system resonant frequency is in the vicinity of 300-420 Hz. With both damping and line filtering, all harmonics are reduced to very low levels.

The harmonic spectra of high-voltage and field-probe-induced current are related, since the probe is capacitively coupled to the high-voltage electrode. Current through a capacitor is $I = \omega CV$, where $\omega = 2\pi \times$ frequency; therefore, the amplitude of the n^{th} harmonic of induced current should be n times greater than the n^{th} harmonic of the high voltage. The data in Table II-1 exhibit this pattern remarkably well.

TABLE II-2. Measured Harmonic Amplitudes for the Rat Exposure System. Measurements are given for each of the two equal-magnitude, oppositely phased voltages (HV-1 and HV-2) used to energize the exposure electrodes, and for the current induced in a probe capacitively coupled to HV-2. Harmonic amplitudes are expressed as fractions of the fundamental (60-Hz) amplitudes. Damping resistance and line filtering were installed for these measurements.

Harmonic	HV-1	HV-2	Induced Current 2
2	0.000	0.000	0.000
3	0.003	0.003	0.006
4	0.000	0.000	0.000
5	0.001	0.001	0.003
6	0.000	0.000	0.000
7	0.000	0.000	0.001
8	0.000	0.000	0.000
9	0.000	0.000	0.001
10	0.000	0.000	0.000
11	0.000	0.000	0.001
Total	0.003	0.003	0.007

Harmonic amplitudes in the Rat Exposure System were very small (Table II-2). Note that the harmonic spectra for the two high-voltages, used to energize adjacent electrodes, were identical in magnitude and phase (phase angles are not given in Table II-2 but were generated during the Fourier analysis).

B. IMPEDANCE MEASUREMENTS: SPECIAL STUDIES HIGH-VOLTAGE SUPPLY

1. Introduction. The substantial levels of harmonic distortion observed in our exposure systems are a result of resonances between the output inductances of the high-voltage supplies and the capacitive loads of the exposure systems and associated coaxial cables (Phillips et al., 1978). In this section, a measurement of the output impedance of the high-voltage system of the Special Studies Exposure System is reported. The purpose of this measurement was to provide data for use in the calculation of the system resonant frequency.

2. Methods. The high-voltage supply of the Special Studies Exposure System consists of a variable autotransformer exciting a high-voltage (HV) transformer. The output impedance of the HV tranformer was calculated from measurements of the open-circuit voltage and short-circuit current (for a constant input voltage). The impedance of the autotransformer/HV transformer combination was also determined (in the same way) as a function of the autotransformer tap setting; a second variable autotransformer was used to excite the autotransformer/HV transformer for this determination.

3. Results. The impedances of the high-voltage transformer and the autotransformer/HV transformer combination were measured for several values of input voltage and, in each case, the impedance was found to be independent of the voltage used.

Measured data for the transformer are given in Table II-3. Measured data for the autotransformer/HV transformer combination are given in Table II-4 for five different settings of the autotransformer tap, corresponding to exposure-system voltages of 10, 20, 30, 40 and 50 kV. In the final column of Table II-4 are shown the calculated resonant frequencies of the system (the measured load capacitance is 370 pF).

4. Discussion. Measurements of the harmonic spectra of the Special Studies Exposure System, when operating at an electrode voltage of 25 kV, were presented earlier in this section (Table II-1). These data show large fifth and seventh harmonic amplitudes. The calculated value for the resonant frequency of the Special Studies System at this voltage is about 360 Hz (Table II-4), which is midway between the fifth and seventh harmonics.

C. VIBRATION MEASUREMENTS

1. Introduction. Animal cages in electric-field exposure systems are subject to electrical forces and may, therefore, vibrate. Measurements are reported here of ambient background and electric-field-induced vibration in both empty and occupied rat cages.

TABLE II-3. Measured Properties of the High-Voltage Transformer of the Special Studies Exposure System

Turns Ratio	400:1
Impedance	103 kΩ
Impedance (100 kV, 5 kVA)	5.2%
Output Inductance*	273 H

* Neglecting resistive contributions

TABLE II-4. Measured Properties of the High-Voltage Supply (Autotransformer/ High-Voltage Transformer) of the Special Studies Exposure System

High-Voltage System Output Voltage (kV)†*	Autotransformer Output Impedance (Ω)	Total System Output Impedance (Ω)	Total System Output Inductance (H)**	Calculated System Resonant Frequency (Hz)***
10	0.23	140,000	370	430
20	0.48	180,000	470	380
30	0.70	210,000	560	350
40	0.80	230,000	620	330
50	0.86	240,000	640	330

† Output voltage controlled by autotransformer tap setting
* Output voltage with the Special Studies Exposure System connected
** Neglecting resistive contributions
*** Capacitance of Special Studies Exposure System = 370 pF

2. Method

Rat Exposure System Measurements. Piezoelectric accelerometers (sensitivity of 1.0 mV/m/sec^2, mass of 14 g, frequency response of 3-10,000 Hz; BBN Instruments Co. Model 508, Cambridge, MA) were used for vibration measurements. In the Rat Exposure System, a special technique for mounting the accelometers was developed (Fig. II-1) because all the exposure electrodes are energized. Since two adjacent electrodes of the Rat Exposure System have equal magnitude but oppositely phased voltages, the plane midway between the two electrodes is at ground potential. The accelerometer (whose case is grounded) was placed in this plane, and attached to the surface of the lower electrode with a 6.4-mm-diameter plexiglass rod (mass = 35 g). The shielded cable connecting the accelerometer to its remote power supply was also placed in the midplane to minimize field perturbations. The accelerometer was enclosed in a grounded conducting sphere (diameter, 8.3 cm), to shield it from the electric field (measurements with and without the sphere were identical). Perturbations in the electric field resulting from the presence of this sphere were calculated to be less than 2% at the surfaces of the exposure electrodes.

The output voltage from the accelerometer, which was proportional to the instantaneous acceleration, was displayed on a Tektronix 5031 storage oscilloscope (frequency response limited to 0-5,000 Hz). For data acquisition, approximately 100 individual traces were accumulated in the storage mode, using the 60-Hz line voltage as a trigger for the horizontal sweep. In this way, random building vibration and electrical

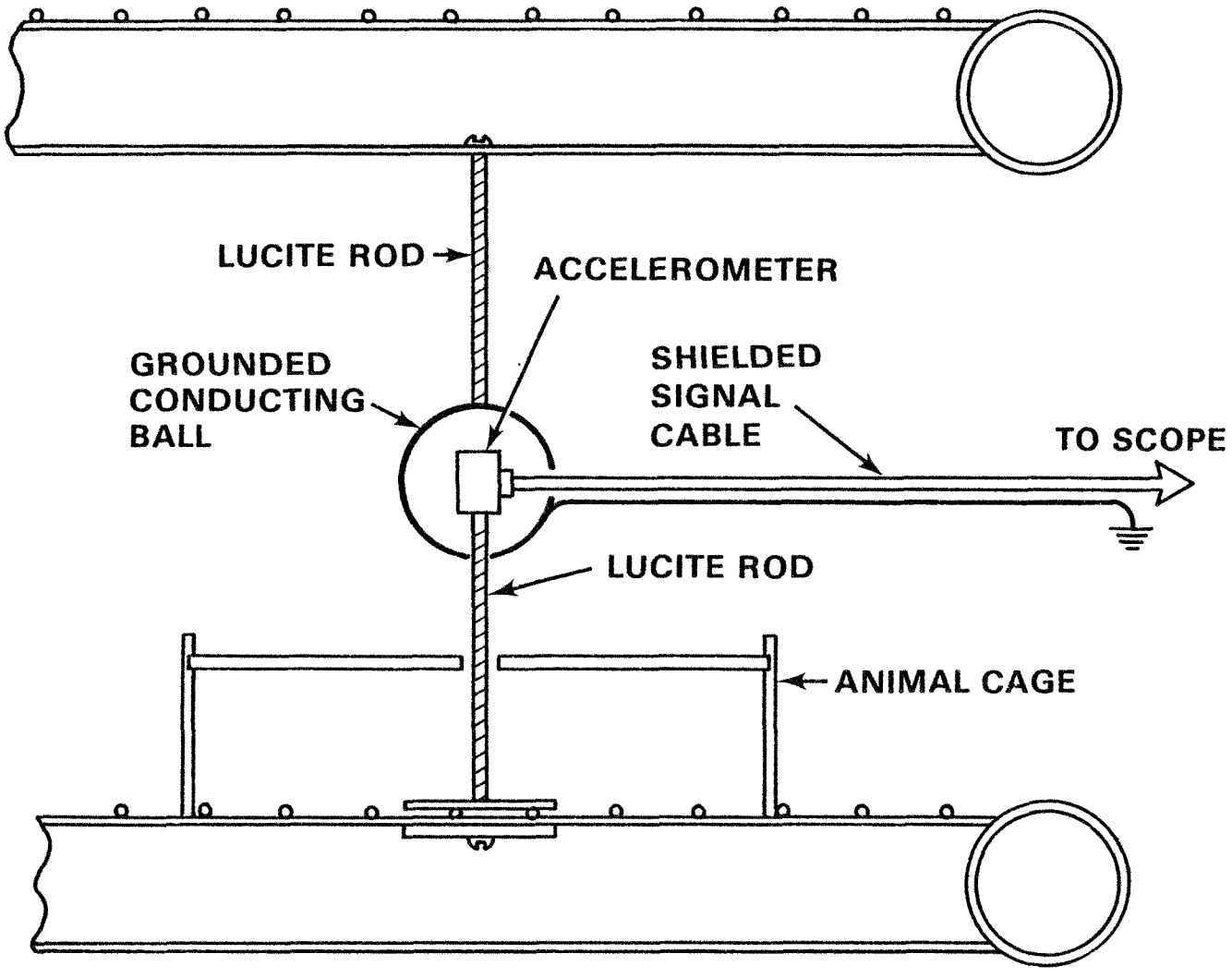


FIGURE II-1. Apparatus for Vibration Measurements in Rat Exposure System

noise (not synchronized with the 60-Hz line) were averaged and recorded as a broadening of the composite trace (consisting of the 100 individual traces). The resulting display was photographed and, later, digitized (Hewlett Packard Model 9865A, Loveland, CO) and Fourier-analyzed (Hewlett-Packard Model HP 9825, Loveland, CO).

Special Studies Exposure System Measurements. The Special Studies Exposure System was used for vibration measurements in cages containing animals, since the lower electrode, on which two 8-compartment cage modules (Phillips and Kaune, 1977) were placed, was grounded. This allowed accelerometers to be placed directly under the floor, out of reach of the rat. Typically, two accelerometers were attached to the bottom sides of the 1/2-in.-mesh screen floor with magnetic mounts and isolation studs (BBN Instrument Co., Cambridge, MA). Vibration data were photographically recorded and analyzed as described above.

3. Results

Measurements in Empty Cages. Measurements of electric-field-induced vibration were complicated by general room vibration. Occasional very high background levels were observed with frequencies close to (but not exactly equal to) 30, 60, and 120 Hz. For example, the building cagewasher, located in a separate room across and about 15 m down the hall from the Rat Exposure System, produced (when it was operating) vibration of the floor of an empty rat cage module with a frequency close to 60 Hz, and rms acceleration of about 0.07 m/sec^2.

The 55-cm x 52-cm x 10-cm-high, plastic (lexan) rat cage modules (consisting of eight 12.5-cm x 25.0-cm rat cages) are mounted on 51-cm x 81-cm pieces of 1/2-in.-mesh stainless steel screen (Phillips and Kaune, 1977). An outer frame of stainless steel encloses the screen floor. The screen floor, by itself, is quite flexible, but is greatly stiffened by the plastic cage, which is fastened to the floor in 12 places with nylon screws.

Measured electric-field-induced vibration of a cage floor (i.e., no plastic cage attached) exposed in the Rat Exposure System contained only 120-Hz components. At an interelectrode voltage of 40 kV (each electrode at 20 kV to ground, electric field = 100 kV/m), the observed rms vibration amplitude was 0.02 m/sec^2.

The electric-field-induced vibration of the floor of one rat compartment of an 8-compartment rat cage module was measured; the lexan cage module was clean, empty, and had its lids in place. Initial measurements showed that both 60- and 120-Hz components were present. The presence of a 60-Hz component was surprising since the pressure exerted by an electric field, E, on a metal electrode is $\varepsilon_0 E^2/2$ ($\varepsilon_0 = 8.85 \times 10^{-12}$ F/m), and contains only static and 120-Hz components (Reitz and Milford, 1960). To gain more information on this unexpected observation, the following tests were made:

1. The plastic cage module was covered with copper foil, which was electrically connected to the cage floor. The 60-Hz component of vibration was eliminated, and 120-Hz vibrations were slightly increased.

2. The cage module was vigorously rubbed with a dry terry-cloth towel, and large increases in the level of 60-Hz vibration were observed.

Based on these observations, we concluded that the 60-Hz component of vibration arose from an interaction between the electric field and static electric charge attached to the plastic animal cages.

Vibration data obtained for six clean cage modules were highly variable across modules. At an interelectrode voltage of 40 kV, corresponding to an electric field inside the animal cage of 95 kV/m, the average rms level of 60-Hz vibration was 0.1 m/sec^2, with maximum and minimum observed values of 0.3 and 0.003 m/sec^2, respectively. The average rms amplitudes of 120-Hz vibration was 0.05 m/sec^2, with maximum values of 0.2 and 0.02 m/sec^2, respectively.

For one cage module, data were obtained with interelectrode voltages of 0, 20, 40, and 60 kV. The resulting 60- and 120-Hz amplitudes are shown in Figure II-2. The dependencies of the 60- and 120-Hz amplitudes on the electrode voltage are linear and quadratic, respectively, as shown in Figure II-2.

Measurements in Full Cages. Two 8-compartment cage modules were placed in the Special Studies Exposure System, and the electric-field-induced vibration of the cage floors was measured as described earlier. Measurements were made on six different sets of modules (all initially clean), each set consisting of one module filled with eight rats and one empty module. These data, as well as the empty cage data presented

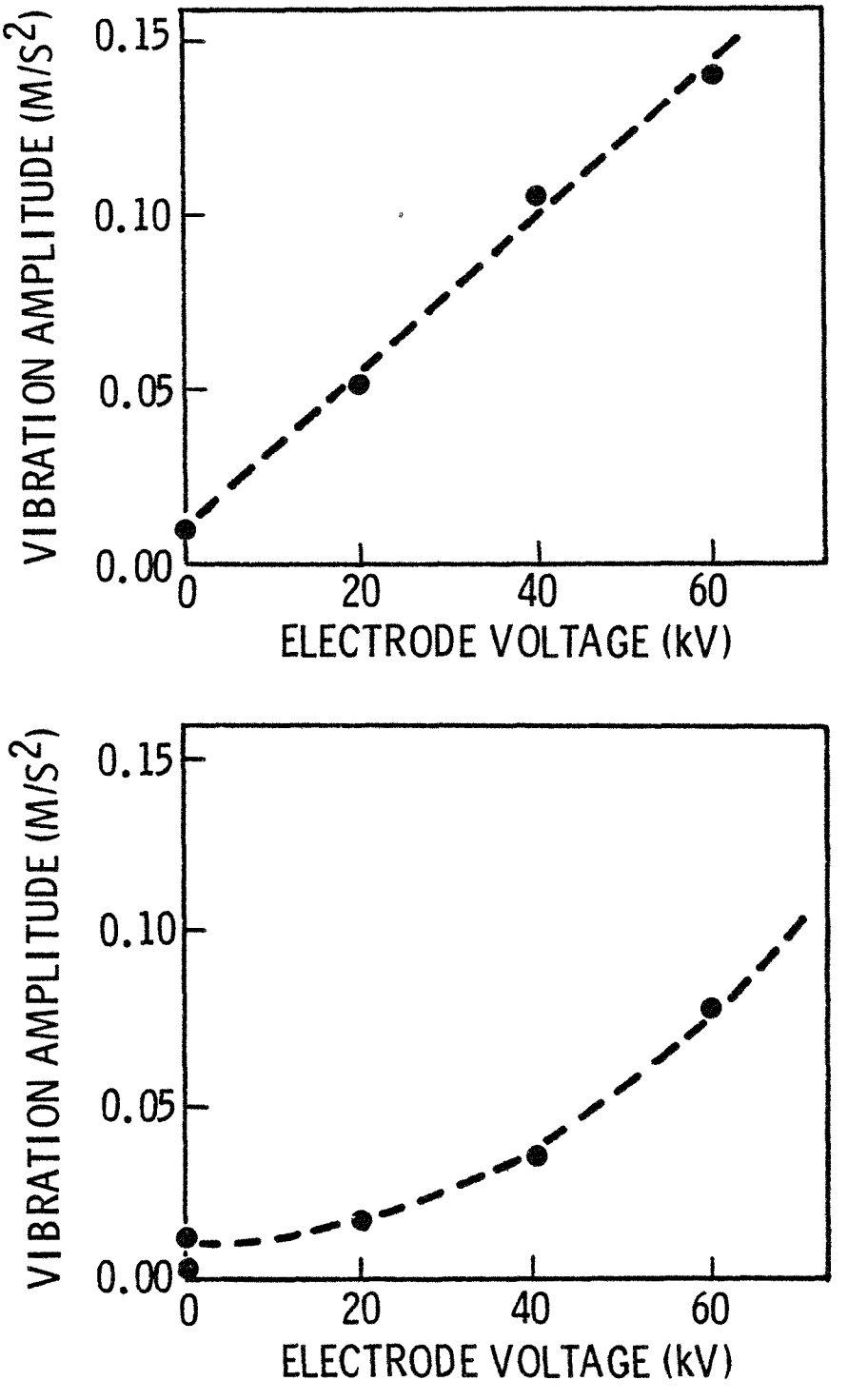


FIGURE II-2. Vibration Amplitudes Measured in Rat Exposure System as a Function of the Interelectrode Voltage, 60-Hz (upper graph) and 120-Hz (lower graph). Ambient background vibration has not been subtracted.

earlier, are summarized in Table II-5. In addition, Table II-5 gives ambient vibration levels (3-5000 Hz), determined by measuring the broadening of the composite oscilloscope trace, as discussed in the Methods section. All vibration levels are given in Table II-5 in terms of peak-to-peak accelerations to facilitate comparisons between background (which cannot easily be specified in terms of rms amplitude) and field-induced vibration.

5. Discussion and Conclusions. Table II-5 demonstrates that electric-field-induced (but not ambient) vibration of the cage floors is substantially lower in occupied than in empty cages. For cages occupied for more than 1 hr, both the 60- and 120-Hz amplitudes are reduced by about a factor of 10. Probable causes for this reduction are: (1) changes in the natural vibration frequencies of the cages due to the added mass of the rats; (2) partial shielding, by the bodies of the rats, of the cage floors from the electric field; (3) alterations of the static charge on the cage walls resulting from contact between the animals and the cages.

The ambient background consists of continuous, low-level, higher-frequency (>100 Hz) noise, with occasional stronger episodes in the frequency range of 30-120 Hz, produced (we believe) by machinery in the laboratory building. When compared against these backgrounds, and against vibration resulting from animal movement, electric-field-induced vibration is small (but is, of course, concentrated at 60 and 120 Hz).

TABLE II-5. Measured Ambient and Electric-Field-Induced Vibration Levels In Empty and Full Rat Cage Modules. Vibration levels expressed as peak-to-peak (p-p) accelerations.

Condition	Location of Measurement	Ambient Background 3-5,000 Hz (p-p, m/sec^2)			Electric-Field-Induced Vibration 60-Hz (p-p, m/sec^2)			120-Hz (p-p, m/sec^2)		
		Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum
Empty Cage Module	Floor of Empty Cage	0.06	0.3	1.0	0.01	0.3	0.8	0.06	0.2	0.6
Full Cage Module, Rats Resting	Floor of Full Cage	0.1	0.2	0.3	0* 0**	0.1* 0.04**	0.6* 0.2**	0* 0**	0.02* 0.015**	0.06* 0.04**
Full Cage Module, Rats Active	Floor of Full Cage	-	-	20	-	-	-	-	-	-

† Ambient background subtracted
* Cage module occupied less than 45 min
** Cage module occupied more than 1 hr

D. ELECTRIC FIELD MEASUREMENTS IN THE SHAM-EXPOSED AREA OF THE RAT EXPOSURE SYSTEM

1. Introduction. The Rat Exposure System consists of four identical exposure racks, separated from each other by two layers of 1-in.-mesh poultry wire (Phillips and Kaune, 1977). Two of the racks are energized to expose animals; the other two hold sham-exposed animals. Field measurements in the sham-exposure area were made to document levels of 60-Hz electric fields to which control animals are exposed.

2. Methods. Measurements were made with a dipole fieldmeter (Polytek Materials Inc. Model FBM 100-7, Scotia, NY) with the insulating handle reduced to a length of 1.25 m. Because of the confined spaces, and the proximity to conducting surfaces, measurements with a large fieldmeter (such as the Polytek) will have significant errors.

3. Results. With the exposure racks energized to interelectrode voltages of 40 kV, which corresponds to a field strength of 100 kV/m (95 kV/m inside the animal cages), the fields in the sham-exposure area were surveyed. Alongside the sham-exposure rack, fields of about 0.5 V/m were observed. Between the electrodes, which are grounded, no change in the background reading of about 0.1 V/m was observed as the exposure racks were energized and de-energized. (The meter was held both vertically and horizontally). Allowing for possible errors associated with the size of the dipole field meter, it can be concluded that an upper limit on leakage of 60-Hz electric fields from the exposure to the sham-exposure area is about 0.2 V/m.

E. ELECTRIC CURRENTS DURING DRINKING

1. Introduction. An animal will have current induced in its body by an alternating electric field. If the animal is grounded, as in the Rat Exposure System, current will pass between the body and ground (called the short-circuit current). Normally, this current will pass through the feet and other parts of the lower body which are in contact with the ground. However, during drinking, an alternative path exists through the animal's mouth and the watering nozzle.

In the Rat Exposure System, distilled water is distributed through a stainless steel manifold under the floor of the rat cage, into which are placed small, demand-operated nozzles (Phillips and Kaune, 1977). The manifold nozzle joints are sealed with rubber grommets; electrical conduction between the nozzle and manifold is through the distilled water and, possibly, along the contaminated surface of the grommet.

An equivalent electrical circuit for drinking rats is shown in Figure II-3. Induced short-circuit current may pass through the impedance, Z_F, between the body (feet, tail, etc.) and ground or, alternatively, through the impedances between the mouth and nozzle, Z_M, and the nozzle and ground, Z_N. The division of short-circuit current between these two paths will depend on relative values of Z_F and $Z_M + Z_N$.

The determination of accurate and representative values of I_M, the current through the mouth during drinking, is complicated by the possibility that the impedances, Z_F and Z_M (Figure II-3), will depend on the experimental methods used. For example, water deprivation prior to an experiment may be used to insure that rats drink during the experiment;

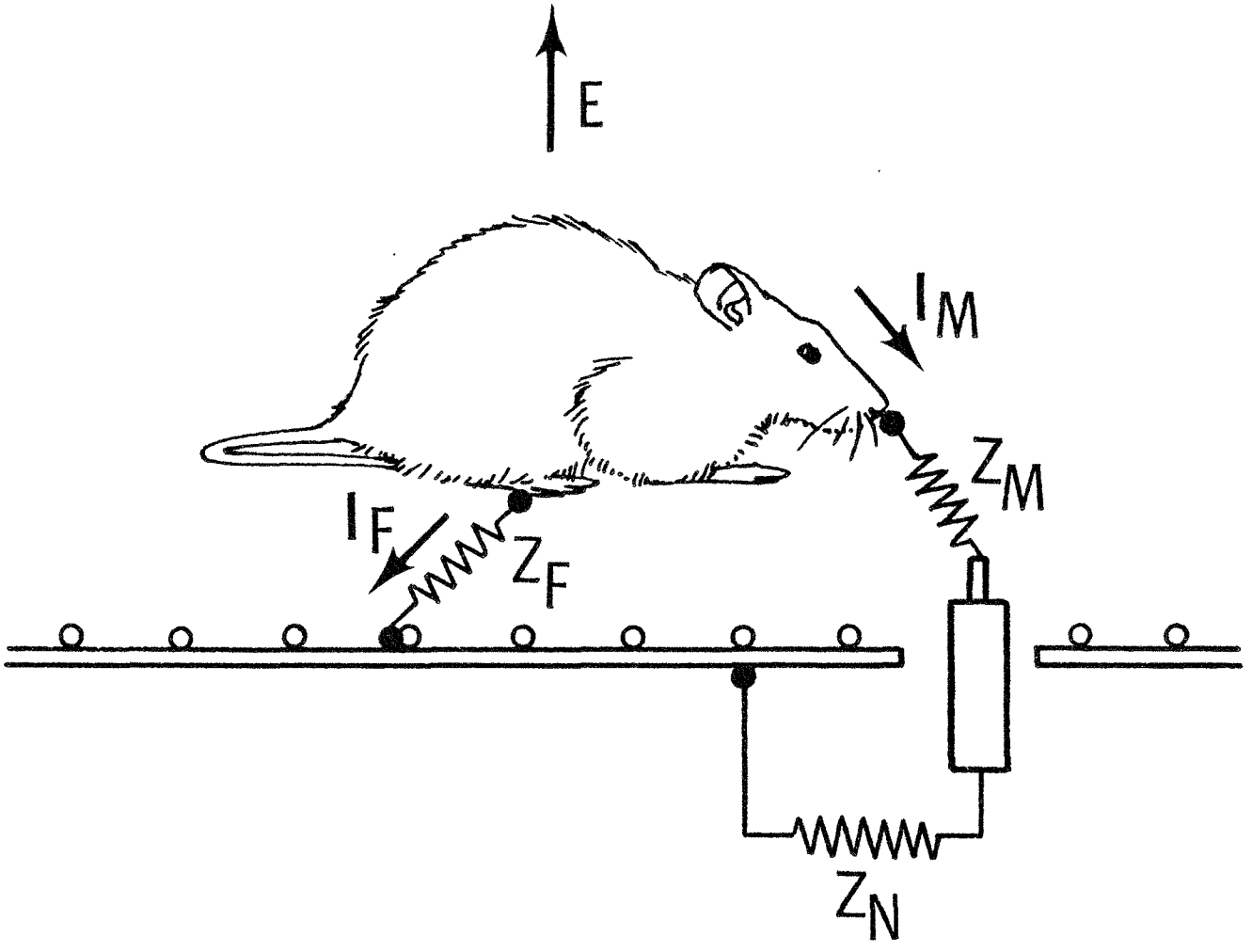


FIGURE II-3. Equivalent Electrical Circuit for a Drinking Rat Exposed to a 60-Hz Electric Field

but it is known that water deprivation is associated with large increases in the direct-current resistance between the feet and ground (Weijnen, 1977), and it seems likely that a similar effect will occur at 60 Hz. The best technique would be to make direct measurements of I_M in the apparatus normally used for rat exposure and with food and water supplied in the normal way (in our experiment, ad libitum). Unfortunately, such a measurement was not practical because: (1) a large number of measurements and animals would be required to obtain meaningful results, and (2) long-term surveillance (or an automated system) would be required to obtain data covering several drinking episodes of one rat only. Instead, we used an alternative procedure: (1) direct measurements of I_M for water-deprived rats were used to set an approximate upper limit on I_M (a higher foot-ground impedance will force more current through the mouth), and (2) measurements of Z_F for rats watered ad libitum are used to estimate I_M.

2. Methods

Measurement of I_M and I_{SC}. The Special Studies Exposure System was used for measurements of the induced current passing through the animal's mouth during drinking. The water manifold of this system is plastic; when used with distilled water, the impedance between a nozzle and ground is very high (>10 megΩ). For measurements of I_M, fixed resistances were placed between the nozzle and ground, and a Tektronix 5031 storage oscilloscope (input impedance = 1 megΩ) was used to monitor the voltage across the resistor.

Eight male Sprague-Dawley rats (weight range, 360-500 g) were tested in the apparatus after they were deprived of water for 1-2 days. After a rat was inserted in the cage, the electrode voltage was raised to 50 kV, corresponding to an electric-field strength inside the exposure cage of about 75 kV/m. Four to six photographs were taken of oscilloscope displays showing the nozzle voltage during 5 sec of drinking. Average measurements of the peak-peak nozzle voltage during drinking were subsequently made for each photograph, and the results for each animal were averaged.

For short-circuit current measurements, each animal was anesthetized, placed in a cage in an approximately normal resting posture, insulated from the floor with a sheet of Mylar, and separately grounded through a low-impedance current-to-voltage converter.

Measurement of Z_F. Sprague-Dawley rats, which had unlimited access to water except during the measuring sessions, were placed in a small plastic cage with a floor of the same material as used in our exposure systems (1/2-in. by 1/2-in. stainless steel screen). At least 24 hr prior to this, a wound clip was placed in the back of the neck of each anesthetized subject. The following procedures were used to make the measurements: (1) A rat was placed in the cage, and a flexible lead was attached to the wound clip to introduce a constant 60-Hz current of 2.1 μA into the subject's body. (2) Sufficient time was allowed for the subject to settle down into normal resting behavior. (3) The voltage between the wound clip and the floor was recorded, every 30 sec, for at least 30 observations.

Current and voltage waveforms were also observed with an oscilloscope to give an approximate measure of the phase of the impedance. The voltage between the wound clip and the floor gave a measurement of the impedance; the resulting data were averaged for each rat.

Measurement of $|Z_N|$. The magnitude of the impedance between a water nozzle and its electrode was measured by introducing a constant 60-Hz current into the nozzle and measuring the voltage between the nozzle and the electrode.

3. Results

I_M and I_{SC}. Measurements of I_M were made for nozzle-to-ground resistances, R_N, of 63 and 200 kΩ, respectively. Eight rats were tested at each resistance; however, data from only six of the rats were obtained for R_N = 63 kΩ, since two rats did not drink. In addition, the short-circuit currents were measured for all eight rats, using the method described above. These data are given in Table II-6.

Z_F. Impedance measurements between the floor and wound clips placed in the necks of four male and four female Sprague-Dawley rats were made, using the procedure described earlier. The results are given in Table II-7. The data are highly variable, both within individual measurement sessions and across animals.

The measured impedance consisted not only of Z_F but also the impedance between the wound clip and the body, as well as internal impedances within the body. The conductivity of body tissue is sufficiently high

TABLE II-6. Measured Short-Circuit Currents, I_{SC}, and Currents Through the Mouth of a Drinking Rat, I_M, Exposed to a 75-kV/m Electric Field. Data are given for two nozzle-to-ground resistances, R_N. Currents are rms values.

R_N (kΩ)	Number of Animals	I_{SC} (μA)		I_M (μA)		I_M/I_{SC}	
		Average	S.D.	Average	S.D.	Average	S.D.
63	6	12.3	0.8	8.2	2.0	0.66	0.17
200	8	12.0	0.8	5.2	1.4	0.43	0.11

TABLE II-7. Measured Impedances Between Sprague-Dawley Rats and a 1/2 in. x 1/2 in. Stainless Steel Screen Floor. The means, standard deviations (S.D.), and numbers of observation (N) are given.

Sex	Weight (g)	N	Body-Floor Impedance	
			Mean (kΩ)	S.D. (kΩ)
Male	590	35	65	57
Male	550	37	46	30
Male	590	36	74	43
Male	540	33	64	33
Female	380	44	264	84
Female	300	35	174	120
Female	350	32	113	25
Female	300	30	451	300

(Geddes and Baker, 1967) that contributions from internal impedances are not significant. To estimate the impedance between the wound clip and the body, pairs of clips were placed about 2 cm apart in the necks of two rats, and the 60-Hz impedance was measured between these two clips. Immediately after placing the clips, impedance for each rat was 11 kΩ. Three days after the clips were inserted, the measured impedances were 5 and 6 kΩ, respectively. We conclude that corrections to the data in Table II-7 for wound-clip-to-body impedances are negligible.

Oscilloscope observations indicated that the phase angle for the impedance, Z_F, is about -30° (estimated error = ± 10°).

The choice of a statistical model for use in the analysis of the foot-to-ground impedance data in Table II-7 is uncertain because of the large variability. For purposes of discussion in this report, we have simply averaged the mean values for animals within a group, using 60 kΩ and 250 kΩ for males and females, respectively.

$|Z_N|$: *Rat Exposure System*. Over a 3-month period (April-July, 1979), impedances between watering nozzles and exposure electrodes in the Rat Exposure System were sampled on a weekly basis. Initially, the conductivity of the distilled water was highly variable. Some of this variability was reduced by cleaning the distilling apparatus more frequently. Prior to this change (made June, 1979), the mean value for $|Z_N|$ was 110 kΩ (S.D. = 80 kΩ, 60 observations); after the change, we found $|Z_N|$ = 135 kΩ (S.D. = 40 kΩ, 28 observations).

Analysis of $|I_M/I_{SC}|$ Data. Table II-6 gives measurements of $|I_M/I_{SC}|$ for two values of nozzle-ground resistance, R_N. These measurements can be used to extract estimates of the magnitude and phase of Z_F.

According to Figure II-3:

$$\left|\frac{I_M}{I_{SC}}\right| = \frac{|Z_F|}{|Z_F + Z_M + R_N|} . \qquad \text{(II-1)}$$

No data are available on Z_M, the impedance of the mouth/nozzle contact. However, it seems reasonable to expect that it will be small relative to R_N, the nozzle-to-ground resistance, and we shall neglect it in Eq. (II-1). Direct solution can then be made for $|Z_F|$ and ϕ_F, the magnitude and phase of Z_F, respectively; this solution, however, has $\cos \phi_F > 1$. As an alternative, we formed the following χ^2 sum, using the data in Table II-6, and Eq. (II-1).

$$\chi^2 = \left(\frac{0.66 - \frac{|Z_F|}{|Z_F + 63\ k\Omega|}}{0.07}\right)^2 + \left(\frac{0.43 - \frac{|Z_F|}{|Z_F + 202\ k\Omega|}}{0.04}\right)^2 .$$

For any value of ϕ_F in the range $-90° \leq \phi_F \leq 0$, an estimate of $|Z_F|$ can then be obtained by minimizing χ^2. The results of the analysis are given in Table II-8. These data show that the best estimates of $|Z_F|$ and ϕ_F are 145 kΩ and 0°, respectively; however, the data are consistent with a relatively wide range of values of ϕ_F, and are certainly consistent with the observation of $\phi_F = -30°$ reported here.

4. Discussion and Conclusions. Data obtained with water-deprived rats can be used to get an approximate upper limit on the fraction of current passing through the mouths of male rats during drinking. Assuming a nozzle-ground impedance of 110-135 kΩ, corresponding to measured values for the Rat Exposure System, and using our best estimate for ϕ_F and $|Z_F|$ (Table II-8), we have

$$\left|\frac{I_M}{I_{SC}}\right| \lesssim 0.6.$$

Applying this result to the Rat Exposure System is done in the following way: (1) for an unperturbed, nominal electric field of E_o, the field inside the lexan exposure cage is 0.95 E_o (Phillips and Kaune, 1977); (2) using data given in Section XV of this report, the short-circuit current induced in a rat will be $I_{SC} = 0.95\ \alpha\ W^n E_o$, where I_{SC} is in μA, $\alpha = 0.0033$, W is the weight of the rat in grams; n = 0.63 and E_o is in kV/m; (3) because of shielding between multiply exposed animals, the short-circuit current is reduced by about 35% (Phillips and Kaune, 1977); (4) thus, for male rats,

$$I_M \lesssim 0.12 \times 10^{-2}\ W^{0.63}\ E_o.$$

For example, we have normally used E_o = 100 kV/m in the Rat Exposure System. Assuming W = 400 g, $I_M \lesssim 5$ μA.

The body/ground impedance data can be used to estimate the I_M for male and female rats who are not water-deprived. The estimates for the Rat

TABLE II-8. Estimates of Magnitude of Impedance, Z_F, Between Male, Water-Deprived Sprague-Dawley Rats and a Screen Floor. For various values of the phase, ϕ_F, of Z_F, estimates of the magnitude of Z_F, $|Z_F|$, were obtained by minimizing a χ^2 expression.

$\cos \phi_F$	Estimated $\lvert Z_F \rvert$ (kΩ)	χ^2
1.00	145	0.34
0.90	140	0.44
0.80	130	0.57
0.70	125	0.74
0.50	110	1.3
0.25	100	2.5
0.00	85	5.1

Exposure System are $I_M \lesssim 0.66 \times 10^{-3} W^n E_o$ for males (weight range, 540-590 g), and $I_M \lesssim 0.14 \times 10^{-2} W^n E_o$ for females (weight range, 300-380 g). These estimates must be used carefully since they are based on limited measurements of the body/ground impedance and may not accurately represent long-term averages. However, one conclusion that does seem reasonably certain is that there is a difference in impedance based on sex, with males evidently better grounded then females.

Direct currents of 1-5 μA that pass through the mouth can be perceived by highly trained and behaviorally motivated rats (Weijnen, 1977); it therefore seems reasonable to expect that 60-Hz currents in this range may also be perceived. Whether such a small 60-Hz current has physiological consequences for the animal is unknown.

The original design of the drinking systems in the Rat and Mouse Exposure Systems was based on the assumption that $|Z_F|$, the impedance between the body of the rat and the floor, is small (≲10 kΩ). In fact, measurements (Table II-7) have shown that this assumption is incorrect. Eq. (II-1) shows that I_M could be decreased by increasing the impedance between the drinking nozzle and ground. Such an increase could be accomplished by using a plastic water-distribution manifold, distilled water, and high-value grounding resistors for each nozzle. Measured nozzle short-circuit currents (Phillips and Kaune, 1979) suggest that the grounding resistor should have a value of about 10 megΩ.

REFERENCES

Geddes, L. A. and L. B. Baker. 1967. The specific resistance of biological material--a compendium of data for the biomedical engineer and physiologist. Med. Biol. Eng. 5: 271-293.

Kaune, W. T. 1979. "A prototype system for exposing small laboratory animals to 60-Hz vertical electric fields: electrical measurements." In: Proceedings of the 18th Annual Hanford Symposium, Biological Effects of Extremely-Low Frequency Electromagnetic Fields, held at Richland, WA, 1978. Springfield, VA, NTIS (in press)

Phillips, R. D., W. T. Kaune, J. R. Decker and D. L. Hjeresen. 1976. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Interim Progess Report. CONS/1830-1, Conservation, Energy Research and Development Administration, Washington, DC.

Phillips, R. D. and W. T. Kaune. 1977. Biological Effects of High Strength Electric Fields. Second Interim Progress Report. CONS/1830-2, Conservation, Energy Research and Development Administration, Washington, DC.

Phillips, R. D., et al. 1978. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Annual Report. HCP/T1830-03, Division of Electrical Energy Systems, U.S. Department of Energy, Washington, DC.

Phillips, R. D., M. F. Gillis, J. L. Beamer, C. H. Allen, W. T. Kaune, J. R. Decker, T. W. Jeffs, and H. A. Ragan. 1979. Biological Effects of Electric Fields on Large Animals. Interim Progress Report. PNL-2280, Battelle, Pacific Northwest Laboratory, Richland, WA. NTIS, Springfield, VA.

Reitz, J. R. and F. J. Milford. 1960. Foundations of Electromagnetic Theory. Reading: Addison-Wesley Publishing Co., Inc., New York, NY.

Schwan, H. P. and C. F. Kay. 1956. Specific resistance of body tissues. Circ. Res., 4: 664-670.

Weijnen, J. A. W. M. 1977. "The recording of licking behavior." In: Weijnen, J. A. W. M. and J. Mendelson, (eds.). Drinking Behavior. New York, Plenum Press.

III. BIOLOGICAL INVESTIGATIONS

ABSTRACT

This section briefly describes the general experimental design of the biological studies in this project and summarizes several features common to most of the experiments described in subsequent sections. Any deviations from the general experimental design will be described in detail in the sections where such deviations occur.

Since our last interim report (April, 1978), experiments have been conducted in 11 major biological areas to screen for effects over a wide range of parameters in mice and rats exposed to a 60-Hz electric field for up to 120 days. For experiments in each major area we usually used separate animal groups; each experiment was directed by professionally trained staff with specialties in the areas under investigation. Rats and mice were exposed to 60-Hz electric fields in systems described in previous interim reports. Extensive measurements have been made to characterize the animal exposure systems. Electric-field uniformity is ±4% over the total cage area in the rat and mouse exposure systems. The exposure systems are free of detectable levels of audible noise and ozone, and there is less than 0.3% harmonic distortion. Animals do not receive spark discharges or other shocks from the housing system or from other animals during exposure to electric fields.

All experiments except those dealing with mutagenesis used Sprague-Dawley rats and/or Swiss-Webster mice. All animals received from the supplier, Hilltop Laboratories, were carefully examined to verify that

they were healthy before being used in experiments. A sample population from each shipment was sacrificed at delivery for virological screening and histopathologic examination. The remaining animals were maintained in standard cages in an isolation room for 1-3 wk, ear-coded, then acclimated for 14 days in cages identical to those used to house animals in the electric-field exposure systems. Temperatures in the isolation and acclimation rooms were maintained at 21°C (20-23°C range). Relative humidity was monitored daily; typically, it was 20-40%. Animals were provided water and food ad libitum during isolation and acclimation.

After acclimation, the animals were randomly subdivided into three groups for subsequent treatment: exposed, sham-exposed and "cage controls" (i.e., maintained in standard cages in the vivarium). During exposure or sham exposure, the environment, housing and husbandry were identical for both groups of animals except for the presence or absence of the electric field. This design reduces the risk of obtaining false positive results and increases the ability to detect subtle effects of exposure. In addition, animals maintained in standard cages ("cage controls") were used in many of the experiments to verify that the exposure cages did not produce effects that might mask subtle effects of exposure. In most experiments the animals were about 60 days of age at the start of exposure.* The field strength used for most exposures was 100 kV/m. The exposure period was about 20 hr/day; this allowed about 4 hr/day for scheduled measurements, cage cleaning, feeding and watering.

* See individual sections for those experiments where animals were exposed in utero or as juveniles.

Temperature and humidity in the exposure rooms were 21°C (19-23°C range) and 30-40%, respectively. Food and water were available to the rats during exposure except in those cases where the exposure duration was $\leq$8 hr. With few exceptions, experiments were double-blind; the principal investigator was unaware of which animals were exposed and which animals were sham-exposed until the measurements had been completed. Most experiments were replicated to insure that the results of a single experiment were not due to some unidentified systematic error in that experiment, or to statistical chance. To reduce Type II errors intrinsic in a replicate design strategy, additional experiments were usually conducted if either of the replicates indicated a statistically significant effect or if a trend of a possible effect was evident across replicates.

IV. HEMATOLOGY AND SERUM CHEMISTRY

Principal Investigator: H. A. Ragan

ABSTRACT

From evaluation of a large number of parameters, there appear to be no unequivocal hematologic or serum chemistry effects of exposing young adult female rats or mice to unperturbed field strengths of ∿ 100 kV/m for 15, 30, 60 or 120 days. Consistent effects were observed on clinical pathology parameters in mice exposed from conception through 77 days of age, and more effects were seen in exposed male mice than in exposed females when compared to their sham-exposed controls. In all studies, statistically significant differences between exposed and sham-exposed animals were seen when evaluated by Student's t test, but the effects were usually not reproducible in replicate experiments. These data are presently being evaluated by more appropriate statistical techniques.

A. INTRODUCTION

As detailed in the 1978 Annual Report (Phillips et al., 1978), rats were exposed for 15, 30, or 60 days, and mice for 30 or 60 days, to an unperturbed 60-Hz electric field of 100 kV/m. Each study was replicated with 10-20 rats or mice exposed (or sham-exposed) per replicate study.

In the rat studies, there were no reproducible changes observed in 15-, 30-, or 60-day exposures, except for a possibly elevated platelet concentration at 60 days of exposure.

The results of exposing mice did not indicate any unequivocal hematologic or serum chemistry changes consistently attributable to exposure to electric fields. In some cases, statistically significant

differences were found between exposed and sham-exposed animals, but these changes were never consistent either across replications or within various exposure durations.

The present report covers continuation of these studies for exposures of rats and mice to 100-kV/m fields for 120 days, and of ferrokinetic studies in mice and rats exposed for 60 days. In addition, mice from the three-generation study (see Section XII, Reproduction and Development) were evaluated for changes in hematologic or serum chemistry parameters. Blood samples were evaluated from three successive generations of mice (F_1, F_2, and F_3) exposed in utero and for 77 days after birth.

B. METHODS

The techniques and procedures used in the clinical pathology evaluations were detailed in the 1978 Annual Report (Phillips et al., 1978). Preliminary statistical evaluations for this report are based on Student's *t* test. More appropriate multivariant statistical analyses of the data are in progress.

C. RESULTS

120-Day Rat Studies. The results of hematologic evaluations for the 120-day exposures are shown in Tables IV-1 and IV-2, and for clinical chemistry in Table IV-3. Values from the exposed and sham-exposed group that are statistically different at $p < 0.05$, using a two-tailed Student's *t* test, are shown in Table IV-4.

TABLE IV-1. Hematologic Values (Mean ± S.E.) in Rats Exposed for 120 Days to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m. Each group consisted of 19-20 rats.

	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Body Wt (g)	312.1 ± 8.6	310.3 ± 7.9	288.9 ± 5.9	282.3 ± 6.2	300.8 ± 5.5	296.3 ± 5.4
Volume Packed Red Cells (ml/dl)	42.8 ± 0.3	44.0 ± 0.4	43.9 ± 0.3	44.9 ± 0.4	43.3 ± 0.3	44.4 ± 0.3
Red Blood Cells (x $10^6/mm^3$)	8.03 ± 0.08	8.13 ± 0.08	8.29 ± 0.06	8.56 ± 0.09	8.15 ± 0.05	8.34 ± 0.07
Hemoglobin (g/dl)	16.6 ± 0.1	16.8 ± 0.1	17.1 ± 0.1	17.5 ± 0.1	16.9 ± 0.1	17.2 ± 0.1
Mean Corpuscular Volume (μm^3)	53.5 ± 0.4	54.2 ± 0.4	55.0 ± 0.3	55.0 ± 0.4	54.2 ± 0.3	54.5 ± 0.3
Mean Corpuscular Hemoglobin ($\mu\mu g$)	20.8 ± 0.1	20.8 ± 0.2	20.8 ± 0.1	20.6 ± 0.1	20.8 ± 0.1	20.7 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	39.2 ± 0.1	38.6 ± 0.2	38.9 ± 0.1	38.8 ± 0.1	39.0 ± 0.1	38.7 ± 0.1
Reticulocyte (x $10^3/mm^3$)	135 ± 10	134 ± 11	115 ± 6	143 ± 7	126 ± 6	139 ± 7
Platelets (x $10^3/mm^3$)	857 ± 34	848 ± 43	877 ± 34	924 ± 61	867 ± 24	913 ± 30
Bone Marrow (x $10^3/mm^3$)	55.0 ± 2.4	53.0 ± 2.4	46.8 ± 1.4	47.2 ± 1.7	51.0 ± 1.6	50.1 ± 1.5

TABLE IV-2. Total Leukocyte Count and Cell Distribution (Mean ± S.E.) in Rats Exposed for 120 Days to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m. Each group consisted of 19-20 rats.

	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	5.4 ± 0.2	5.8 ± 0.2	6.1 ± 0.2	7.1 ± 0.3	5.7 ± 0.2	6.4 ± 0.2
Neutrophils (%)	18.2 ± 2.1	17.1 ± 1.8	15.7 ± 1.8	12.7 ± 1.4	16.9 ± 1.4	14.9 ± 1.2
Neutrophils (x$10^3/mm^3$)	0.97 ± 0.11	0.97 ± 0.10	0.95 ± 0.12	0.91 ± 0.12	0.96 ± 0.08	0.94 ± 0.08
Lymphocyte (%)	76.8 ± 2.2	78.9 ± 1.8	80.5 ± 2.0	83.8 ± 1.6	78.6 ± 1.5	81.4 ± 1.3
Lymphocyte (x $10^3/mm^3$)	4.15 ± 0.23	4.57 ± 0.20	4.91 ± 0.22	5.93 ± 0.28	4.52 ± 0.17	5.25 ± 0.20
Monocytes (%)	1.3 ± 0.2	1.2 ± 0.2	0.6 ± 0.1	1.0 ± 0.2	0.09 ± 0.5	1.1 ± 0.1
Monocytes (x $10^3/mm^3$)	0.06 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Eosinophils (%)	3.7 ± 0.4	2.9 ± 0.4	3.3 ± 0.6	2.6 ± 0.3	3.5 ± 0.3	2.7 ± 0.2
Eosinophils (x $10^3/mm^3$)	0.19 ± 0.01	0.16 ± 0.02	0.19 ± 0.02	0.17 _ 0.02	0.19 ± 0.02	0.17 + 0.02

TABLE IV-3. Serum Chemistry Values (Mean ± S.E.) in Rats Exposed for 120 Days to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m. Each group consisted of 19-20 rats.

	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Total Protein (g/dl)	6.80 ± 0.07	6.96 ± 0.21	6.32 ± 0.10	6.49 ± 0.08	6.59 ± 0.07	6.64 ± 0.09
Albumin (g/dl)	3.34 ± 0.04	3.40 ± 0.09	3.12 ± 0.07	3.28 ± 0.08	3.23 ± 0.04	3.34 ± 0.06
Globulins						
Alpha (g/dl)	1.62 ± 0.04	1.61 ± 0.04	1.43 ± 0.04	1.48 ± 0.04	1.52 ± 0.03	1.54 ± 0.03
Beta (g/dl)	1.12 ± 0.02	1.15 ± 0.02	1.04 ± 0.03	1.04 ± 0.03	1.08 ± 0.02	1.09 ± 0.02
Gamma (g/dl)	0.72 ± 0.02	0.65 ± 0.03	0.73 ± 0.02	0.69 ± 0.03	0.73 ± 0.02	0.67 ± 0.02
SGOT* (IU)	353 ± 35	376 ± 21	411 ± 64	418 ± 43	382 ± 36	399 ± 25
BUN** (mg/dl)	22.5 ± 1.0	23.0 ± 0.7	24.8 ± 0.8	25.5 ± 1.2	23.6 ± 0.7	24.3 ± 0.7
Glucose (mg/dl)	157 ± 11	151 ± 6	108 ± 4	114 ± 5	133 ± 7	132 ± 5
Alkaline Phosphatase (BLB Units)	3.3 ± 0.2	3.4 ± 0.2	3.4 ± 0.2	3.9 ± 0.3	3.4 ± 0.1	3.6 ± 0.2
Triglyceride (mg/dl)	133.0 ± 6.0	146.3 ± 6.7	124.3 ± 8.2	143.3 ± 8.0	128.7 ± 5.0	144.8 ± 5.2
Cholesterol (mg/dl)	74.3 ± 3.9	74.9 ± 4.5	60.0 ± 4.3	78.4 ± 5.1	68.1 ± 3.0	76.6 ± 3.3
Iron (μg/dl)	259 ± 13	275 ± 11	243 ± 9	279 ± 14	247 ± 8	275 ± 9
TIBC*** (μg/dl)	465 ± 13	487 ± 13	469 ± 22	462 ± 16	467 ± 12	478 ± 10

* Serum glutamic oxaloacetic transaminase
** Blood urea nitrogen
*** Total iron binding capacity

TABLE IV-4. Statistical Differences in Clinical Pathology Parameters Between Rats Exposed in a 100-kV/m Electric Field for 120 days and their Sham-Exposed Controls.

Experiment	N/Group	Parameter	Mean ± S.E.		p<
			Exposed	Sham-Exposed	
1	20	VPRC*	42.8 ± 0.3	44.0 ± 0.4	0.05
		MCHC**	39.2 ± 0.1	38.6 ± 0.2	0.02
2	20	Red Blood Cells	8.29 ± 0.06	8.56 ± 0.09	0.02
		Hemoglobin	17.1 ± 0.1	17.5 ± 0.1	0.05
		Reticulocytes	115 ± 6	143 ± 7	0.01
		White Blood Cells	6.1 ± 0.2	7.1 ± 0.3	0.02
		Lymphocytes	4.91 ± 0.22	5.93 ± 0.28	0.01
		Monocytes	0.04 ± 0.01	0.07 ± 0.01	0.05
		Cholesterol	60.0 ± 4.3	78.4 ± 5.1	0.02
		Iron	243 ± 9	279 ± 14	0.02

* Volume Packed Red Cells
** Mean Corpuscular Hemoglobin Concentration

The mean volume of packed red cells (VPRC) in the exposed group was significantly lower ($p < 0.05$) than in controls in Experiment #1, and was also lower in Experiment #2 (but not statistically significant). There was a concomitant decrease in red cell count and in hemoglobin concentration, reflecting the interdependence among these red blood cell parameters. Both parameters were significantly lower in the exposed animals as compared to controls in Experiment #2, with a trend in the same direction in Experiment #1. A highly significant ($p < 0.01$) decrease in reticulocytes is compatible with the finding of a decrease in erythrocyte count, and is to be expected if the decrease in red blood cell count is due to a depression in bone-marrow production. However, the decreased reticulocyte concentration was seen only in Experiment #2; in Experiment #1, the mean reticulocyte values were identical in the two groups.

The pertinent finding in the evaluation of effects on leukocytes was in mean white cell count, which was significantly decreased ($p < 0.02$) in Experiment #2 and somewhat reduced in Experiment #1. This effect was due to the reduction in circulating lymphocyte concentrations, since the other major white-cell type, i.e. neutrophils, was not affected in either replication.

The only statistically significant difference ($p < 0.02$) in serum chemistry parameters was in the lower cholesterol levels in the exposed rats of Experiment #2. Interestingly, serum triglyceride levels were also lower (but not statistically significant) in the exposed rats than

in the sham-exposed group of both replicates. There was also a trend to slightly lower total serum proteins in the exposed rats of both replicates, due primarily to a slight reduction in serum albumin concentrations.

120-Day Mouse Studies. Hematologic data for mice are shown in Tables IV-5 and IV-6; clinical chemistry values are shown in Table IV-7, and some selected organ weights are also shown in Table IV-7. Values from the exposed and sham-exposed mice that are statistically different at $p \leq 0.05$ are shown in Table IV-8.

In Experiment #1, platelet concentrations were significantly lower ($p < 0.05$) in the exposed mice. Leukocyte values were higher ($p < 0.02$) in this group, due primarily to a significant increase ($p < 0.05$) in lymphocytes. In Experiment #2, there were no significant differences observed in hematologic parameters between the two treatment groups.

In Experiment #1, there were no significant differences in clinical chemistry parameters between the exposed and sham-exposed mice. In Experiment #2, serum glucose concentrations were greater in the exposed mice ($p < 0.02$); serum proteins were lower ($p < 0.02$), due primarily to a significant ($p < 0.05$) decrease in the concentration of beta globulins. Albumin and alpha globulins were also lower in this group, which contributed to the decrease in protein values, but these differences were not statistically significant.

TABLE IV-5. Hematologic Values (Mean ± S.E.) in Female Mice Exposed for 120 Days to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m. Each group consisted of 20 mice.

	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	39.2 ± 0.4	40.0 ± 0.4	38.4 ± 0.4	37.6 ± 0.3	38.8 ± 0.3	38.8 ± 0.3
Red Blood Cells (x 10^6/mm^3)	8.53 ± 0.13	8.50 ± 0.09	8.45 ± 0.06	8.28 ± 0.09	8.49 ± 0.07	8.39 ± 0.06
Hemoglobin (g/dl)	14.4 ± 0.2	14.6 ± 0.2	14.3 ± 0.2	13.9 ± 0.2	14.3 ± 0.1	14.2 ± 0.1
Mean Corpuscular Volume (m^3)	46.4 ± 0.4	47.4 ± 0.3	47.3 ± 0.3	47.2 ± 0.3	46.9 ± 0 3	47.3 ± 0.2
Mean Corpuscular Hemoglobin (g)	17.0 ± 0.2	17.2 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	17.0 ± 0.1	17.1 ± 0.1
Mean Corpuscular Hemoglobin Concentration ( )	37.0 ± 0.3	36.7 ± 0.2	37.1 ± 0.1	36.8 ± 0.2	37.0 ± 0.2	36.8 ± 0.1
Reticulocytes (x 10^3/mm^3)	256 ± 27	288 ± 29	225 ± 15	188 ± 15	245 ± 16	238 ± 18
Platelets (x 10^3/mm^3)	1050 ± 28	1211 ± 61	1144 ± 40	1172 ± 65	1098 ± 25	1192 ± 44
Bone Marrow (x 10^3/mm^3)	12.5 ± 0.5	13.8 ± 0.6	11.2 ± 0.5	11.2 ± 0.5	11.9 ± 0.4	12.5 ± 0.4

TABLE IV-6. Total Leukocyte Count and Cell Distributions (Mean ± S.E.) in Female Mice Exposed for 120 Days to a 60-Hz Electric Field Strength of 100 kV/m. Each group consisted of 20 mice.

	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	7.3 + 0.5	5.6 ± 0.4	7.1 ± 0.7	7.8 ± 0.5	7.1 ± 0.4	6.7 ± 0.3
Neutrophils ( )	20.1 + 2.6	17.9 ± 1.4	19.4 ± 1.4	15.5 ± 0.9	20.0 ± 1 5	16.7 ± 0.9
Neutrophils (x$10^3/mm^3$)	1.44 + 0.17	1.03 ± 0.11	1.41 + 0.20	1.19 ± 0.10	1.42 ± 0.13	1.13 ± 0.08
Lymphocytes ( )	77.4 ± 2.6	78.9 + 1.6	78.2 + 1.5	82.3 ± 1.0	77.8 ± 1.5	80.6 + 1.0
Lymphocytes (x$10^3/mm^3$)	5.55 + 0.48	4.39 + 0.26	5.49 + 0.55	6.42 ± 0.39	5.52 ± 0.36	5.40 ± 0.28
Monocytes ( )	1.5 + 0.3	1.5 + 0.3	1.1 + 0.3	1.3 ± 0.2	1 3 ± 0.2	1.4 ± 0.2
Monocytes (x$10^3/mm^3$)	0.11 + 0.02	0.09 + 0.02	0.09 + 0.02	0.10 ± 0.02	0.10 ± 0.02	0.09 + 0.01
Eosinophils ( )	1.0 + 0.2	1.8 + 0.4	1.3 + 0.4	1.0 ± 0.2	1.2 ± 0.2	1.4 ± 0.2
Eosinophils (x$10^3/mm^3$)	0.08 + 0.02	0.11 + 0.04	0.12 + 0.04	0.08 ± 0.02	0.10 ± 0.02	0.10 ± 0.02

TABLE IV-7. Some Serum Chemistry and Organ Weight Values (Mean ± S.E.) in Female Mice Exposed for 120 Days to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m. Each group consisted of 20 mice.

	Experiment 1*		Experiment 2**		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	---	---	159 + 2	158 + 1	159 + 2	158 + 1
Potassium (meq/L)	---	---	6.3 + 0.6	6.4 + 0.4	6.3 + 0.6	6.4 + 0.4
Blood Urea Nitrogen (mg/dl)	21.6 + 0.7	20.2 + 0.6	30.3 + 1.5	28.9 + 0.9	26.3 + 1.1	24.5 + 0.9
SGOT*** (IU)	258 + 32	335 + 50	---	---	258 + 32	335 + 50
Triglyceride (mg/dl)	179 + 9	160 + 9	129 + 7	139 + 7	150 + 7	148 + 6
Glucose (mg/dl)	90 + 10	100 + 6	138 + 8	113 + 7	117 + 7	106 + 5
Total Protein (g/dl)	6.84 + 0.15	6.84 + 0.17	5.80 + 0.11	6.28 + 0.15	6.32 + 0.12	6.56 + 0.12
Albumin (g/dl)	3.48 + 0.10	3.50 + 0.13	2.95 + 0.08	3.16 + 0.15	3.21 + 0.08	3.38 + 0.08
Globulins						
Alpha (g/dl)	1.36 + 0.04	1.30 + 0.05	1.08 + 0.06	1.16 + 0.04	1.24 + 0.03	1.23 + 0.04
Beta (g/dl)	1.29 + 0.04	1.33 + 0.04	1.04 + 0.06	1.21 + 0.04	1.19 + 0.03	1.27 + 0.03
Gamma (g/dl)	0.71 + 0.03	0.71 + 0.05	0.64 + 0.03	0.64 + 0.04	0.67 + 0.02	0.68 + 0.03
Weights						
Total Wt (g)	31.3 + 0.5	31.6 + 0.5	29.4 + 0.6	30.9 + 0.6	30.6 + 0.4	31.3 + 4
Liver wt (g)	1.42 + 0.04	1.40 + 0.03	1.27 + 0.27	1.32 + 0.03	1.34 + 0.03	1.36 + 0.02
Spleen (mg)	129.1 + 6.1	119.8 + 6.6	132.0 + 6.2	133.0 + 7.7	130.6 + 4.3	126.4 + 5.1
Femur (mg)	65.7 + 1.9	64.0 + 1.5	79.3 + 2.1	82.3 + 2.1	72.8 + 1.8	73.2 + 1.9

* Experiment #1 - Hematology and chemistry done separately on two different groups of mice.
** Experiment #2 - Hematology and chemistry done on same group of animals.
*** Serum glutamic oxaloacetic transaminase.

TABLE IV-8. Statistical Differences in Clinical Pathology Parameters Between Mice Exposed to a 100-kV/m Electric Field for 120 Days and their Sham-Exposed Controls.

Experiment	N/Group	Parameter	Mean ± S.E.		p<
			Exposed	Sham-Exposed	
1	20	Platelets	1050 ± 28	1211 ± 61	0.05
		White Blood Cells	7.3 ± 0.5	5.6 ± 0.4	0.02
		Lymphocytes	5.55 ± 0.48	4.39 ± 0.26	0.05
2	20	Glucose	138 ± 8	113 ± 7	0.02
		Protein	5.80 ± 0.11	6.28 ± 0.15	0.02
		β-Globulins	1.04 ± 0.06	1.21 ± 0.04	0.05

Three-Generation Mouse Study. Animals used for these clinical pathology evaluations were from the three-generation teratology study (Cf Section XII, Reproduction and Development). They were from F_1, F_2, and F_3 generations, conceived, born, and maintained in the electric field or sham-exposure facility until ∿ 77 days of age, at which time they were killed to evaluate hematologic and serum chemistry parameters. With the exception of Experiment #1 chemistry studies, these groups contained both male and female mice, whereas all previous clinical pathology studies were with female mice only.

F_1 Generation. Hematologic data for each sex and for both replications are shown in Tables IV-9 and IV-10, and clinical chemistry results are shown in Table IV-11. Mean values of the exposed groups that are statistically different from the sham-exposed mice are shown in Table IV-12.

No significant differences were observed in erythrocyte parameters in either sex in either of the two experiments. Bone-marrow cellularity was increased in exposed male mice of the second experiment. This appeared to be a spurious finding since no consistent trends were evident toward increased marrow cellularity in the females, or in either sex in the first experiment.

The most striking effect in the electric-field-exposed mice was on white-cell concentrations. Highly significant ($p < 0.01$) elevations were observed in the total leukocyte count, due to increases in all white-cell types. This effect was seen in both sexes and in both experiments.

TABLE IV-9. Hematologic Values (Mean ± S.E.) in F_1 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 10 mice; each female group (see continuation page) consisted of 7-10 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	42.2 ± 0.7	42.8 ± 0.6	40.3 ± 0.6	40.7 ± 0.9	41.3 ± 0.5	41.8 ± 0.6
Red Blood Cells (x $10^6/mm^3$)	8.86 ± 0.18	8.95 ± 0.12	8.39 ± 0.13	8.53 ± 0.22	8.62 ± 0.12	8.74 ± 0.13
Hemoglobin (g/dl)	14.9 ± 0.2	15.2 ± 0.2	14.3 ± 0.3	14.6 ± 0.3	14.6 ± 0.2	14.9 ± 0.2
Mean Corpuscular Volume (μm^3)	48.0 ± 0.4	48.0 ± 0.3	48.3 ± 0.2	48.0 ± 0.4	48.3 ± 0.2	48.2 ± 0.2
Mean Corpuscular Hemoglobin ($\mu\mu g$)	16.9 ± 0.2	17.1 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.0 ± 0.1	17.2 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.5 ± 0.3	35.7 ± 0.2	35.8 ± 0.2	36.3 ± 0.2	35.7 ± 0.2	36.0 ± 0.1
Reticulocytes (x $10^3/mm^3$)	237 ± 18	220 ± 10	192 ± 18	153 ± 12	215 ± 14	187 ± 11
Platelets (x $10^3/mm^3$)	1138 ± 77	1333 ± 59	1098 ± 59	1140 ± 67	1118 ± 47	1236 ± 49
Bone Marrow (x $10^3/mm^3$)	12.7 ± 0.6	14.2 ± 0.6	13.4 ± 0.6	10.9 ± 0.5	13.1 ± 0.4	12.6 ± 0.6

TABLE IV-9. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	39.9 ± 0.7	41.0 ± 0.7	41.5 ± 0.5	42.4 ± 0.8	40.5 ± 0.5	41.6 ± 0.5
Red Blood Cells (x 10^6/mm^3)	8.28 ± 0.12	8.52 ± 0.12	8.84 ± 0.19	8.89 ± 0.14	8.51 ± 0.12	8.73 ± 0.11
Hemoglobin (g/dl)	14.4 ± 0.3	14.7 ± 0.2	15.0 ± 0.3	15.2 ± 0.2	14.6 ± 0.2	14.9 ± 0.2
Mean Corpuscular Volume (μm^3)	48.6 ± 0.4	48.5 ± 0.3	47.6 ± 0.5	47.5 ± 0.7	48.2 ± 0.3	48.1 ± 0.3
Mean Corpuscular Hemoglobin ($\mu\mu g$)	17.4 ± 0.2	17.4 ± 0.1	17.1 ± 0.1	17.1 ± 0.2	17.3 ± 0.1	17.2 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	36.3 ± 0.1	36.2 + 0.2	36.5 ± 0.3	36.3 ± 0.1	36.4 ± 0.1	36.2 ± 0.1
Reticulocytes (x 10^3/mm^3)	200 ± 24	168 ± 13	213 ± 19	206 ± 23	205 ± 16	185 ± 13
Platelets (x 10^3/mm^3)	943 ± 58	1101 ± 65	1000 ± 107	1034 ± 75	966 ± 54	1071 ± 48
Bone Marrow (x 10^3/mm^3)	11.4 ± 0.5	12.1 ± 0.9	11.7 ± 0.6	11.4 ± 0.9	11.5 ± 0.4	11.8 ± 0.6

TABLE IV-10. Total Leukocyte Count and Cell Distribution (Mean ± S.E.) in F_1 Generation. Mice exposed to a 60-Hz electric field at an unperturbed field strength of 100 kV/m from conception to approximately 77 days of age. Each male group consisted of 10 mice; each female group (see continuation page) consisted of 7-10 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	6.3 ± 0.7	3.9 ± 0.3	9.1 ± 0.6	5.1 ± 0.5	7.7 ± 0.6	4.5 ± 0.3
Neutrophils (%)	21.8 ± 3.0	14.6 ± 2.3	20.8 ± 3.2	24.9 ± 2.4	21.3 ± 2.2	19.7 ± 2.0
Neutrophils (x $10^3/mm^3$)	1.42 ± 0.26	0.59 ± 0.11	1.83 ± 0.26	1.26 ± 0.16	1.63 ± 0.19	0.93 ± 0.12
Lymphocytes (/)	75.5 ± 3.4	83.9 ± 2.3	76.8 ± 3.2	72.9 ± 2.2	76.2 ± 2.3	78.4 ± 2.0
Lymphocytes (x $10^3/mm^3$)	4.70 ± 0.55	3.29 ± 0.21	6.94 ± 0.71	3.72 ± 0.36	5.85 ± 0.48	3.51 ± 0.21
Monocytes (%)	1.6 ± 0.4	0.6 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 0.3	0.4 ± 0.1
Monocytes (x $10^3/mm^3$)	0.10 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.02	0.02 ± 0.01
Eosinophils (%)	1.1 ± 0.3	0.9 ± 0.2	2.3 ± 0.4	2.2 ± 0.4	1.7 ± 0.3	1.5 ± 0.3
Eosinophils (x $10^3/mm^3$)	0.08 ± 0.02	0.03 ± 0.01	0.19 ± 0.03	0.11 ± 0.03	0.13 ± 0.02	0.07 ± 0.02

TABLE IV-10. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	5.9 ± 0.5	4.0 ± 0.2	8.8 ± 1.2	4.9 ± 0.4	7.1 ± 0.7	4.4 ± 0.2
Neutrophils (%)	13.9 ± 0.7	11.8 ± 1.0	21.0 ± 1.9	13.6 ± 1.9	16.8 ± 1.2	12.6 ± 1.0
Neutrophils (x $10^3/mm^3$)	0.83 ± 0.09	0.47 ± 0.05	1.78 ± 0.16	0.68 ± 0.11	1.22 ± 0.14	0.56 ± 0.06
Lymphocytes (%)	83.9 ± 0.8	86.7 ± 1.2	76.3 ± 2.0	84.6 ± 1.8	80.8 ± 1.3	85.8 ± 1.0
Lymphocytes (x $10^3/mm^3$)	4.91 ± 0.46	3.42 ± 0.18	6.83 ± 1.05	4.14 ± 0.29	5.70 ± 0.54	3.74 ± 0.18
Monocytes (%)	1.1 ± 0.3	0.4 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.7 ± 0.2	0.3 ± 0.1
Monocytes (x $10^3/mm^3$)	0.06 ± 0.02	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.01	0.04 ± 0.01	0.01 ± 0.01
Eosinophils (%)	1.1 ± 0.2	1.2 ± 0.3	2.5 ± 0.9	1.6 ± 0.3	1.7 ± 0.4	1.4 ± 0.2
Eosinophils (x $10^3/mm^3$)	0.07 ± 0.02	0.05 ± 0.01	0.22 ± 0.06	0.08 ± 0.01	0.13 ± 0.03	0.06 ± 0.01

TABLE IV-11. Serum Chemistry Values (Mean ± S.E.) in F_1 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 10 mice; each female group (see continuation page) consisted of 7-10 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	---	---	156.9 ± 0.6	156.6 ± 2.0	156.9 ± 0.6	156.6 ± 2.0
Potassium (meq/L)	---	---	4.7 ± 0.2	5.6 ± 0.3	4.7 ± 0.2	5.6 ± 0.3
Blood Urea Nitrogen (mg/dl)	30.0 ± 2	29.0 ± 1	45.0 ± 2.5	37.6 ± 1.1	38.1 ± 2.4	34.0 ± 1.4
Cholesterol (mg/dl)	---	---	169.8 ± 7.3	160.2 ± 13.3	169.8 ± 7.3	160.0 ± 13.3
Triglycerides(mg/dl)	174.0 ± 4.0	161.0 ± 20.8	184.9 ± 7.2	202.9 ± 18.6	179.4 ± 4.4	183.3 ± 15.3
Total Protein (g/dl)	5.51 ± 0.06	5.71 ± 0.13	5.56 ± 0.12	6.01 ± 0.11	5.53 ± 0.07	5.88 ± 0.09
Albumin (g/dl)	2.73 ± 0.04	3.05 ± 0.09	2.76 ± 0.07	3.15 ± 0.10	2.75 ± 0.04	3.11 ± 0.07
Globulins						
Alpha (g/dl)	1.46 ± 0.04	1.38 ± 0.06	1.37 ± 0.05	1.39 ± 0.04	1.41 ± 0.03	1.39 ± 0.04
Beta (g/dl)	0.85 ± 0.02	0.85 ± 0.04	0.91 ± 0.05	0.94 ± 0.03	0.88 ± 0.03	0.90 ± 0.03
Gamma (g/dl)	0.46 ± 0.02	0.42 ± 0.04	0.52 ± 0.03	0.53 ± 0.04	0.49 ± 0.02	0.48 ± 0.03

TABLE IV-11. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	---	---	155.1 ± 1.4	154.4 ± 0.7	155.1 ± 1.4	154.4 ± 0.7
Potassium (meq/L)	---	---	4.7 ± 0.2	4.4 ± 0.2	4.7 ± 0.2	4.4 ± 0.2
Blood Urea Nitrogen (mg/dl)	---	---	38.3 ± 1.9	29.6 ± 1.2	38.3 ± 1.9	29.6 ± 1.2
Cholesterol (mg/dl)	---	---	113.7 ± 9.1	120.5 ± 8.9	113.7 ± 9.1	120.5 ± 8.9
Triglycerides(mg/dl)	---	---	145.5 ± 6.3	158.0 ± 17.7	145.5 ± 6.3	158.0 ± 17.7
Total Protein (g/dl)	---	---	5.74 ± 0.11	5.78 ± 0.14	5.74 ± 0.11	5.78 ± 0.14
Albumin (g/dl)	---	---	3.38 ± 0.07	3.56 ± 0.05	3.38 ± 0.07	3.56 ± 0.05
Globulins						
Alpha (g/dl)	---	---	0.97 ± 0.05	0.95 ± 0.06	0.97 ± 0.05	0.95 ± 0.06
Beta (g/dl)	---	---	0.96 ± 0.03	0.90 ± 0.04	0.96 ± 0.03	0.90 ± 0.04
Gamma (g/dl)	---	---	0.43 ± 0.03	0.37 ± 0.02	0.43 ± 0.03	0.37 ± 0.02

TABLE IV-12. Statistical Differences in Clinical Pathology Parameters Between F_1 Generation Mice Exposed to 100-kV/m, 60-Hz Electric Field from Conception to Approximately 77 Days of Age as Compared to Sham-Exposed Controls.

		MEAN ± S.E.					
		MALE			FEMALE		
Experiment	Parameter	Exposed	Sham-Exposed	p<	Exposed	Sham-Exposed	p<
1*	White Blood Cells				5.9 ± 0.5	4.0 ± 0.2	0.01
	Neutrophils	1.42 ± 0.26	0.59 ± 0.11	0.01	0.83 ± 0.09	0.47 ± 0.05	0.01
	Lymphocytes	4.70 ± 0.55	3.29 ± 0.21	0.05	4.91 ± 0.46	3.42 ± 0.18	0.01
	Monocytes	0.10 ± 0.03	0.02 ± 0.01	0.02			
	Eosinophils	0.08 ± 0.02	0.03 ± 0.01	0.05			
2**	Marrow Cellularity	13.4 ± 0.6	10.9 ± 0.5	0.01			
	White Blood Cells	9.1 ± 0.6	5.1 ± 0.5	0.001	8.8 ± 1.2	4.9 ± 0.4	0.01
	Neutrophils				1.78 ± 0.16	0.68 ± 0.11	0.001
	Lymphocytes	6.94 ± 0.71	3.72 ± 0.36	0.001	6.83 ± 1.05	4.14 ± 0.29	0.05
	Eosinophils				0.22 ± 0.06	0.08 ± 0.01	0.05
	Potassium	4.7 ± 0.2	5.6 ± 0.3	0.02			
	Blood Urea Nitrogen	45.0 ± 2.5	37.6 ± 1.1	0.02	38.3 ± 1.9	29.6 ± 1.2	0.01
	Total Protein	5.56 ± 0.12	6.01 ± 0.11	0.02			
	Albumin	2.76 ± 0.07	3.15 ± 0.10	0.01	3.38 ± 0.07	3.56 ± 0.05	0.05

*Experiment 1 - 10 males and 10 females in each exposed and sham-exposed group
**Experiment 2 - 10 males in each group, 7 females in each exposed and 8 females in each sham-exposed group; no female group for clinical chemistry in Experiment 1

Serum potassium levels were measured in both sexes of Experiment #2, and were significantly reduced ($p < 0.02$) in the exposed male group. Blood urea nitrogen (BUN) levels of exposed animals were increased in both sexes in Experiment #2, but not different from sham-exposed animals in Experiment #1. Total serum protein values were decreased in the males of both replicates. This reduction in serum protein was due exclusively to a decrease in serum albumin levels ($p < 0.001$) of the exposed mice.

F_2 Generation. Hematologic data for both sexes of the F_2 generation are shown in Tables IV-13 and IV-14, and the serum chemistry data are shown in Table IV-15. Significant differences between the exposed and sham-exposed groups of the replicate experiments are shown in Table IV-16.

In the F_2 females, there were no statistically significant differences noted between the exposed and sham-exposed mice in either experiment. However, in the F_2 males, several differences between the exposed and sham-exposed groups were seen: Some of the red cell indices were different, but not consistently so; i.e., mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in Experiment #1, and mean corpuscular volume (MCV) and MCH in Experiment #2. Blood urea nitrogen was markedly elevated in exposed males of Experiment #1, but was similar between the two groups in Experiment #2. Leukocyte counts in the exposed males of Experiment #2 were significantly greater ($p < 0.001$) than in the sham-exposed males. This was due to absolute elevations of both neutrophils ($p < 0.05$) and lymphocytes ($p < 0.001$).

TABLE IV-13. Hematologic Values (Mean ± S.E.) in F_2 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 10-20 mice; each female group (see continuation page) consisted of 6-20 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	40.6 ± 1.0	39.4 ± 0.6	39.1 ± 0.6	39.7 ± 0.5	39.6 ± 0.6	39.6 ± 0.4
Red Blood Cells (x 10^6/mm^3)	8.77 ± 0.25	8.65 ± 0.13	8.40 ± 0.11	8.71 ± 0.13	8.52 ± 0.11	8.68 ± 0.09
Hemoglobin (g/dl)	14.8 ± 0.4	14.8 ± 0.3	14.5 ± 0.2	14.7 ± 0.2	14.6 ± 0.2	14.7 ± 0.2
Mean Corpuscular Volume (μm^3)	47.5 ± 0.3	46.6 ± 0.4	48.4 ± 0.3	47.3 ± 0.3	48.1 ± 0.2	47.0 ± 0.2
Mean Corpuscular Hemoglobin ($\mu\mu g$)	16.8 ± 0.1	17.1 ± 0.1	17.4 ± 0.1	17.0 ± 0.1	17.2 ± 0.1	17.0 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	36.3 ± 0.1	37.6 ± 0.3	36.9 ± 0.1	36.7 ± 0.1	36.7 ± 0.1	37.1 ± 0.1
Reticulocytes (x 10^3/mm^3)	219 ± 12	250 ± 20	246 ± 13	236 ± 18	237 ± 10	242 ± 14
Platelets (x 10^3/mm^3)	1223 ± 68	1049 ± 74	906 ± 57	994 ± 102	1011 ± 52	1019 ± 64
Bone Marrow (x 10^3/mm^3)	12.1 ± 0.6	12.8 ± 0.7	12.8 ± 0.4	12.2 ± 0.7	12.6 ± 0.4	12.4 ± 0.5

TABLE IV-13. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	41.2 ± 0.3	39.7 ± 0.6	39.1 ± 0.4	40.2 ± 0.5	39.5 ± 0.4	40.0 ± 0.4
Red Blood Cells (x 10^6/mm^3)	8.87 ± 0.13	8.63 ± 0.15	8.42 ± 0.09	8.71 ± 0.10	8.52 ± 0.08	8.68 ± 0.09
Hemoglobin (g/dl)	15.3 ± 0.1	15.0 ± 0.3	14.8 ± 0.2	15.2 ± 0.2	14.9 ± 0.1	15.1 ± 0.2
Mean Corpuscular Volume (μm^3)	47.8 ± 0.5	47.1 ± 0.3	48.0 ± 0.2	48.0 ± 0.4	48.0 ± 0.2	47.6 ± 0.3
Mean Corpuscular Hemoglobin ($\mu\mu g$)	17.2 ± 0.2	17.3 ± 0.5	17.8 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.5 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	37.0 ± 0.2	37.7 ± 0.3	37.8 ± 0.1	37.7 ± 0.3	37.6 ± 0.1	37.7 ± 0.2
Reticulocytes (x 10^3/mm^3)	258 ± 30	207 ± 34	240 ± 27	233 ± 26	244 ± 22	221 ± 20
Platelets (x 10^3/mm^3)	1076 ± 132	1144 ± 86	955 ± 88	1047 ± 125	983 ± 73	1091 ± 77
Bone Marrow (x 10^3/mm^3)	9.8 ± 0.3	10.8 ± 0.5	10.1 ± 0.5	10.3 ± 0.5	10.0 ± 0.4	10.5 ± 0.4

TABLE IV-14. Total Leukocyte Count and Cell Distribution (Mean ± S.E.) in F_2 Generation Mice Exposed to 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 10-20 mice; each female group (see continuation page) consisted of 6-20 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	5.9 ± 0.5	5.1 ± 0.3	7.6 ± 0.5	4.3 ± 0.3	7.1 ± 0.4	4.7 ± 0.2
Neutrophils (%)	18.1 ± 1.8	15.3 ± 1.7	19.9 ± 1.8	16.8 ± 3.8	19.3 ± 1.3	16.1 ± 2.2
Neutrophils (x $10^3/mm^3$)	1.04 ± 0.12	0.76 ± 0.08	1.46 ± 0.15	0.81 ± 0.29	1.32 ± 0.11	0.79 ± 0.16
Lymphocytes (%)	80.4 ± 1.9	82.9 ± 1.7	77.8 ± 1.8	81.3 ± 3.9	78.6 ± 1.4	82.0 ± 2.2
Lymphocytes (x $10^3/mm^3$)	4.78 ± 0.47	4.27 ± 0.33	5.99 ± 0.50	3.37 ± 0.17	5.59 ± 0.38	3.78 ± 0.20
Monocytes (%)	0.30 ± 0.11	0.40 ± 0.16	0.53 ± 0.13	0.17 ± 0.10	0.45 ± 0.10	0.27 ± 0.09
Monocytes (x $10^3/mm^3$)	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.01 ± 0.005
Eosinophils (%)	1.25 ± 0.03	1.40 ± 0.39	1.78 ± 0.30	1.67 ± 0.33	1.60 ± 0.23	1.55 ± 0.25
Eosinophils (x $10^3/mm^3$)	0.07 ± 0.33	0.08 ± 0.03	0.12 ± 0.02	0.07 ± 0.01	0.10 ± 0.01	0.77 ± 0.01

TABLE IV-14. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	3.6 ± 0.6	5.1 ± 0.5	8.1 ± 0.5	6.9 ± 0.6	7.1 ± 0.5	6.1 ± 0.4
Neutrophils (%)	20.3 ± 2.8	16.9 ± 1.7	15.0 ± 1.2	13.3 ± 0.8	16.2 ± 1.2	15.0 ± 1.0
Neutrophils (x $10^3/mm^3$)	0.69 ± 0.10	0.83 ± 0.08	1.20 ± 0.13	0.92 ± 0.10	1.08 ± 0.11	0.88 ± 0.06
Lymphocytes (%)	79.1 ± 2.7	82.7 ± 1.9	82.8 ± 1.1	84.5 ± 0.8	82.0 ± 1.1	83.7 ± 1.0
Lymphocytes (x $10^3/mm^3$)	2.89 ± 0.52	4.26 ± 0.48	6.70 ± 0.41	5.86 ± 0.51	5.82 ± 0.46	5.13 ± 0.39
Monocytes (%)	0.17 ± 0.11	0.20 ± 0.11	0.60 ± 0.05	0.50 ± 0.15	0.29 ± 0.06	0.36 ± 0.10
Monocytes (x $10^3/mm^3$)	0.01 ± 0.004	0.01 ± 0.003	0.03 ± 0.002	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Eosinophils (%)	0.50 ± 0.13	0.25 _ 0.08	1.83 ± 0.32	1.67 ± 0.25	1.52 ± 0.27	1.02 ± 0.21
Eosinophils (x $10^3/mm^3$)	0.02 ± 0.005	0.01 ± 0.003	0.18 ± 0.03	0.14 ± 0.02	0.14 ± 0.03	0.08 ± 0.02

TABLE IV-15. Serum Chemistry Values (Mean ± S.E.) in F_2 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 10-20 mice; each female group (see continuation page) consisted of 6-20 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	155.9 ± 1.2	154.9 ± 1.2	150.5 ± 1.8	152.4 ± 3.3	152.3 ± 1.3	153.5 ± 1.9
Potassium (meq/L)	6.4 ± 0.4	6.0 ± 0.4	5.9 ± 0.4	5.5 ± 0.3	6.1 ± 0.3	5.7 ± 0.2
Blood Urea Nitrogen (mg/dl)	41.3 ± 0.8	28.7 ± 1.2	34.9 ± 1.4	35.5 ± 1.7	37.2 ± 1.1	32.2 ± 1.3
Cholesterol (mg/dl)	---	---	---	---	---	---
Triglycerides (mg/dl)	158 ± 11	192 ± 18	181 ± 9	181 ± 11	174 ± 8	186 ± 10
Total Protein (g/dl)	5.15 ± 0.11	5.37 ± 0.07	5.02 ± 0.09	5.12 ± 0.14	5.06 ± 0.07	5.23 ± 0.08
Albumin (g/dl)	2.59 ± 0.09	2.78 ± 0.05	2.62 ± 0.05	2.65 ± 0.09	2.61 ± 0.04	2.71 ± 0.05
Globulins						
Alpha (g/dl)	1.26 ± 0.04	1.20 ± 0.05	1.22 ± 0.03	1.25 ± 0.04	1.23 ± 0.02	1.23 ± 0.03
Beta (g/dl)	0.95 ± 0.04	0.99 ± 0.03	0.81 ± 0.02	0.78 ± 0.04	0.85 ± 0.02	0.88 ± 0.04
Gamma (g/dl)	0.35 ± 0.02	0.40 ± 0.02	0.37 ± 0.02	0.44 ± 0.03	0.37 ± 0.01	0.42 ± 0.01

TABLE IV-15. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	157.8 ± 2.2	156.3 ± 1.1	149.9 ± 2.8	153.0 ± 1.4	151.7 ± 2.3	154.5 ± 1.0
Potassium (meq/L)	5.8 ± 0.7	5.4 ± 0.3	5.3 ± 0.2	5.4 ± 0.3	5.4 ± 0.2	5.4 ± 0.2
Blood Urea Nitrogen (mg/dl)	33.7 ± 5.5	26.1 ± 2.0	32.4 ± 1.3	32.1 ± 1.6	32.7 ± 1.7	29.2 ± 1.4
Cholesterol (mg/dl)	---	---	---	---	---	---
Triglycerides (mg/dl)	123 ± 16	128 ± 10	142 ± 6	126 ± 7	138 ± 6	127 ± 6
Total Protein (g/dl)	5.66 ± 0.18	5.94 ± 0.15	5.25 ± 0.10	5.22 ± 0.13	5.35 ± 0.09	5.55 ± 0.12
Albumin (g/dl)	3.17 ± 0.14	3.26 ± 0.09	2.90 ± 0.06	2.98 ± 0.09	2.95 ± 0.05	3.11 ± 0.07
Globulins						
Alpha (g/dl)	0.96 ± 0.04	1.09 ± 0.05	0.91 ± 0.02	0.90 ± 0.04	0.92 ± 0.02	0.99 ± 0.04
Beta (g/dl)	1.19 ± 0.07	1.09 ± 0.05	0.92 ± 0.03	0.86 ± 0.04	0.97 ± 0.03	0.96 ± 0.04
Gamma (g/dl)	0.45 ± 0.05	0.51 ± 0.03	0.52 ± 0.03	0.47 ± 0.02	0.51 ± 0.03	0.49 ± 0.02

TABLE IV-16. Statistical Differences in Clinical Pathology Parameters Between F_2 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age as Compared to Sham-Exposed Controls.

		Mean ± S.E.*					
		MALE			FEMALE		
Experiment	Parameter	Exposed	Sham-Exposed	p<	Exposed	Sham-Exposed	p<
1	Mean Corpuscular Hemoglobin	16.8 ± 0.1	17.1 ± 0.1	0.01			
	Mean Corpuscular Hemoglobin Concentration	36.3 ± 0.1	37.6 ± 0.3	0.001	No significant differences in any parameters		
	Blood Urea Nitrogen	41.3 ± 0.8	28.7 ± 1.2	0.001			
	Albumin	2.59 ± 0.09	2.78 ± 0.05	0.02			
2	Mean Corpuscular Volume	48.4 ± 0.3	47.3 ± 0.3	0.05			
	Mean Corpuscular Hemoglobin Concentration	17.4 ± 0.1	17.0 ± 0.1	0.02			
	Leukocytes	7.6 ± 0.5	4.3 ± 0.3	0.001			
	Neutrophils	1.46 ± 0.15	0.81 ± 0.29	0.05			
	Lymphocytes	5.99 ± 0.50	3.37 ± 0.17	0.001			

*Experiment 1 - 10 males and 10 females in each exposed and sham-exposed group
Experiment 2 - 10 males in each group, 7 females in each exposed and 8 females in each sham-exposed group; no female group for clinical chemistry in Experiment 1

F_3 Generation. Hematologic data for each sex in the two experiments are shown in Tables IV-17 and IV-18, and clinical chemistry results in Table IV-19. Statistically significant differences between the exposed and sham-exposed groups for both replications are shown in Table IV-20.

As observed in studies of F_1 and F_2 generations, more significant differences are seen in exposed F_3 males than in exposed females when compared to the sham-exposed mice. Some erythrocyte parameters, i.e., VPRC, hemoglobin concentration and mean corpuscular volumes were elevated in exposed males of both experiments, as were platelet concentrations. In contrast to results from F_1 and F_2 generations, leukocyte concentrations were lower in exposed males of Experiment #2, due to decreased concentrations of both neutrophils and lymphocytes. Serum sodium concentrations were increased in exposed males of Experiment #1 and in exposed females in Experiment #2, whereas mean BUN values were elevated in exposed females of Experiment #1 and exposed males in Experiment #2. No other consistent effects on the F_3 generation were seen, either between the sexes or in replicate experiments.

Body and Organ Weights. Weights from F_1, F_2 and F_3 generations are shown in Table IV-21. There were no consistent differences in body or organ weights between exposed and sham-exposed males or females within generations or across replicate studies.

TABLE IV-17. Hematologic Values (Mean ± S.E.) in F_3 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 16-20 mice; each female group (see continuation page) consisted of 7-12 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	40.9 ± 0.6	36.6 ± 0.7	41.2 ± 0.3	39.5 ± 0.5	41.2 ± 0.3	38.3 ± 0.5
Red Blood Cells (x $10^6/mm^3$)	8.79 ± 0.14	8.14 ± 0.15	8.72 ± 0.08	8.48 ± 0.1	8.75 ± 0.07	8.33 ± 0.09
Hemoglobin (g/dl)	14.6 ± 0.2	13.5 ± 0.2	14.4 ± 0.1	13.9 ± 0.2	14.5 ± 0.1	13.7 ± 0.1
Mean Corpuscular Volume (μm^3)	46.8 ± 0.3	45.0 ± 0.3	47.5 ± 0.2	46.8 ± 0.2	47.2 ± 0.2	46.0 ± 0.2
Mean Corpuscular Hemoglobin ($\mu\mu g$)	16.8 ± 0.1	16.8 ± 0.1	16.8 ± 0.1	16.5 ± 0.1	16.8 ± 0.1	16.7 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.6 ± 0.2	36.9 ± 0.3	34.8 ± 0.1	34.9 ± 0.2	35.1 ± 0.1	35.8 ± 0.2
Reticulocytes (x $10^3/mm^3$)	241 ± 20	186 ± 14	237 ± 13	233 ± 11	239 ± 11	213 ± 9
Platelets (x $10^3/mm^3$)	1292 ± 42	1019 ± 65	1216 ± 38	974 ± 46	1249 ± 28	993 ± 38
Bone Marrow (x $10^3/mm^3$)	13.9 ± 0.5	14.3 ± 0.4	15.3 ± 0.4	13.4 ± 0.4	14.7 ± 0.3	13.8 ± 0.3

TABLE IV-17. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Volume Packed Red Cells (ml/dl)	42.7 ± 0.2	42.7 ± 0.4	40.3 ± 0.4	39.9 ± 0.3	41.3 ± 0.4	41.1 ± 0.4
Red Blood Cells (x 10^6/mm^3)	9.10 ± 0.11	9.00 ± 0.10	8.51 ± 0.11	8.73 ± 0.08	8.75 ± 0.11	8.88 ± 0.07
Hemoglobin (g/dl)	15.3 ± 0.1	15.1 ± 0.1	14.6 ± 0.1	14.6 ± 0.1	14.9 ± 0.1	14.9 ± 0.1
Mean Corpuscular Volume (μm^3)	47.3 ± 0.6	47.0 ± 0.3	47.5 ± 0.5	45.8 ± 0.3	47.4 ± 0.4	46.5 ± 0.3
Mean Corpuscular Hemoglobin ($\mu\mu g$)	17.0 ± 0.2	17.0 ± 0.1	17.3 ± 0.1	16.9 ± 0.1	17.2 ± 0.1	16.9 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.7 ± 0.3	35.8 ± 0.2	36.1 ± 0.2	36.5 ± 0.2	35.9 ± 0.2	36.1 ± 0.2
Reticulocytes (x 10^3/mm^3)	281 ± 27	248 ± 17	225 ± 29	183 ± 29	248 ± 21	218 ± 17
Platelets (x 10^3/mm^3)	1111 ± 75	1035 ± 59	1095 ± 73	1027 ± 59	1102 ± 51	1032 ± 41
Bone Marrow (x 10^3/mm^3)	10.2 ± 0.6	11.9 ± 0.6	9.5 ± 0.6	8.4 ± 0.4	9.8 ± 0.4	10.3 ± 0.5

TABLE IV-18. Total Leukocyte Count and Cell Distribution (Mean ± S.E.) in F_3 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 15-20 mice; each female group (see continuation page) consisted of 7-12 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	6.0 ± 0.4	6.3 ± 0.4	4.6 ± 0.2	6.2 ± 0.5	5.2 ± 0.2	6.2 ± 0.3
Neutrophils (%)	17.4 ± 1.6	18.0 ± 1.4	14.4 ± 0.8	20.5 ± 1.7	15.7 ± 0.8	19.4 ± 1.1
Neutrophils (x $10^3/mm^3$)	1.00 ± 0.08	1.13 ± 0.12	0.66 ± 0.05	1.35 ± 0.22	0.81 ± 0.05	1.25 ± 0.13
Lymphocytes (%)	79.2 ± 1.4	79.7 ± 1.2	83.4 ± 0.9	77.1 ± 1.7	81.6 ± 0.9	78.2 ± 1.1
Lymphocytes (x $10^3/mm^3$)	4.79 ± 0.37	5.04 ± 0.37	3.86 ± 0.18	4.73 ± 0.32	4.26 ± 0.20	4.86 ± 0.24
Monocytes (%)	2.0 ± 0.3	0.9 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.2	0.5 ± 0.1
Monocytes (x $10^3/mm^3$)	0.13 ± 0.02	0.06 ± 0.02	0.01 ± 0.01	0.01 ± 0.004	0.06 ± 0.01	0.03 ± 0.01
Eosinophils (%)	1.5 ± 0.3	1.4 ± 0.2	2.0 ± 0.4	2.2 ± 0.3	1.8 ± 0.2	1.8 ± 0.2
Eosinophils (x $10^3/mm^3$)	0.09 ± 0.02	0.10 ± 0.02	0.09 ± 0.02	0.13 ± 0.02	0.09 ± 0.01	0.11 ± 0.01

TABLE IV-18. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
White Blood Cells (x $10^3/mm^3$)	4.9 ± 0.5	5.8 ± 0.5	6.1 ± 0.8	6.0 ± 0.7	5.6 ± 0.5	5.9 ± 0.4
Neutrophils (%)	13.2 ± 1.8	15.6 ± 2.1	14.0 ± 1.0	13.1 ± 1.3	13.7 ± 0.9	14.5 ± 1.3
Neutrophils (x $10^3/mm^3$)	0.63 ± 0.09	1.01 ± 0.27	0.84 ± 0.10	0.82 ± 0.16	0.76 ± 0.07	0.92 ± 0.16
Lymphocytes (%)	84.0 ± 1.8	82.0 ± 2.0	84.1 ± 1.1	84.9 ± 1.5	84.1 ± 1.0	83.3 ± 1.3
Lymphocytes (x $10^3/mm^3$)	4.10 ± 0.50	4.69 ± 0.29	5.14 ± 0.67	5.01 ± 0.58	4.71 ± 0.4	4.83 ± 0.03
Monocytes (%)	1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	0.7 ± 0.2	1.0 ± 0.2	0.9 ± 0.2
Monocytes (x $10^3/mm^3$)	0.11 ± 0.05	0.06 ± 0.01	0.06 ± 0.02	0.04 ± 0.02	0.08 ± 0.02	0.05 ± 0.01
Eosinophils (%)	1.6 + 0.04	1.1 ± 0.3	1.1 ± 0.3	1.4 ± 0.3	1.3 ± 0.2	1.3 ± 0.2
Eosinophils (x $10^3/mm^3$)	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.03	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.01

TABLE IV-19. Serum Chemistry Values (Mean ± S.E.) in F_3 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age. Each male group consisted of 15-20 mice; each female group (see continuation page) consisted of 7-12 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	160.4 ± 0.7	155.6 ± 0.6	153.9 ± 0.3	153.6 ± 0.6	156.7 ± 0.7	154.4 ± 0.5
Potassium (meq/L)	6.4 ± 0.4	6.1 ± 0.6	5.8 ± 0.2	6.5 ± 0.4	6.1 ± 0.2	6.3 ± 0.3
Blood Urea Nitrogen (mg/dl)	37.0 ± 1.6	32.1 ± 2.4	44.1 ± 1.5	36.6 ± 1.8	40.9 ± 1.3	34.6 ± 1.5
Cholesterol (mg/dl)	121 ± 8	111 ± 4	116 ± 4	115 ± 5	118 ± 4	113 ± 3
Triglycerides (mg/dl)	197 ± 6	176 ± 5	146 ± 7	164 ± 7	164 ± 7	169 ± 5
Total Protein (g/dl)	5.55 ± 0.15	5.69 ± 0.11	5.12 ± 0.06	5.56 ± 0.11	5.30 ± 0.08	5.62 ± 0.08
Albumin (g/dl)	2.62 ± 0.06	2.64 ± 0.07	2.56 ± 0.05	2.80 ± 0.06	2.58 ± 0.04	2.73 ± 0.05
Globulins						
Alpha (g/dl)	1.38 ± 0.05	1.45 ± 0.04	1.32 ± 0.03	1.32 ± 0.04	1.34 ± 0.03	1.38 ± 0.03
Beta (g/dl)	1.03 ± 0.04	1.09 ± 0.05	0.88 ± 0.02	0.96 ± 0.04	0.94 ± 0.03	1.01 ± 0.03
Gamma (g/dl)	0.52 ± 0.03	0.51 ± 0.03	0.37 ± 0.01	0.50 ± 0.02	0.43 ± 0.02	0.50 ± 0.02

TABLE IV-19. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Sodium (meq/L)	150.3 ± 2.7	150.4 ± 0.9	158.9 ± 0.8	153.8 ± 0.4	155.4 ± 1.6	152.0 ± 0.6
Potassium (meq/L)	6.3 ± 0.4	5.2 ± 0.2	6.1 ± 0.4	5.7 ± 0.4	6.2 ± 0.3	5.4 ± 0.2
Blood Urea Nitrogen (mg/dl)	32.7 ± 0.9	30.2 ± 0.6	31.2 ± 3.1	33.0 ± 3.5	32.0 ± 1.5	30.9 ± 1.0
Cholesterol (mg/dl)	79 ± 6	85 ± 6	79 ± 8	80 ± 6	79 ± 6	83 ± 4
Triglycerides (mg/dl)	173 ± 13	168 ± 13	129 ± 6	120 ± 11	146 ± 8	145 ± 10
Total Protein (g/dl)	5.81 ± 0.15	5.67 ± 0.11	5.34 ± 0.12	5.61 ± 0.14	5.53 ± 0.11	5.64 ± 0.09
Albumin (g/dl)	3.18 ± 0.07	3.06 ± 0.07	2.94 ± 0.07	3.26 ± 0.08	3.04 ± 0.06	3.15 ± 0.06
Globulins						
Alpha (g/dl)	1.08 ± 0.06	1.05 ± 0.03	0.98 ± 0.04	0.94 ± 0.03	1.02 ± 0.03	1.00 ± 0.02
Beta (g/dl)	1.06 ± 0.07	1.00 ± 0.05	1.03 ± 0.06	1.00 ± 0.07	1.04 ± 0.04	1.00 ± 0.04
Gamma (g/dl)	0.51 ± 0.04	0.56 ± 0.03	0.40 ± 0.02	0.42 ± 0.03	0.44 ± 0.02	0.50 ± 0.03

TABLE IV-20. Statistical Differences (Mean ± S.E.) in Clinical Pathology Parameters Between F_3 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m from Conception to Approximately 77 Days of Age as Compared to Sham-Exposed Controls.

Experiment	Parameter	MALE			FEMALE		
		Exposed	Sham-Exposed	p<	Exposed	Sham-Exposed	p<
1	VPRC*	40.9 ± 0.6	36.6 ± 0.7	0.001			
	Red Blood Cells	8.79 ± 0.14	8.14 ± 0.15	0.001			
	Hemoglobin	14.6 ± 0.2	13.5 ± 0.2	0.001			
	MCV**	46.8 ± 0.3	45.0 ± 0.3	0.001			
	MCHC***	35.6 ± 0.7	36.9 ± 0.3	0.01			
	Reticulocytes	241 ± 20	186 ± 14	0.05			
	Platelets	1292 ± 42	1019 ± 65	0.01			
	Neutrophils				0.63 ± 0.09	1.01 ± 0.27	0.01
	Monocytes	0.13 ± 0.02	0.06 ± 0.02	0.05			
	Sodium	160.4 ± 0.7	155.6 ± 0.6	0.001			
	Triglycerides	197 ± 6	176 ± 5	0.02			
	Potassium				6.3 ± 0.4	5.2 ± 0.2	0.05
	Blood Urea Nitrogen				32.7 ± 0.9	30.2 ± 0.6	0.05
2	VPRC*	41.2 ± 0.3	39.5 ± 0.5	0.01			
	Hemoglobin	14.4 ± 0.1	13.9 ± 0.2	0.05			
	MCV**	47.5 ± 0.2	46.8 ± 0.2	0.05	47.5 ± 0.5	45.8 ± 0.3	0.01
	MCH****	16.8 ± 0.1	16.5 ± 0.1	0.01	17.3 ± 0.1	16.9 ± 0.1	0.05
	Platelets	1216 ± 38	974 ± 46	0.001			
	Bone Marrow	15.3 ± 0.4	13.4 ± 0.4	0.01			
	Leukocytes	4.6 ± 0.2	6.2 ± 0.5	0.01			
	Neutrophils	0.66 ± 0.05	1.35 ± 0.22	0.01			
	Lymphocytes	3.86 ± 0.18	4.73 ± 0.32	0.05			
	Blood Urea Nitrogen	44.1 ± 1.5	36.6 ± 1.8	0.01			

* Volume Packed Red Cells
** Mean Corpuscular Volume
*** Mean Corpuscular Hemoglobin Concentration
**** Mean Corpuscular Hemoglobin

TABLE IV-21. Weights (Mean ± S.E.) from F_1, F_2 and F_3 Generation Mice Exposed to a 60-Hz Electric Field at an Unperturbed Field Strength of 100 kV/m fron Conception to Approximately 77 Days of Age. Each male group consisted of 10-19 mice; each female group (see continuation page) consisted of 6-20 mice.

	MALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
F_1 Total Wt (g)	29.6 ± 0.4	31.7 ± 0.5	30.6 ± 0.6	29.4 ± 0.6	29.9 ± 0.3	30.8 ± 0.4
F_1 Spleen Wt (mg)	100.2 ± 8.8	96.1 ± 3.7	106.9 ± 7.7	89.8 ± 5.0	103.5 ± 5.7	92.9 ± 3.1
F_2 Total Wt (g)	31.4 ± 0.8	29.7 ± 0.5	31.5 ± 0.5	30.5 ± 0.5	31.5 ± 0.4	30.1 ± 0.4
F_2 Spleen Wt (mg)	102.8 ± 2.5	103.3 ± 3.6	116.2 ± 2.8	105.9 ± 5.1	111.7 ± 2.8	104.7 ± 3.2
F_3 Total Wt (g)	30.6 ± 0.5	32.1 ± 0.6	33.8 ± 0.4	31.2 ± 0.5	32.4 ± 0.4	31.6 ± 0.4
F_3 Spleen Wt (mg)	116.4 ± 3.5	109.4 ± 5.0	87.6 ± 3.4	104.6 ± 5.8	99.9 ± 3.4	106.7 ± 3.9

TABLE IV-21. (Continued)

	FEMALE					
	Experiment 1		Experiment 2		Composite	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
F_1 Total Wt (g)	25.9 ± 0.4	25.9 ± 0.4	23.8 ± 0.8	23.8 ± 0.5	25.0 ± 0.5	24.9 ± 0.4
F_1 Spleen Wt (mg)	120.7 ± 4.8	112.7 ± 5.3	98.5 ± 4.3	91.2 ± 4.6	111.5 ± 4.2	104.3 ± 4.0
F_2 Total Wt (g)	22.7 ± 0.5	25.0 ± 0.5	25.4 ± 0.3	23.6 ± 0.3	24.8 ± 0.4	24.3 ± 0.3
F_2 Spleen Wt (mg)	100.0 ± 1.22	116.7 ± 5.5	107.0 ± 2.9	110.7 ± 7.5	105.1 ± 3.3	113.4 ± 4.7
F_3 Total Wt (g)	24.7 ± 0.4	25.5 ± 0.6	25.7 ± 0.5	23.9 ± 0.3	25.3 ± 0.3	24.8 ± 0.4
F_3 Spleen Wt (mg)	136.0 ± 5.9	114.6 ± 5.0	100.2 ± 4.6	88.4 ± 6.4	115.5 ± 5.5	102.7 ± 4.8

Iron Kinetics.

Mice. Two groups of mice were exposed or sham-exposed to the electric field for 60 days in a replicate design. At the end of that time they received ∿ 5 μCi of ^{59}Fe chloride by intravenous injection, and the plasma clearance of ^{59}Fe was determined 15, 30, 60 and 90 min later. The results are shown in Figure IV-1. No statistically significant differences were seen at any of the four sampling periods between the exposed and sham-exposed mice.

Rats. Plasma iron clearance of ^{59}Fe in rats was determined following 60-day exposures in a replicate design similar to that for mice (Figure IV-2). No statistically significant differences were seen in the plasma clearance of ^{59}Fe between the exposed and sham-exposed groups. However, the half-clearance time appears to be about twofold greater in the exposed rats than in the sham-exposed groups.

The rats from the replicate of the above study were killed 7 days after the ^{59}Fe injection in order to evaluate the uptake of ^{59}Fe into developing erythrocytes. The percent of injected ^{59}Fe present per milliliter of packed erythrocytes at 7 days following injection was 7.52 ± 0.24% (mean ± 1 SEM) in the exposed rats and 7.10 ± 0.22% in the sham-exposed group. The difference was not statistically significant at the 5% level.

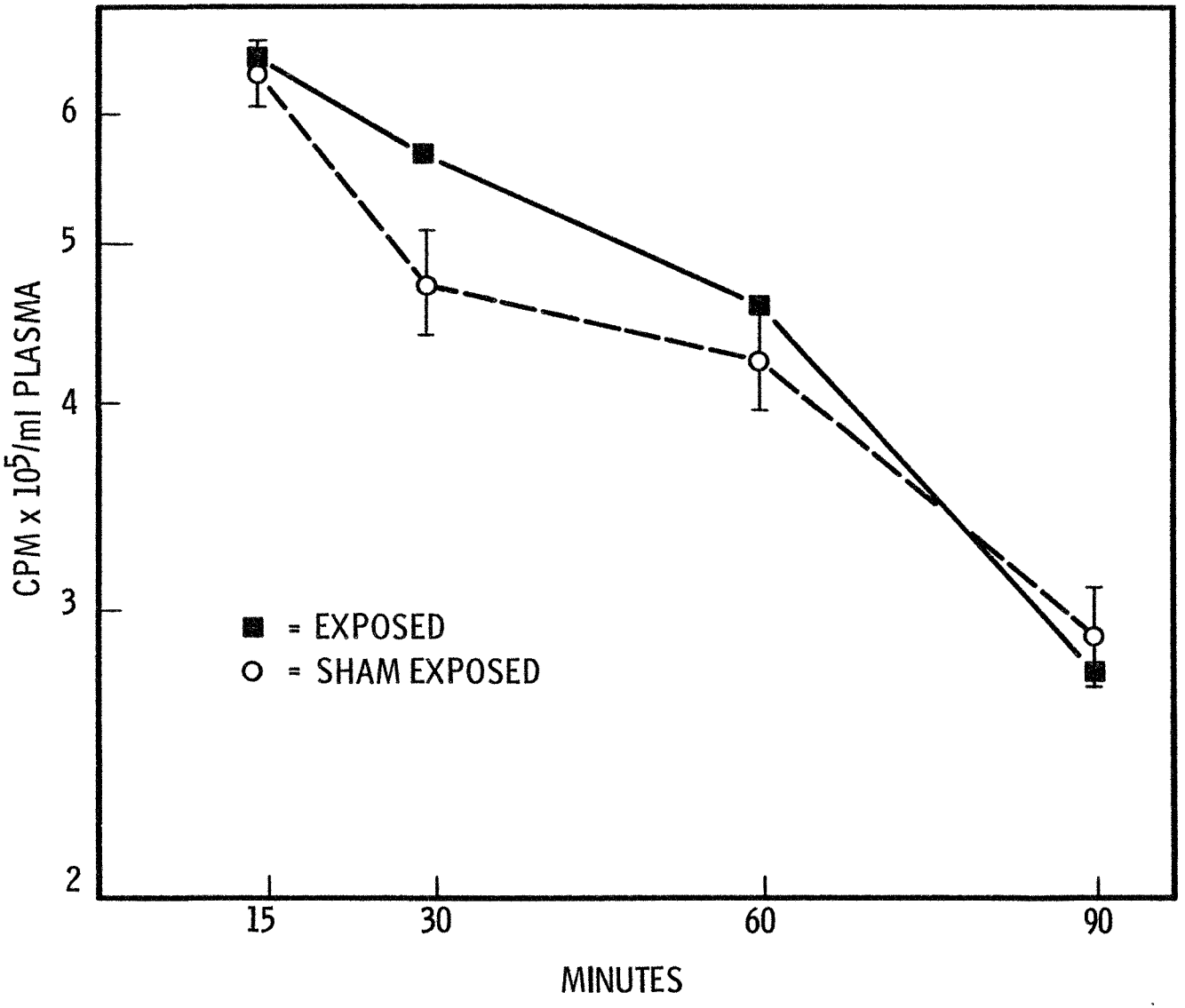


FIGURE IV-1. Plasma Clearance of ^{59}Fe in Mice Exposed and Sham-Exposed for 60 Days to a 60-Hz Electric Field of 100 kV/m. Each point represents 14-20 mice per group (mean $\pm$ 1 S.E. for sham-exposed animals).

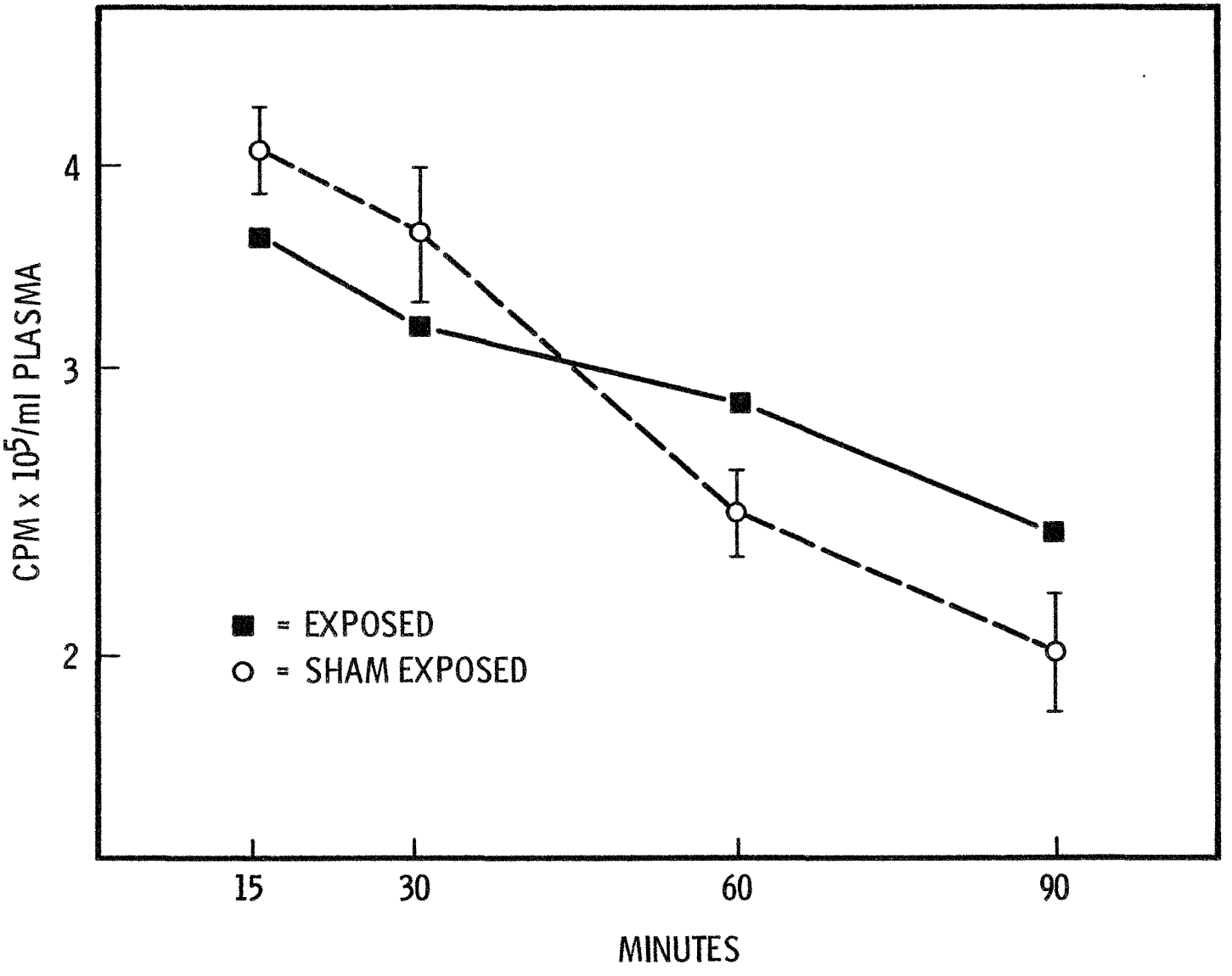


FIGURE IV-2. Plasma Iron Clearance of ^{59}Fe in Rats Exposed or Sham-Exposed for 60 Days to a 60-Hz Electric Field of 100 kV/m. Each point represents 8-9 rats per group (mean ± 1 S.E. for sham-exposed animals).

D. DISCUSSION

Occupational exposure of linemen to electric fields has been reported to result in a variety of general, nondescript symptomatology. However, there have been only a few studies in man regarding clinical pathologic effects of electric-field exposures, some indicating that alterations may occur in the hematopoietic and other major organ systems. Singewald et al. (1973) and Kouwenhoven et al. (1967) reported on extensive medical examinations of linemen working on 138- and 345-kV transmission systems, in which no clinical pathologic effects were observed over a prolonged time period. Hauf (1976) found increased reticulocyte and neutrophil concentrations in men exposed to 20 kV/m for 3 hr under controlled laboratory conditions. He felt these changes were the result of nonspecific excitation effects. Other blood and serum parameters remained within normal limits. Asanova and Rakov (1966) reported increased reticulocyte and neutrophil counts in Soviet linemen working in 400-kV substations.

Results from studies with nonhuman species have yielded conflicting results regarding clinical pathologic effects. Problems are encountered in interpreting these results because of variations in exposure conditions, durations of exposure, field strengths and the probability that secondary electric-field effects (e.g., shocks, ozone, noise, etc.) caused some alterations in the exposed animals. The results of studies by other investigators on blood and serum chemistry of electric-field-exposed animals are summarized in Table IV-22. In brief, at field strengths of 10-100 kV/m there are indications of increased concentrations of neutrophils and eosinophils, depression of lymphocytes,

TABLE IV-22. Clinical Pathologic Effects of Electric-Field Exposures Reported in Various Species

Author	Species	kV/m	Duration	Red Cell Parameters	Reticulocytes	Leukocytes	Neutrophils	Lymphocytes	Serum Constituents
Marino et al. (1976, 1977)	rat	15	30 d						SGOT* ↑ Albumin ↑ γ globulins ↓
Dumansky et al. (1976)	rat	0.1-5	4 m			eos ↓			glucose ↑ BUN ↑ α & β globulins ↑ LDH** ↑
Meda et al. (1972)	rat	10	6 h			eos ↑	↑	↓	
Bayer et al. (1977)	rat	100	1 y			↓			
Le Bars and Andre (1976)	rat	50	30-100 d	-	=	=	=	=	BUN*** ↑
Blanchi et al. (1973)	mouse	10	55 d			eos ↑	↑	↓	
Poznaniak et al. (1977)	mouse	25-50	6 w	↓		↑			Corticosteroids ↑
Knickerbocker et al. (1967)	mouse	157	1500 h				=	=	Marrow =
Le Bars and Andre (1976)	rabbit	50	30-100 d			↑	↑	↓	BUN*** ↑ Ca ↑ glucose ↓

- no change
- ↑ increase
- ↓ decrease

* Serum glutamic oxaloacetic transaminase
** Lactic dehydrogenese
*** Blood urea nitrogen

increases in some serum constituents (i.e., glutamic oxaloacetic transaminase, lactic dehydrogenase, glucose, calcium, BUN), and alterations in concentrations of serum protein fractions.

Under the conditions of our exposures of rats and mice to 60-Hz fields of about 100 kV/m, we have obtained effects similar to those in studies where effects were reported by other investigators. However, when tested in replicate exposures, the effects have not been consistent or reproducible in young adult female rats or in mice exposed to the electric field for various periods up to 120 days.

Examination of clinical pathology parameters in mice from the three-generation study reveals what may be more consistent and reproducible effects. Mean leukocyte concentrations were increased in exposed mice from several studies, as were BUN concentrations. In general, exposed male mice had more parameters that differed significantly when compared to the sham-exposed mice than did the exposed female mice.

It appears, from evaluation of a large number of parameters, that electric-field exposures, under our conditions, may indeed have an effect on the hematopoietic system. Potential effects on leukocyte numbers were particularly evident, although the effect was not always in the same direction. However, all these data should be considered preliminary, since all data are being evaluated in depth by a biostatistician using multivariant analyses. These methods may reveal significant trends in effects, and/or may reject effects that presently appear different when examined individually by the Student's t test.

E. FUTURE PLANS

Emphasis will be directed to sophisticated statistical evaluations of the voluminous data from the various experiments. These will then be interpreted as to potential health effects on the hematopoietic and other organ systems, and open literature publications will be prepared. Future research directions on hematologic effects are dependent on the results of these interpretations.

An experiment is in progress to examine the hematopoietic and serum chemistry of male and female rats exposed in utero and for 77 days after birth. This study is being made to determine whether the effects observed in the F_1 generation of the mouse study also occur in rats. A similar study is in progress using miniature swine as the test subjects (Phillips et al., 1979).

REFERENCES

Asanova, T. P. and A. I. Rakov. 1966. Health condition of workers in the electrical field of outdoor 400 kV and 500 kV switchyards. Gig. Tr. Prof. Zakol. 5: 50-52. Translated by G. Knickerbocker, Available as Special Publication #10, 1974, IEEE Power Engineering Society, Piscataway, NJ.

Bayer, A., J. Brinkman and G. Wittke. 1977. Experimental research on rats for determining the effect of electrical ac fields on living beings. Elektrizitaetswirtschaft 4:77-81.

Blanchi, D., L. Cedrini, F. Ceria, E. Meda and G. G. Re. 1973. Exposure of mammalians to strong 50 Hz electric fields. I. Effects on the proportion of the different leukocyte types. Arch. Fisiol 70:30-32.

Dumansky, Y. D., V. M. Popovich and Y. V. Prokhvatilo. 1976. Hygienic evaluation of electromagnetic field generated by high-voltage power lines. Gig. I. Sanit 8: 19-23. (Transl. UDC 613-647).

Hauf, R. 1976. Influence of 50-Hz alternating electric and magnetic fields on human beings. Rev. Gen. Electr. Special Issue, July: 31-49.

Knickerbocker, G., W. Kouwenhoven and H. Barnes. 1976. Exposure of mice to a strong ac electric field. An experimental study. IEEE Trans. PAS-86: 498-505.

Kouwenhoven, W. B., O. R. Langworthy, M. L. Singewald and G. G. Knickerbocker. 1967. Medical evaluation of men working in ac electric fields, IEEE Trans. PAS-86: 506-511.

LeBars, H. and G. André. 1976. Biological effects of an electric field on rats and rabbits. Rev. Gen. Electr. Special issue, July; 91-97.

Marino, A. A., T. J. Berger, B. P. Austin, R. O. Becker and F. X. Hart. 1976. Evaluation of electrochemical information transfer system. I. Effect of electric fields on living organisms. J. Electrochem. Soc. 123:1199-1200.

Marino, A. A. and R. O. Becker. 1977. Biological effects of extremely low frequency electric and magnetic fields: A Review. Physiol. Chem. Phys. 9: 131-147.

Marino, A. A., T. J. Berger, B. P. Austin, R. O. Becker and F. X. Hart. 1977. In vivo bioelectrochemical changes associated with exposure to extremely low frequency electric fields. Physiol. Chem. Phys. 9: 433-441.

Meda, E., V. Carrescia, and F. Kappa. 1972. Experimental results from exposure of animals to ac electric fields. Presented at the Second International Colloquium on the Prevention of Potential Risks Due to Electricity. Köln, Germany, November, 1972.

Phillips, R. D., et al. 1978. Biological Effects of 60-Hz Electric Fields on Small Laboratory Animals. Annual Report. HCP/TI830-3, Division of Electrical Energy Systems, U.S. Department of Energy.

Phillips, R. D., M. F. Gillis, W. T. Kaune, J. L. Beamer, J. R. Decker, C. H. Allen, T. W. Jeffs and H. A. Ragan. 1979. Effects of Electric Fields on Large Animals. Interim Report. PNL-2280, Battelle, Pacific Northwest Laboratories, Richland, WA. NTIS, Springfield, VA.

Poznaniak, D. T., H. B. Graves and G. W. McKee. 1977. Biological effects of high-intensity 60-Hz electric fields on the growth and development of plants and animals. In: International Microwave Power Institute Symposium, Minneapolis, May 25-27. J. Microwave Power 12:41-2 (abstract).

Singewald, M. L., O. R. Langworthy and W. B. Kouwenhoven. 1973. Medical follow-up study of high voltage linemen working in ac electric fields. IEEE Trans. PAS-92: 1307-1309.

V. IMMUNOLOGY

Principal Investigators: J. E. Morris and H. A. Ragan

ABSTRACT

Two studies were conducted to measure effects of exposure to 60-Hz electric fields at 100 kV/m on humoral and cellular functions of the immune system of Swiss-Webster mice. In humoral experiments, the primary antibody response to keyhole limpet hemocyanin (KLH) was measured in mice exposed for 30 or 60 days. No significant effect was observed in the levels of precipitating antibody in exposed mice when compared to sham-exposed controls.

In cellular studies, in vivo assays of delayed and contact hypersensitivity showed instances of statistically significant decreases in skin thickness in animals which were exposed to the electric field for 30 days; however, no statistically significant change was observed after 120 days of exposure. Cell-mediated immunity was also evaluated by corresponding in vitro assays which revealed no significant difference in mitogen-stimulation response of spleen cells from mice exposed to 100-kV/m electric fields for 90 or 150 days when compared to sham-exposed animals.

A. INTRODUCTION

In last year's Annual Report (Phillips et al., 1978), we presented quantitative data on several major components of the humoral and cellular aspects of the immune system. These measurements included number and distribution of peripheral-blood lymphocytes, as well as relative concentrations of T and B cells in the lymphocyte populations. We also analyzed serum immunoglobulin levels and complement activity. There was no statistically significant difference between exposed and sham-exposed

mice in any of the measurements. However, preliminary functional measurements of in vivo cell-mediated immunity with delayed hypersensitivity assays indicated a reduced response to KLH, as measured by skin thickness in mice exposed for 30 days.

Experiments made during this last year extended in vivo measurements of the cellular components of immune mechanisms, and included in vitro assays (mitogen stimulation) that correlate with cell-mediated immunity. The function of the humoral aspect of the immune system was measured with primary antibody responses of mice exposed to a 100-kV/m, 60-Hz electric field for 30 or 60 days.

B. METHODS

Animals and Exposure Conditions. For the primary antibody response studies, male Swiss-Webster mice were exposed for $\sim$ 20 hr a day to 60-Hz, 100-kV/m, unperturbed electric fields for 30 or 60 days. For in vitro cell-mediated immunity studies (mitogen stimulation), Swiss-Webster male mice were exposed for 90 or 150 days; they were divided into exposed, sham-exposed and cage control groups. For in vivo cell-mediated immunity studies, female Swiss-Webster mice were exposed for 30 or 120 days. In all experiments, differences between means of exposed and sham-exposed animals were compared by the Student's t test. A p value less than 0.05 was considered to be significant.

Radioiodination of KLH. Keyhole limpet hemocyanin (KLH) was tagged with ^{125}I by the chloramine-T technique (Greenwood et al., 1963). Free

^{125}I was removed by passing the iodination solution through a 0.7 x 23-cm column of Dowex AG 1-X8 (20-50 mesh), equilibrated with PBS (0.15 M NaCl, 0.01 M sodium phosphate, pH 7.2), for removal of trace amounts of mitogens at selected concentrations. Prior to gamma-counting, cells were washed three times with distilled water and twice with a 3% solution of acetic acid.

Primary Antibody Response. The levels of serum-precipitating antibody were measured in mice after exposure for 30 or 60 days. The experimental procedure followed was to challenge the mice with 5 mg of KLH intraperitoneally (Rittenberg and Amkraut, 1966; Rittenberg and Pratt, 1969) 14 days before the end of exposure and then test for precipitating antibody at the end of the exposure period (i.e., at 14 days after challenge).

A direct-binding, double-isotope, radioimmune titering assay (Jaton et al., 1973) was used for measuring serum-precipitating antibody. In the assay, 10 µl of cocktail containing ^{125}I-labeled KLH and ^{22}Na were mixed with 25 µl of mouse immune serum and 10 µl of unlabeled KLH (concentration range, 0.38 µg to 700 µg). Following incubation for 4 hr at 37°C and for 2 days at 4°C, the tubes were centrifuged and a portion of the supernatant was aspirated. The tubes containing precipitated KLH-antibody complex were then counted in a Beckman-310 gamma counter. Sodium-22 was used as a volume marker in the assay system (Gotschlich, 1971).

Preparation of Spleen Cells. Spleens were aseptically removed and placed in sterile RPMI 1640 tissue culture medium (pH 7.3 $\pm$ 0.1). Cell suspensions were prepared by mincing the tissues with scissors, then gently pushing the cells through wire screens in order to obtain single-cell suspensions. Cell clumps and debris were removed by gravity sedimentation. The supernatant solution was centrifuged, and cell pellets were resuspended in 2-4 ml of medium. Total and differential cell counts, as well as viability with trypan blue, were determined. The cells were then diluted with supplemented RPMI-1640 (containing 10% heat-inactivated fetal bovine serum) to give a final density of 2.2×10^6 viable cells per ml in the mitogen assay system (Bianco et al., 1970).

In Vitro Cell-Mediated Immunity. Mitogen stimulation of spleen lymphocytes was used as an in vitro measure of cell-mediated immunity. The following T- and B-cell-specific mitogens were utilized: Concanavalin-A (Con-A) and phytohemagglutinin (PHA), which are plant lectins that stimulate T-cell populations; lipopolysaccharide (LPS), a B-cell-activating endotoxin; and pokeweed mitogen (PWM), a plant lectin, mainly specific for B cells, but which also stimulates a small portion of the T-cell population (O'Brien et al., 1978; Oppenheim and Rosenstreich, 1976).

Lymphocyte activation was measured by the incorporation of ^{125}I-iododeoxyuridine (^{125}I•IUDR), a gamma-emitting thymidine analog (Le Mevel et al., 1973; Oldham and Herberman, 1973), in newly synthesized DNA. Two µCi of ^{125}I•IUDR were added to all tubes for the final 20 hr

of the culture period. Fluorodeoxyuridine (1×10^{-6} M final concentration), a known inhibitor of thymidylate synthetase, was added simultaneously with the ^{125}I·IUDR pulse to preferentially increase the DNA incorporation of the pulse (Langenbach et al., 1972; Zimmerman and Kern, 1973). The extent of labeled IUDR incorporated by the lymphocytes was measured following 2 days of cell incubation in the presence of the various mitogens at selected concentrations. Prior to gamma counting, cells were washed three times with distilled water and twice with 3% acetic acid.

In Vivo Cell-Mediated Immunity. Cell-mediated immunity was evaluated in female Swiss-Webster mice after 30 or 120 days of exposure by measuring delayed hypersensitivity response to KLH and contact hypersensitivity to dinitrofluorobenzene (DNFB), using methods described in last year's Annual Report (Phillips et al., 1978).

C. RESULTS

Primary Antibody Response. The primary antibody response in male mice exposed for 30 or 60 days to 100 kV/m are shown in Table V-1. The levels of serum antibodies to KLH in mice 14 days after challenge are expressed as the maximum amount (micrograms) of KLH precipitated by 25 µl of immune serum. No significant differences in antibody levels were observed between exposed and sham-exposed groups.

In Vitro Cell-Mediated Immunity (Mitogen Stimulation). The data for both the 90-day and 150-day studies are reported as a stimulation index,

TABLE V-1. Primary Antibody Response in Male Mice Exposed for 30 or 60 Days to a 60-Hz, 100-kV/m Electric Field

Group	Exposure Duration (Days)	N	Response (μg KLH Precipitated per 25 μl serum) Mean ± S.E.
Exposed	30	12	28.1 ± 5.4
Sham-exposed	30	11	24.3 ± 4.3
Cage control	30	10	20.5 ± 2.8
Exposed	60	11	30.9 ± 3.4
Sham-exposed	60	10	33.8 ± 0.6
Cage control	60	8	24.3 ± 4.0

which is the ratio of cpm incorporated in stimulated spleen cells divided by cpm incorporated in unstimulated spleen cells.

The results of the 90-day exposure are shown in Table V-2. The response of spleen cells from the exposed, sham-exposed, and cage-control mice were comparable for the three mitogen tests (Con-A, LPS and PWM) at the various concentrations used.

In the 150-day exposure (Table V-3), mitogen stimulation of spleen cells was induced with Con-A, LPS, PWM and PHA. The stimulation indexes obtained for Con-A, LPS and PWM for the three different exposure groups are not significantly different. The mean stimulation indexes obtained for PHA-treated spleen cells appear to be higher for the exposed group than for sham-exposed controls; however, evaluation of the data indicates the difference is not statistically significant.

In Vivo Cell-Mediated Immunity. Skin responses following sensitization of mice to KLH and DNFB are shown in Table V-4. The study included data collected on three groups of animals exposed for 30 days and one group exposed for 120 days. There were instances of statistically significant mean decreases in skin thickness ($p < 0.001$, $p < 0.01$) in two of the three 30-day-exposed groups when compared to corresponding sham-exposed groups; however, the individual variability in the responses was large. No significant differences were observed in animals exposed for 120 days.

In the hypersensitivity studies conducted with DNFB, one of three replicate studies of 30-day-exposed animals showed a significant decrease ($p < 0.01$) in response. No significant difference in the DNFB response was seen in animals exposed for 120 days.

TABLE V-2. In Vitro Mitogen-Stimulated Spleen Lymphocyte Studies in Male Mice Exposed for 90 Days to 60-Hz, 100-kV/m Electric Fields

Mitogen	Amount (μg)	Stimulation Index* Cage Control (N = 15) Mean ± S.E.	Exposed (N = 18) Mean ± S.E.	Sham-Exposed (N = 18) Mean ± S.E.	P Values** Exposed/Sham Exposed
Concanavalin-A	0.9	37.2 ± 3.7	36.0 ± 3.7	36.1 ± 2.9	0.97
	1.9	73.4 ± 6.9	65.5 ± 6.9	72.1 ± 5.8	0.47
	2.8	79.2 ± 8.8	72.7 ± 8.4	68.1 ± 5.1	0.65
Lipopolysaccharide	5.0	5.6 ± 0.6	5.1 ± 0.4	5.1 ± 0.4	0.99
	10.0	5.0 ± 0.6	4.7 ± 0.4	4.8 ± 0.4	0.70
	15.0	4.4 ± 0.4	4.2 ± 0.4	4.6 ± 0.4	
Pokeweed Mitogen	2.5	11.2 ± 1.1	10.6 ± 1.3	10.3 ± 0.9	0.87
	5.0	12.2 ± 1.2	11.0 ± 1.4	10.9 ± 0.8	0.98
	10.0	13.2 ± 1.4	11.7 ± 1.3	11.5 ± 1.0	0.91

* Stimulation Index (ratio of cpm incorporated in stimulated cells to cpm incorporated in unstimulated cells).
** p values determined using Student's t test.

TABLE V-3. In Vitro Mitogen-Stimulated Spleen Lymphocyte Studies in Male Mice Exposed for 150 Days to 60-Hz, 100-kV/m Electric Fields

Mitogen	Amount (μg)	Stimulation Index*			
		Cage Control (N = 9) Mean ± S.E.	Exposed (N = 9) Mean ± S.E.	Sham-Exposed (N = 9) Mean ± S.E.	P Values** Exposed/Sham Exposed
Concanavalin-A	0.9	97.9 ± 11.1	87.7 ± 18.9	95.8 ± 20.1	0.77
	1.9	132.5 ± 18.0	118.7 ± 24.0	98.7 ± 16.9	0.51
	2.8	102.2 ± 19.7	83.3 ± 18.2	61.3 ± 13.3	0.35
Lipopolysaccharide	5.0	9.9 ± 1.7	10.3 ± 2.2	12.7 ± 1.7	0.39
	10.0	13.3 ± 2.1	14.1 ± 2.9	15.5 ± 2.5	0.70
	15.0	12.5 ± 2.1	16.4 ± 5.2	16.1 ± 2.5	0.95
Pokeweed Mitogen	2.5	18.8 ± 4.2	12.2 ± 2.4	13.8 ± 3.2	0.70
	5.0	21.8 ± 6.0	14.1 ± 2.4	12.8 ± 3.7	0.78
	10.0	21.4 ± 8.0	11.7 ± 2.5	11.4 ± 3.1	0.97
Phytohemagglutinin	20 μl	104.6 ± 13.8	101.5 ± 22.9	68.2 ± 12.1	0.22
	40 μl	84.2 ± 17.0	87.1 ± 21.3	52.8 ± 11.8	0.19

* Stimulation Index (ratio of cpm incorporated in stimulated cells to cpm incorporated in unstimulated cells).
** p values obtained using Student's t test.

TABLE V-4. In Vivo Cell-Mediated Immunity of Mice Exposed to a 60-Hz Electric Field at 100 kV/m for 30 or 120 Days. Values shown are mean ± S.E.

Date	Group	Exposure Time (Days)	Increase in Skin Thickness (μm)* KLH	N	DNFB	N
8/8/77	Exposed	30	215.6 ± 26.3**	20	246.4 ± 18.2	20
	Sham-Exposed		382.2 ± 34.5	20	248.9 ± 12.2	21
11/14/77	Exposed	30	309.9 ± 24.9	24	195.6 ± 12.5	24
	Sham-Exposed		365.8 ± 40.3	22	185.4 ± 11.7	23
9/28/78	Exposed	30	340.4 ± 44.3***	19	94.0 ± 11.3***	10
	Sham-Exposed		657.9 ± 91.4	20	162.6 ± 12.5	10
5/23/78	Exposed	120	335.3 ± 36.4	20	165.1 ± 20.9	10
	Sham-Exposed		388.6 ± 30.1	20	243.8 ± 38.6	8

* Mean ± Standard Error
** $p < 0.001$
*** $p < 0.01$

D. DISCUSSION

In the immunological studies conducted during the past year, the emphasis has been on functional measurements of immune system components.

In our analysis of data resulting from studies of the humoral component of the immune system, using the primary antibody response of mice exposed for 30 or 60 days and challenged with KLH, no significant difference in the amount of precipitating antibody was observed. This functional response to a T-cell-dependent antigen (KLH) indicated that B cells were capable of producing antibodies, and that the required T-cell function for this response was intact.

In last year's Annual Report, we described some preliminary studies directed at measuring the cellular component of the immune system with in vivo assays that correlate with cell-mediated immunity. These studies suggested that following exposure of mice to 100 kV/m, there was a reduced delayed-hypersensitivity response to KLH, as evaluated on the basis of skin thickness. These studies (30-day exposure) have been repeated, with one experiment showing a significant reduction in response ($p < 0.01$); the second experiment showed no significant decrease, although a trend toward a decrease was apparent. No significant difference in response was observed in animals exposed for 120 days.

Coupled with the in vivo cell-mediated immunity studies were in vitro mitogen-stimulation assays. In animals exposed for 90 or 150 days, no significant differences were observed. Because of the failure to replicate skin-thickness measurements across sham-exposed groups, and also because of the high degree of variability in the in vivo assays, additional

experiments will be performed to establish the validity and reliability of the test measurements. These additional studies may also resolve the apparent contradiction observed between the two assay systems that served as measures of cell-mediated immunity.

E. FUTURE PLANS

In an effort to resolve the discrepancy observed between the results of in vivo and in vitro assays which correlate with cell-mediated immunity, an experiment combining both types of assays will be conducted on animals for 30 or 120 days. At each of these time points, delayed hypersensitivity to KLH will be measured, as well as the responsiveness of spleen cells to the previously tested mitogens. We will also include in the in vitro studies an analysis of the stimulation of spleen cell cultures by the challenging antigen, KLH. These stimulation studies will provide a means of comparing the in vivo responsiveness to KLH with the KLH responses measured in in vitro asssays.

To complete our screening studies of possible effects of 60-Hz, 100-kV/m electric fields on the immune system, we will perform some preliminary studies related to natural immunity. This component of the immune system is not T- or B-cell-related, nor does it appear to be actively acquired. Natural immunity in mice appears shortly after birth (Herberman and Holden, 1978; Koren and Williams, 1978), increases for the first 6 months of life, and declines thereafter with age. Effectiveness is specific for each animal species, and shows variability among strains within species.

REFERENCES

Bianco, C., R. Patrick, and V. Nussensweig. 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. J. Exp. Med. 132:702-720.

Gotschlich, E. C. 1971. A simplification of the radioactive antigen-binding test by a double label technique. J. Immunol. 107: 910-911.

Greenwood, F. C., W. M. Hunter, and J. S. Glaser. 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. 89: 114-123.

Herberman, R. B. and H. T. Holden. 1978. "Natural Cell-Mediated Immunity," pp. 302-377. In: Advances in Cancer Research. G. Klein and S. Weinhouse (eds.), Academic Press, New York.

Jaton, J., D. G. Dietmar, A. D. Strosberg, E. Haber, and J. E. Morris. 1973. Restricted rabbit antibodies: Amino acid sequences of rabbit H chains of allotype a1, a2, and a3 in the region 80 to 94. J. Immunol. 111: 1838-1843.

Koren, H. S. and M. S. Williams. 1978. Natural killing and antibody-dependent cellular cytotoxicity are mediated by different mechanisms and by different cells. J. Immunol. 121: 1956-1960.

Langenbach, R. J., P. V. Danenberg, and C. Heidelberger. 1972. Thymidylate synthetase: Mechanism of Inhibition by 5-fluoro-2'-deoxyuridylate. Biochem. Biophys. Res. Comm. 48: 1565-1571.

Le Mevel, B. P., R. K. Oldham, S. A. Wells, and R. B. Herberman. 1973. An evaluation of ^{125}I-iododeoxyuridine as a cellular label for in vitro assays: Kinetics of incorporation and toxicity. J. Natl. Cancer Inst. 51: 1551-1558.

O'Brien, R. L., J. W. Parker, and J. F. P. Dixon. 1978. "Mechanisms of lymphocyte transformation," p. 201. In: Progress in Molecular and Subcellular Biology. F. E. Hahan, H. Kersten, W. Kersten, and W. Szybalski (eds.), Springer-Verlag, New York.

Oldham, R. K. and R. B. Herberman. 1973. Evaluation of cell-mediated cytotoxic reactivity against tumor associated antigens with ^{125}I-iododeoxyuridine labeled target cells. J. Immunol. 111: 1862-1871.

Oppenheim, J. J. and D. L. Rosenstreich. 1976. "Lymphocyte transformation: Utilization of automatic harvesters," Chapter 54. In: In Vitro Methods in Cell-Mediated and Tumor Immunity, B. R. Bloom and J. R. David (eds.), Academic Press, New York, NY.

Phillips, et al., 1978. Biological Effects of Electric Fields on Small Laboratory Animals. Annual Report. Report HCP/T1830-3, Division of Electrical Energy Systems, U.S. Department of Energy, Washington, DC.

Rittenberg, M. B. and A. A. Amkraut. 1966. Immunogenicity of trinitrophenyl-hemocyanin. Production of primary and secondary antihapten precipitins. J. Immunol. 97: 421-430.

Rittenberg, M. B. and K. L. Pratt. 1969. Antinitrophenyl (TDP) plaque assay. Primary response of Balb-C mice to soluble and particulate immunogen. J. Immunol. 132: 575-581.

Zimmerman, D. H. and M. Kern. 1973. Differentiation of lymphoid cells: The in vitro emergence of immunoglobulin M-secreting cells. J. Immunol. 111: 761-769.

VI. PATHOLOGY

Principal Investigator: R. A. Renne

ABSTRACT

A histopathological survey of 23 tissues of rats and mice exposed to a 100-kV/m, 60-Hz electric field for 120 days revealed no differences from sham-exposed controls that could be attributed to the exposure.

A. INTRODUCTION

Previous reports from this project (Phillips and Kaune, 1977; Phillips, et al., 1978) have described detailed pathology studies of tissues from rodents exposed to 60-Hz, 100-kV/m electric fields for 30 days, in which no apparent effect of exposure was observed. However, since a Soviet study (Dumansky et al., 1976) described lesions in several organs that were apparently related to exposure to lower-strength fields (2- to 5-kV/m) for periods of 4 mo, we conducted another study in which detailed gross and histopathologic examinations were performed on groups of rats and mice exposed to 60-Hz, 100-kV/m electric fields for the same time period as that of the Soviet study (120 days).

B. METHODS

Twenty male Sprague-Dawley rats, and 20 male and 20 female Swiss-Webster mice, were exposed to an unperturbed, 60-Hz electric field of

100 kV/m for approximately 20.5 hr/day for 120 days. Twenty other male rats, and 40 mice (20 males, 20 females), maintained in otherwise identical conditions but without exposure to the electric field, served as sham-exposed controls. An additional 20 male rats and 10 mice of each sex served as cage controls. These cage-control animals were housed in shoebox cages in another room of the vivarium. All animals were numbered with an ear-notch code; the pathologist did not know which animals were exposed or sham-exposed until all gross and histopathologic evaluations had been completed, at which time the code was broken.

Following the exposure period, all animals were anesthetized with ether, sacrificed, and complete necropsies were performed. At necropsy, body weight and weight of heart, kidneys, liver, brain, testes, adrenals, and pituitary were recorded for each rat. Body weight and weight of heart, kidneys, liver, and brain were recorded for each mouse. Weight of testes was also recorded for each male mouse. These data are reported and discussed in Section VII, which deals with metabolic status and growth.

Representative sections from the following tissues were fixed in 10% formalin, processed, sectioned at 5 μm and stained with hematoxylin and eosin for light microscopy: lungs, liver, spleen, pancreas, mandibular lymph node, salivary gland, kidney, stomach, duodenum, colon, mesenteric lymph node, heart, thoracic aorta, thymus, adrenal, thyroid, trachea, esophagus, prostate, seminal vesicle, bladder, testis, uterus, ovary, brain, spinal cord, sciatic nerve, skeletal muscle, pituitary, skin, eye, and sternal bone marrow.

A replicate study of the 120-day rat exposure was completed in November, 1978. Necropsy, organ-weighing procedures, and fixation of tissues were performed in the same manner as in the rat study described above. Tissues were not processed for histopathology, but were held in 10% formalin until histopathology results from the initial 120-day rat exposure were complete. This was done to enable us to examine tissues from this second 120-day study to confirm any effects attributable to electric-field exposure in the first 120-day study.

C. RESULTS

Summaries of lesions observed by microscopic examination of tissues from exposed, sham-exposed, and cage-control rats and mice are presented in Tables VI-1 (rats) and VI-2 (mice). Relatively few lesions were observed in any group, and no effect of exposure to the electric field was apparent in either rats or mice.

D. DISCUSSION AND CONCLUSIONS

The increased incidence of prostatitis in rats exposed to a 60-Hz electric field at 100 kV/m for 30 days, observed in an earlier study at this laboratory (Phillips and Kaune, 1977), was not observed in this study. In this study, incidence of prostatitis in rats was 20% (4/20) in cage controls, 15% (3/20) in exposed rats, and 20% (4/20) in sham-exposed rats, with a mean severity of between 1.0 (minimal) and 2.0 (mild) in all three groups. In the earlier study, the increased incidence of

TABLE VI-1. Summary of Microscopic Lesions (No. Lesions/No. Animals in Group) Observed in Male Rats After 120-Day Exposure to 60-Hz, 100-kV/m Electric Field

	Exposed	Sham-Exposed	Cage Control
Urinary Bladder			
Proteinaceous material in lumen	5/20	7/20	3/20
Epithelial hyperplasia, focal	1/20	0/20	0/20
Lung			
Acute congestion	0/20	0/20	2/20
Calcification, vascular wall	0/20	0/20	2/20
Alveolar macrophage aggregates	3/20	4/20	2/20
Interstitial pneumonitis	2/20	2/20	1/20
Focal suppurative alveolitis	1/20	3/20	1/20
Focal fibrosis	0/20	1/20	0/20
Liver			
Cytoplasmic vacuolation of hepatocytes (mean severity)*	4/20 (1.0)	3/20 (1.0)	7/20 (2.0)
Pericholangitis, diffuse, multifocal	0/20	1/20	0/20
Kidney			
Chronic nephritis (mean severity)*	2/20 (1.0)	2/20 (1.0)	2/20 (1.0)
Dilatation, renal pelvis (mean severity)*	6/20 (2.33)	2/20 (2.0)	3/20 (1.33)
Prostate			
Chronic prostatitis, multifocal (mean severity)*	3/20 (1.33)	4/20 (1.5)	4.20 (1.75)
Large Bowel			
Nematodiasis	3/20	1/20	1/20
Thyroid			
Ultimobranchial rest	4/20	2/20	0/20
Mesenteric Lymph Node			
Lymphoid hyperplasia	1/20	0/20	0/20
Trachea			
Chronic tracheitis	0/20	1/20	0/20

* Severity rating: (1) minimal; (2) mild; (3) moderate; (4) severe.

TABLE VI-2. Summary of Microscopic Lesions (No. Lesions/No. Animals in Group) Observed in Mice After 120-Day Exposure to 60-Hz, 100-kV/m Electric Field

	Exposed		Sham-Exposed		Cage Controls	
	Male	Female	Male	Female	Male	Female
Kidney/Lymphocytic Aggregates	7/20	10/17	5/20	11/20	5/10	7/10
Liver/Inflammation	1/20	1/17	1/20	2/20	0/10	2/10
Liver/Necrosis, Hepatocytes	1/20	1/17	0/20	0/20	0/10	1/10
Liver/Cytoplasmic Vacuolation, Hepatocytes	2/20	0/17	0/20	1/20	0/10	0/10
Lung or Pleura/ Inflammation	1/20	1/17	0/20	0/20	1/10	3/10
Vagina or Uterus/ Inflammation	---	1/17	---	0/20	---	3/10
Ovary/Cyst	---	0/13	---	3/18	---	0/20

prostatitis in exposed rats--11/25 (35%) compared with 11/50 (22%) in sham-exposed rats--was not statistically significant. Based on the data from the past and present studies, the prostatitis appears to be unrelated to exposure to the electric field.

Our data also differ from those reported by Dumansky et al. (1976), in which dystrophy and vascular changes were reported in the brain, liver, kidneys, adrenal glands, and thyroid glands of male rats exposed to a much lower-strength electric field (2-5 kV/m) for the same duration as in this study. These differences in results may be due to different exposure or environmental conditions, different strains of animals, or other differences between the studies that are not readily apparent.

Our conclusion, based on the data presented in this report and previous reports from this laboratory, is that no effects (detectable with the standard light-microscopic techniques used in this study) are produced by exposure to electric fields under the conditions we have described.

E. FUTURE PLANS

With the completion of the evaluation of rat and mouse tissues from the 120-day exposure, no further routine histopathologic screening is planned unless, or until, results of other studies in progress or of future studies indicate a requirement for pathologic examination of tissues. Pathologic examination may be performed on individual animals from other studies at the investigator's request, if clinical signs or test results indicate the need.

REFERENCES

Dumansky, Y. D., V. M. Popovich and Y. V. Prokhvatilo. 1976. Hygienic evaluation of electromagnetic field generated by high-voltage power lines. Gig. I. Sanit. 8: 19-23. (Transl. UDC: 613-647.)

Phillips, R. D. and W. T. Kaune. 1977. Biological Effects of High Strength Electric Fields. Second Interim Report. CONS/1830-2, Conservation Division, Energy Research and Development Administration, Washington, DC.

Phillips, R. D., et al. 1978. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Annual Report. Report HCP/T1830-3, Division of Electrical Energy Systems, U.S. Department of Energy Systems, Washington, DC. NTIS, Springfield, VA.

VII. METABOLIC STATUS AND GROWTH

Principal Investigators: R. D. Phillips and J. H. Chandon

ABSTRACT

In a series of experiments, body and organ weights were determined in juvenile (age 26 days) and young adult (age 56 days) male and female rats, and in young adult (age 60 days) male and female mice that were exposed or sham exposed to 60-Hz electric fields for 30-120 days. In addition, food and water consumption was measured daily during the 30- to 120-day exposure periods. In another experiment, oxygen consumption and carbon dioxide production rates were measured in rats during electric-field exposure. It was found that exposure to 60-Hz electric fields had no effect on growth or metabolic parameters of rats or mice.

A. INTRODUCTION

Several reports appear in the literature that indicate exposure to 50- or 60-Hz electric fields depresses growth in rats (Marino et al., 1976a; Cerretelli and Malaguti, 1976; LeBars and André, 1976), mice (Marino et al., 1976b) and rabbits (LeBars and André, 1976). It is not known whether the effects on growth are the result of direct interactions of the electric field with the subject, or are responses to secondary factors associated with the generation of such fields in the laboratory (i.e. corona, ozone, spark discharge, audible noise or field-induced cage vibration). In an attempt to resolve this question, experiments were initiated in this project in 1977 to assess growth and metabolic status of rats and mice exposed to 60-Hz electric fields in an exposure

system documented to be free of secondary factors. The goal was to determine whether direct interaction of the animal with the electric field would alter growth and metabolic processes.

In the 1978 Annual Report (Phillips et al., 1978), the results of experiments were described in which growth and metabolic parameters were measured in young adult rats exposed ∿ 20 hr/day to 100 kV/m for 31 days. Exposure had no effect on body weight, organ weight, food consumption, water consumption, oxygen consumption rate, carbon dioxide production rate or metabolic rate. Since the 1978 Annual Report, experiments to assess possible effects of exposure on growth and metabolic status were expanded to include mice as well as rats, juvenile animals as well as young adults, and longer durations of exposure. The results of these additional experiments are given in this report.

B. METHODS

Table VII-1 lists the experiments conducted on growth and metabolic status since the last Annual Report. The table shows the animal species, sex, age, electric-field strength, duration of exposure and parameters measured. The methods and procedures used to make the various growth and metabolic determinations were given in the 1978 Annual Report (Phillips et al., 1978).

The method for making measurements of oxygen consumption and carbon dioxide production rates in rats after the completion of an exposure regimen was described in the last Annual Report. However, a special procedure, described below, was developed to make these measurements during exposure.

TABLE VII-1. Experiments on Growth and Metabolic Status Conducted Since 1978 Annual Report

Species	Sex	N/Sex/ Group/Exp.	Age at Start of Exposure (Days)	Field Strength (kV/m)	Duration of Exposure	Parameters
Rat	Males and Females	12	26	100	30 d	Body weight, food consumption
Rat	Males	20	56	100	120 d	Body weight, organ weight, food and water consumption
Mouse	Males and Females	20	60	100	120 d	Body weight and food consumption
Rat	Males	8	86	100	1 h	Oxygen consumption and carbon dioxide production rates

Sixteen rats were pair-matched by body weight. One rat from each pair was then randomly assigned to an exposed or sham-exposed group. All animals were acclimated to special restraint holders (Bollman type) for 24 hr over a 3-week period, as follows:

Week 1: 3 hr/day on Monday, Wednesday and Friday

Week 2: Same as week 1

Week 3: 3 hr/day on Monday, Wednesday, Friday and Sunday.

This amount of acclimation has been shown to be adequate to minimize stressing the animal as a result of the restraint and measurement technique (Phillips and Kimeldorf, 1963; Phillips et al., 1975).

Electric-field exposures and respiratory gas exchange measurements were made 2 days after the last acclimation session. On the day of exposure, each pair of animals (an exposed and a sham-exposed) was placed on the lower, grounded electrode of the Special Studies Exposure System (Phillips and Kaune, 1977). A Faraday cage was placed over the sham-exposed animal. Pre-exposure baseline measurements were made of oxygen consumption and carbon dioxide production rates for 0.5 hr. During this time, the high-voltage disconnect lever and the high-voltage relay were in the "ON" position, with the output voltage set at 0 (0-kV/m field strength). This was done to minimize the possibility of the animal responding to an onset noise when the system was energized. After the pre-exposure measurements had been taken, the upper electrode was rapidly energized to produce a field (unperturbed) of 100 kV/m at the location of the rat on the lower electrode. The respiratory gas measurements were continued during the 1-hr exposure period and for 0.5 hr following exposure.

C. RESULTS

Body Weight, Organ Weight, and Food and Water Consumption

Body weights and food consumption were measured on juvenile (26 days of age) male and female rats that were chronically exposed ($\sim$ 20 hr/day) to a 60-Hz electric field for 30 days (N = 12 exposed and 12 sham-exposed rats of each sex). Body weights are shown in Figures VII-1 and VII-2. Exposure had no effect on body growth of either sex. Food consumption (not shown) was the same among exposed and sham-exposed rats within each sex.

Body weight, and food and water consumption of young adult male rats exposed or sham-exposed (N = 20/group) to 100 kV/m for 120 days are shown in Figure VII-3. After about 34 days of exposure, mean body weight of the exposed group was significantly ($p < 0.05$, repeated measures t test) lower than that of sham-exposed controls. There was no apparent effect of exposure on food or water consumption. A replicate of this experiment (Figure VII-4) failed to confirm the effect of exposure on body weight.

Organ weights of male rats after 120 days of exposure are shown in Tables VII-2 (first experiment) and VII-3 (replicate experiment). The smaller organ weights noted in the first experiment were not seen in the replicate experiment.

Body weights and food consumption were determined in male and female mice during exposure to 60-Hz electric fields at 100 kV/m for 120 days (N = 20 exposed and 20 sham-exposed male mice, and 16 exposed and 20 sham-exposed female mice). No effects of exposure were evident on

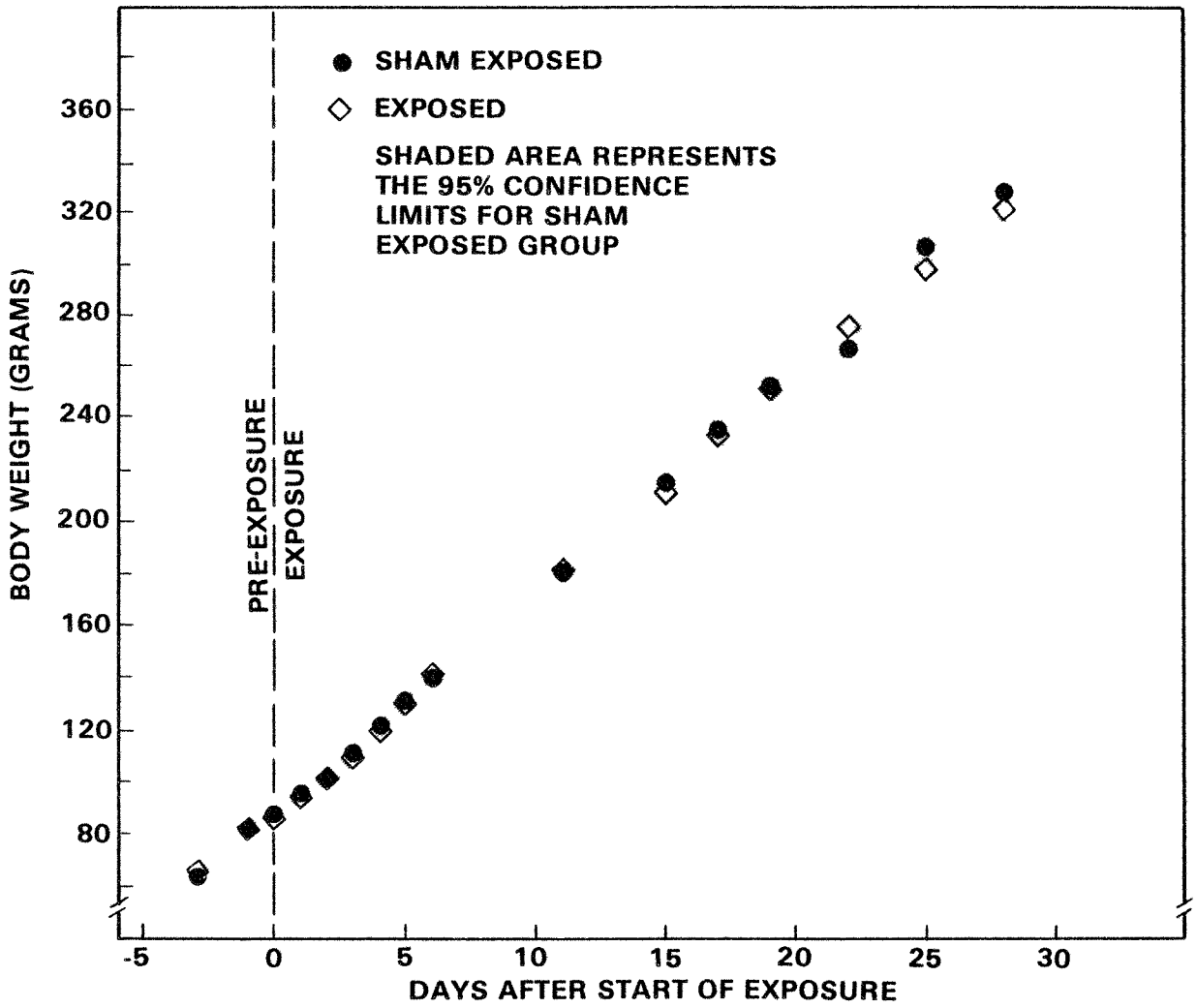


FIGURE VII-1. Body Weight of Juvenile (26 days of age) Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 30 Days. N = 12/group.

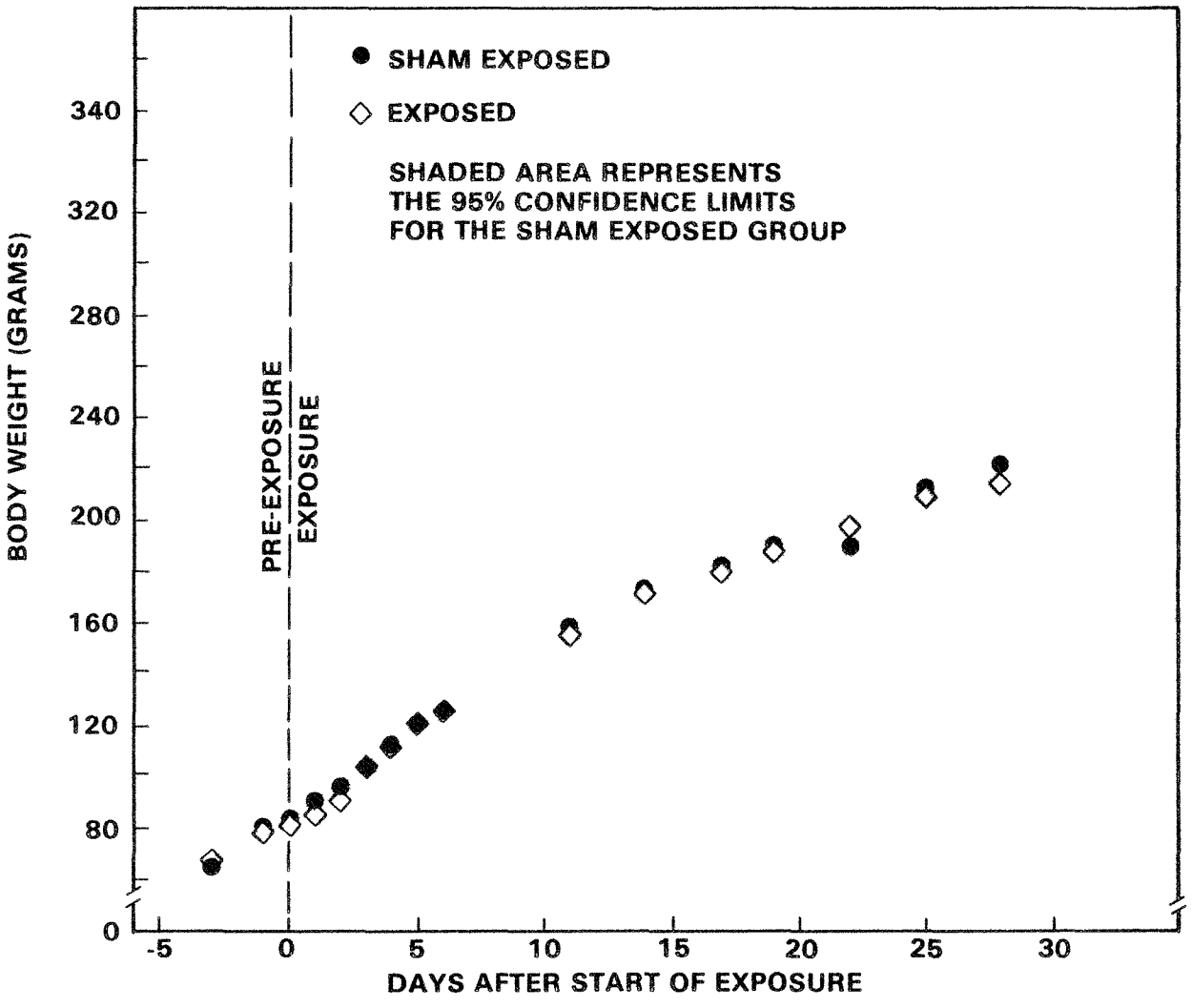


Figure VII-2. Body Weight of Juvenile (26 days of age) Female Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 30 Days. N = 12/group

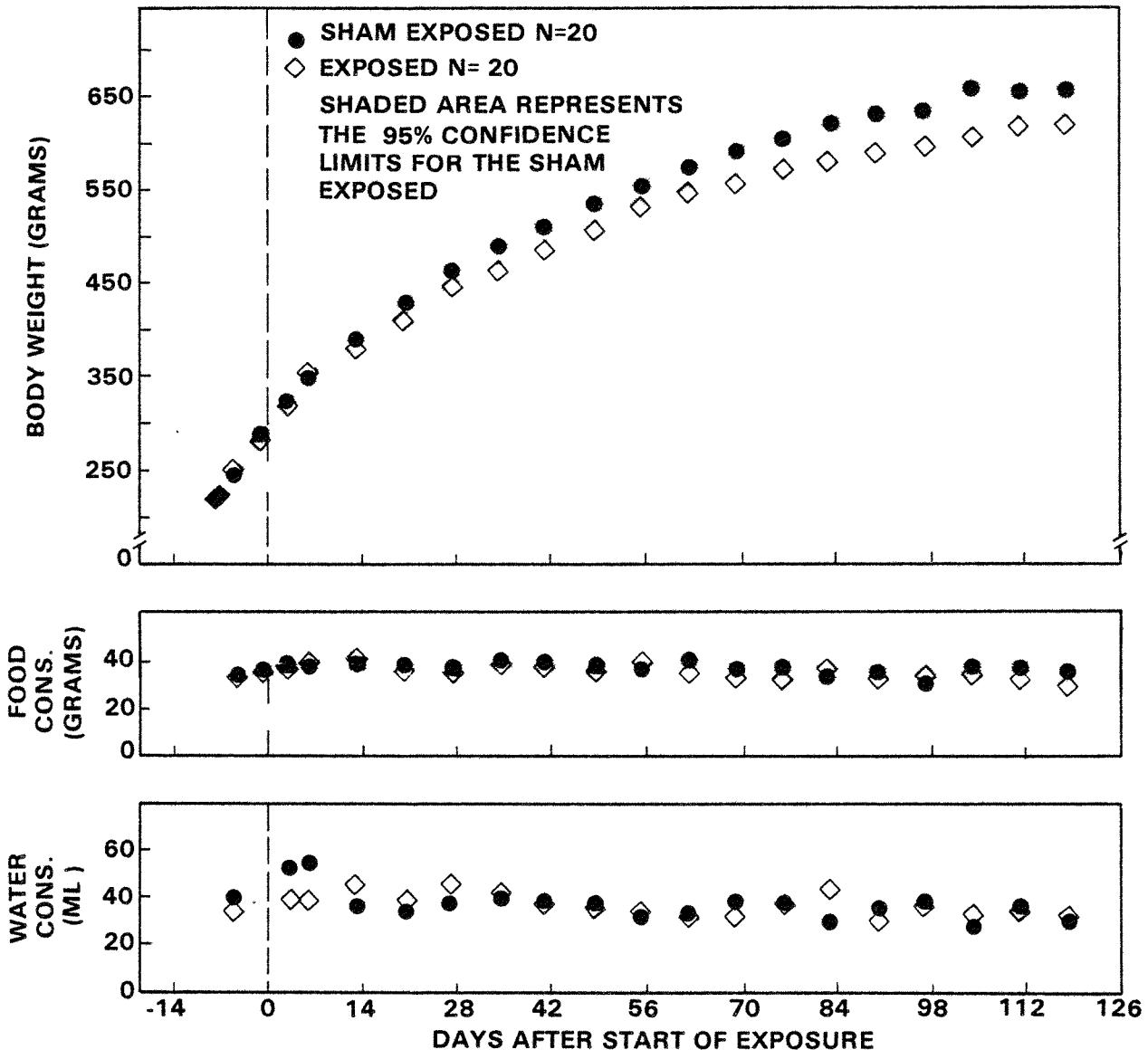


FIGURE VII-3. Body Weight, Food Consumption and Water Consumption of Male Rats (56 Days of Age) Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 120 days (First Experiment)

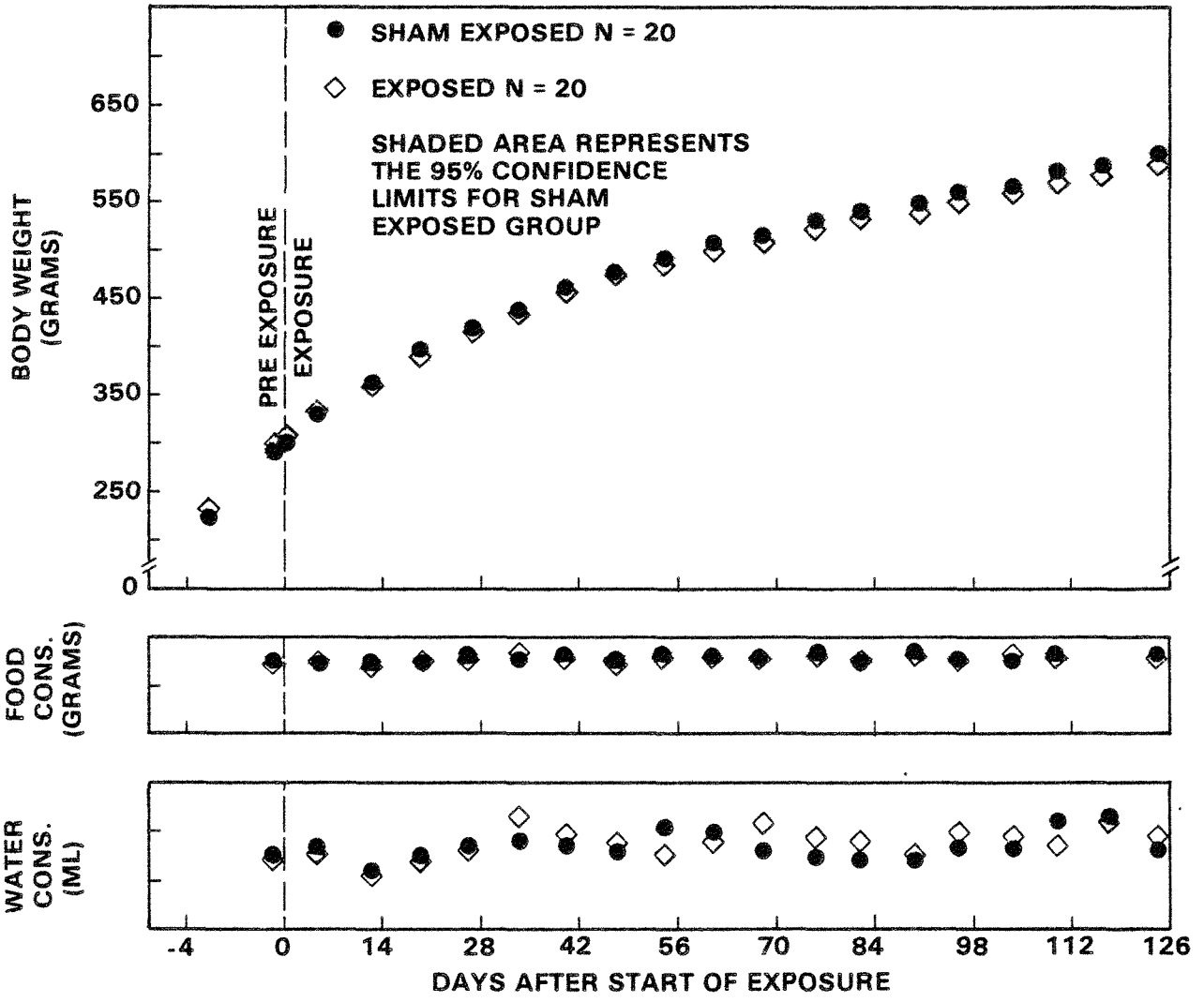


FIGURE VII-4. Body Weight, Food Consumption and Water Consumption of Male Rats (56 Days of Age) Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 120 days (Replicate Experiment)

TABLE VII-2. Body and Organ Weights (grams) of Adult Male Rats After Exposure or Sham Exposure to a 60-Hz Electric Field at 100 kV/m for 120 Days (First Experiment)

	Exposed		Sham-Exposed		
	N	Mean ± S.E.	N	Mean ± S.E.	p Value
Body Weight	20	623* ± 14	20	661 ± 12	0.04
Heart	20	1.71 ± 0.05	20	1.82 ± 0.07	N.S.
Left Kidney	20	1.78*± 0.03	20	1.93 ± 0.05	0.02
Right Kidney	20	1.76*± 0.04	20	1.98 ± 0.05	0.002
Liver	20	18.0 ± 1.0	20	19.4 ± 1.4	N.S.
Brain	20	2.07*± 0.03	20	2.17 ± 0.04	0.04
Left Testis	20	1.94 ± 0.04	20	1.99 ± 0.04	N.S.
Right Testis	20	1.96 ± 0.04	20	1.98 ± 0.04	N.S.
Left Adrenal	18	0.025 ± 0.002	18	0.029 ± 0.003	N.S.
Right Adrenal	19	0.023 ± 0.001	19	0.028 ± 0.02	N.S.
Pituitary	17	0.010 ± 0.001	17	0.009 ± 0.001	N.S.

*Significantly different from sham-exposed control group at $p \leq 0.05$ (Student's t test). N.S. = not significant.

TABLE VII-3. Body and Organ Weights (grams) of Adult Male Rats After Exposure or Sham Exposure to a 60-Hz Electric Field at 100 kV/m for 120 Days (Replicate Experiment)

	Exposed		Sham-Exposed		
	N	Mean ± S.E.	N	Mean ± S.E.	p Value
Body Weight	19	585 ± 16	20	588 ± 14	N.S.
Heart	20	1.68 ± 0.04	20	1.68 ± 0.05	N.S.
Kidneys	20	3.82 ± 0.08	20	3.68 ± 0.07	N.S.
Liver	20	20.5 ± 0.8	19	20.4 ± 0.5	N.S.
Brain	20	2.17 ± 0.03	20	2.19 ± 0.03	N.S.
Testes	20	3.79 ± 0.10	20	3.75 ± 0.08	N.S.
Adrenals	20	0.057 ± 0.004	20	0.053 ± 0.003	N.S.
Pituitary	18	0.0011 ± 0.0010	19	0.0010 ± 0.0009	N.S.

N.S. = not significant

body weight or food consumption in either sex (Figure VII-5 and VII-6). Organ weights at the end of 120 days of exposure were comparable among exposed and sham-exposed groups within each sex (Table VII-4).

Oxygen Consumption and Carbon Dioxide Production Rates During Exposure

Oxygen consumption, carbon dioxide production and respiratory gas exchange ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) were determined in eight exposed and eight sham-exposed rats during exposure to a 60-Hz electric field at 100 kV/m for 1 hr. The results are shown in Table VII-5. Values among exposed and sham-exposed rats during the 0.5-hr period prior to the start of exposure were comparable. No differences occurred between groups either during the 1-hr exposure period or during the 0.5-hr period after the termination of exposure.

D. DISCUSSION

Chronic exposure of rats or mice to 60-Hz electric fields at 100 kV/m for 30-120 days had no effects on growth or on gross indices of metabolic status. Our results are consistent with those of Adey et al. (1979), Dumansky et al. (1976) and Smith et al. (1979), but fail to confirm other reports in the literature (Marino et al., 1976a,b; LeBars and André, 1976; Cerretelli and Malaguti, 1976).

There may be a simple explanation for the apparent discrepancy in results among the various studies of electric-field effects on growth. In our experiment, and in those by Adey et al., Dumansky et al., and

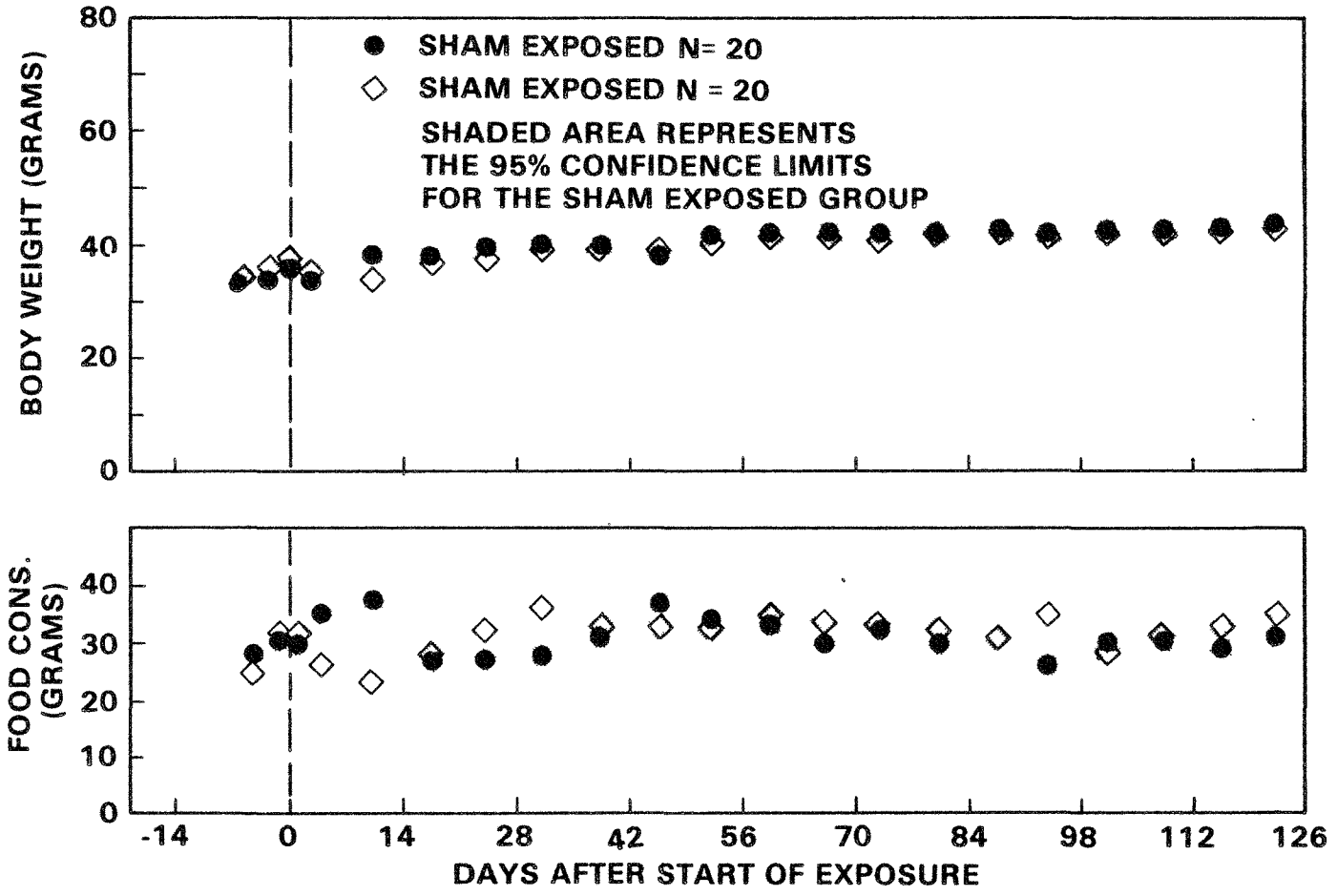


FIGURE VII-5. Body Weight and Food Consumption of Male Mice (60 Days of Age) Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 120 Days

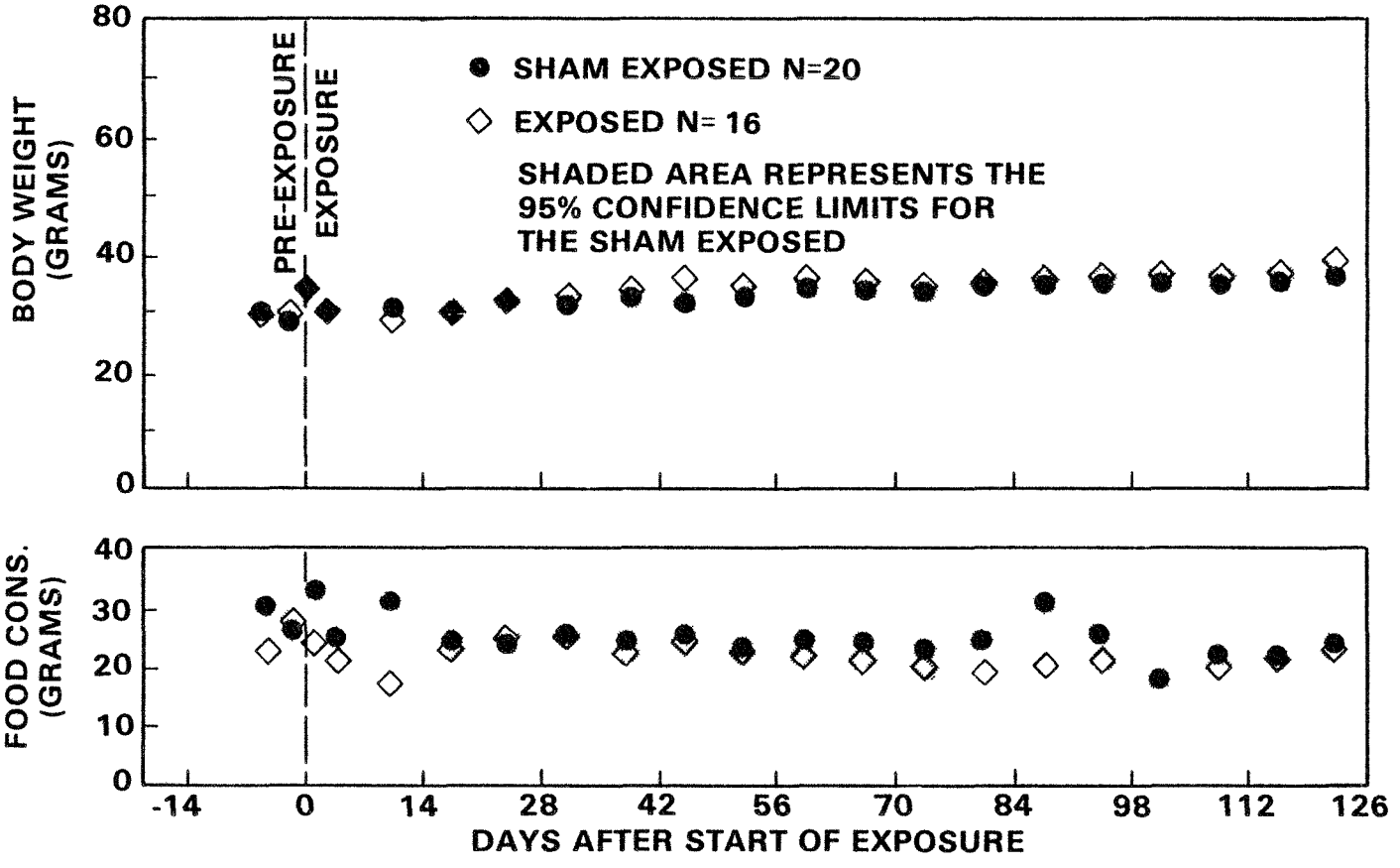


FIGURE VII-6. Body Weight and Food Consumption of Female Mice (60 Days of Age) Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 120 Days

TABLE VII-4. Body and Organ Weights (grams) of Male and Female Mice After Exposure or Sham Exposure to a 60-Hz Electric Field at 100 kV/m for 120 Days. N = 20/sex/group.

	Mean ± S.E.			
	Males		Females	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Body Weight	38.8 ± 0.5	38.7 ± 1.0	35.0*±0.9	31.7 ± 0.7
Heart	0.204 ± 0.005	0.202 ± 0.008	0.151 ± 0.004	0.145 ± 0.003
Left Kidney	0.429 ± 0.008	0.412 ± 0.014	0.275 ± 0.006	0.262 ± 0.008
Right Kidney	0.450 ± 0.006	0.421 ± 0.018	0.275 ± 0.008	0.263 ± 0.007
Liver	2.60 ± 0.05	2.5 ± 0.07	2.16 ± 0.07	2.07 ± 0.06
Brain	0.500 ± 0.009	0.474 ± 0.009	0.503 ± 0.012	0.487 ± 0.005
Left Testis	0.151 ± 0.008	0.151 ± 0.008	---	---
Right Testis	0.147 ± 0.004	0.158 ± 0.008	---	---

*Signficantly ($p < 0.05$) greater than sham-exposed controls (Student's t test).

TABLE VII-5. Mean (± S.E.) Oxygen Consumption, Carbon Dioxide Production and Respiratory Gas Exchange Ratio (RGER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$) of Rats Before (0.5 hr), During (1 hr) and After (0.5 hr) Exposure or Sham Exposure to a 60-Hz Electric Field at 100 kV/m (N = 8/group).

		Exposed	Sham Exposed
Before Exposure (0.5 hr)	O_2 (ml/min)	9.7 ± 0.4	9.4 ± 0.2
	CO_2 (ml/min)	8.4 ± 0.2	8.4 ± 0.2
	RGER	0.87 ± 0.01	0.89 ± 0.01
During Exposure (1 hr)	O_2 (ml/min)	8.9 ± 0.3	8.6 ± 0.2
	CO_2 (ml/min)	8.0 ± 0.3	7.7 ± 0.3
	RGER	0.90 ± 0.01	0.90 ± 0.01
After Exposure (0.5 hr)	O_2 (ml/min)	9.0 ± 0.3	8.6 ± 0.2
	CO_2	8.0 ± 0.3	7.7 ± 0.3
	RGER	0.88 ± 0.01	0.89 ± 0.02

Smith et al., great care was taken to make sure that the animals did not receive spark discharges when they drank water during exposure. It is not clear that such care was taken in those studies where effects on growth were found.

An animal subjected to spark discharges when it drinks would probably be stressed, drink and eat less than normal, and therefore have a lower growth rate than normal. Although such an explanation to account for the discrepancies in the literature is speculative, there are several pieces of evidence to support such a hypothesis. In the Marino experiment (1976), water consumption among exposed rats was depressed, and the exposed rats showed several signs of stress, including larger than normal adrenal glands at the end of a 30-day exposure period. These effects could have been caused by the animal receiving spark discharges when it drank (Miller and Kaufmann, 1978). Such a hypothesis, of course, needs to be tested.

E. FUTURE PLANS

We plan to test the hypothesis that rats subjected to spark discharges will exhibit a reduced water intake, a lower growth rate and signs of stress. The magnitude and characteristics of the spark discharges will mimic those to which the ungrounded animal is subjected when exposed to a 60-Hz electric field while drinking from a water supply at a different electrical potential than its body.

REFERENCES

Adey, W. R., S. M. Bawin and P. M. Sagan. 1979. Effects of 60-Hz Environmental Electric Fields on the Mammalian Central Nervous System. Presented at US/USSR Scientific Exchange Program Symposium on the Biological Effects of Static and Extremely Low Frequency Electromagnetic Fields, June 11-15, 1979, Seattle, WA.

Cerretelli, P. and C. Malaguti. 1976. Research carried out in Italy by ENEL on the effects of high voltage electric fields. Rev. Gen. Electr. Special Issue, July: 65-74.

Dumansky, Y. D., V. M. Popovich and Y. V. Prokhvatilo. 1976. Hygienic evaluation of electromagnetic field generated by high-voltage power lines. Gig. I. Sanit. 8: 19-23 (Transl. UDC: 613-647.)

LeBars, H. and G. André. 1976. Biological effects of an electric field on rats and rabbits. Rev. Gen. Electr. Special Issue, July: 91-97.

Marino, A. A., R. O. Becker and B. Ullrich. 1976a. The effect of continuous exposure to low frequency electric fields on three generations of mice: A pilot study. Experientia 32: 565-566.

Marino, A. A., T. J. Berger, B. P. Austin, R. O. Becker and F. X. Hart. 1976b. Evaluation of electrochemical information transfer system. I. Effect of electric fields on living organisms. J. Electrochem. Soc. 123(8): 1199-1200.

Miller, M. W. and G. E. Kaufmann. 1978. High voltage overhead. Environment 20: 60-15, 32-36.

Phillips, R. D. and D. J. Kimeldorf. 1963. The effect of whole-body X-irradiation of blood pressure in the rat. Radiat. Res. 18: 86-95.

Phillips, R. D., E. L. Hunt, R. D. Castro and N. W. King. 1975. Thermoregulatory, metabolic and cardiovascular response of rats to microwaves. J. Appl. Physiol. 38: 630-635.

Phillips, R. D., et al. 1978. Biological Effects of 60-Hz Electric Fields on Small Laboratory Animals. Annual Report. HCP/T1830-03, Division of Electrical Energy Systems, U.S. Department of Energy, Washington, D.C.

Smith, M. T., J. A. D'Andrea and O. P. Gandhi. 1979. Behavioral effects of strong 60-Hz electric fields in rats. J. Microwave Power 14: 223-228.

VIII. BONE GROWTH

Principal Investigator: B. J. McClanahan

ABSTRACT

Exposure of juvenile male and female rats to 60-Hz electric fields at 100 kV/m for 30 days did not alter the normal skeletal growth patterns as measured by the amount of bone formed in their tibias.

A. INTRODUCTION

Artificially induced electric currents have been shown to influence the behavior of bone cells. Several investigations have demonstrated that currents produced by implanted electrodes projecting into the medullary canal caused increased osteogenesis around the cathode and osteoclasia around the anode. External application of balanced ac fields was noted by McElhaney, et al. (1968), and by Martin and Gutman (1978), to reduce osteoporosis in immobilized femora in rats. Bassett, et al. (1974) reported that fracture repair in dogs was accelerated by electromagnetic fields applied externally at the fracture site.

The purpose of this investigation was to determine the influence of a uniform, externally applied 60-Hz electric field on the normal skeletal growth pattern of juvenile rats. In the first replicate, young male rats were exposed to 100 kV/m for 30 days. Bone growth, which was measured during the last 10 days of exposure, was the same in both the

exposed and sham-exposed groups of animals (Phillips et al., 1978). The data presented here complete the skeletal measurements made on animals from the second replicate, which was extended to include female as well as male rats.

B. METHODS

Twenty-four male and 24 female, 21-day-old, Sprague-Dawley rats (12 exposed and 12 sham-exposed of each sex) were placed in exposure cages for a 5-day acclimation period. A 30-day exposure to 60 Hz at 100 kV/m was started when the animals were 26 days old. Body weights were measured at intervals during the exposure period. On exposure days 21, 22, 28, and 29 each animal was injected intramuscularly with oxytetracycline hydrochloride (Terramycin) to label the skeleton, as described in the last Annual Report (Phillips et al., 1978).

The animals were killed after 30 days of exposure. Bone-growth measurements were made on cross sections of tibias cut at the tibiofibular junction. The specimens were fixed in neutral buffered formalin and dehydrated in acetone. They were then embedded (not decalcified) in ethyl butyl methacrylate and 40- to 50-μm sections were cut with a slow-speed diamond-blade saw. The sections were examined under a Zeiss epi-illumination, fluorescent microscope with a reticule calibrated with a stage micrometer. Differences between exposed and sham-exposed means were compared by the Student's t test; $p < 0.05$ was considered to be significant.

C. RESULTS

Table VIII-1 lists the parameters which were measured on the cross sections of the tibias. There were no differences between exposed and sham-exposed groups in the growth fronts examined or in the total area of the cortical bone or marrow space of the specimens. The more rapid growth rate of the male compared to the female rats is most markedly reflected in the area of the cortical bone and in the amount of bone deposited on the periosteal surface. Since the endosteal surface is in a net resorptive state in growing animals, the difference between sexes in quantity of bone deposited on the endosteum is less than on the periosteum.

Body weights of exposed and sham-exposed male and female rats before and during the 30-day exposure period are shown in Table VIII-2. Exposure to 100 kV/m had no effect on weight gain of either sex.

D. DISCUSSION AND CONCLUSIONS

As was demonstrated in the first replicate (Phillips et al., 1978), exposure of young rats to 60-Hz electric fields for 30 days did not alter body weight or the skeletal growth parameters which were measured. While the growth rates in the first replicate (7.0 to 7.3 μm/day on periosteal surfaces, and 2.1 to 2.3 μm/day on endosteal surfaces) and the area of cortical bone (3.3 mm^2) were not as large as in the second replicate, no differences between exposed and sham-exposed groups were detected in either replicate. In an investigation on the influence of an external

TABLE VIII-1. Influence of Exposure to 60-Hz Electric Field at 100 kV/m on Bone Growth of Juvenile Male (M) and Female (F) Rats

Measurement*	Sex	Exposed	Sham-Exposed
Periosteal Growth (μm/day)	M	8.5 ± 0.6	8.9 ± 0.2
	F	5.5 ± 0.6	4.7 ± 0.4
Endosteal Growth (μm/day)	M	3.1 ± 0.6	3.2 ± 0.1
	F	2.6 ± 0.2	2.8 ± 0.6
Cortical Area (mm^2)	M	3.6 ± 0.1	3.5 ± 0.2
	F	2.9 ± 0.1	2.9 ± 0.1
Medullary Area (mm^2)	M	1.6 ± 0.1	1.5 ± 0.1
	F	1.3 ± 0.1	1.3 ± 0.1

*Mean ± S.E.

TABLE VIII-2. Body Weights of Male and Female Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m. Rats were 26 days of age at the start of exposure. (N = 12 rats/sex/group).

Time From Start of Exposure (Days)	Body Weights (grams)*			
	Males		Females	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
-3	66.9 ± 0.9	64.4 ± 0.6	67.1 ± 1.0	66.0 ± 0.7
-1	82.7 ± 1.0	82.7 ± 1.1	77.2 ± 0.9	79.0 ± 1.0
0	85.0 ± 1.5	88.9 ± 1.5	81.0 ± 1.3	83.4 ± 1.0
1	92.2 ± 1.5	96.1 ± 1.6	85.5 ± 1.4	89.9 ± 1.2
2	99.4 ± 2.0	103.9 ± 1.6	92.9 ± 1.7	97.9 ± 1.4
3	112.8 ± 1.7	112.5 ± 1.8	104.6 ± 1.7	104.9 ± 1.4
4	120.3 ± 1.8	121.9 ± 1.9	110.1 ± 1.8	111.3 ± 1.7
5	130.8 ± 2.2	131.6 ± 1.8	118.5 ± 2.0	120.3 ± 1.9
6	141.7 ± 2.5	140.5 ± 2.1	126.9 ± 2.4	126.3 ± 1.8
11	184.6 ± 4.1	181.4 _ 4.4	154.3 ± 3.9	158.7 ± 2.7
14	209.8 ± 5.4	217.5 ± 4.7	170.2 ± 4.9	173.3 ± 3.1
17	233.3 ± 6.3	236.9 ± 5.9	179.6 ± 5.4	183.2 ± 3.2
19	251.8 ± 6.8	254.3 ± 6.2	188.2 ± 5.8	190.4 ± 3.8
22	276.2 ± 7.6	269.1 ± 7.1	197.9 ± 6.7	190.7 ± 5.8
25	299.5 ± 7.6	306.4 ± 8.5	208.6 ± 4.4	213.1 ± 5.2
28	321.0 ± 10.8	328.7 ± 9.1	215.7 ± 7.1	222.2 ± 6.2

* Mean ± S.E.

ac field which appeared to increase bone growth and strength of femora immobilized in plaster casts, McElhaney et al. (1968) concluded that structural rather than compositional elements were altered by the field. In a study in which the effect of an electromagnetic field on repair of fibular osteotomies was examined, Basset et al. (1974) noted that cortical bone appeared to be unaffected. In another phase of their study, in which ^{45}Ca was injected into the animals, there was an inverse relationship between stiffness and ^{45}Ca uptake, suggesting that there may be an increased rate of structural and tissue maturation in the stimulated animals. Bone strength (resistance to deflection and/or torsion, breaking strength, etc.) was not measured in our study. Since tetracyclines are considered to be deposited in the mineral matrix at the time of mineralization, they are probably indicative only of mineralization rates. They would not reflect alterations in molecular orientation that could influence responses to mechanical stress.

E. FUTURE PLANS

Experiments have been initiated to examine the possible influence of exposure to 60-Hz electric fields on fracture repair in rats. Resistance to deflection and breaking strength of incompletely healed fibular fractures will be used to assess the effect of exposure on fracture repair.

REFERENCES

Bassett, C. A. L., R. J. Pawluk and A. A. Pilla. 1974. Acceleration of fracture repair by electromagnetic fields. A surgically noninvasive method. Ann. NY Acad. Sci. 238: 242-262.

Martin, R. B. and W. Gutman. 1978. The effect of electric fields on osteoporosis of disuse. Calcif. Tiss. Res. 25: 23-27.

McElhaney, J. H., R. Stalnaker and R. Bullard. 1968. Electric fields and bone loss of disuse. J. Biochem. 1: 47-52.

Phillips, R. D., et al. 1978. Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Annual Report. HCP/T1830-03, Division of Electrical Energy Systems, U.S. Department of Energy, Washington, D.C.

IX. ENDOCRINOLOGY

Principal Investigator: M. J. Free

ABSTRACT

The effects of 60-Hz electric fields on the endocrine system were studied in young adult male rats exposed for 30 and 120 days, and on weanling rats exposed from 20-56 days of age. Exposure of young adults to 100 kV/m for 30 days had no effects on serum levels of corticosterone, growth hormone (GH), or thyroid-stimulating hormone (TSH), but variance of serum corticosterone levels among exposed animals was lower than that of sham-exposed animals. Exposure of young adult male rats for 120 days had no effect on serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin or TSH, but serum levels of testosterone and corticosterone were significantly lower in exposed animals. Exposure of weanling rats to 100 kV/m from 20 to 56 days of age had no effect on the growth rate of reproductive and endocrine organs, nor on the serum levels of FSH, LH or prolactin. However, inconsistent differences in serum corticosterone levels were noted between exposed and sham-exposed animals during the 36-day exposure period, and testosterone levels in exposed rats were lower than those of sham-exposed rats at the end of the exposure period.

A. INTRODUCTION

Initial studies in rats after 30 days of exposure to 60-Hz electric fields at 100 kV/m have shown no significant effects on body or organ weights, plasma levels of adrenocortical steroids, testosterone, thyroxine, luteinizing hormone (LH) or follicle-stimulating hormone, testicular venous blood levels of testosterone or 5-α reduced androgens (see Annual Report, Phillips et al., 1978). Since that time, data on the following parameters have become available from these original 30-day exposed rats:

1. Growth hormone
2. Thyroid-stimulating hormone
3. Corticosterone.

In addition, the following studies have been completed:

1. Effect of 120-day exposure to 60-Hz electric field at 100 kV/m on serum hormone levels
2. Effect of different sampling procedures on serum hormone levels in adult male rats
3. Effect of chronic electric-field exposure on endocrine development of male rats from weaning to 56 days of age.

B. METHODS

All blood samples for studies involving electric-field exposure were taken by heart puncture under halothane/N_2O/O_2 anesthesia within 2.5 min of removal of the animal from the cage. Samples from the 30-day exposure studies were heparinized, centrifuged and stored as plasma. Samples from all other studies were permitted to clot, and were centrifuged and stored as serum.

Corticosterone assays were performed by the dextran-coated-charcoal, competitive-binding method (Murphy, 1967). These assays, as well as growth hormone and thyroxine stimulating hormone radioimmunoassays, were carried out at the University of Rochester, in Dr. S. Michaelson's laboratory, using reagents supplied by A. F. Parlow and the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Distribution Program. All other hormones (i.e., those in

Experiments 1, 2 and 3) were assayed, using radioimmunoassay techniques, by Dr. Hsien-Chen Cheng, Department of Gynecology and Obstetrics, University of Kansas.

All animals were assigned a code so that the treatment groups remained unidentified to all those participating in the sampling and assay procedures. Data were analyzed statistically using an analysis of variance and Student's t test. A p value less than 0.05 was considered to be significant.

C. RESULTS

Effect of 30-Day Exposure. Effects of a 30-day exposure on growth hormone (GH) and thyroid stimulating hormone (TSH) in two separate groups of adult male rats are given in Table IX-1. No significant effects of electric-field exposure were observed in either experiment. These results are consistent with those reported previously for organ and body weights, and for thyroxine and reproductive hormones (see 1978 Annual Report).

Corticosterone assays (by the dextran-coated-charcoal method) were performed on aliquots of plasma from the same 30-day-exposed adult male rats used for previously reported fluorometric measurements of cortical steroids (see 1978 Annual Report). This provided an opportunity to compare the two assays, as well as to check for a possible effect of 30-day exposure to the 100-kV/m electric field. The results are given in Table IX-2. There were no statistically significant differences between the means for exposed and sham-exposed animals with either assay. However, the results of an F-test revealed that variance among exposed

TABLE IX-1. Circulating Growth Hormone and Thyroid Stimulating Hormone Levels of Adult Male Rats Exposed to a 60-Hz Electric Field at 100 kV/m for 30 Days. Levels measured in plasma by radioimmune assay.

	Growth Hormone* (ng/ml)		Thyroid Stimulating Hormone (μ Units/ml)	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
First Experiment				
N	17	18	17	18
Mean	181	271	176	169
SE	59	78	15	12
Replicate Experiment				
N	17	18	---	---
Mean	218	181	---	---
SE	67	54	---	---

*Several values from samples in each group exceeded 1000--beyond the calibration curve for the assay--and are not included in the means or estimates of variances shown above.

TABLE IX-2. Plasma Adrenocortical Steroid Levels Measured by Two Methods in Adult Male Rats Exposed to a 60-Hz Electric Field at 100 kV/m for 30 days

	Exposed		Sham-Exposed	
	N	Mean ± S.E.	N	Mean ± S.E.
Adrenal Cortical Steroids (Fluorometric Assay) (ng/ml)	20	150 ± 17	20	193 ± 32
Corticosterone (Dextran-Coated-Charcoal Assay) (ng/ml)	17	43 ± 11	18	83 ± 22

rats was significantly less ($p < 0.05$) than among sham-exposed rats. This lower variance of adrenocortical levels in exposed animals has also been observed in one of two experiments in a separate study (see Section X, Cardiovascular Function), in which means for radioimmune assays were 35 $\pm$ 8 ng/ml for exposed rats and 287 $\pm$ 138 ng/ml for sham-exposed rats in the first experiment, and 42.1 $\pm$ 11.6 ng/ml for exposed rats and 35.6 $\pm$ 9.5 ng/ml for sham-exposed rats in the second experiment.

The fluorometric technique for assaying corticosterone levels in rat plasma does not appear to have the specificity of the dextran-coated-charcoal technique. Values obtained by the fluorometric assay were almost three times greater than the values obtained by the dextran-coated-charcoal method. However, the correlation coefficient (0.788) suggested a moderately close relationship between values obtained by the two methods.

Effects of 120-Day Exposure. The effects of 120 days of electric-field exposure at 100 kV/m on rat serum hormone levels are shown in Table IX-3. Both testosterone and corticosterone levels were significantly lower ($p < 0.05$) in the exposed group. Serum levels of FSH, LH, prolactin and TSH were comparable in exposed and sham-exposed animals.

Effect of Different Sampling Procedures on Serum Hormone Levels. In this experiment, blood was taken from adult male rats by five different methods. All samples were taken within 2.5 min of removing the rat from

TABLE IX-3. Serum Hormone Levels in Adult Male Rats Exposed to a 60-Hz Electric Field at 100 kV/m for 120 Days

	Exposed		Sham-Exposed	
	N	Mean ± S.E.	N	Mean ± S.E.
FSH* ng/ml	20	330 ± 30	19	401 ± 32
LH** ng/ml	20	23 ± 2	19	22 ± 2
Prolactin ng/ml	20	37 ± 6	19	30 ± 3
Testosterone ng/ml	20	1.5 ± 0.2***	18	2.2 ± 0.3
TSH**** μ Units/ml	20	137 ± 14	19	148 ± 16
Corticosterone ng/ml	20	52 ± 10***	19	91 ± 16

* Follicle stimulating hormone
** Luteinizing hormone
*** $p < 0.05$
**** Thryoid stimulating hormone

the cage. Rats used for each sampling procedure were taken simultaneously from the cage rack to avoid possible bias by progressive disturbance effects. The results are given in Table IX-4. Killing by concussion and, to a lesser extent, by ether increased the level of pituitary hormones in serum. Testosterone levels were also higher after killing by concussion and, surprisingly, by use of halothane. Since the time between removing the animal from the cage and completion of sampling was less than 2.5 min, the testosterone levels are unlikely to reflect LH action on the Leydig cells of the testes. Rather, it appears that intracellular and/or extracellular reserves of testosterone must pass from the testis, or metabolism of the steroid must be sharply curtailed as a result of the procedures.

A possible source of error in this experiment is the variable resulting from having a different technician handle the rats at each sampling station. For this reason, the experiment will be repeated using a single technician for all animal sacrifices and sampling.

Effect of Exposure During Early Development on the Endocrine System of Male Rats. The effect of exposing male rats to a 60-Hz electric field at 100 kV/m from 20 to 56 days of age is shown in Figures IX-1 to IX-14. Although changes in hormone levels from the two groups of animals may be out of synchronization at some stages of development, the overall pattern of development was similar in exposed and sham-exposed rats. Growth rate of reproductive and endocrine organs during this early development and growth period were the same in exposed and sham-exposed animals.

TABLE IX-4. Effect of Blood-Sampling Procedures on Serum Hormone Levels (Mean ± S.E.)

Sacrificing and Sampling Procedure	FSH* (ng/ml)	LH** (ng/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)	Corticosterone (ng/ml)
Guillotine N = 12	199 ± 21	14 ± 7	10 ± 3	2.4 ± 0.3	32 ± 13
Halothane/N_2O/O_2 + Heart Puncture N = 12	177 ± 10	8 ± 1	13 ± 3	5.7 ± 1.0(1,2)	41 ± 16
Ethrane/N_2O/O_2 + Heart Puncture N = 11	199 ± 33	8 ± 1	14 ± 3	3.0 ± 0.3	18 ± 7
Ether + Heart Puncture N = 11	241 ± 9(3)	17 ± 3(2,3)	16 ± 3	3.4 ± 0.4	19 ± 12
Concussion + Heart Puncture N = 12	295 ± 85	25 ± 7(2,4)	24 ± 6(5)	4.9 ± 0.8(1,2)	42 ± 16

*Follicle stimulating hormone
**Luteinizing hormone

(1) Different from guillotine, $p \leq 0.01$
(2) Different from ethrane, $p \leq 0.05$
(3) Different from halothane, $p \leq 0.01$
(4) Different from halothane, $p \leq 0.05$
(5) Different from guillotine, $p \leq 0.05$

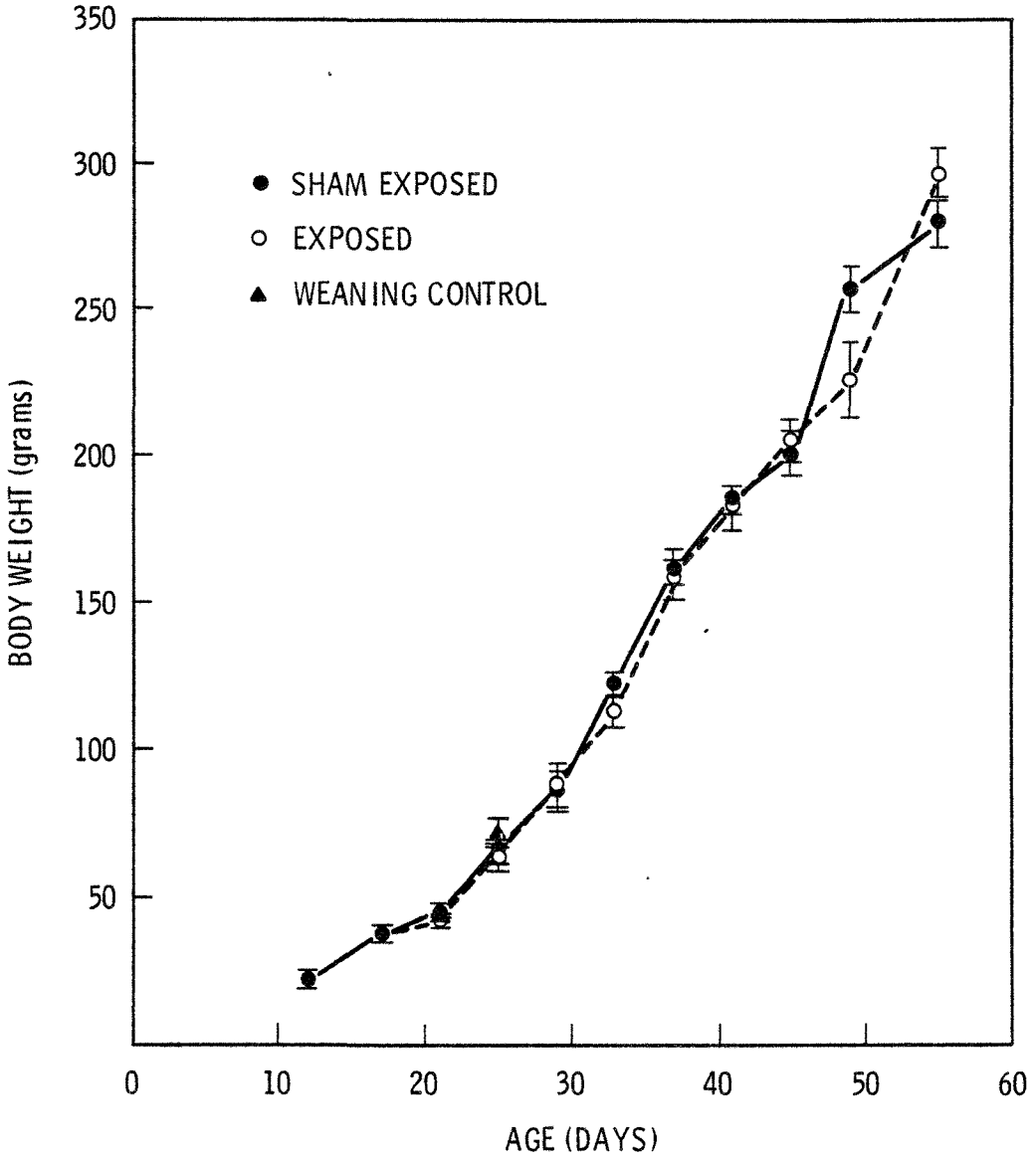


FIGURE IX-1. Mean (± S.E.) Body Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 days of Age. N = 8 rats/group at each measurement time.

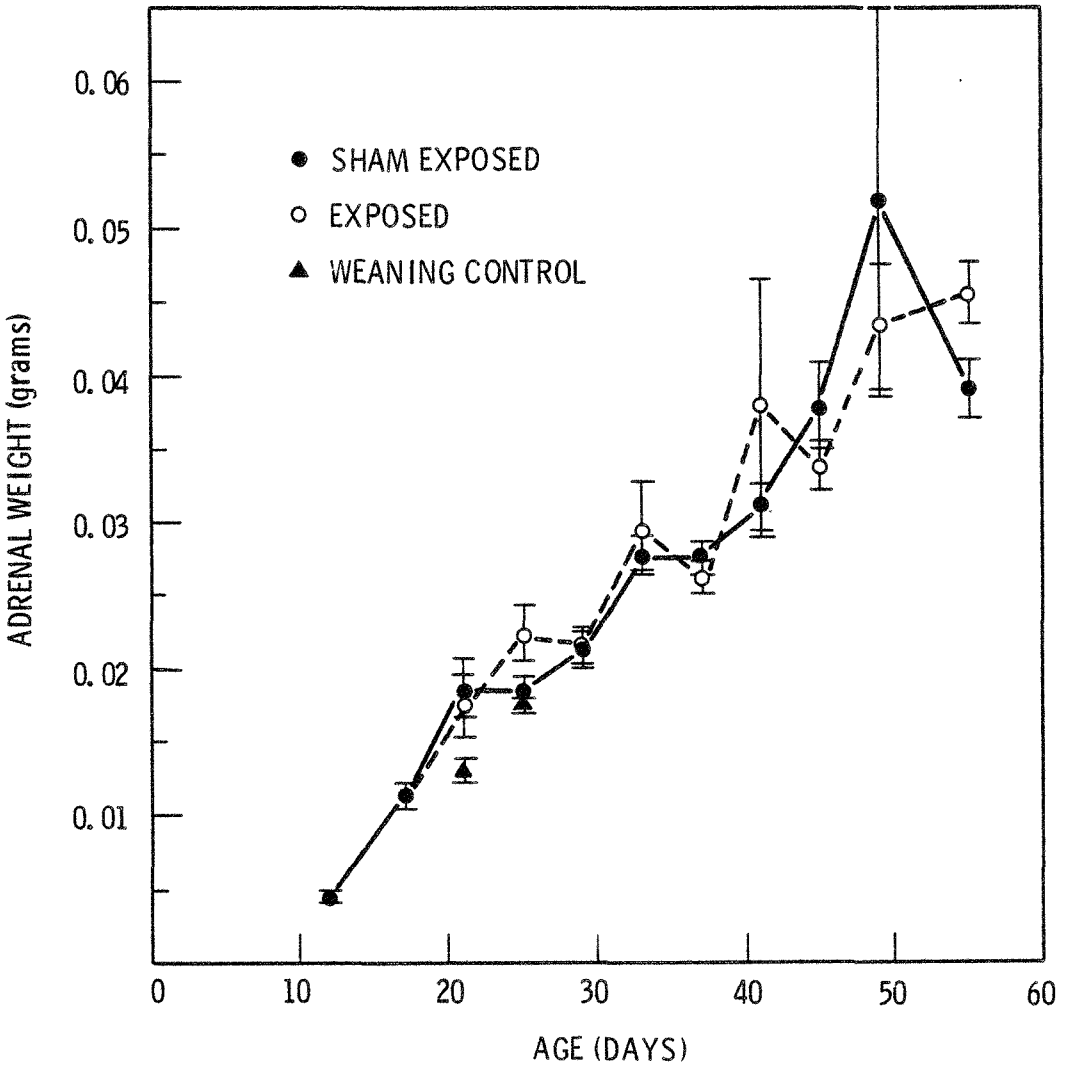


FIGURE IX-2. Mean (± S.E.) Adrenal Weights of Juvenile Male Rats Exposed or Sham-Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 days of Age. N = 8 rats/group at each measurement time.

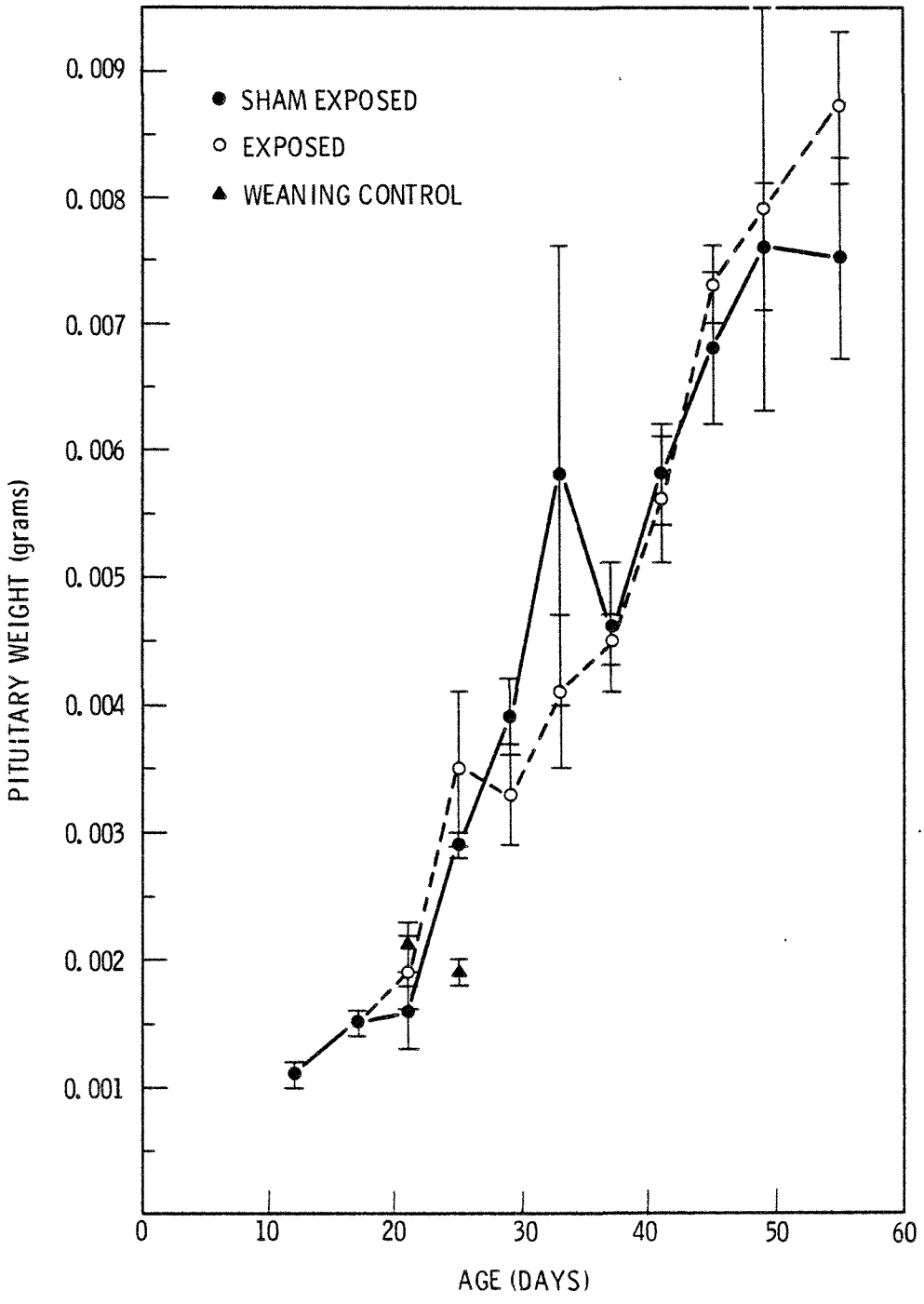


FIGURE IX-3. Mean (± S.E.) Pituitary Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 days of Age. N = 8 rats/group at each measurement time.

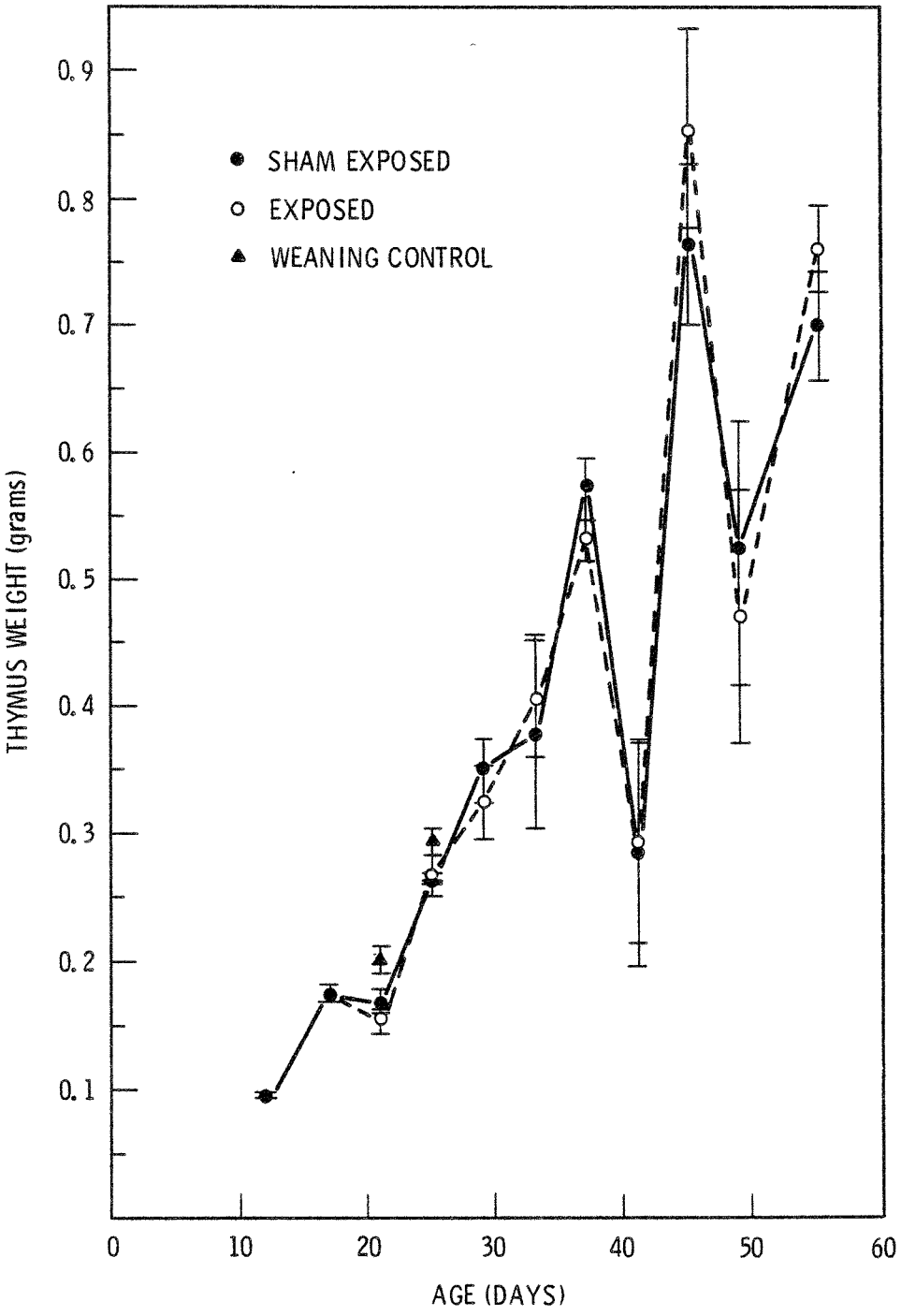


FIGURE IX-4. Mean (± S.E.) Thymus Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

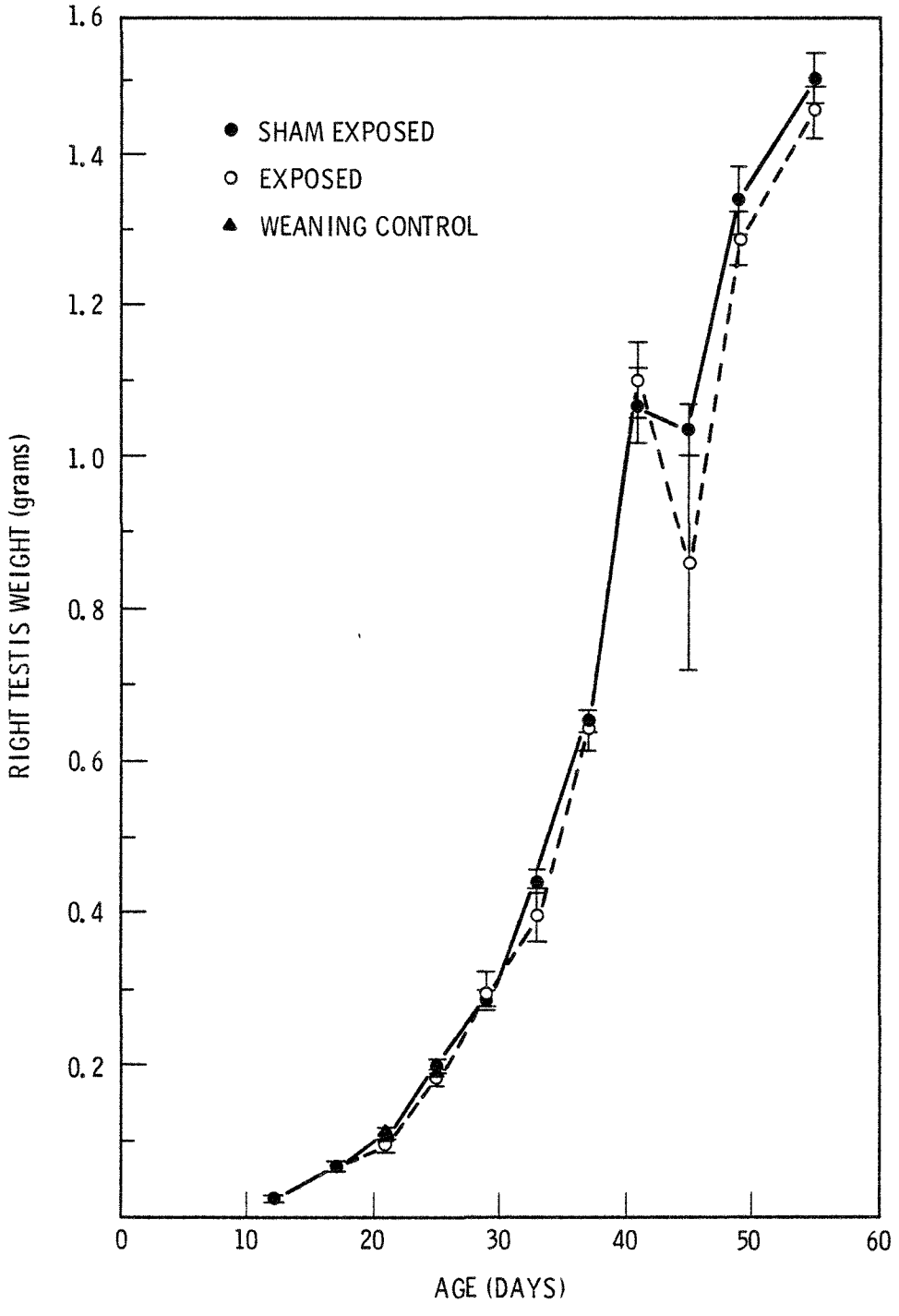


FIGURE IX-5. Mean (± S.E.) Right Testis Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

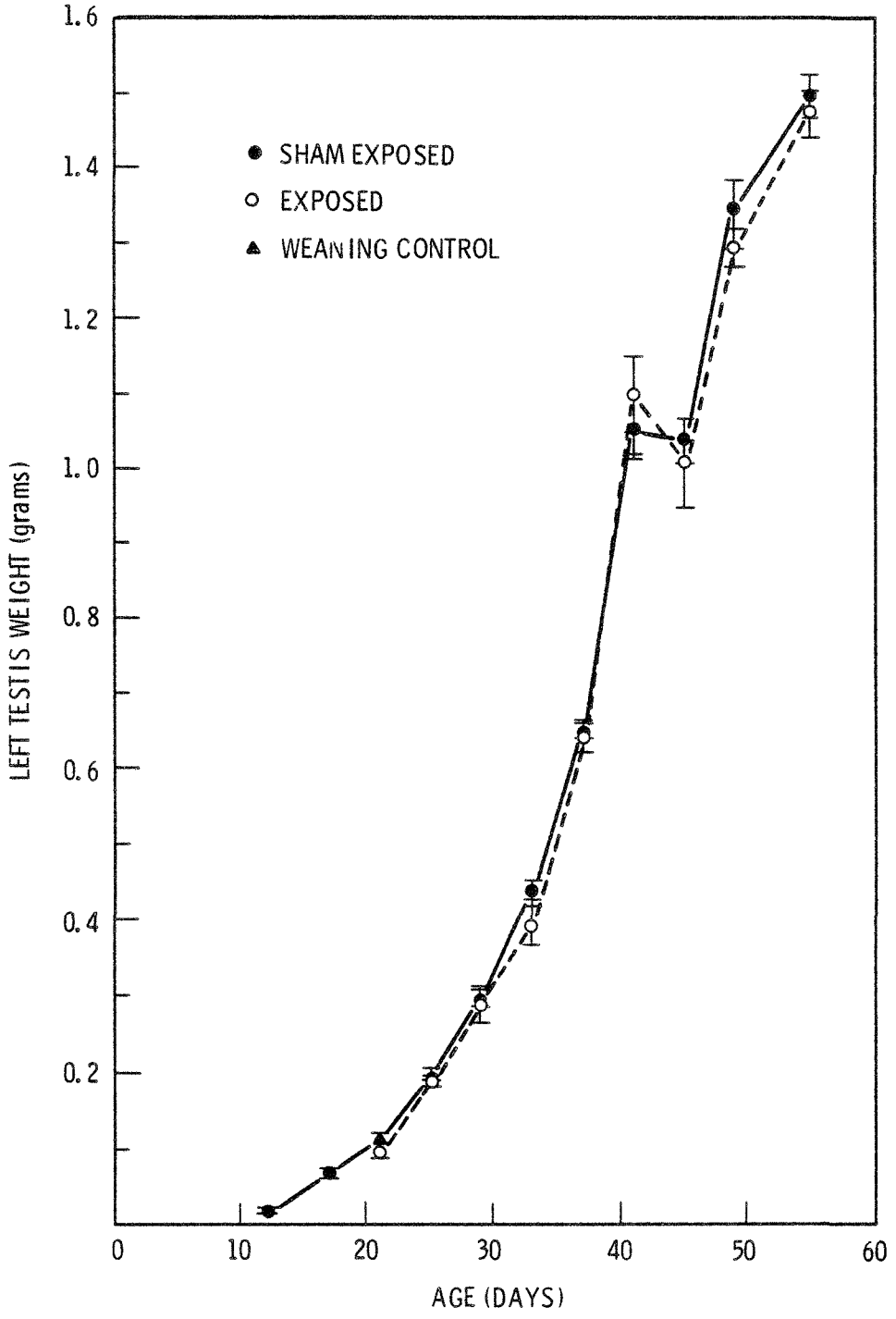


FIGURE IX-6. Mean (± S.E.) Left Testis Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m for 20 to 56 Days of Age. N = 8 rats/group at each measurement time.