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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

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

**Pacific Northwest Laboratory
Richland, Washington 99352
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ON SMALL LABORATORY ANIMALS

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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 μ A.

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 ~ 60 kV/m) is about 8.6 μ A 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 hormone, 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 postnatal 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.

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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

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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 $10\ \Omega$ 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.

Harmonic	No Damping Resistor; No Filtering		10- Ω Damping Resistor; No Filtering		10- Ω Damping Resistor; Input Power Filtered	
	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.

<u>Harmonic</u>	<u>HV-1</u>	<u>HV-2</u>	<u>Induced Current 2</u>
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 transformer 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 k Ω
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 accelerometers 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 $\epsilon_0 E^2/2$ ($\epsilon_0 = 8.85 \times 10^{-12} \text{ F/m}$), 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					
		Minimum	Average	Maximum	60-Hz (p-p, m/sec ²)			120-Hz (p-p, m/sec ²)		
					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 ($>10 \text{ meg}\Omega$). For measurements of I_M , fixed resistances were placed between the nozzle and ground, and a Tektronix 5031 storage oscilloscope (input impedance = $1 \text{ meg}\Omega$) 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_p . 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 μ A 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 k Ω , respectively. Eight rats were tested at each resistance; however, data from only six of the rats were obtained for $R_N = 63$ k Ω , 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 (k Ω)	Number of Animals	I_{SC} (μ A)		I_M (μ A)		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.

<u>Sex</u>	<u>Weight (g)</u>	<u>N</u>	<u>Body-Floor Impedance</u>	
			<u>Mean (kΩ)</u>	<u>S.D. (kΩ)</u>
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 k Ω . Three days after the clips were inserted, the measured impedances were 5 and 6 k Ω , 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 = $\pm 10^\circ$).

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 k Ω and 250 k Ω 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 k Ω (S.D. = 80 k Ω , 60 observations); after the change, we found $|Z_N| = 135$ k Ω (S.D. = 40 k Ω , 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:

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

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).

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

For any value of ϕ_F in the range $-90^\circ \leq \phi_F \leq 0$, 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 k Ω 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 $\phi_F = -30^\circ$ 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 k Ω , 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_0 , the field inside the lexan exposure cage is $0.95 E_0$ (Phillips and Kaune, 1977); (2) using data given in Section XV of this report, the short-circuit current induced in a rat will be $I_{SC} = 0.95 \alpha W^n E_0$, where I_{SC} is in μA , $\alpha = 0.0033$, W is the weight of the rat in grams; $n = 0.63$ and E_0 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,

$$I_M \lesssim 0.12 \times 10^{-2} W^{0.63} E_0.$$

For example, we have normally used $E_0 = 100$ kV/m in the Rat Exposure System. Assuming $W = 400$ g, $I_M \lesssim 5 \mu A$.

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.

<u>$\cos \phi_F$</u>	<u>Estimated Z_F (kΩ)</u>	<u>χ^2</u>
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 $I_M \lesssim 0.66 \times 10^{-3} W^n E_0$ for males (weight range, 540-590 g), and $I_M \lesssim 0.14 \times 10^{-2} W^n E_0$ 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 than females.

Direct currents of 1-5 μ A 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 k Ω). 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 meg Ω .

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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 $\pm 4\%$ 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 ≤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 $\pm$ 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 $\pm$ 8.6	310.3 $\pm$ 7.9	288.9 $\pm$ 5.9	282.3 $\pm$ 6.2	300.8 $\pm$ 5.5	296.3 $\pm$ 5.4
Volume Packed Red Cells (ml/dl)	42.8 $\pm$ 0.3	44.0 $\pm$ 0.4	43.9 $\pm$ 0.3	44.9 $\pm$ 0.4	43.3 $\pm$ 0.3	44.4 $\pm$ 0.3
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.03 $\pm$ 0.08	8.13 $\pm$ 0.08	8.29 $\pm$ 0.06	8.56 $\pm$ 0.09	8.15 $\pm$ 0.05	8.34 $\pm$ 0.07
Hemoglobin (g/dl)	16.6 $\pm$ 0.1	16.8 $\pm$ 0.1	17.1 $\pm$ 0.1	17.5 $\pm$ 0.1	16.9 $\pm$ 0.1	17.2 $\pm$ 0.1
Mean Corpuscular Volume (μm^3)	53.5 $\pm$ 0.4	54.2 $\pm$ 0.4	55.0 $\pm$ 0.3	55.0 $\pm$ 0.4	54.2 $\pm$ 0.3	54.5 $\pm$ 0.3
Mean Corpuscular Hemoglobin (pg)	20.8 $\pm$ 0.1	20.8 $\pm$ 0.2	20.8 $\pm$ 0.1	20.6 $\pm$ 0.1	20.8 $\pm$ 0.1	20.7 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	39.2 $\pm$ 0.1	38.6 $\pm$ 0.2	38.9 $\pm$ 0.1	38.8 $\pm$ 0.1	39.0 $\pm$ 0.1	38.7 $\pm$ 0.1
Reticulocyte ($\times 10^3/\text{mm}^3$)	135 $\pm$ 10	134 $\pm$ 11	115 $\pm$ 6	143 $\pm$ 7	126 $\pm$ 6	139 $\pm$ 7
Platelets ($\times 10^3/\text{mm}^3$)	857 $\pm$ 34	848 $\pm$ 43	877 $\pm$ 34	924 $\pm$ 61	867 $\pm$ 24	913 $\pm$ 30
Bone Marrow ($\times 10^3/\text{mm}^3$)	55.0 $\pm$ 2.4	53.0 $\pm$ 2.4	46.8 $\pm$ 1.4	47.2 $\pm$ 1.7	51.0 $\pm$ 1.6	50.1 $\pm$ 1.5

TABLE IV-2. Total Leukocyte Count and Cell Distribution (Mean $\pm$ 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 ($\times 10^3/\text{mm}^3$)	5.4 $\pm$ 0.2	5.8 $\pm$ 0.2	6.1 $\pm$ 0.2	7.1 $\pm$ 0.3	5.7 $\pm$ 0.2	6.4 $\pm$ 0.2
Neutrophils (%)	18.2 $\pm$ 2.1	17.1 $\pm$ 1.8	15.7 $\pm$ 1.8	12.7 $\pm$ 1.4	16.9 $\pm$ 1.4	14.9 $\pm$ 1.2
Neutrophils ($\times 10^3/\text{mm}^3$)	0.97 $\pm$ 0.11	0.97 $\pm$ 0.10	0.95 $\pm$ 0.12	0.91 $\pm$ 0.12	0.96 $\pm$ 0.08	0.94 $\pm$ 0.08
Lymphocyte (%)	76.8 $\pm$ 2.2	78.9 $\pm$ 1.8	80.5 $\pm$ 2.0	83.8 $\pm$ 1.6	78.6 $\pm$ 1.5	81.4 $\pm$ 1.3
Lymphocyte ($\times 10^3/\text{mm}^3$)	4.15 $\pm$ 0.23	4.57 $\pm$ 0.20	4.91 $\pm$ 0.22	5.93 $\pm$ 0.28	4.52 $\pm$ 0.17	5.25 $\pm$ 0.20
Monocytes (%)	1.3 $\pm$ 0.2	1.2 $\pm$ 0.2	0.6 $\pm$ 0.1	1.0 $\pm$ 0.2	0.09 $\pm$ 0.5	1.1 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.06 $\pm$ 0.01	0.07 $\pm$ 0.01	0.04 $\pm$ 0.01	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.07 $\pm$ 0.01
Eosinophils (%)	3.7 $\pm$ 0.4	2.9 $\pm$ 0.4	3.3 $\pm$ 0.6	2.6 $\pm$ 0.3	3.5 $\pm$ 0.3	2.7 $\pm$ 0.2
Eosinophils ($\times 10^3/\text{mm}^3$)	0.19 $\pm$ 0.01	0.16 $\pm$ 0.02	0.19 $\pm$ 0.02	0.17 $\pm$ 0.02	0.19 $\pm$ 0.02	0.17 $\pm$ 0.02

TABLE IV-3. Serum Chemistry Values (Mean $\pm$ 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 $\pm$ 0.07	6.96 $\pm$ 0.21	6.32 $\pm$ 0.10	6.49 $\pm$ 0.08	6.59 $\pm$ 0.07	6.64 $\pm$ 0.09
Albumin (g/dl)	3.34 $\pm$ 0.04	3.40 $\pm$ 0.09	3.12 $\pm$ 0.07	3.28 $\pm$ 0.08	3.23 $\pm$ 0.04	3.34 $\pm$ 0.06
Globulins						
Alpha (g/dl)	1.62 $\pm$ 0.04	1.61 $\pm$ 0.04	1.43 $\pm$ 0.04	1.48 $\pm$ 0.04	1.52 $\pm$ 0.03	1.54 $\pm$ 0.03
Beta (g/dl)	1.12 $\pm$ 0.02	1.15 $\pm$ 0.02	1.04 $\pm$ 0.03	1.04 $\pm$ 0.03	1.08 $\pm$ 0.02	1.09 $\pm$ 0.02
Gamma (g/dl)	0.72 $\pm$ 0.02	0.65 $\pm$ 0.03	0.73 $\pm$ 0.02	0.69 $\pm$ 0.03	0.73 $\pm$ 0.02	0.67 $\pm$ 0.02
SGOT* (IU)	353 $\pm$ 35	376 $\pm$ 21	411 $\pm$ 64	418 $\pm$ 43	382 $\pm$ 36	399 $\pm$ 25
BUN** (mg/dl)	22.5 $\pm$ 1.0	23.0 $\pm$ 0.7	24.8 $\pm$ 0.8	25.5 $\pm$ 1.2	23.6 $\pm$ 0.7	24.3 $\pm$ 0.7
Glucose (mg/dl)	157 $\pm$ 11	151 $\pm$ 6	108 $\pm$ 4	114 $\pm$ 5	133 $\pm$ 7	132 $\pm$ 5
Alkaline Phosphatase (BLB Units)	3.3 $\pm$ 0.2	3.4 $\pm$ 0.2	3.4 $\pm$ 0.2	3.9 $\pm$ 0.3	3.4 $\pm$ 0.1	3.6 $\pm$ 0.2
Triglyceride (mg/dl)	133.0 $\pm$ 6.0	146.3 $\pm$ 6.7	124.3 $\pm$ 8.2	143.3 $\pm$ 8.0	128.7 $\pm$ 5.0	144.8 $\pm$ 5.2
Cholesterol (mg/dl)	74.3 $\pm$ 3.9	74.9 $\pm$ 4.5	60.0 $\pm$ 4.3	78.4 $\pm$ 5.1	68.1 $\pm$ 3.0	76.6 $\pm$ 3.3
Iron (μ g/dl)	259 $\pm$ 13	275 $\pm$ 11	243 $\pm$ 9	279 $\pm$ 14	247 $\pm$ 8	275 $\pm$ 9
TIBC*** (μ g/dl)	465 $\pm$ 13	487 $\pm$ 13	469 $\pm$ 22	462 $\pm$ 16	467 $\pm$ 12	478 $\pm$ 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.

<u>Experiment</u>	<u>N/Group</u>	<u>Parameter</u>	<u>Mean $\pm$ S.E.</u>		<u>p<</u>
			<u>Exposed</u>	<u>Sham-Exposed</u>	
1	20	VPRC*	42.8 $\pm$ 0.3	44.0 $\pm$ 0.4	0.05
		MCHC**	39.2 $\pm$ 0.1	38.6 $\pm$ 0.2	0.02
2	20	Red Blood Cells	8.29 $\pm$ 0.06	8.56 $\pm$ 0.09	0.02
		Hemoglobin	17.1 $\pm$ 0.1	17.5 $\pm$ 0.1	0.05
		Reticulocytes	115 $\pm$ 6	143 $\pm$ 7	0.01
		White Blood Cells	6.1 $\pm$ 0.2	7.1 $\pm$ 0.3	0.02
		Lymphocytes	4.91 $\pm$ 0.22	5.93 $\pm$ 0.28	0.01
		Monocytes	0.04 $\pm$ 0.01	0.07 $\pm$ 0.01	0.05
		Cholesterol	60.0 $\pm$ 4.3	78.4 $\pm$ 5.1	0.02
		Iron	243 $\pm$ 9	279 $\pm$ 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 $\pm$ 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 $\pm$ 0.4	40.0 $\pm$ 0.4	38.4 $\pm$ 0.4	37.6 $\pm$ 0.3	38.8 $\pm$ 0.3	38.8 $\pm$ 0.3
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.53 $\pm$ 0.13	8.50 $\pm$ 0.09	8.45 $\pm$ 0.06	8.28 $\pm$ 0.09	8.49 $\pm$ 0.07	8.39 $\pm$ 0.06
Hemoglobin (g/dl)	14.4 $\pm$ 0.2	14.6 $\pm$ 0.2	14.3 $\pm$ 0.2	13.9 $\pm$ 0.2	14.3 $\pm$ 0.1	14.2 $\pm$ 0.1
Mean Corpuscular Volume (m^3)	46.4 $\pm$ 0.4	47.4 $\pm$ 0.3	47.3 $\pm$ 0.3	47.2 $\pm$ 0.3	46.9 $\pm$ 0.3	47.3 $\pm$ 0.2
Mean Corpuscular Hemoglobin (g)	17.0 $\pm$ 0.2	17.2 $\pm$ 0.1	17.1 $\pm$ 0.1	17.0 $\pm$ 0.1	17.0 $\pm$ 0.1	17.1 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	37.0 $\pm$ 0.3	36.7 $\pm$ 0.2	37.1 $\pm$ 0.1	36.8 $\pm$ 0.2	37.0 $\pm$ 0.2	36.8 $\pm$ 0.1
Reticulocytes ($\times 10^3/\text{mm}^3$)	256 $\pm$ 27	288 $\pm$ 29	225 $\pm$ 15	188 $\pm$ 15	245 $\pm$ 16	238 $\pm$ 18
Platelets ($\times 10^3/\text{mm}^3$)	1050 $\pm$ 28	1211 $\pm$ 61	1144 $\pm$ 40	1172 $\pm$ 65	1098 $\pm$ 25	1192 $\pm$ 44
Bone Marrow ($\times 10^3/\text{mm}^3$)	12.5 $\pm$ 0.5	13.8 $\pm$ 0.6	11.2 $\pm$ 0.5	11.2 $\pm$ 0.5	11.9 $\pm$ 0.4	12.5 $\pm$ 0.4

TABLE IV-6. Total Leukocyte Count and Cell Distributions (Mean $\pm$ 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 ($\times 10^3/\text{mm}^3$)	7.3 $\pm$ 0.5	5.6 $\pm$ 0.4	7.1 $\pm$ 0.7	7.8 $\pm$ 0.5	7.1 $\pm$ 0.4	6.7 $\pm$ 0.3
Neutrophils ()	20.1 $\pm$ 2.6	17.9 $\pm$ 1.4	19.4 $\pm$ 1.4	15.5 $\pm$ 0.9	20.0 $\pm$ 1.5	16.7 $\pm$ 0.9
Neutrophils ($\times 10^3/\text{mm}^3$)	1.44 $\pm$ 0.17	1.03 $\pm$ 0.11	1.41 $\pm$ 0.20	1.19 $\pm$ 0.10	1.42 $\pm$ 0.13	1.13 $\pm$ 0.08
Lymphocytes ()	77.4 $\pm$ 2.6	78.9 $\pm$ 1.6	78.2 $\pm$ 1.5	82.3 $\pm$ 1.0	77.8 $\pm$ 1.5	80.6 $\pm$ 1.0
Lymphocytes ($\times 10^3/\text{mm}^3$)	5.55 $\pm$ 0.48	4.39 $\pm$ 0.26	5.49 $\pm$ 0.55	6.42 $\pm$ 0.39	5.52 $\pm$ 0.36	5.40 $\pm$ 0.28
Monocytes ()	1.5 $\pm$ 0.3	1.5 $\pm$ 0.3	1.1 $\pm$ 0.3	1.3 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.2
Monocytes ($\times 10^3/\text{mm}^3$)	0.11 $\pm$ 0.02	0.09 $\pm$ 0.02	0.09 $\pm$ 0.02	0.10 $\pm$ 0.02	0.10 $\pm$ 0.02	0.09 $\pm$ 0.01
Eosinophils ()	1.0 $\pm$ 0.2	1.8 $\pm$ 0.4	1.3 $\pm$ 0.4	1.0 $\pm$ 0.2	1.2 $\pm$ 0.2	1.4 $\pm$ 0.2
Eosinophils ($\times 10^3/\text{mm}^3$)	0.08 $\pm$ 0.02	0.11 $\pm$ 0.04	0.12 $\pm$ 0.04	0.08 $\pm$ 0.02	0.10 $\pm$ 0.02	0.10 $\pm$ 0.02

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

<u>Experiment</u>	<u>N/Group</u>	<u>Parameter</u>	<u>Mean $\pm$ S.E.</u>		<u>p<</u>
			<u>Exposed</u>	<u>Sham-Exposed</u>	
1	20	Platelets	1050 $\pm$ 28	1211 $\pm$ 61	0.05
		White Blood Cells	7.3 $\pm$ 0.5	5.6 $\pm$ 0.4	0.02
		Lymphocytes	5.55 $\pm$ 0.48	4.39 $\pm$ 0.26	0.05
2	20	Glucose	138 $\pm$ 8	113 $\pm$ 7	0.02
		Protein	5.80 $\pm$ 0.11	6.28 $\pm$ 0.15	0.02
		β -Globulins	1.04 $\pm$ 0.06	1.21 $\pm$ 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 $\pm$ S.E.) in F₁ 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 $\pm$ 0.7	42.8 $\pm$ 0.6	40.3 $\pm$ 0.6	40.7 $\pm$ 0.9	41.3 $\pm$ 0.5	41.8 $\pm$ 0.6
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.86 $\pm$ 0.18	8.95 $\pm$ 0.12	8.39 $\pm$ 0.13	8.53 $\pm$ 0.22	8.62 $\pm$ 0.12	8.74 $\pm$ 0.13
Hemoglobin (g/dl)	14.9 $\pm$ 0.2	15.2 $\pm$ 0.2	14.3 $\pm$ 0.3	14.6 $\pm$ 0.3	14.6 $\pm$ 0.2	14.9 $\pm$ 0.2
Mean Corpuscular Volume (μm^3)	48.0 $\pm$ 0.4	48.0 $\pm$ 0.3	48.3 $\pm$ 0.2	48.0 $\pm$ 0.4	48.3 $\pm$ 0.2	48.2 $\pm$ 0.2
Mean Corpuscular Hemoglobin (μg)	16.9 $\pm$ 0.2	17.1 $\pm$ 0.1	17.2 $\pm$ 0.1	17.3 $\pm$ 0.1	17.0 $\pm$ 0.1	17.2 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.5 $\pm$ 0.3	35.7 $\pm$ 0.2	35.8 $\pm$ 0.2	36.3 $\pm$ 0.2	35.7 $\pm$ 0.2	36.0 $\pm$ 0.1
Reticulocytes ($\times 10^3/\text{mm}^3$)	237 $\pm$ 18	220 $\pm$ 10	192 $\pm$ 18	153 $\pm$ 12	215 $\pm$ 14	187 $\pm$ 11
Platelets ($\times 10^3/\text{mm}^3$)	1138 $\pm$ 77	1333 $\pm$ 59	1098 $\pm$ 59	1140 $\pm$ 67	1118 $\pm$ 47	1236 $\pm$ 49
Bone Marrow ($\times 10^3/\text{mm}^3$)	12.7 $\pm$ 0.6	14.2 $\pm$ 0.6	13.4 $\pm$ 0.6	10.9 $\pm$ 0.5	13.1 $\pm$ 0.4	12.6 $\pm$ 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 $\pm$ 0.7	41.0 $\pm$ 0.7	41.5 $\pm$ 0.5	42.4 $\pm$ 0.8	40.5 $\pm$ 0.5	41.6 $\pm$ 0.5
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.28 $\pm$ 0.12	8.52 $\pm$ 0.12	8.84 $\pm$ 0.19	8.89 $\pm$ 0.14	8.51 $\pm$ 0.12	8.73 $\pm$ 0.11
Hemoglobin (g/dl)	14.4 $\pm$ 0.3	14.7 $\pm$ 0.2	15.0 $\pm$ 0.3	15.2 $\pm$ 0.2	14.6 $\pm$ 0.2	14.9 $\pm$ 0.2
Mean Corpuscular Volume (μm^3)	48.6 $\pm$ 0.4	48.5 $\pm$ 0.3	47.6 $\pm$ 0.5	47.5 $\pm$ 0.7	48.2 $\pm$ 0.3	48.1 $\pm$ 0.3
Mean Corpuscular Hemoglobin (μg)	17.4 $\pm$ 0.2	17.4 $\pm$ 0.1	17.1 $\pm$ 0.1	17.1 $\pm$ 0.2	17.3 $\pm$ 0.1	17.2 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	36.3 $\pm$ 0.1	36.2 $\pm$ 0.2	36.5 $\pm$ 0.3	36.3 $\pm$ 0.1	36.4 $\pm$ 0.1	36.2 $\pm$ 0.1
Reticulocytes ($\times 10^3/\text{mm}^3$)	200 $\pm$ 24	168 $\pm$ 13	213 $\pm$ 19	206 $\pm$ 23	205 $\pm$ 16	185 $\pm$ 13
Platelets ($\times 10^3/\text{mm}^3$)	943 $\pm$ 58	1101 $\pm$ 65	1000 $\pm$ 107	1034 $\pm$ 75	966 $\pm$ 54	1071 $\pm$ 48
Bone Marrow ($\times 10^3/\text{mm}^3$)	11.4 $\pm$ 0.5	12.1 $\pm$ 0.9	11.7 $\pm$ 0.6	11.4 $\pm$ 0.9	11.5 $\pm$ 0.4	11.8 $\pm$ 0.6

TABLE IV-10. Total Leukocyte Count and Cell Distribution (Mean $\pm$ S.E.) in F₁ 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 ($\times 10^3/\text{mm}^3$)	6.3 $\pm$ 0.7	3.9 $\pm$ 0.3	9.1 $\pm$ 0.6	5.1 $\pm$ 0.5	7.7 $\pm$ 0.6	4.5 $\pm$ 0.3
Neutrophils (%)	21.8 $\pm$ 3.0	14.6 $\pm$ 2.3	20.8 $\pm$ 3.2	24.9 $\pm$ 2.4	21.3 $\pm$ 2.2	19.7 $\pm$ 2.0
Neutrophils ($\times 10^3/\text{mm}^3$)	1.42 $\pm$ 0.26	0.59 $\pm$ 0.11	1.83 $\pm$ 0.26	1.26 $\pm$ 0.16	1.63 $\pm$ 0.19	0.93 $\pm$ 0.12
Lymphocytes (%)	75.5 $\pm$ 3.4	83.9 $\pm$ 2.3	76.8 $\pm$ 3.2	72.9 $\pm$ 2.2	76.2 $\pm$ 2.3	78.4 $\pm$ 2.0
Lymphocytes ($\times 10^3/\text{mm}^3$)	4.70 $\pm$ 0.55	3.29 $\pm$ 0.21	6.94 $\pm$ 0.71	3.72 $\pm$ 0.36	5.85 $\pm$ 0.48	3.51 $\pm$ 0.21
Monocytes (%)	1.6 $\pm$ 0.4	0.6 $\pm$ 0.2	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.9 $\pm$ 0.3	0.4 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.10 $\pm$ 0.03	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.06 $\pm$ 0.02	0.02 $\pm$ 0.01
Eosinophils (%)	1.1 $\pm$ 0.3	0.9 $\pm$ 0.2	2.3 $\pm$ 0.4	2.2 $\pm$ 0.4	1.7 $\pm$ 0.3	1.5 $\pm$ 0.3
Eosinophils ($\times 10^3/\text{mm}^3$)	0.08 $\pm$ 0.02	0.03 $\pm$ 0.01	0.19 $\pm$ 0.03	0.11 $\pm$ 0.03	0.13 $\pm$ 0.02	0.07 $\pm$ 0.02

TABLE IV-10. (Continued)

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

TABLE IV-11. Serum Chemistry Values (Mean $\pm$ S.E.) in F₁ 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 $\pm$ 0.6	156.6 $\pm$ 2.0	156.9 $\pm$ 0.6	156.6 $\pm$ 2.0
Potassium (meq/L)	---	---	4.7 $\pm$ 0.2	5.6 $\pm$ 0.3	4.7 $\pm$ 0.2	5.6 $\pm$ 0.3
Blood Urea Nitrogen (mg/dl)	30.0 $\pm$ 2	29.0 $\pm$ 1	45.0 $\pm$ 2.5	37.6 $\pm$ 1.1	38.1 $\pm$ 2.4	34.0 $\pm$ 1.4
Cholesterol (mg/dl)	---	---	169.8 $\pm$ 7.3	160.2 $\pm$ 13.3	169.8 $\pm$ 7.3	160.0 $\pm$ 13.3
Triglycerides(mg/dl)	174.0 $\pm$ 4.0	161.0 $\pm$ 20.8	184.9 $\pm$ 7.2	202.9 $\pm$ 18.6	179.4 $\pm$ 4.4	183.3 $\pm$ 15.3
Total Protein (g/dl)	5.51 $\pm$ 0.06	5.71 $\pm$ 0.13	5.56 $\pm$ 0.12	6.01 $\pm$ 0.11	5.53 $\pm$ 0.07	5.88 $\pm$ 0.09
Albumin (g/dl)	2.73 $\pm$ 0.04	3.05 $\pm$ 0.09	2.76 $\pm$ 0.07	3.15 $\pm$ 0.10	2.75 $\pm$ 0.04	3.11 $\pm$ 0.07
Globulins						
Alpha (g/dl)	1.46 $\pm$ 0.04	1.38 $\pm$ 0.06	1.37 $\pm$ 0.05	1.39 $\pm$ 0.04	1.41 $\pm$ 0.03	1.39 $\pm$ 0.04
Beta (g/dl)	0.85 $\pm$ 0.02	0.85 $\pm$ 0.04	0.91 $\pm$ 0.05	0.94 $\pm$ 0.03	0.88 $\pm$ 0.03	0.90 $\pm$ 0.03
Gamma (g/dl)	0.46 $\pm$ 0.02	0.42 $\pm$ 0.04	0.52 $\pm$ 0.03	0.53 $\pm$ 0.04	0.49 $\pm$ 0.02	0.48 $\pm$ 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 $\pm$ 1.4	154.4 $\pm$ 0.7	155.1 $\pm$ 1.4	154.4 $\pm$ 0.7
Potassium (meq/L)		---	---	4.7 $\pm$ 0.2	4.4 $\pm$ 0.2	4.7 $\pm$ 0.2	4.4 $\pm$ 0.2
Blood Urea Nitrogen (mg/dl)		---	---	38.3 $\pm$ 1.9	29.6 $\pm$ 1.2	38.3 $\pm$ 1.9	29.6 $\pm$ 1.2
Cholesterol (mg/dl)		---	---	113.7 $\pm$ 9.1	120.5 $\pm$ 8.9	113.7 $\pm$ 9.1	120.5 $\pm$ 8.9
Triglycerides(mg/dl)		---	---	145.5 $\pm$ 6.3	158.0 $\pm$ 17.7	145.5 $\pm$ 6.3	158.0 $\pm$ 17.7
Total Protein (g/dl)		---	---	5.74 $\pm$ 0.11	5.78 $\pm$ 0.14	5.74 $\pm$ 0.11	5.78 $\pm$ 0.14
Albumin (g/dl)		---	---	3.38 $\pm$ 0.07	3.56 $\pm$ 0.05	3.38 $\pm$ 0.07	3.56 $\pm$ 0.05
Globulins							
Alpha (g/dl)		---	---	0.97 $\pm$ 0.05	0.95 $\pm$ 0.06	0.97 $\pm$ 0.05	0.95 $\pm$ 0.06
Beta (g/dl)		---	---	0.96 $\pm$ 0.03	0.90 $\pm$ 0.04	0.96 $\pm$ 0.03	0.90 $\pm$ 0.04
Gamma (g/dl)		---	---	0.43 $\pm$ 0.03	0.37 $\pm$ 0.02	0.43 $\pm$ 0.03	0.37 $\pm$ 0.02

TABLE IV-12. Statistical Differences in Clinical Pathology Parameters Between F₁ 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.

Experiment	Parameter	MEAN ± S.E.					
		MALE			FEMALE		
		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.

F₂ Generation. Hematologic data for both sexes of the F₂ 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 F₂ females, there were no statistically significant differences noted between the exposed and sham-exposed mice in either experiment. However, in the F₂ 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 $\pm$ S.E.) in F₂ 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 $\pm$ 1.0	39.4 $\pm$ 0.6	39.1 $\pm$ 0.6	39.7 $\pm$ 0.5	39.6 $\pm$ 0.6	39.6 $\pm$ 0.4
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.77 $\pm$ 0.25	8.65 $\pm$ 0.13	8.40 $\pm$ 0.11	8.71 $\pm$ 0.13	8.52 $\pm$ 0.11	8.68 $\pm$ 0.09
Hemoglobin (g/dl)	14.8 $\pm$ 0.4	14.8 $\pm$ 0.3	14.5 $\pm$ 0.2	14.7 $\pm$ 0.2	14.6 $\pm$ 0.2	14.7 $\pm$ 0.2
Mean Corpuscular Volume (μm^3)	47.5 $\pm$ 0.3	46.6 $\pm$ 0.4	48.4 $\pm$ 0.3	47.3 $\pm$ 0.3	48.1 $\pm$ 0.2	47.0 $\pm$ 0.2
Mean Corpuscular Hemoglobin ($\mu\mu\text{g}$)	16.8 $\pm$ 0.1	17.1 $\pm$ 0.1	17.4 $\pm$ 0.1	17.0 $\pm$ 0.1	17.2 $\pm$ 0.1	17.0 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	36.3 $\pm$ 0.1	37.6 $\pm$ 0.3	36.9 $\pm$ 0.1	36.7 $\pm$ 0.1	36.7 $\pm$ 0.1	37.1 $\pm$ 0.1
Reticulocytes ($\times 10^3/\text{mm}^3$)	219 $\pm$ 12	250 $\pm$ 20	246 $\pm$ 13	236 $\pm$ 18	237 $\pm$ 10	242 $\pm$ 14
Platelets ($\times 10^3/\text{mm}^3$)	1223 $\pm$ 68	1049 $\pm$ 74	906 $\pm$ 57	994 $\pm$ 102	1011 $\pm$ 52	1019 $\pm$ 64
Bone Marrow ($\times 10^3/\text{mm}^3$)	12.1 $\pm$ 0.6	12.8 $\pm$ 0.7	12.8 $\pm$ 0.4	12.2 $\pm$ 0.7	12.6 $\pm$ 0.4	12.4 $\pm$ 0.5

TABLE IV-13. (Continued)

		FEMALE					
		Experiment 1		Experiment 2		Composite	
		Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
G	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 ($\times 10^6/\text{mm}^3$)	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 (μg)	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 ($\times 10^3/\text{mm}^3$)	258 ± 30	207 ± 34	240 ± 27	233 ± 26	244 ± 22	221 ± 20
	Platelets ($\times 10^3/\text{mm}^3$)	1076 ± 132	1144 ± 86	955 ± 88	1047 ± 125	983 ± 73	1091 ± 77
	Bone Marrow ($\times 10^3/\text{mm}^3$)	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 $\pm$ S.E.) in F₂ 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 ($\times 10^3/\text{mm}^3$)	5.9 $\pm$ 0.5	5.1 $\pm$ 0.3	7.6 $\pm$ 0.5	4.3 $\pm$ 0.3	7.1 $\pm$ 0.4	4.7 $\pm$ 0.2
Neutrophils (%)	18.1 $\pm$ 1.8	15.3 $\pm$ 1.7	19.9 $\pm$ 1.8	16.8 $\pm$ 3.8	19.3 $\pm$ 1.3	16.1 $\pm$ 2.2
Neutrophils ($\times 10^3/\text{mm}^3$)	1.04 $\pm$ 0.12	0.76 $\pm$ 0.08	1.46 $\pm$ 0.15	0.81 $\pm$ 0.29	1.32 $\pm$ 0.11	0.79 $\pm$ 0.16
Lymphocytes (%)	80.4 $\pm$ 1.9	82.9 $\pm$ 1.7	77.8 $\pm$ 1.8	81.3 $\pm$ 3.9	78.6 $\pm$ 1.4	82.0 $\pm$ 2.2
Lymphocytes ($\times 10^3/\text{mm}^3$)	4.78 $\pm$ 0.47	4.27 $\pm$ 0.33	5.99 $\pm$ 0.50	3.37 $\pm$ 0.17	5.59 $\pm$ 0.38	3.78 $\pm$ 0.20
Monocytes (%)	0.30 $\pm$ 0.11	0.40 $\pm$ 0.16	0.53 $\pm$ 0.13	0.17 $\pm$ 0.10	0.45 $\pm$ 0.10	0.27 $\pm$ 0.09
Monocytes ($\times 10^3/\text{mm}^3$)	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01	0.01 $\pm$ 0.005
Eosinophils (%)	1.25 $\pm$ 0.03	1.40 $\pm$ 0.39	1.78 $\pm$ 0.30	1.67 $\pm$ 0.33	1.60 $\pm$ 0.23	1.55 $\pm$ 0.25
Eosinophils ($\times 10^3/\text{mm}^3$)	0.07 $\pm$ 0.33	0.08 $\pm$ 0.03	0.12 $\pm$ 0.02	0.07 $\pm$ 0.01	0.10 $\pm$ 0.01	0.77 $\pm$ 0.01

TABLE IV-14. (Continued)

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

TABLE IV-15. Serum Chemistry Values (Mean $\pm$ S.E.) in F₂ 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 $\pm$ 1.2	154.9 $\pm$ 1.2	150.5 $\pm$ 1.8	152.4 $\pm$ 3.3	152.3 $\pm$ 1.3	153.5 $\pm$ 1.9
Potassium (meq/L)	6.4 $\pm$ 0.4	6.0 $\pm$ 0.4	5.9 $\pm$ 0.4	5.5 $\pm$ 0.3	6.1 $\pm$ 0.3	5.7 $\pm$ 0.2
Blood Urea Nitrogen (mg/dl)	41.3 $\pm$ 0.8	28.7 $\pm$ 1.2	34.9 $\pm$ 1.4	35.5 $\pm$ 1.7	37.2 $\pm$ 1.1	32.2 $\pm$ 1.3
Cholesterol (mg/dl)	---	---	---	---	---	---
Triglycerides (mg/dl)	158 $\pm$ 11	192 $\pm$ 18	181 $\pm$ 9	181 $\pm$ 11	174 $\pm$ 8	186 $\pm$ 10
Total Protein (g/dl)	5.15 $\pm$ 0.11	5.37 $\pm$ 0.07	5.02 $\pm$ 0.09	5.12 $\pm$ 0.14	5.06 $\pm$ 0.07	5.23 $\pm$ 0.08
Albumin (g/dl)	2.59 $\pm$ 0.09	2.78 $\pm$ 0.05	2.62 $\pm$ 0.05	2.65 $\pm$ 0.09	2.61 $\pm$ 0.04	2.71 $\pm$ 0.05
Globulins						
Alpha (g/dl)	1.26 $\pm$ 0.04	1.20 $\pm$ 0.05	1.22 $\pm$ 0.03	1.25 $\pm$ 0.04	1.23 $\pm$ 0.02	1.23 $\pm$ 0.03
Beta (g/dl)	0.95 $\pm$ 0.04	0.99 $\pm$ 0.03	0.81 $\pm$ 0.02	0.78 $\pm$ 0.04	0.85 $\pm$ 0.02	0.88 $\pm$ 0.04
Gamma (g/dl)	0.35 $\pm$ 0.02	0.40 $\pm$ 0.02	0.37 $\pm$ 0.02	0.44 $\pm$ 0.03	0.37 $\pm$ 0.01	0.42 $\pm$ 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 $\pm$ 2.2	156.3 $\pm$ 1.1	149.9 $\pm$ 2.8	153.0 $\pm$ 1.4	151.7 $\pm$ 2.3	154.5 $\pm$ 1.0
Potassium (meq/L)	5.8 $\pm$ 0.7	5.4 $\pm$ 0.3	5.3 $\pm$ 0.2	5.4 $\pm$ 0.3	5.4 $\pm$ 0.2	5.4 $\pm$ 0.2
Blood Urea Nitrogen (mg/dl)	33.7 $\pm$ 5.5	26.1 $\pm$ 2.0	32.4 $\pm$ 1.3	32.1 $\pm$ 1.6	32.7 $\pm$ 1.7	29.2 $\pm$ 1.4
Cholesterol (mg/dl)	---	---	---	---	---	---
Triglycerides (mg/dl)	123 $\pm$ 16	128 $\pm$ 10	142 $\pm$ 6	126 $\pm$ 7	138 $\pm$ 6	127 $\pm$ 6
Total Protein (g/dl)	5.66 $\pm$ 0.18	5.94 $\pm$ 0.15	5.25 $\pm$ 0.10	5.22 $\pm$ 0.13	5.35 $\pm$ 0.09	5.55 $\pm$ 0.12
Albumin (g/dl)	3.17 $\pm$ 0.14	3.26 $\pm$ 0.09	2.90 $\pm$ 0.06	2.98 $\pm$ 0.09	2.95 $\pm$ 0.05	3.11 $\pm$ 0.07
Globulins						
Alpha (g/dl)	0.96 $\pm$ 0.04	1.09 $\pm$ 0.05	0.91 $\pm$ 0.02	0.90 $\pm$ 0.04	0.92 $\pm$ 0.02	0.99 $\pm$ 0.04
Beta (g/dl)	1.19 $\pm$ 0.07	1.09 $\pm$ 0.05	0.92 $\pm$ 0.03	0.86 $\pm$ 0.04	0.97 $\pm$ 0.03	0.96 $\pm$ 0.04
Gamma (g/dl)	0.45 $\pm$ 0.05	0.51 $\pm$ 0.03	0.52 $\pm$ 0.03	0.47 $\pm$ 0.02	0.51 $\pm$ 0.03	0.49 $\pm$ 0.02

TABLE IV-16. Statistical Differences in Clinical Pathology Parameters Between F₂ 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	Mean ± S.E.*					
		MALE			FEMALE		
		Exposed	Sham-Exposed	p<	Exposed	Sham-Exposed	p<
1	Mean Corpuscular Hemoglobin	16.8 ± 0.1	17.1 ± 0.1	0.01	No significant differences in any parameters		
	Mean Corpuscular Hemoglobin Concentration	36.3 ± 0.1	37.6 ± 0.3	0.001			
	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

F₃ 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 F₁ and F₂ generations, more significant differences are seen in exposed F₃ 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 F₁ and F₂ 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 F₃ generation were seen, either between the sexes or in replicate experiments.

Body and Organ Weights. Weights from F₁, F₂ and F₃ 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 $\pm$ S.E.) in F₃ 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 $\pm$ 0.6	36.6 $\pm$ 0.7	41.2 $\pm$ 0.3	39.5 $\pm$ 0.5	41.2 $\pm$ 0.3	38.3 $\pm$ 0.5
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.79 $\pm$ 0.14	8.14 $\pm$ 0.15	8.72 $\pm$ 0.08	8.48 $\pm$ 0.1	8.75 $\pm$ 0.07	8.33 $\pm$ 0.09
Hemoglobin (g/dl)	14.6 $\pm$ 0.2	13.5 $\pm$ 0.2	14.4 $\pm$ 0.1	13.9 $\pm$ 0.2	14.5 $\pm$ 0.1	13.7 $\pm$ 0.1
Mean Corpuscular Volume (μm^3)	46.8 $\pm$ 0.3	45.0 $\pm$ 0.3	47.5 $\pm$ 0.2	46.8 $\pm$ 0.2	47.2 $\pm$ 0.2	46.0 $\pm$ 0.2
Mean Corpuscular Hemoglobin (μg)	16.8 $\pm$ 0.1	16.8 $\pm$ 0.1	16.8 $\pm$ 0.1	16.5 $\pm$ 0.1	16.8 $\pm$ 0.1	16.7 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.6 $\pm$ 0.2	36.9 $\pm$ 0.3	34.8 $\pm$ 0.1	34.9 $\pm$ 0.2	35.1 $\pm$ 0.1	35.8 $\pm$ 0.2
Reticulocytes ($\times 10^3/\text{mm}^3$)	241 $\pm$ 20	186 $\pm$ 14	237 $\pm$ 13	233 $\pm$ 11	239 $\pm$ 11	213 $\pm$ 9
Platelets ($\times 10^3/\text{mm}^3$)	1292 $\pm$ 42	1019 $\pm$ 65	1216 $\pm$ 38	974 $\pm$ 46	1249 $\pm$ 28	993 $\pm$ 38
Bone Marrow ($\times 10^3/\text{mm}^3$)	13.9 $\pm$ 0.5	14.3 $\pm$ 0.4	15.3 $\pm$ 0.4	13.4 $\pm$ 0.4	14.7 $\pm$ 0.3	13.8 $\pm$ 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 $\pm$ 0.2	42.7 $\pm$ 0.4	40.3 $\pm$ 0.4	39.9 $\pm$ 0.3	41.3 $\pm$ 0.4	41.1 $\pm$ 0.4
Red Blood Cells ($\times 10^6/\text{mm}^3$)	9.10 $\pm$ 0.11	9.00 $\pm$ 0.10	8.51 $\pm$ 0.11	8.73 $\pm$ 0.08	8.75 $\pm$ 0.11	8.88 $\pm$ 0.07
Hemoglobin (g/dl)	15.3 $\pm$ 0.1	15.1 $\pm$ 0.1	14.6 $\pm$ 0.1	14.6 $\pm$ 0.1	14.9 $\pm$ 0.1	14.9 $\pm$ 0.1
Mean Corpuscular Volume (μm^3)	47.3 $\pm$ 0.6	47.0 $\pm$ 0.3	47.5 $\pm$ 0.5	45.8 $\pm$ 0.3	47.4 $\pm$ 0.4	46.5 $\pm$ 0.3
Mean Corpuscular Hemoglobin (μg)	17.0 $\pm$ 0.2	17.0 $\pm$ 0.1	17.3 $\pm$ 0.1	16.9 $\pm$ 0.1	17.2 $\pm$ 0.1	16.9 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	35.7 $\pm$ 0.3	35.8 $\pm$ 0.2	36.1 $\pm$ 0.2	36.5 $\pm$ 0.2	35.9 $\pm$ 0.2	36.1 $\pm$ 0.2
Reticulocytes ($\times 10^3/\text{mm}^3$)	281 $\pm$ 27	248 $\pm$ 17	225 $\pm$ 29	183 $\pm$ 29	248 $\pm$ 21	218 $\pm$ 17
Platelets ($\times 10^3/\text{mm}^3$)	1111 $\pm$ 75	1035 $\pm$ 59	1095 $\pm$ 73	1027 $\pm$ 59	1102 $\pm$ 51	1032 $\pm$ 41
Bone Marrow ($\times 10^3/\text{mm}^3$)	10.2 $\pm$ 0.6	11.9 $\pm$ 0.6	9.5 $\pm$ 0.6	8.4 $\pm$ 0.4	9.8 $\pm$ 0.4	10.3 $\pm$ 0.5

TABLE IV-18. Total Leukocyte Count and Cell Distribution (Mean $\pm$ S.E.) in F3 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.

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

TABLE IV-18. (Continued)

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

TABLE IV-19. Serum Chemistry Values (Mean + S.E.) in F₃ 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 $\pm$ 2.7	150.4 $\pm$ 0.9	158.9 $\pm$ 0.8	153.8 $\pm$ 0.4	155.4 $\pm$ 1.6	152.0 $\pm$ 0.6
	Potassium (meq/L)	6.3 $\pm$ 0.4	5.2 $\pm$ 0.2	6.1 $\pm$ 0.4	5.7 $\pm$ 0.4	6.2 $\pm$ 0.3	5.4 $\pm$ 0.2
	Blood Urea Nitrogen (mg/dl)	32.7 $\pm$ 0.9	30.2 $\pm$ 0.6	31.2 $\pm$ 3.1	33.0 $\pm$ 3.5	32.0 $\pm$ 1.5	30.9 $\pm$ 1.0
	Cholesterol (mg/dl)	79 $\pm$ 6	85 $\pm$ 6	79 $\pm$ 8	80 $\pm$ 6	79 $\pm$ 6	83 $\pm$ 4
∞	Triglycerides (mg/dl)	173 $\pm$ 13	168 $\pm$ 13	129 $\pm$ 6	120 $\pm$ 11	146 $\pm$ 8	145 $\pm$ 10
	Total Protein (g/dl)	5.81 $\pm$ 0.15	5.67 $\pm$ 0.11	5.34 $\pm$ 0.12	5.61 $\pm$ 0.14	5.53 $\pm$ 0.11	5.64 $\pm$ 0.09
	Albumin (g/dl)	3.18 $\pm$ 0.07	3.06 $\pm$ 0.07	2.94 $\pm$ 0.07	3.26 $\pm$ 0.08	3.04 $\pm$ 0.06	3.15 $\pm$ 0.06
	Globulins						
	Alpha (g/dl)	1.08 $\pm$ 0.06	1.05 $\pm$ 0.03	0.98 $\pm$ 0.04	0.94 $\pm$ 0.03	1.02 $\pm$ 0.03	1.00 $\pm$ 0.02
	Beta (g/dl)	1.06 $\pm$ 0.07	1.00 $\pm$ 0.05	1.03 $\pm$ 0.06	1.00 $\pm$ 0.07	1.04 $\pm$ 0.04	1.00 $\pm$ 0.04
	Gamma (g/dl)	0.51 $\pm$ 0.04	0.56 $\pm$ 0.03	0.40 $\pm$ 0.02	0.42 $\pm$ 0.03	0.44 $\pm$ 0.02	0.50 $\pm$ 0.03

TABLE IV-20. Statistical Differences (Mean $\pm$ S.E.) in Clinical Pathology Parameters Between F₃ 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 $\pm$ 0.6	36.6 $\pm$ 0.7	0.001			
	Red Blood Cells	8.79 $\pm$ 0.14	8.14 $\pm$ 0.15	0.001			
	Hemoglobin	14.6 $\pm$ 0.2	13.5 $\pm$ 0.2	0.001			
	MCV**	46.8 $\pm$ 0.3	45.0 $\pm$ 0.3	0.001			
	MCHC***	35.6 $\pm$ 0.7	36.9 $\pm$ 0.3	0.01			
	Reticulocytes	241 $\pm$ 20	186 $\pm$ 14	0.05			
	Platelets	1292 $\pm$ 42	1019 $\pm$ 65	0.01			
	Neutrophils				0.63 $\pm$ 0.09	1.01 $\pm$ 0.27	0.01
	Monocytes	0.13 $\pm$ 0.02	0.06 $\pm$ 0.02	0.05			
	Sodium	160.4 $\pm$ 0.7	155.6 $\pm$ 0.6	0.001			
	Triglycerides	197 $\pm$ 6	176 $\pm$ 5	0.02			
	Potassium				6.3 $\pm$ 0.4	5.2 $\pm$ 0.2	0.05
	Blood Urea Nitrogen				32.7 $\pm$ 0.9	30.2 $\pm$ 0.6	0.05
2	VPRC*	41.2 $\pm$ 0.3	39.5 $\pm$ 0.5	0.01			
	Hemoglobin	14.4 $\pm$ 0.1	13.9 $\pm$ 0.2	0.05			
	MCV**	47.5 $\pm$ 0.2	46.8 $\pm$ 0.2	0.05	47.5 $\pm$ 0.5	45.8 $\pm$ 0.3	0.01
	MCH****	16.8 $\pm$ 0.1	16.5 $\pm$ 0.1	0.01	17.3 $\pm$ 0.1	16.9 $\pm$ 0.1	0.05
	Platelets	1216 $\pm$ 38	974 $\pm$ 46	0.001			
	Bone Marrow	15.3 $\pm$ 0.4	13.4 $\pm$ 0.4	0.01			
	Leukocytes	4.6 $\pm$ 0.2	6.2 $\pm$ 0.5	0.01			
	Neutrophils	0.66 $\pm$ 0.05	1.35 $\pm$ 0.22	0.01			
	Lymphocytes	3.86 $\pm$ 0.18	4.73 $\pm$ 0.32	0.05			
	Blood Urea Nitrogen						
		44.1 $\pm$ 1.5	36.6 $\pm$ 1.8	0.01			

* Volume Packed Red Cells

** Mean Corpuscular Volume

*** Mean Corpuscular Hemoglobin Concentration

**** Mean Corpuscular Hemoglobin

TABLE IV-21. Weights (Mean $\pm$ S.E.) from F₁, F₂ and F₃ 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 10-19 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 $\pm$ 0.4	31.7 $\pm$ 0.5	30.6 $\pm$ 0.6	29.4 $\pm$ 0.6	29.9 $\pm$ 0.3	30.8 $\pm$ 0.4
F ₁ Spleen Wt (mg)	100.2 $\pm$ 8.8	96.1 $\pm$ 3.7	106.9 $\pm$ 7.7	89.8 $\pm$ 5.0	103.5 $\pm$ 5.7	92.9 $\pm$ 3.1
F ₂ Total Wt (g)	31.4 $\pm$ 0.8	29.7 $\pm$ 0.5	31.5 $\pm$ 0.5	30.5 $\pm$ 0.5	31.5 $\pm$ 0.4	30.1 $\pm$ 0.4
F ₂ Spleen Wt (mg)	102.8 $\pm$ 2.5	103.3 $\pm$ 3.6	116.2 $\pm$ 2.8	105.9 $\pm$ 5.1	111.7 $\pm$ 2.8	104.7 $\pm$ 3.2
F ₃ Total Wt (g)	30.6 $\pm$ 0.5	32.1 $\pm$ 0.6	33.8 $\pm$ 0.4	31.2 $\pm$ 0.5	32.4 $\pm$ 0.4	31.6 $\pm$ 0.4
F ₃ Spleen Wt (mg)	116.4 $\pm$ 3.5	109.4 $\pm$ 5.0	87.6 $\pm$ 3.4	104.6 $\pm$ 5.8	99.9 $\pm$ 3.4	106.7 $\pm$ 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 $\sim 5 \mu\text{Ci}$ 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 $7.52 \pm 0.24\%$ (mean ± 1 SEM) in the exposed rats and $7.10 \pm 0.22\%$ 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 ⁵⁹Fe 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 $\pm$ 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 ↑ globulins ↓
Dumansky et al. (1976)	rat	0.1-5	4 m			eos ↓			glucose ↑ BUN ↑ & 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 ↓	↑	↓	
Poznanski 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 dehydrogenase

*** Blood urea nitrogen

increases in some serum constituents (i.e., glutamic oxaloacetic trans-aminase, 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 multivariate 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).

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V. IMMUNOLOGY

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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 ~ 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 ± 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

<u>Group</u>	<u>Exposure Duration (Days)</u>	<u>N</u>	<u>Response[*]</u> (μ g KLH Precipitated per 25 μ l serum)	
			<u>Mean</u>	<u>S.E.</u>
Exposed	30	12	28.1 $\pm$	5.4
Sham-exposed	30	11	24.3 $\pm$	4.3
Cage control	30	10	20.5 $\pm$	2.8
Exposed	60	11	30.9 $\pm$	3.4
Sham-exposed	60	10	33.8 $\pm$	0.6
Cage control	60	8	24.3 $\pm$	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 (μ g)	Stimulation Index*			P Values** Exposed/Sham Exposed
		Cage Control	Exposed	Sham- Exposed	
		(N = 15) Mean $\pm$ S.E.	(N = 18) Mean $\pm$ S.E.	(N = 18) Mean $\pm$ S.E.	
Concanavalin-A	0.9	37.2 $\pm$ 3.7	36.0 $\pm$ 3.7	36.1 $\pm$ 2.9	0.97
	1.9	73.4 $\pm$ 6.9	65.5 $\pm$ 6.9	72.1 $\pm$ 5.8	0.47
	2.8	79.2 $\pm$ 8.8	72.7 $\pm$ 8.4	68.1 $\pm$ 5.1	0.65
Lipopolysaccharide	5.0	5.6 $\pm$ 0.6	5.1 $\pm$ 0.4	5.1 $\pm$ 0.4	0.99
	10.0	5.0 $\pm$ 0.6	4.7 $\pm$ 0.4	4.8 $\pm$ 0.4	0.70
	15.0	4.4 $\pm$ 0.4	4.2 $\pm$ 0.4	4.6 $\pm$ 0.4	
Pokeweed Mitogen	2.5	11.2 $\pm$ 1.1	10.6 $\pm$ 1.3	10.3 $\pm$ 0.9	0.87
	5.0	12.2 $\pm$ 1.2	11.0 $\pm$ 1.4	10.9 $\pm$ 0.8	0.98
	10.0	13.2 $\pm$ 1.4	11.7 $\pm$ 1.3	11.5 $\pm$ 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 (μ g)	Stimulation Index*			P Values** Exposed/Sham Exposed
		Cage Control	Exposed	Sham- Exposed	
		(N = 9)	(N = 9)	(N = 9)	
		Mean $\pm$ S.E.	Mean $\pm$ S.E.	Mean $\pm$ S.E.	
Concanavalin-A	0.9	97.9 $\pm$ 11.1	87.7 $\pm$ 18.9	95.8 $\pm$ 20.1	0.77
	1.9	132.5 $\pm$ 18.0	118.7 $\pm$ 24.0	98.7 $\pm$ 16.9	0.51
	2.8	102.2 $\pm$ 19.7	83.3 $\pm$ 18.2	61.3 $\pm$ 13.3	0.35
Lipopolysaccharide	5.0	9.9 $\pm$ 1.7	10.3 $\pm$ 2.2	12.7 $\pm$ 1.7	0.39
	10.0	13.3 $\pm$ 2.1	14.1 $\pm$ 2.9	15.5 $\pm$ 2.5	0.70
	15.0	12.5 $\pm$ 2.1	16.4 $\pm$ 5.2	16.1 $\pm$ 2.5	0.95
Pokeweed Mitogen	2.5	18.8 $\pm$ 4.2	12.2 $\pm$ 2.4	13.8 $\pm$ 3.2	0.70
	5.0	21.8 $\pm$ 6.0	14.1 $\pm$ 2.4	12.8 $\pm$ 3.7	0.78
	10.0	21.4 $\pm$ 8.0	11.7 $\pm$ 2.5	11.4 $\pm$ 3.1	0.97
Phytohemagglutinin	20 μ l	104.6 $\pm$ 13.8	101.5 $\pm$ 22.9	68.2 $\pm$ 12.1	0.22
	40 μ l	84.2 $\pm$ 17.0	87.1 $\pm$ 21.3	52.8 $\pm$ 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 $\pm$ S.E.

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

* Mean $\pm$ 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 assays.

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.

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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 μ m 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

	<u>Exposed</u>	<u>Sham-Exposed</u>	<u>Cage Control</u>
<u>Urinary Bladder</u>			
Proteinaceous material in lumen	5/20	7/20	3/20
Epithelial hyperplasia, focal	1/20	0/20	0/20
<u>Lung</u>			
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
<u>Liver</u>			
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
<u>Kidney</u>			
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)
<u>Prostate</u>			
Chronic prostatitis, multifocal (mean severity)*	3/20 (1.33)	4/20 (1.5)	4/20 (1.75)
<u>Large Bowel</u>			
Nematodiasis	3/20	1/20	1/20
<u>Thyroid</u>			
Ultimobranchial rest	4/20	2/20	0/20
<u>Mesenteric Lymph Node</u>			
Lymphoid hyperplasia	1/20	0/20	0/20
<u>Trachea</u>			
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

	<u>Exposed</u>		<u>Sham-Exposed</u>		<u>Cage Controls</u>	
	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
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.

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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

<u>Species</u>	<u>Sex</u>	<u>N/Sex/ Group/Exp.</u>	<u>Age at Start of Exposure (Days)</u>	<u>Field Strength (kV/m)</u>	<u>Duration of Exposure</u>	<u>Parameters</u>
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 (~ 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		p Value
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Body Weight	20	623* $\pm$ 14	20	661 $\pm$ 12	0.04
Heart	20	1.71 $\pm$ 0.05	20	1.82 $\pm$ 0.07	N.S.
Left Kidney	20	1.78* $\pm$ 0.03	20	1.93 $\pm$ 0.05	0.02
Right Kidney	20	1.76* $\pm$ 0.04	20	1.98 $\pm$ 0.05	0.002
Liver	20	18.0 $\pm$ 1.0	20	19.4 $\pm$ 1.4	N.S.
Brain	20	2.07* $\pm$ 0.03	20	2.17 $\pm$ 0.04	0.04
Left Testis	20	1.94 $\pm$ 0.04	20	1.99 $\pm$ 0.04	N.S.
Right Testis	20	1.96 $\pm$ 0.04	20	1.98 $\pm$ 0.04	N.S.
Left Adrenal	18	0.025 $\pm$ 0.002	18	0.029 $\pm$ 0.003	N.S.
Right Adrenal	19	0.023 $\pm$ 0.001	19	0.028 $\pm$ 0.02	N.S.
Pituitary	17	0.010 $\pm$ 0.001	17	0.009 $\pm$ 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		p Value
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Body Weight	19	585 $\pm$ 16	20	588 $\pm$ 14	N.S.
Heart	20	1.68 $\pm$ 0.04	20	1.68 $\pm$ 0.05	N.S.
Kidneys	20	3.82 $\pm$ 0.08	20	3.68 $\pm$ 0.07	N.S.
Liver	20	20.5 $\pm$ 0.8	19	20.4 $\pm$ 0.5	N.S.
Brain	20	2.17 $\pm$ 0.03	20	2.19 $\pm$ 0.03	N.S.
Testes	20	3.79 $\pm$ 0.10	20	3.75 $\pm$ 0.08	N.S.
Adrenals	20	0.057 $\pm$ 0.004	20	0.053 $\pm$ 0.003	N.S.
Pituitary	18	0.0011 $\pm$ 0.0010	19	0.0010 $\pm$ 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 $\pm$ S.E.			
	Males		Females	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Body Weight	38.8 $\pm$ 0.5	38.7 $\pm$ 1.0	35.0* $\pm$ 0.9	31.7 $\pm$ 0.7
Heart	0.204 $\pm$ 0.005	0.202 $\pm$ 0.008	0.151 $\pm$ 0.004	0.145 $\pm$ 0.003
Left Kidney	0.429 $\pm$ 0.008	0.412 $\pm$ 0.014	0.275 $\pm$ 0.006	0.262 $\pm$ 0.008
Right Kidney	0.450 $\pm$ 0.006	0.421 $\pm$ 0.018	0.275 $\pm$ 0.008	0.263 $\pm$ 0.007
Liver	2.60 $\pm$ 0.05	2.5 $\pm$ 0.07	2.16 $\pm$ 0.07	2.07 $\pm$ 0.06
Brain	0.500 $\pm$ 0.009	0.474 $\pm$ 0.009	0.503 $\pm$ 0.012	0.487 $\pm$ 0.005
Left Testis	0.151 $\pm$ 0.008	0.151 $\pm$ 0.008	---	---
Right Testis	0.147 $\pm$ 0.004	0.158 $\pm$ 0.008	---	---

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

TABLE VII-5. Mean ($\pm$ 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).

		<u>Exposed</u>	<u>Sham Exposed</u>
Before Exposure (0.5 hr)	O ₂ (ml/min)	9.7 $\pm$ 0.4	9.4 $\pm$ 0.2
	CO ₂ (ml/min)	8.4 $\pm$ 0.2	8.4 $\pm$ 0.2
	RGER	0.87 $\pm$ 0.01	0.89 $\pm$ 0.01
During Exposure (1 hr)	O ₂ (ml/min)	8.9 $\pm$ 0.3	8.6 $\pm$ 0.2
	CO ₂ (ml/min)	8.0 $\pm$ 0.3	7.7 $\pm$ 0.3
	RGER	0.90 $\pm$ 0.01	0.90 $\pm$ 0.01
After Exposure (0.5 hr)	O ₂ (ml/min)	9.0 $\pm$ 0.3	8.6 $\pm$ 0.2
	CO ₂	8.0 $\pm$ 0.3	7.7 $\pm$ 0.3
	RGER	0.88 $\pm$ 0.01	0.89 $\pm$ 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.

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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 tibio-fibular 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- μ m sections were cut with a slow-speed diamond-blade saw. The sections were examined under a Zeiss epillumination, 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 $\mu\text{m}/\text{day}$ on periosteal surfaces, and 2.1 to 2.3 $\mu\text{m}/\text{day}$ 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

<u>Measurement*</u>	<u>Sex</u>	<u>Exposed</u>	<u>Sham-Exposed</u>
Periosteal Growth ($\mu\text{m}/\text{day}$)	M	8.5 ± 0.6	8.9 ± 0.2
	F	5.5 ± 0.6	4.7 ± 0.4
Endosteal Growth ($\mu\text{m}/\text{day}$)	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 $\pm$ 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 $\pm$ 0.9	64.4 $\pm$ 0.6	67.1 $\pm$ 1.0	66.0 $\pm$ 0.7
-1	82.7 $\pm$ 1.0	82.7 $\pm$ 1.1	77.2 $\pm$ 0.9	79.0 $\pm$ 1.0
0	85.0 $\pm$ 1.5	88.9 $\pm$ 1.5	81.0 $\pm$ 1.3	83.4 $\pm$ 1.0
1	92.2 $\pm$ 1.5	96.1 $\pm$ 1.6	85.5 $\pm$ 1.4	89.9 $\pm$ 1.2
2	99.4 $\pm$ 2.0	103.9 $\pm$ 1.6	92.9 $\pm$ 1.7	97.9 $\pm$ 1.4
3	112.8 $\pm$ 1.7	112.5 $\pm$ 1.8	104.6 $\pm$ 1.7	104.9 $\pm$ 1.4
4	120.3 $\pm$ 1.8	121.9 $\pm$ 1.9	110.1 $\pm$ 1.8	111.3 $\pm$ 1.7
5	130.8 $\pm$ 2.2	131.6 $\pm$ 1.8	118.5 $\pm$ 2.0	120.3 $\pm$ 1.9
6	141.7 $\pm$ 2.5	140.5 $\pm$ 2.1	126.9 $\pm$ 2.4	126.3 $\pm$ 1.8
11	184.6 $\pm$ 4.1	181.4 $\pm$ 4.4	154.3 $\pm$ 3.9	158.7 $\pm$ 2.7
14	209.8 $\pm$ 5.4	217.5 $\pm$ 4.7	170.2 $\pm$ 4.9	173.3 $\pm$ 3.1
17	233.3 $\pm$ 6.3	236.9 $\pm$ 5.9	179.6 $\pm$ 5.4	183.2 $\pm$ 3.2
19	251.8 $\pm$ 6.8	254.3 $\pm$ 6.2	188.2 $\pm$ 5.8	190.4 $\pm$ 3.8
22	276.2 $\pm$ 7.6	269.1 $\pm$ 7.1	197.9 $\pm$ 6.7	190.7 $\pm$ 5.8
25	299.5 $\pm$ 7.6	306.4 $\pm$ 8.5	208.6 $\pm$ 4.4	213.1 $\pm$ 5.2
28	321.0 $\pm$ 10.8	328.7 $\pm$ 9.1	215.7 $\pm$ 7.1	222.2 $\pm$ 6.2

* Mean $\pm$ 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.

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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/N₂O/O₂ 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)	
	<u>Exposed</u>	<u>Sham- Exposed</u>	<u>Exposed</u>	<u>Sham- Exposed</u>
<u>First Experiment</u>				
N	17	18	17	18
Mean	181	271	176	169
SE	59	78	15	12
<u>Replicate Experiment</u>				
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

	<u>Exposed</u>		<u>Sham-Exposed</u>	
	<u>N</u>	<u>Mean $\pm$ S.E.</u>	<u>N</u>	<u>Mean $\pm$ S.E.</u>
Adrenal Cortical Steroids (Fluorometric Assay) (ng/ml)	20	150 $\pm$ 17	20	193 $\pm$ 32
Corticosterone (Dextran-Coated-Charcoal Assay) (ng/ml)	17	43 $\pm$ 11	18	83 $\pm$ 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 ± 8 ng/ml for exposed rats and 287 ± 138 ng/ml for sham-exposed rats in the first experiment, and 42.1 ± 11.6 ng/ml for exposed rats and 35.6 ± 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

	<u>Exposed</u>		<u>Sham-Exposed</u>	
	<u>N</u>	<u>Mean $\pm$ S.E.</u>	<u>N</u>	<u>Mean $\pm$ S.E.</u>
FSH* ng/ml	20	330 $\pm$ 30	19	401 $\pm$ 32
LH** ng/ml	20	23 $\pm$ 2	19	22 $\pm$ 2
Prolactin ng/ml	20	37 $\pm$ 6	19	30 $\pm$ 3
Testosterone ng/ml	20	1.5 $\pm$ 0.2***	18	2.2 $\pm$ 0.3
TSH**** μ Units/ml	20	137 $\pm$ 14	19	148 $\pm$ 16
Corticosterone ng/ml	20	52 $\pm$ 10***	19	91 $\pm$ 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 $\pm$ 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 $\pm$ 21	14 $\pm$ 7	10 $\pm$ 3	2.4 $\pm$ 0.3	32 $\pm$ 13
Halothane/N ₂ O/O ₂ + Heart Puncture N = 12	177 $\pm$ 10	8 $\pm$ 1	13 $\pm$ 3	5.7 $\pm$ 1.0 ^(1,2)	41 $\pm$ 16
Ethrane/N ₂ O/O ₂ + Heart Puncture N = 11	199 $\pm$ 33	8 $\pm$ 1	14 $\pm$ 3	3.0 $\pm$ 0.3	18 $\pm$ 7
155 Ether + Heart Puncture N = 11	241 $\pm$ 9 ⁽³⁾	17 $\pm$ 3 ^(2,3)	16 $\pm$ 3	3.4 $\pm$ 0.4	19 $\pm$ 12
Concussion + Heart Puncture N = 12	295 $\pm$ 85	25 $\pm$ 7 ^(2,4)	24 $\pm$ 6 ⁽⁵⁾	4.9 $\pm$ 0.8 ^(1,2)	42 $\pm$ 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 ($\pm$ 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 ($\pm$ 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 ($\pm$ 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 ($\pm$ 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 ($\pm$ 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 ($\pm$ 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.

FIGURE IX-7. Mean (+ S.E.) Seminal Vesicle 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-8. Mean ($\pm$ S.E.) Prostate Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-9. Mean ($\pm$ S.E.) Epididymis Weights of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-10. Mean ($\pm$ S.E.) Serum Prolactin Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-11. Mean ($\pm$ S.E.) Luteinizing Hormone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-12. Mean ($\pm$ S.E.) Testosterone Concentration of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-13. Mean ($\pm$ S.E.) Follicle Stimulating Hormone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

FIGURE IX-14. Mean ($\pm$ S.E.) Corticosterone Concentrations of Juvenile Male Rats Exposed or Sham Exposed to a 60-Hz Electric Field at 100 kV/m from 20 to 56 Days of Age. N = 8 rats/group at each measurement time.

D. DISCUSSION

Exposure of rats to 60-Hz electric fields at 100 kV/m for 30 or 120 days appears to reduce the mean level and the variability of circulating adrenocorticoids in adult rats. Similar effects may occur when rats are exposed during early development, i.e., 20-56 days of age. Currently, we have no evidence that the rats are stressed, and based on the results of other measures (see Section VII and X), it seems unlikely that the apparent lower cortical steroid levels are representative of the exhaustion stage of adaptation to extreme stress. No other endocrine parameters have shown consistently significant differences or notable trends as a result of electric-field exposure.

E. FUTURE PLANS

A replicate of the experiment in which rats are exposed for 120 days is in progress. In addition, the experiment to examine different sampling procedures on serum hormone levels will be replicated. Future studies will concentrate on neurotransmitters and brain biochemistry.

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X. CARDIOVASCULAR FUNCTION

Principal Investigators: D. I. Hilton and R. D. Phillips

ABSTRACT

Electrocardiogram (ECG), heart rate, blood pressure and vascular reactivity measurements were made on rats exposed to 60-Hz electric fields. Heart-rate measurements were made on animals after exposure to 100 kV/m for 1 or 4 mo. Blood pressure and vascular reactivity measurements were made on animals after exposure to 100 kV/m for 35 days or 4 mo. In addition, to test for physiological reserve capabilities, rats were exposed to 100 kV/m for 1 mo and then subjected to cold stress. Heart rate, deep colonic temperature and skin temperature were recorded before, during and after cold exposure. Plasma corticosterone and thyrotropin levels were also measured, both in rats exposed to electric fields alone and in those exposed to electric fields and then subjected to cold stress. Blood pressure and heart-rate measurements were also made on rats during exposure to electric fields. No significant differences in heart rates, ECG patterns, blood pressure or vascular reactivity were found between exposed and sham-exposed animals during exposure or after 1 or 4 mo of exposures. We also found that the physiological and endocrine response to cold stress did not differ between electric-field-exposed animals and sham-exposed controls.

A. INTRODUCTION

A number of investigators have reported effects of high-strength electric fields on the cardiovascular system. Clinical investigations in the USSR (Asanova, 1966) revealed that substation workers who are occupationally exposed to electric fields exhibit signs of unstable pulse and arterial blood pressure. Cerretelli and Malaguti (1976) reported that arterial blood pressure in rabbits exposed to 50-Hz fields at 80 kV/m for 500 hr tended to be higher than in controls. Coate and Teeters (1970) measured blood pressure in dogs exposed to 45-Hz or 75-Hz electric

fields at 10 kV/m, a magnetic field of 1 gauss and a 0.5-mA ground current. He found that the animals had significantly elevated arterial pressure after 3 wk of exposure. The response of the cardiovascular system to controlled hemorrhage was measured in dogs exposed to 15-kV/m fields for 5 hr (Gann, 1976, personal communication). Electric-field-exposed animals, when allowed to hemorrhage, showed lower mean arterial and pulse pressures than controls. In addition, heart-rate values of exposed animals were considerably higher than those of controls during bleeding.

Studies have also been made to assess whether electric-field exposure could produce stress-related physiological responses. Marino et al. (1977) reported lower serum corticosterone levels and enlarged adrenals and pituitaries in rats exposed to 15-kV/m fields for 1 mo. Investigations by Poznaniak et al. (1977) showed increased levels of serum corticosterone in mice in response to the electric field being turned on and off at 25 or 50 kV/m.

Many of the reported effects of electric-field exposure on cardiovascular function might be responses to secondary factors associated with the electric-field exposure systems. These factors include: 1) corona discharges resulting in audible noise and ozone production; 2) discharge shocks to the animals because of differences in electrical potential between the animal and the caging, feeding or watering systems; 3) audible noise from the transformer; 4) electric-field-induced vibration of the electrodes and/or caging system. Since reported effects may be responses to secondary field factors, we are presently investigating the possibility

of cardiovascular and endocrine changes in rats exposed to 60-Hz electric fields, using an exposure facility that is free of secondary factors such as corona, ozone, shocks and significant cage vibration.

B. METHODS

Techniques for obtaining electrocardiograms and heart-rate measurements from rats were described in the last Annual Report (Phillips et al., 1978). Two additional studies involving heart-rate measurements were conducted during this past year.

Heart rates were measured on 20 female Sprague-Dawley rats after exposure to a 100-kV/m electric field for 1 mo. The main purpose of this experiment was to obtain cardiovascular data on female animals comparable to those described in males in the last report, since the longer-duration studies (i.e., 120 days) required the use of females.* These females, like the males in the previous experiments (Phillips et al., 1978), were acclimated to the restraint holders used during physiological measurements in order to minimize the possibility of stress-related effects of confinement. Heart rates were measured within 1 hr after the end of the electric-field exposure. Heart-rate measurements were also taken on female rats after exposure to a 100-kV/m field for 4 mo.

Blood pressure and vascular reactivity were measured on rats that were exposed to 100-kV/m fields for 35 or 120 days. Methods for these procedures were modified from those reported by Phillips and Kimeldorf

*Adult males (older than ~ 100 days of age) are too large to fit in the holders used to contain the animal during measurements.

(1963). In both experiments, all the animals underwent the same acclimation in the restraint holder as was used for ECG and heart-rate measurements. After the last exposure session, each animal was anesthetized with ethrane (Enflurane, Ohio Medical Products) and the left femoral artery and vein were cannulated with polyethelene tubing (I.D., 0.58 mm; O.D., 0.965 mm). The arterial cannula was connected to a pressure transducer (Micron, MP-15) and pressures were recorded on a polygraph (Grass Model 7). After surgical procedures, the incision was infused with a 2% solution of lidocaine HCl. Each animal was then placed in a restraint holder and allowed to recover for 1 hr. Systolic, diastolic, pulse and mean systemic pressures were monitored continuously for 1 hr. Heart rates were determined by counting pressure pulses per min.

After the recovery period, when the animals were fully conscious, a solution of 5% dextrose in sterile water was given as a control injection. After baseline measurements were obtained, a single injection of phenylephrine (Neo-Synephrine HCl, Winthrop), 1 μ g in 1 ml of 5% dextrose in sterile water, was given and the change in mean systemic pressure was recorded during the injection. Vascular reactivity was scored as the increase in the mean systemic arterial blood pressure that was caused by the phenylephrine injection. Diastolic, systolic and pulse pressures, and heart-rate values were recorded $\sim$ 10 sec after injection. Fifteen minutes after the first injection a second identical dose of phenylephrine was given, and measurements of the same parameters were made.

Two experiments were conducted to assess for physiological reserve capabilities of rats subjected to cold stress. In both experiments, rats were exposed or sham exposed to a 100-kV/m electric field for 1 mo.

In the first experiment, animals were acclimated to restraint holders during the last 7 days of exposure (~ 2 hr/day). These acclimations were carried out during the 4 hr/day when the exposure system was de-energized for animal care. After the last day of exposure, each animal was placed in a restraint holder, and heart rate, deep colonic temperature and skin temperature were monitored. Deep colonic temperature was recorded using a thermistor probe inserted, via the rectum, 4 cm into the descending colon. Skin temperature was recorded with a surface thermistor probe attached to the dorsal surface at the base of the tail. Baseline values were taken for 30 min (at 5-min intervals). Each animal was then placed in a cold box ($-13 \pm 1^\circ\text{C}$) for a period of 1 hr and recordings of all parameters were continued. Measurements were also continued for 1 hr after cold exposure.

In the second experiment, 20 male rats were exposed and 20 were sham exposed to a 100-kV/m field for 1 mo. Basal plasma corticosterone and thyrotropin levels were measured in 10 exposed and 10 sham-exposed animals within 1 hr after exposure. The remaining animals in the exposed and sham-exposed groups ($N = 10/\text{group}$) were placed in a cold box ($-13 \pm 1^\circ\text{C}$) for 1 hr prior to blood sampling.

The same experimental procedures were used for obtaining blood from animals maintained at room temperature as for those exposed to cold stress for 1 hr. Exposed and sham-exposed animals were taken alternately from the electric-field exposure room to another room, where they were anesthetized with a fluothane (Halothane, Ayerst)/ NO_2/O_2 mixture. Blood was drawn via intracardiac puncture, and immediately

centrifuged. The plasma portion was frozen and stored for later analysis. A 3-min time limit was imposed for the procedure, from the time of first handling the animal to the time it took to obtain the blood sample. All sampling (basal and cold stress) was made between 8:00 a.m. and 11:30 a.m. The plasma from each rat was divided into two samples; one sample was analyzed fluorometrically for corticosterone by Battelle's analytical chemistry laboratory, using a modified procedure of Steenburg and Thomasson (1964). The other sample was analyzed at the University of Rochester, using a radioimmune assay technique (Murphy, 1967). Concentrations of thyrotropin were measured at the University of Rochester, by Nancy Lebda, using reagents supplied by Dr. A. F. Parlow of the National Institute of Arthritis, Metabolism and Digestive Diseases, Rat Pituitary Hormone Distribution Program.

Blood pressure and heart-rate measurements were also made on rats during exposure to a 100-kV/m field. The procedure for obtaining blood pressure has already been described. To avoid 60-Hz interference with the electrical signals generated from the blood-pressure transducer, the entire length of the transducer cable was wrapped in grounded, conductive wire braid. After the animals were allowed to recover from surgery, 300 units of heparin sodium (Panheprin, Abbott) were introduced into the femoral artery to help prevent clotting in the arterial cannulae during the exposure and recording period. A Faraday shield was placed over the sham-exposed animal, and measurements of heart rate, pulse and mean blood pressures were recorded simultaneously on exposed and sham-exposed animals. After 0.5 hr of baseline measurements, the field was energized to 100 kV/m for 1 hr while measurements

were continued. Cardiovascular parameters were also recorded for 0.5 hr after terminating exposure.

C. RESULTS

Results for ECG and heart-rate measurements on rats exposed to electric fields at 80 kV/m for 8 hr, 40 hr or 1 mo were presented in the last Annual Report. No significant differences were found between exposed and sham-exposed groups.

Figures X-1 and X-2 show the heart-rate measurements taken on female rats exposed to 100-kV/m electric fields for 1 and 4 mo, respectively. Statistical analyses of the data (Student's t test) showed no significant differences between exposed and sham-exposed groups in either experiment.

Tables X-1 and X-2 show the blood pressure and vascular reactivity measurements made on rats exposed or sham-exposed to 100-kV/m electric fields for 35 days or 4 mo, respectively. Statistical analysis of the data from the 35-day exposure showed no significant differences in any of the data between exposed or sham-exposed controls (Student's t test). The only significant difference between exposed and sham-exposed groups after 120 days of exposure was in the baseline pulse pressure prior to the second phenylephrine injection [not shown in Table X-1; mean pulse pressure of the exposed group (56 ± 1 mm Hg) was significantly ($p < 0.01$) higher than that of the sham-exposed group (51 ± 1 mm Hg)]. However, the increases in mean pressure in response to the first and second injections of phenylephrine were not significantly different between exposed

FIGURE X-1. Mean Heart Rates ($\pm$ S.E.) of Rats After 1 Mo Exposure (■) or Sham Exposure (o) to 60-Hz Electric Fields at 100 kV/m. N = 20/group.

FIGURE X-2. Mean Heart Rates ($\pm$ S.E.) of Rats After 4 Mo Exposure (■) or Sham Exposure (o) to 60-Hz Electric Fields at 100 kV/m. N = 20/group.

TABLE X-1. Cardiovascular Measurements* of Rats Exposed (N = 12) or Sham-Exposed (N = 13) to a 60-Hz, 100-kV/m, Electric Field for ~ 20 hr/day for 35 Days*

	Mean $\pm$ S.E.	
	Exposed	Sham-Exposed
Heart Rate (beats/min)	343 $\pm$ 9	362 $\pm$ 12
Diastolic Pressure (mm Hg)	95 $\pm$ 2	97 $\pm$ 2
Systolic Pressure (mm Hg)	155 $\pm$ 3	160 $\pm$ 3
Pulse Pressure (mm Hg)	60 $\pm$ 2	63 $\pm$ 3
Mean Pressure (mm Hg)	115 $\pm$ 2	117 $\pm$ 3
Increase in Mean Pressure (mm Hg) - 1st phenylephrine	34 $\pm$ 2	34 $\pm$ 2
Increase in Mean Pressure (mm Hg) - 2nd phenylephrine	37 $\pm$ 2	35 $\pm$ 2

*Baseline values are shown for each parameter, with additional values showing the change (increase) in mean blood pressure in response to the first and second doses of phenylephrine.

TABLE X-2. Cardiovascular Measurements* of Rats Exposed (N = 12) or Sham-Exposed (N = 13) to a 60-Hz, 100-kV/m Electric Field for ~ 20 hr/day for 120 Days*

	Mean $\pm$ S.E.	
	Exposed	Sham-Exposed
Heart Rate (beats/min)	386 $\pm$ 5	401 $\pm$ 13
Systolic Pressure (mm Hg)	153 $\pm$ 3	154 $\pm$ 2
Diastolic Pressure (mm Hg)	99 $\pm$ 2	102 $\pm$ 2
Pulse Pressure (mm Hg)	54 $\pm$ 2	52 $\pm$ 2
Mean Pressure (mm Hg)	118 $\pm$ 2	119 $\pm$ 3
Increase in Mean Pressure (mm Hg) - 1st phenylephrine	43 $\pm$ 2	44 $\pm$ 2
Increase in Mean Pressure (mm Hg) - 2nd phenylephrine	42 $\pm$ 1	45 $\pm$ 1

*Baseline values are shown for each parameter, with additional values showing the change (increase) in mean blood pressure in response to the first and second doses of phenylephrine.

and sham-exposed groups. Mean systolic/diastolic pressures in exposed and sham-exposed groups were, respectively, 157/102 and 159/105 after the first injection, and 159/105 and 157/104 after the second injection.

Figures X-3 through X-5 show heart rate, skin temperature and colonic temperature, respectively, of animals exposed to acute cold after exposure or sham exposure to a 100-kV/m electric field. The responses of exposed and sham-exposed animals to cold stress were identical. Recovery after cold exposure was also the same in both groups of rats.

Table X-3 shows the plasma corticosterone and thyrotropin levels in rats exposed to 100-kV/m fields for 1 mo and in rats subjected to cold stress after electric-field exposure for 1 mo.

Mean plasma corticosterone and thyrotropin levels measured in rats exposed or sham-exposed to electric fields were not significantly different (unequal variance t test, $p < 0.05$). Corticosterone and thyrotropin levels in rats subjected to cold stress after electric-field exposure were not significantly different from controls.

The apparent large difference in mean concentration of corticosterone (by radioimmunological assay) between exposed and sham-exposed animals was not statistically significant because of the very high variance within the sham-exposed group. However, variances were statistically different (F-test, $p < 0.05$) between exposed and sham-exposed groups not exposed to cold stress. Accordingly, this part of the experiment (without cold stress) was repeated. The results are shown in Table X-4. We failed to replicate the previous findings. Both mean values and variances were the same in exposed and sham-exposed groups.

FIGURE X-3. Mean ($\pm$ S.E.) Heart Rates of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed ($\blacksquare$) or sham exposed ($\circ$) to 60-Hz electric fields at 100 kV/m for 30 days prior to the cold stress test. $N = 10/\text{group}$.

FIGURE X-4. Mean ($\pm$ S.E.) Skin Temperature of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed ($\blacksquare$) or sham exposed ($\circ$) to 60-Hz electric fields at 100 kV/m for 30 days prior to cold stress test. $N = 10/\text{group}$.

FIGURE X-5. Mean ($\pm$ S.E.) Deep Colonic (Core) Temperature of Rats Before, During and After Being Subjected to Low Temperature (-13°C). Animals were exposed ($\blacksquare$) or sham exposed ($\circ$) to 60-Hz electric fields at 100 kV/m for 30 days prior to the cold stress test. $N = 10/\text{group}$.

TABLE X-3. Mean ($\pm$ S.E.) Plasma Corticosterone and Thyrotropin Levels in Rats Exposed or Sham-Exposed to a 100-kV/m Electric Field for 1 Mo. Blood sampling was done within 1 hr postexposure. In addition to electric-field exposure, half the animals (10 exposed, 10 sham-exposed) were also exposed to cold stress ($-13 \pm 1^\circ\text{C}$) for 1 hr prior to blood sampling.

	<u>Exposed</u>	<u>Sham-Exposed</u>	<u>Exposed + Cold Stress</u>	<u>Sham-Exposed + Cold Stress</u>
N	10	10	10	10
Body Weight (g)	446 $\pm$ 14	428 $\pm$ 15	411 $\pm$ 11	426 $\pm$ 7
Plasma Corticosterone, Fluorometric Technique (ng/ml)	123 $\pm$ 17	175 $\pm$ 45	570 $\pm$ 32	534 $\pm$ 36
Plasma Corticosterone, Radioassay Technique (ng/ml)	35 $\pm$ 8	287 $\pm$ 138	620 $\pm$ 28	619 $\pm$ 40
Plasma Thyrotropin (μ units/ml)	920 $\pm$ 93	1122 $\pm$ 145	546 $\pm$ 46	577 $\pm$ 32

TABLE X-4. Mean ($\pm$ S.E.) Plasma Corticosterone Concentrations (Radioimmune Assay) in Rats Exposed or Sham-Exposed to a 100-kV/m Electric Field for 1 Mo.

	Corticosterone (ng/ml)		
	N	Mean	S.E.
Exposed	20	42.1	11.6
Sham-Exposed	20	35.6	9.5

Heart rate values and blood pressures of rats during exposure or sham exposure to a 60-Hz electric field at 100 kV/m for 1 hr are shown in Table X-5. Values for both exposed and sham-exposed groups were comparable during the 0.5 hr preceding exposure. Electric-field exposure had no effect on heart rate or blood pressure during the 1-hr exposure period, nor during the 0.5-hr period after exposure.

D. DISCUSSION AND CONCLUSIONS

Electric-field exposure for periods up to and including 1 mo of duration at 80 or 100 kV/m did not affect the ECG wave pattern or heart rate of rats. Exposures for 1 hr, 35 days or 4 mo at 100 kV/m did not affect heart rate, blood pressure or vascular reactivity. Exposure to a 100-kV/m field for 1 mo did not affect the levels of plasma corticosterone or thyrotropin, and had no effect on the corticosterone, thyrotropin, skin temperature, colonic temperature or heart-rate response to cold stress.

Many of these results cannot be directly compared to other investigators' findings. Cerretelli and Malaguti (1976), Gann (personal communication, 1976), Coate and Teeters (1970) and Poznaniak et al. (1977) each used different species of animals in their studies. Also, in one of those studies, 45-, 50- and 75-Hz frequencies were used, whereas our study used a 60-Hz frequency exclusively. In the experiment by Coate and Teeters (1970), the animals were exposed not only to electric fields, but also to a magnetic field and a ground current. The observed effects might have been due to any one or a combination of these three components.

TABLE X-5. Heart Rate, Mean Blood Pressure, Systolic Pressure, Diastolic Pressure and Pulse Pressure of Rats Before, During and After Exposure or Sham Exposure (N = 12/group) to a 100-kV/m Electric Field for 1 hr*

	Pre-exposure Baseline		During Exposure		Postexposure	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Heart Rate (beats/min)	419 $\pm$ 8	417 $\pm$ 12	406 $\pm$ 8	417 $\pm$ 10	395 $\pm$ 8	408 $\pm$ 10
Mean Arterial Blood Pressure (mm Hg)	121 $\pm$ 3	122 $\pm$ 2	120 $\pm$ 3	118 $\pm$ 2	119 $\pm$ 3	115 $\pm$ 2
191 Systolic Blood Pressure (mm Hg)	155 $\pm$ 3	158 $\pm$ 3	151 $\pm$ 3	152 $\pm$ 2	151 $\pm$ 3	148 $\pm$ 2
Diastolic Blood Pressure (mm Hg)	104 $\pm$ 2	103 $\pm$ 2	102 $\pm$ 2	102 $\pm$ 1	102 $\pm$ 3	100 $\pm$ 1
Pulse Pressure	52 $\pm$ 2	54 $\pm$ 2	49 $\pm$ 3	50 $\pm$ 2	49 $\pm$ 3	48 $\pm$ 2

*Values before, during and after exposure are the respective means of measurements made over 0.5-, 1- and 0.5-hr intervals.

Marino et al. (1977) reported that exposure of rats to a 60-Hz, 15-kV/m electric field for 30 days resulted in physiological stress. In one of two experiments that we conducted, we found that the exposed group exhibited lower (but not significantly different) corticosterone levels in the plasma. Also, in one of our two experiments, variance among sham-exposed rats was significantly higher than among exposed rats.

E. FUTURE PLANS

In view of our failure to detect any effects of 60-Hz electric field exposure on cardiovascular function, no further research in this area is planned.

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XI. NEUROPHYSIOLOGY

Principal Investigator: R. A. Jaffe

ABSTRACT

Following 30 days of electric-field exposure, superior cervical sympathetic ganglia, vagus and sciatic nerves were removed from rats anesthetized with urethan, placed in a temperature-controlled chamber ($37.5 \pm 4^{\circ}\text{C}$) and superfused with a modified mammalian Ringer's solution equilibrated with 95% O_2 and 5% CO_2 . Several parameters and tests were used to characterize synaptic transmission and peripheral nerve function. These included amplitude, area, and configuration of the postsynaptic or whole-nerve compound action potential; conduction velocity; accommodation; refractory period; strength-duration curves; conditioning-test response; frequency response; post-tetanic response; and high-frequency-induced fatigue. The results of the neurophysiologic tests and measurements so far completed indicate that synaptic transmission is significantly and consistently affected by chronic (30-day) exposure to a 60-Hz, high-voltage electric field. In addition, there are trends in the data that suggest a generalized increase in neuronal excitability in exposed animals.

A. INTRODUCTION

Several reports in the literature on biological effects of electric fields have suggested that mammalian and avian nervous systems are affected by exposure to extremely low-frequency (16-60 Hz) electric fields (Adey and Bawin, 1977; Carter and Graves, 1976; Dumansky et al., 1976). The purpose of this study is to examine specific components of the mammalian (rat) nervous system in three broad areas (synaptic transmission, peripheral nerve function, neuromuscular function), in order to detect any possible effects of chronic (30-day) exposure to a 100-kV/m, 60-Hz electric field. The experiments are designed to detect potential

effects prior to the development of obvious clinical signs, thereby providing realistic estimates of threshold exposure levels.

In the last Annual Report (Phillips et al., 1978) data were presented which suggested that synaptic transmission in superior cervical sympathetic ganglia of rats was affected by exposure of the whole animal for 30 days to a 100-kV/m, 60-Hz electric field. Specifically, we reported that animals exposed to an electric field showed a slight but detectable shift in the conditioning-test (C-T) ratio ($p < 0.05$ @ 300 msec; $p < 0.08$ overall), which suggested increased excitability. There was also an increase in time-to-half-amplitude during fatigue testing ($p < 0.05$), which suggested a decrease in susceptibility to fatigue by high-frequency stimulation. These findings prompted us to repeat the experiments in order to verify the results. In this report, results from the replicate experiments on synaptic transmission are presented.

Results from experiments on peripheral nerve function also are presented in this report. Data from these experiments strongly suggested that the conduction velocity of the action potential in unmyelinated nerve fibers was increased (+10%; $p < 0.01$) following 30 days of exposure to a 100-kV/m, 60-Hz electric field. This increase in conduction velocity was accompanied by a shift in the strength-duration curve consistent with increased excitability of the nerve membrane. However, the results of two replicate experiments on unmyelinated (vagal) nerve fibers failed to confirm the increased conduction velocity observed in the first experiment. Similar experiments performed on myelinated

nerve fibers failed to reveal any difference between exposed and sham-exposed animals. Details of these experiments are discussed below.

B. SYNAPTIC TRANSMISSION REPLICATE STUDY

1. Methods. A total of 46 male Sprague-Dawley (Hilltop) rats were examined. One group of 24 rats was exposed to a 100-kV/m, 60-Hz electric field for 30 days. The other group (22 rats) was maintained in identical housing without the electric field (sham-exposed). The exposure system has been described in detail in previous reports (Phillips and Kaune, 1977; Phillips et al., 1978).

Superior cervical sympathetic ganglia, including segments of the cervical sympathetic trunk (CST) and internal carotid nerve (ICN), were removed from 24 electric-field-exposed and 22 sham-exposed rats. Rats were anesthetized with urethan (1.25 g/kg) and the ganglia were removed within 2 hr after completion of the 30-day exposure period for C-T ratio measurements, and between 4 and 6 hr postexposure for the second ganglion fatigue study. Ganglia were maintained in vitro in a specially constructed, temperature-controlled lucite chamber, where they were continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% O₂ and 5% CO₂.

Suction electrodes were used for both stimulation of CST and recording of ICN activity. Presynaptic nerve fibers were stimulated supramaximally by square-wave pulses of 0.3-msec duration. A signal-averaging computer was used to obtain detailed time and amplitude

information during the course of individual experiments. In addition, postsynaptic compound action potentials were recorded photographically for subsequent analyses.

Several parameters were used to compare the properties of synaptic transmission in the two groups:

- (1) Characteristics of the postsynaptic compound action potential [including amplitude, area, configuration, maximum rate of rise (dV/dt), maximum rate of fall ($-dV/dt$) and time relationships]
- (2) Conduction velocity of the compound action potential--measured between the stimulating and recording electrodes, therefore including synaptic delay
- (3) Conditioning-test response--an adaptation of the method used by Whitcomb and Santolucito (1976), which measured the excitability and the refractory period of the ganglionic synapses. This is done by applying two stimuli to the preganglionic nerve and measuring the postganglionic action potential heights as a function of the time interval between the stimuli
- (4) High-stimulus, frequency-induced fatigue--preganglionic nerve fibers were stimulated at 20 Hz for 60 min while the amplitude of the postganglionic action potential was monitored. Recovery at a stimulus frequency of 0.5 Hz was measured during the subsequent 10 min. In order to duplicate the original experiment on second ganglia, the ganglia used for this fatigue test were left in the urethan-anesthetized animal 4-6 hr before removal and subsequent fatigue testing.

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.

2. Results. No significant differences between electric-field-exposed and sham-exposed animals were found among the parameters used to characterize the postsynaptic compound action potentials except for the rate of rise, dV/dt (Table XI-1). Measurements of the conduction velocity (reduced by inclusion of synaptic delay) are shown in Table XI-2. No significant differences were found between exposed and sham-exposed animals.

The C-T response ratio was different in this replicate study, as in the first study, for electric-field-exposed animals compared to sham-exposed animals (Figure XI-1). Mean values for the postsynaptic compound action potential height ratio at specific conditioning-test intervals are shown in Tables XI-3a and 3b. The tables differ in the method used to acquire the data: In Table XI-3a, C-T ratios were determined by direct measurement of traces on a storage oscilloscope; while in Table XI-3b, C-T ratios were determined by a computer average at four separate trials at each time interval. Although the data presented in both tables are qualitatively identical, slight differences in statistical significance are apparent. In the original experiment (see Phillips, et al., 1978), the C-T ratio at only one of the 11 C-T intervals was significantly ($p < 0.05$) increased for electric-field-exposed animals compared to the sham-exposed controls; whereas in this replicate experiment, seven of the 11 C-T ratios were significantly ($p < 0.05$) increased. A statistical

TABLE XI-1. Characteristics of the Postsynaptic Compound Action Potential Recorded from Rat Superior Cervical Sympathetic Ganglia Following 30 days Exposure to a 60-Hz, 100-kV/m Electric Field.

	Exposed		Sham-Exposed		p*
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Amplitude (mV)	40	8.65 $\pm$ 0.29	30	8.22 $\pm$ 0.30	0.311
Area (mV·msec)	40	46.6 $\pm$ 1.7	30	44.3 $\pm$ 1.8	0.268
Rate of Rise (+dV/dt) (V/sec)	40	5.00 $\pm$ 0.18	30	4.37 $\pm$ 0.21	0.024
Time before Peak to Maximum +dV/dt (msec)	40	2.2 $\pm$ 0.1	30	2.1 $\pm$ 0.1	0.309
Rate of Fall (-dV/dt)(V/sec)	40	-4.08 $\pm$ 0.30	30	-4.10 $\pm$ 0.19	0.948
Time after Peak to Maximum -dV/dt (msec)	40	2.4 $\pm$ 0.1	30	2.4 $\pm$ 0.1	0.789

* p values determined using Student's *t* test

TABLE XI-2. Conduction Velocity (Including Synaptic Delay) of the Postsynaptic Compound Action Potential Recorded from the Internal Carotid Nerve of the Rat Superior Cervical Sympathetic Ganglion Following 30 Days Exposure to a 60-Hz, 100-kV/m Electric Field.

	Exposed		Sham-Exposed		p*
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Peak Conduction Velocity (m/sec)	40	0.40 $\pm$ 0.01	29	0.38 $\pm$ 0.01	0.233
Chamber Temperature (°C)	40	32.1 $\pm$ 0.1	30	32.1 $\pm$ 0.1	0.540
Inflection Conduction Velocity (m/sec)	40	0.69 $\pm$ 0.01	29	0.69 $\pm$ 0.02	0.868

*p values determined using Student's *t* test

FIGURE XI-1. Comparison of Conditioning-Test Response Ratios of Rats Exposed and Sham Exposed to 60-Hz Electric Fields at 100 kV/m

TABLE XI-3a. Mean Values ($\pm$ S.E.) for the Postsynaptic Compound Action Potential (Spike) Height Ratio at Specific Conditioning-Test Intervals. Conditioning stimulus rate = 0.5 Hz. Ratios determined by direct measurement from CRT.

<u>Conditioning-Test Interval (msec)</u>		<u>C-T Ratio, Exposed</u>	<u>C-T Ratio, Sham-Exposed</u>
25	Mean	1.06*	1.00
	$\pm$ S.E.	0.01	0.03
	N	17	10
50	Mean	1.08	1.03
	$\pm$ S.E.	0.02	0.02
	N	19	11
100	Mean	0.89	0.86
	$\pm$ S.E.	0.02	0.03
	N	19	11
150	Mean	0.81	0.76
	$\pm$ S.E.	0.02	0.02
	N	19	11
200	Mean	0.80	0.75
	$\pm$ S.E.	0.02	0.02
	N	19	11
300	Mean	0.88*	0.82*
	$\pm$ S.E.	0.02	0.02
	N	19	11
400	Mean	0.95*	0.91*
	$\pm$ S.E.	0.01	0.01
	N	19	11
500	Mean	0.98*	0.94*
	$\pm$ S.E.	0.005	0.007
	N	19	11
600	Mean	0.99*	0.97*
	$\pm$ S.E.	0.004	0.005
	N	19	11
700	Mean	1.00*	0.98*
	$\pm$ S.E.	0.003	0.004
	N	19	11
800	Mean	1.00*	0.99*
	$\pm$ S.E.	0.002	0.003
	N	19	11

*Significantly different (Student's t test, $p < 0.05$)

TABLE XI-3b. Mean Values ($\pm$ S.E.) for the Postsynaptic Compound Action Potential (Spike) Height Ratio at Specific Conditioning-Test Intervals. Conditioning stimulus rate = 0.5 Hz. Ratios determined by computer average of four separate trials at each interval.

<u>Conditioning-Test, Interval (msec)</u>		<u>C-T Ratio, Exposed</u>	<u>C-T Ratio, Sham-Exposed</u>
25	Mean	1.06	1.03
	$\pm$ S.E.	0.01	0.03
	N	17	9
50	Mean	1.08	1.03
	$\pm$ S.E.	0.02	0.03
	N	19	10
100	Mean	0.90	0.84
	$\pm$ S.E.	0.02	0.02
	N	19	10
150	Mean	0.81	0.75
	$\pm$ S.E.	0.02	0.02
	N	19	10
200	Mean	0.80*	0.74*
	$\pm$ S.E.	0.02	0.02
	N	19	10
300	Mean	0.88*	0.82*
	$\pm$ S.E.	0.02	0.02
	N	19	10
400	Mean	0.95*	0.91*
	$\pm$ S.E.	0.01	0.01
	N	19	10
500	Mean	0.98*	0.94*
	$\pm$ S.E.	0.005	0.008
	N	19	10
600	Mean	0.99*	0.97*
	$\pm$ S.E.	0.005	0.005
	N	19	10
700	Mean	0.99*	0.98*
	$\pm$ S.E.	0.003	0.006
	N	19	10
800	Mean	1.00*	0.99*
	$\pm$ S.E.	0.002	0.002
	N	19	8

*Significantly different (Student's t test, $p < 0.05$)

analysis of the original C-T ratio curves yielded a 92% chance that the curves were different ($p = 0.08$, not statistically significant). An analysis of the curves in the present experiment, however, indicates that they are significantly different ($p < 0.05$; multivariate test of the coefficients, obtained from fitting orthogonal polynomials to the curves).

Synaptic fatigue was tested by stimulating the presynaptic nerve trunk at 20 Hz for 60 min. The amplitude of the postsynaptic compound action potential was measured at intervals throughout the period of high-frequency stimulation (20 Hz) and during a 10-min recovery period, at the control stimulus frequency of 0.5 Hz. These data are presented in Table XI-4, reported as the ratio of the spike amplitude during fatigue and recovery to the spike amplitude measured prior to the start of the test. There were no significant differences between electric-field-exposed and sham-exposed animals. In addition, the calculated values for times-to-half-amplitude (exposed = 6.7 ± 1.3 min; sham-exposed = 10.6 ± 3.1 min) were not significantly different for either group. The variability of the times-to-half-amplitude for the exposed group, however, was significantly less (F-test; $p < 0.001$) than that for the sham-exposed group.

3. Discussion and Conclusions. The difference in the C-T response ratio between exposed and sham-exposed animals suggested in the original experiment is substantiated in the present experiment. The shape of the

TABLE XI-4. Fatigue Response to High-Frequency Stimulation. Superior cervical sympathetic ganglia were stimulated presynaptically at 20 Hz for 60 Min. The amplitude of the postsynaptic compound action potential was measured (computer averaged) at intervals throughout the period of stimulation. Data are expressed as the ratio of the 20-Hz spike amplitude to the amplitude of the spike during 0.5-Hz control stimulation. Stimulus frequency during recovery = 0.5 Hz.

<u>Time (min)</u>	<u>FATIGUE</u>											<u>RECOVERY</u>			
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>10</u>	<u>15</u>	<u>20</u>	<u>30</u>	<u>45</u>	<u>60</u>	<u>1</u>	<u>2</u>	<u>5</u>	<u>10</u>
<u>Exposed</u>															
Mean	0.79	0.70	0.63	0.56	0.54	0.44	0.40	0.42	0.41	0.41	0.39	0.68	0.75	0.82	0.84
S.E.	0.03	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.02	0.04
N	18	19	18	17	18	17	18	19	19	20	20	18	19	20	20
<u>Sham-Exposed</u>															
Mean	0.80	0.72	0.67	0.60	0.58	0.47	0.44	0.43	0.44	0.45	0.45	0.67	0.74	0.80	0.79
S.E.	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.03	0.02	0.03	0.03	0.03
N	19	18	17	16	18	17	18	19	19	19	19	16	18	19	19
p	NS*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*NS = not significant ($p > 0.05$)

C-T curve is determined by two overlapping phenomena: relative refractory period and recruitment from subliminally excited groups of postsynaptic neurons. Thus, as the interval between the conditioning and test stimuli decreases, increasing numbers of neurons enter their relative refractory periods and fail to elicit a postsynaptic response, causing the amplitude of the test spike to decrease relative to the conditioning spike. At the shorter C-T intervals, however, temporal summation of stimuli permits recruitment of subliminally excited neurons and the test spike amplitude increases, eventually exceeding the amplitude of the conditioning spike. The upward shift in the C-T curve seen in electric-field-exposed animals is consistent with a hypothesis of a persistent field-induced increase in neuronal excitability.

In the original experiment, the exposed and sham-exposed fatigue curves for ganglia that were allowed to remain in the urethan-anesthetized animal for several hours ("second" ganglia) were not significantly different. The times-to-half-amplitude, however, were significantly different (7 exposed = 16.2 ± 5.5 min; 8 sham-exposed = 2.8 ± 0.7 min; $p < 0.05$). In the present study, under conditions duplicating those of the original experiment (see interexperiment comparison of standard parameters, Table XI-5), this difference was not confirmed. It is likely that the results from the original second ganglion fatigue experiment were spurious. In that experiment, the fatigue curves for several of the exposed animals, although rapidly decreasing toward half-amplitude in the first few minutes, failed to actually reach half-amplitude for

TABLE XI-5. Mean Values ($\pm$ S.E.) for Standard Parameters Measured at the Time of the Original Post-Synaptic Compound Action Potential Experiment (I) Compared with the same Parameters Measured During the Replicate Experiment (II). (N shown in parentheses.)

	I		II	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Action Potential Amplitude (mV)	7.6 ± 0.6 (12)	7.2 ± 0.5 (15)	8.6 ± 0.3 (40)	8.2 ± 0.3 (30)
Action Potential Area (mV·msec)	36.7 ± 3.4 (12)	35.6 ± 3.3 (15)	46.6 ± 1.7 (40)	44.3 ± 1.8 (30)
Inflection Conduction Velocity (m/sec)	0.78 ± 0.03 (11)	0.85 ± 0.08 (12)	0.69 ± 0.01 (40)	0.69 ± 0.02 (29)
Peak Conduction Velocity (m/sec) (Including synaptic delay)	0.60 ± 0.02 (11)	0.63 ± 0.04 (11)	0.40 ± 0.01 (40)	0.38 ± 0.01 (29)
Chamber Temperature (°C)	32.1 ± 0.1 (11)	31.9 ± 0.1 (12)	32.1 ± 0.1 (40)	32.1 ± 0.1 (30)
Age of Rats (days)	83.6 ± 0.6 (14)	84.2 ± 0.6 (15)	88.8 ± 0.6 (24)	88.3 ± 0.5 (22)

30-45 min. Thus, had the time-to-0.4-amplitude been selected for comparison, no difference would have been observed. Results from the present fatigue experiment on "second" ganglia closely resemble those obtained with "first" ganglia reported for the original experiment in the 1978 Annual Report. The fatigue curves for electric-field-exposed and sham-exposed animals are not significantly different.

C. PERIPHERAL NERVE FUNCTION: ORIGINAL AND REPLICATE STUDIES

1. Methods. Thirty-six male Sprague-Dawley (Hilltop) rats, 77 to 84 days of age, were exposed to a 100-kV/m, 60-Hz electric field for 30 days, while an equal number were maintained in identical housing without the electric field (sham-exposed). The exposure system has been described in detail in previous reports (Phillips and Kaune, 1977; Phillips et al., 1978).

A segment of a cervical vagus nerve was removed from each of 18 electric-field-exposed and 18 sham-exposed rats. The sciatic nerve, including a segment of the tibial nerve, was removed from the remaining 18 electric-field-exposed and 18 sham-exposed rats. Rats were anesthetized with urethan (1.25 g/kg) and the nerves were removed within 2-5 hr after the completion of the 30-day exposure period. Nerves were maintained in vitro in a specially constructed, temperature-controlled ($37.5 \pm 0.3^{\circ}\text{C}$) lucite chamber, where they were continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% O_2 and 5% CO_2 .

Platinum-iridium electrodes were used to stimulate and record activity in sciatic nerves, and suction electrodes were used to stimulate and record activity in vagus nerves. Compound action potentials were recorded photographically for subsequent analyses. In addition, a signal-averaging computer was used to obtain detailed time and amplitude information during the course of individual experiments.

Several parameters were used to compare peripheral nerve function in the exposed and sham-exposed groups:

- (1) Characteristics of the compound action potential [including amplitude, area, configuration, rate of rise (dV/dt), and rate of fall ($-dV/dt$)]
- (2) Conduction velocity of the compound action potential, measured at the beginning of the compound action potential (inflection conduction velocity), and at the peak of the compound action potential (peak conduction velocity); the signal-averaging computer was used to obtain an "averaged" ($N = 16$) peak conduction velocity.
- (3) Absolute and relative refractory periods--measured by applying two supramaximal stimuli to the nerve and measuring the height of the second compound action potential as a function of the time interval between the stimuli
- (4) Accommodation index--stimulating current was applied as a slowly increasing ramp function with a fixed duration (5 msec for sciatic nerves, 25 msec for vagus nerves); the amplitude

of the stimulus ramp necessary to elicit a threshold response was defined as the accommodation index

- (5) Strength-duration curve--the stimulus strength (μA) necessary to elicit a threshold response at fixed stimulus durations
- (6) Changes in poststimulus excitability--nerves were repeatedly stimulated with two identical submaximal stimuli. The interval between the stimuli (conditioning-test interval) was adjusted until the first compound action potential (conditioning response) was double its original amplitude. The interval was then increased until the second compound action potential (test response) was at: (1) one-half its control amplitude, (2) lowest amplitude and (3) highest amplitude relative to its control amplitude.

In the replicate study, a group of 14 male, Sprague-Dawley (Hill-top) rats was exposed to a 100-kV/m, 60-Hz electric field for 30 days while an equal number was maintained in identical housing without the electric field (sham-exposed). The exposure system in the replicate was the same as in the original study. Segments of one or both cervical vagus nerves were removed 2-5 hr following the completion of the 30-day exposure period.

In the replicate experiment, the following parameters were examined:

- (1) Characteristics of the compound action potential [including amplitude, area, configuration, rate of rise (dV/dt), and rate of fall ($-dV/dt$)]

- (2) Conduction velocity of the compound action potential, measured at the peak of the B component and at the peak of the C component; this differs from the original experiment in that the inflection conduction velocity was not measured. Instead, conduction velocity was measured at the more precisely definable peak of the B component. A signal-averaging computer was used to obtain all conduction velocities.
- (3) Strength-duration-curve--the stimulus strength (μA) necessary to elicit a threshold response at fixed stimulus durations was measured.

Unless otherwise noted, differences between means of exposed and sham-exposed animals were compared by the Student's t test (unpaired, unequal or equal variance). Differences were considered to be significant if $p < 0.05$.

2. Results: Original Experiment. Significant differences between electric-field-exposed and sham-exposed animals were observed among the properties used to characterize peripheral nerve function. Table XI-6 contains mean values for the maximum amplitude and area of the compound action potential, as well as relative values for the maximum rates of potential change during the rising and falling phases. The maximum rate at which the compound action potential decreases appears to be reduced by electric-field exposure, although this effect is significant only for vagus (unmyelinated) nerve fibers. Conduction velocity data (Table XI-7)

TABLE XI-6. Properties of the Compound Action Potential in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

	Exposed		Sham-Exposed		
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	p
<u>Vagus Nerve</u>					
Maximum Amplitude (mV)	18	1.87 $\pm$ 0.14	15	2.12 $\pm$ 0.13	NS*
Area (mV•msec)	18	11.56 $\pm$ 1.10	14	11.32 $\pm$ 1.12	NS
Maximum dV/dt Rise (V/sec)	17	6.16 $\pm$ 0.61	16	7.62 $\pm$ 0.94	NS
Maximum dV/dt Fall (V/sec)	9	-1.91 $\pm$ 0.21	7	-2.39 $\pm$ 0.40	<0.05
<u>Sciatic Nerve</u>					
Maximum Amplitude (mV)	14	9.78 $\pm$ 0.75	14	8.30 $\pm$ 0.84	NS
Area (mV•msec)	15	2.42 $\pm$ 0.15	15	2.06 $\pm$ 0.26	NS
Maximum dV/dt Rise (V/sec)	5	17.0 $\pm$ 1.8	7	15.5 $\pm$ 1.6	NS
Maximum dV/dt Fall (V/sec)	5	-6.70 $\pm$ 0.62	7	-8.66 $\pm$ 2.87	NS

*NS = Not statistically significant ($p > 0.05$, Student's t test)

TABLE XI-7. Conduction Velocities of Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

	Exposed		Sham-Exposed		
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	p
<u>Vagus Nerve</u>					
Inflection Conduction Velocity (m/sec)	18	0.90 $\pm$ 0.02	17	0.82 $\pm$ 0.02	0.0097
Peak Conduction Velocity (m/sec)	18	0.74 $\pm$ 0.01	17	0.66 $\pm$ 0.02	0.0011
Computer Averaged Conduction Velocity (m/sec)	18	0.72 $\pm$ 0.01	17	0.66 $\pm$ 0.02	0.0021
Nerve Chamber Temperature (°C)	18	37.6 $\pm$ 0.04	17	37.5 $\pm$ 0.04	NS*
<u>Sciatic Nerve</u>					
Inflection Conduction Velocity (m/sec)	13	108 $\pm$ 15	13	121 $\pm$ 17	NS
Peak Conduction Velocity (m/sec)	13	51 $\pm$ 6	12	50 $\pm$ 5	NS
Computer Averaged Conduction Velocity (m/sec)	12	50 $\pm$ 4	11	55 $\pm$ 3	NS
Nerve Chamber Temperature (°C)	17	37.4 $\pm$ 0.1	14	37.5 $\pm$ 0.1	NS

*NS = Not statistically significant ($p > 0.05$, Student's t test)

indicate that electric-field exposure significantly increases conduction velocity in unmyelinated (vagus) nerve fibers but has no effect on myelinated (sciatic) nerve fibers.

In Table XI-8, refractory period and accommodation index measurements [parameters (3) and (4)] from both vagus and sciatic nerves are compared. No significant differences were found between exposed and sham-exposed animals for any of these parameters. The magnitude of the stimulus current necessary for threshold stimulation at fixed stimulus durations [parameter (5)] is shown in Table XI-9. These data were used to plot the strength-duration curves shown in Figure XI-2 for both vagus and sciatic nerves. Preliminary statistical evaluation of these curves using a repeated measures analysis suggests the possibility that chronic electric-field exposure may preferentially affect excitability in nonmyelinated (vagus) nerve fibers. No significant effects of electric-field exposure were observed on poststimulus excitability [parameter (6)] in either vagus or sciatic nerves (Table XI-10).

3. Results: Replicate Experiment. No significant differences were observed between electric-field-exposed and sham-exposed animals in any of the parameters used to characterize unmyelinated nerve fiber (C-fiber) function. Specific properties of the compound action potential are presented in Table XI-11. In the original experiment, the maximum rate at which the action potential returned to its resting level (dV/dt fall, Table XI-6, original experiment) was significantly slower among electric-field-exposed animals. In the replicate experiment (dV/dt C,

TABLE XI-8. Refractory Period and Accommodation Measured in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric Field (or Sham) Exposure

	Exposed		Sham-Exposed		
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	p
<u>Vagus Nerve</u>					
Absolute Refractory Period (msec)	18	1.11 $\pm$ 0.02	17	1.13 $\pm$ 0.05	NS*
50% Relative Refractory Period (msec)	18	1.94 $\pm$ 0.26	17	1.79 $\pm$ 0.12	NS
Accommodation Index	14	3.36 $\pm$ 0.25	12	3.23 $\pm$ 0.23	NS
<u>Sciatic Nerve</u>					
Absolute Refractory Period, (msec)	16	0.38 $\pm$ 0.02	16	0.35 $\pm$ 0.01	NS
50% Relative Refractory Period (msec)	16	0.51 $\pm$ 0.02	16	0.52 $\pm$ 0.02	NS
Accommodation Index	13	2.47 $\pm$ 0.15	14	2.38 $\pm$ 0.06	NS

*NS = Not statistically significant ($p > 0.05$, Student's t test)

TABLE XI-9. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure. Data are presented as mean $\pm$ S.E. with N shown in parentheses.

Stimulus Duration (msec)	Stimulus Strength (μ A)			
	Vagus Nerve		Sciatic Nerve	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
5	11.7 $\pm$ 1.0 (18)	12.6 $\pm$ 0.8 (17)	5.0 $\pm$ 0.8 (15)	4.3 $\pm$ 0.4 (17)
2	12.8 $\pm$ 1.0 (18)	13.8 $\pm$ 0.8 (17)	4.9 $\pm$ 0.8 (15)	4.3 $\pm$ 0.4 (17)
1	16.8 $\pm$ 1.2 (18)	18.6 $\pm$ 1.0 (17)	5.0 $\pm$ 0.8 (15)	4.3 $\pm$ 0.4 (17)
0.5	27.4 $\pm$ 2.0 (18)	29.7 $\pm$ 1.7 (17)	5.8 $\pm$ 0.8 (15)	5.1 $\pm$ 0.5 (17)
0.2	52.0 $\pm$ 3.4 (18)	58.1 $\pm$ 3.6 (17)	8.9 $\pm$ 1.1 (15)	8.5 $\pm$ 1.0 (17)
0.1	85.8 $\pm$ 5.6 (18)	96.7 $\pm$ 5.9 (17)	14.6 $\pm$ 2.1 (15)	13.7 $\pm$ 1.8 *17)
0.08	110 $\pm$ 10 (18)	130 $\pm$ 12 (17)	18.4 $\pm$ 2.5 (15)	17.6 $\pm$ 2.2 (17)
0.06	152 $\pm$ 18 (17)	191 $\pm$ 20 (17)	23.3 $\pm$ 3.0 (15)	22.9 $\pm$ 2.6 (17)
0.04	261 $\pm$ 23 (17)	307 $\pm$ 21 (17)	32.0 $\pm$ 3.8 (15)	32.5 $\pm$ 3.3 (17)
0.02	504 $\pm$ 33 (17)	573 $\pm$ 34 (17)	57.8 $\pm$ 6.4 (15)	58.8 $\pm$ 5.7 (17)
0.01	854 $\pm$ 57 (17)	972 $\pm$ 59 (17)	90.4 $\pm$ 9.3 (14)	97.4 $\pm$ 12.6 (16)
Chronaxie (msec)	0.66 $\pm$ 0.05 (18)	0.65 $\pm$ 0.04 (17)	0.25 $\pm$ 0.03 (6)	0.24 $\pm$ 0.03 (8)

FIGURE XI-2. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations. Data are presented as means for exposed and sham-exposed vagal and sciatic nerves.

TABLE XI-10. Effects of Poststimulus Excitability in Peripheral Nerves from Rats Following 30 Days of 60-Hz Electric-Field (or Sham) Exposure

	Exposed		Sham-Exposed		p
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
<u>Conditioning-Test Interval (msec)</u>					
	<u>Vagus Nerve</u>				
2X Conditioning Response	18	1.26 $\pm$ 0.08	17	1.33 $\pm$ 0.06	NS*
0.5X Test Response	11	11.23 $\pm$ 1.59	15	10.29 $\pm$ 0.63	NS
Minimum Test Response	17	6.66 $\pm$ 0.47	17	6.54 $\pm$ 0.48	NS
Maximum Test Response	18	47 $\pm$ 3	17	46 $\pm$ 2	NS
	<u>Sciatic Nerve</u>				
2X Conditioning Response	13	0.30 $\pm$ 0.02	14	0.32 $\pm$ 0.02	NS
0.5X Test Response	13	2.43 $\pm$ 0.21	14	2.69 $\pm$ 0.30	NS
Minimum Test Response	13	1.30 $\pm$ 0.10	14	1.30 $\pm$ 0.09	NS
Maximum Test Response	13	3.64 $\pm$ 0.10	14	3.79 $\pm$ 0.15	NS

*NS = Not statistically significant ($p > 0.05$, Student's t test)

TABLE XI-11. Properties of the Compound Action Potential in the Vagus Nerves of Rats Following 30 Days of Exposure or Sham Exposure to a 100-kV/m, 60-Hz Electric Field (Replicate Experiment).

	Exposed		Sham-Exposed		p
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Maximum Amplitude: B Component (mV)	24	1.43 $\pm$ 0.10	26	1.68 $\pm$ 0.11	NS*
Maximum Amplitude: C Component (mV)	24	1.76 $\pm$ 0.14	26	2.10 $\pm$ 0.16	NS
Maximum dV/dt B (V/sec)	24	4.01 $\pm$ 0.31	27	4.20 $\pm$ 0.37	NS
Maximum dV/dt B-C (V/sec)	23	-6.91 $\pm$ 0.49	27	-8.12 $\pm$ 0.72	NS
Maximum dV/dt C (V/sec)	24	1.74 $\pm$ 0.15	27	1.99 $\pm$ 0.27	NS

*NS = not significant; $p > 0.05$

Table XI-11, replicate experiment) we were unable to confirm this effect, although the general relationship was maintained. Other differences between exposed and sham-exposed animals, while not statistically significant, were consistent in both experiments.

No difference was observed in the conduction velocities of the principal components of the C-fiber compound action potential (Table XI-12). This is in marked contrast to observations made in the original study, in which conduction velocities were significantly ($p < 0.01$) increased in electric-field-exposed animals. Animal ages and body weights, as well as nerve-chamber temperatures, were compared for the two experiments. No differences were found that could account for the absence of the effect in the replicate experiment or for the presence of the effect in the original experiment.*

Strength-duration curves for the two principal components of the vagal compound action potential are shown in Figure XI-3. No significant differences between exposed and sham-exposed animals were observed (Table XI-13). As in the original experiment, the strength-duration curve for unmyelinated (vagal) nerve fibers in electric-field-exposed animals is shifted to the left, relative to the sham-exposed controls. The chronaxies are correspondingly shorter for exposed animals than for sham-exposed animals (Table XI-13).

* This experiment was replicated a third time; the results were the same as in the second replicate. Exposure of rats to 100 kV/m for 30 days had no significant effect on conduction velocities of the principal components of the C-fiber compound action potential.

TABLE XI-12. Conduction Velocities of Components of the Compound Action Potential in Vagus Nerves of Rats Following 30 Days of Exposure or Sham Exposure to a 100-kV/m, 60-Hz Electric Field (Replicate Experiment). Conduction velocities were calculated from computer averages of 16 action potentials in each individual nerve.

	Exposed		Sham-Exposed		p
	N	Mean $\pm$ S.E.	N	Mean $\pm$ S.E.	
Peak Conduction Velocity: B Component (m/sec)	24	0.95 $\pm$ 0.01	26	0.95 $\pm$ 0.01	NS*
Peak Conduction Velocity: C Component (m/sec)	24	0.70 $\pm$ 0.01	26	0.70 $\pm$ 0.01	NS
Chamber Temperature ($^{\circ}$ C)	25	37.5 $\pm$ 0.1	26	37.5 $\pm$ 0.1	NS
Age of Rats (Days)	15	84.7 $\pm$ 0.4	14	84.9 $\pm$ 0.4	NS

*NS = not significant; $p > 0.05$

FIGURE XI-3. Strength-Duration Curves for the Two Principal Components of the Vagal Compound Action Potential for Both Exposed and Sham-Exposed Nerves

TABLE XI-13. Stimulus Strength Required for Threshold Stimulation at Fixed Stimulus Durations in the Vagus Nerve of Rats Following 30 Days of 60-Hz Electric Field or Sham Exposure (Replicate Experiment). Data are presented as mean $\pm$ standard error with N shown in parentheses.

Stimulus Duration (msec)	Stimulus Strength (μ A)			
	Exposed: B	Sham-Exposed: B	Exposed: C	Sham-Exposed: C
5	7.3 $\pm$ 0.3 (23)	7.4 $\pm$ 0.5 (27)	6.1 $\pm$ 0.3 (24)	5.8 $\pm$ 0.4 (27)
2	7.7 $\pm$ 0.3 (23)	7.9 $\pm$ 0.5 (27)	6.6 $\pm$ 0.3 (24)	6.5 $\pm$ 0.4 (27)
1	11.2 $\pm$ 0.5 (23)	11.7 $\pm$ 0.7 (27)	9.4 $\pm$ 0.4 (24)	9.5 $\pm$ 0.5 (27)
0.5	19.1 $\pm$ 0.9 (23)	20.2 $\pm$ 1.2 (27)	14.9 $\pm$ 0.7 (24)	15.6 $\pm$ 1.0 (27)
0.2	42.9 $\pm$ 2.2 (23)	45.8 $\pm$ 2.9 (27)	31.0 $\pm$ 1.8 (24)	33.1 $\pm$ 2.1 (27)
0.1	82.1 $\pm$ 4.8 (23)	85.9 $\pm$ 5.6 (27)	53.6 $\pm$ 3.7 (24)	56.6 $\pm$ 4.2 (27)
0.08	102.2 $\pm$ 5.9 (23)	109.1 $\pm$ 7.3 (27)	66.0 $\pm$ 4.4 (24)	68.2 $\pm$ 4.9 (27)
0.06	137.0 $\pm$ 8.5 (23)	143.9 $\pm$ 9.8 (27)	85.1 $\pm$ 5.7 (24)	89.2 $\pm$ 6.9 (27)
0.04	209.4 $\pm$ 12.6 (23)	217.7 $\pm$ 13.9 (27)	127.3 $\pm$ 9.4 (24)	132.3 $\pm$ 10.6 (27)
0.02	431.3 $\pm$ 24.3 (23)	450.6 $\pm$ 28.1 (27)	270.8 $\pm$ 17.4 (24)	282.6 $\pm$ 19.2 (27)
0.01	780.4 $\pm$ 43.3 (23)	828.5 $\pm$ 52.1 (27)	478.5 $\pm$ 28.6 (24)	491.8 $\pm$ 30.2 (27)
Chronaxie (msec)	0.80 $\pm$ 0.04 (23)	0.82 $\pm$ 0.03 (26)	0.73 $\pm$ 0.04 (24)	0.81 $\pm$ 0.04 (26)

4. Discussion and Conclusions. The increased conduction velocity in unmyelinated nerve fibers from electric-field-exposed animals reported in the original experiment was not observed in the replicate experiment. This discrepancy cannot be explained on the basis of available information. The conditions of the replicate experiments were designed to closely duplicate those of the original experiment. Improvements in nerve chamber design, instrumentation and technique occurred during the interval between the two experiments. Presumably as a result of these subtle changes, the configuration of the compound action potential differed in the two experiments. Neither procedural improvements nor configurational changes in the action potential could reasonably be used to account for the absence of the effect in the replicate experiment. It is interesting to note that the conduction velocities for exposed and sham-exposed animals measured in the replicate experiment fall exactly in between the corresponding conduction velocities measured in the original experiment.

The hypothesis of increased neuronal excitability continues to be supported by the shift in the strength-duration curves and decreased chronaxie found in electric-field-exposed animals. Trends in other parameters (maximum amplitude, maximum rates of potential change), while consistent with those found in the original experiment, are not obviously compatible with this hypothesis.

The question of whether or not chronic exposure to a 60-Hz electric field can cause persistent changes in peripheral nerve function remains unanswered. Additional experiments, perhaps of longer duration, will be required if we are to answer the question.

D. FUTURE PLANS

Experiments on neuromuscular function are scheduled for completion in December 1979, with the data analysis to be completed in February 1980.

Because of the confirmed effects of electric fields on synaptic transmission (C-T ratio), additional experiments will be conducted to examine the basic mechanisms whereby changes in the C-T ratio are produced, and to determine if the effects are reversible. These experiments will provide some of the additional information necessary to assess the health consequences of exposure to 60-Hz electric fields.

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XII. REPRODUCTION AND DEVELOPMENT

Principal Investigator: M. R. Sikov

ABSTRACT

Two experiments were conducted to assess the effects of exposure to uniform 60-Hz electric fields (100 kV/m) on reproduction, and on fetal and postnatal growth and development in the rat. In one experiment (replicate experiment) exposure of the pregnant rat was begun on day 0 of gestation and continued until the resulting offspring reached 8 days of age. It was found that exposure did not affect reproductive performance in either males or females, nor did it affect the survival, size or morphology of the fetuses. In the other experiment, exposure of the pregnant female began at 17 days of gestation and continued through 25 days of postnatal life. These 30-day exposures produced few statistically significant effects on the growth, survival or neuromuscular development of the offspring. Accelerated development of motile behavior was observed in offspring in the first experiment. This difference was not observed in the later experiment. Tests of reproductive integrity of the offspring did not disclose any deficits.

Mice conceived, born, and raised in a 60-Hz (100 kV/m), electric field for three successive generations were compared with an identical sham-exposed group. Since the study is still in progress, no statistical analyses of the data are available. However, the presence of the electric field had no apparent effect on conception, litter size, mortality, or body weights up to 10 wk of age in any of the three generations studied.

A. INTRODUCTION

The previous Annual Report described two experiments in which we studied the effect of exposure to 100-kV/m electric fields on the growth and development of rats. The first experiment, which involved prenatal exposure and teratologic evaluation, had been completed and was reported in detail (Phillips, et al., 1978). Certain aspects of the second experiment, which involved exposure from the day of conception through 8 days of

postnatal age, also had been completed, and preliminary results were reported (Phillips, et al., 1978). Statistical analyses of these data, as well as additional measurements on the same animals, have now been completed and will be presented in this report. A third experiment, to evaluate the effects of exposure during the perinatal and juvenile period, was also performed during the past year.

Marino et al. (1976) reported a significant effect attributable to exposure to 60-Hz electric fields over three successive generations. The offspring of mice exposed to a vertical electric field at 15 kV/m were reported to show decreased body weights and increased mortality rate for all three generations. Mice exposed to a horizontal electric field at 10 kV/m showed decreased body weights for two successive generations. The authors indicate that the presence of grounding micro-currents may have influenced the results, although they suggest alternative mechanisms.

We designed an experiment to determine whether effects similar to those reported by Marino et al. would be observed under conditions in which such discharges did not occur. The rats in this study were grounded and exposed to 100 kV/m, in contrast to rats of Marino's study which were ungrounded and exposed to 15 kV/m. This study is still in progress, with data from the third generation incomplete at this time; therefore, only preliminary results will be reported here.

B. METHODS

Rat Experiment 2. The procedures were the same as those described in the 1978 Annual Report. Exposure extended from 0 days of gestation

(d.g.) and continued through 8 days of age. In this experiment, four female offspring (age $\sim$ 2 mo) from each litter that had been exposed or sham-exposed in utero and for 8 days after birth, together with one randomly selected male (similarly exposed or sham-exposed), were reintroduced into the sham-exposure or exposure field to determine whether deficits in reproductive performance had been induced. At 7 wk of age, one-half of the remaining males from the first two replicates were tested for susceptibility to audiogenic seizures by exposure to a 110- to 114-db white noise for 1 min. The remaining animals in that group were handled in an identical fashion except that they were not subjected to the sound. Blood samples, obtained after completion of the seizure test, were furnished to other investigators to determine whether changes in corticosteroid levels had been produced by the stress of the seizure test, and whether these changes differed between sham-exposed and exposed groups. The animals which were not subjected to the seizure test were killed and necropsied, which included weighing several organs; tissues were preserved for histopathologic examination. Blood samples were taken from other animals for more extensive hematologic and hormonal analyses by other investigators.

The males of the third replicate were given Metrazol prior to testing to heighten their susceptibility to audiogenic seizures. The males from the fourth replicate were killed at 28 days of age to determine whether transient early alterations in organ weights had been induced.

Rat Experiment 3. This experiment, which was performed to evaluate the effects of exposure during late gestation and during postnatal development, was performed in five replicates. Within each replicate, one male and four female rats were caged together; vaginal smears from females were examined daily. The day of a sperm-positive vaginal smear was defined as day 0 of gestation. At 17 d.g., females were transferred to littering cages [which were identical to the mating cages used in Experiment 1 and contained Antron-III (an electrical conductor) for nesting (Phillips and Kaune, 1977)] and randomly selected for placement in the electric field or sham field. The dams delivered and reared their litters during exposure, and were removed from the field when the pups reached 21 days of age. The offspring were removed from the field 4 days later, at 25 days of age.

At delivery, the interval since mating was recorded, as well as the number of living and stillborn offspring. At 1 day of age, litters were reduced to eight pups (evenly divided between the sexes when possible); litters of fewer than six were eliminated. At that time, as well as at several subsequent times, pups were weighed and examined for gross abnormalities. Offspring were subjected (at various times) to a battery of tests to evaluate reflex development and general neurological status. The measures, which are commonly employed in our laboratory (Sikov et al., 1977), were described in the previous Annual Report.

Mouse Multigeneration Experiment. The exposure system consisted of two identical units: one for vertical field exposure and the other

for sham exposure (Phillips and Kaune, 1977). The exposure system is designed to minimize microcurrents through the watering system when the animals drink. Preconceived bias was eliminated from the studies because the exposed and sham-exposed groups were not identified to the investigators until all measurements had been completed. Exposure to the 60-Hz (100-kV/m) electric field was continuous except for an approximately 4-hr daily shutdown period for animal care and data collection. The exposure room was on a 12-hr light/12-hr dark cycle, and had an average temperature of 21°C and a relative humidity of 30% during this study.

The experimental design may be seen in Table XII-5. Swiss-Webster mice, obtained from Hilltop Laboratories, were used for the F_0 generation. Male and female animals were acclimated to the housing system in the exposure room for 5 days before the system was energized. Subsequently, all mice were conceived, born and raised with the exposure system energized.

The study involved two replicates (A and B), with an exposed group and a sham-exposed group in each replicate. Each replicate started with 16 exposed and 16 sham-exposed female mice (F_0). Two female mice were housed with one male for mating for a period of 1 wk; females were checked daily for evidence of mating. A few days before delivery, eight pregnant females were randomly selected from each group and housed individually with a small amount of conductive nesting material (Antron-III).

After delivery, the pups (F_1) were counted, sexed, weighed and marked with an identification code. Each litter was reduced to eight pups (four females and four males, when possible) and the pups were weighed

weekly for 10 wk. They were checked for tooth eruption, eye-opening, and testes descent or vaginal opening at appropriate ages, and weaned at 21 days of age.

At 10 weeks of age, 16 exposed and 16 sham-exposed females (two from each litter, if possible) were randomly selected from among the F_1 animals. Breeding males were selected (one from each litter, if possible) so that there was a maximum of genetic mixing. There were no sibling matings, and reciprocal crosses were avoided; males and females from each litter were represented whenever possible. After selection of the breeding animals, the remaining mice were provided to another investigator for hematologic evaluation (see Section IV, Hematology and Serum Chemistry).

The breeding and evaluation procedures were successively repeated until a total of three offspring generations had been obtained. A fourth generation was used for detailed neuromuscular evaluations. Statistical tests on the data have been deferred until completion of the study.

C. RESULTS

Experiment 2. As was reported previously (Phillips et al., 1978), there were no differences between exposed and sham-exposed groups in litter sizes, birth weights, and subsequent growth and survival. The data obtained when the animals were bred showed no detectable differences attributable to the original or subsequent exposure, or to interactions between them.

Most results obtained from tests of neuromuscular and neurological development did not differ between the sham-exposed and exposed groups. The only notable difference was the greater percentage of exposed offspring showing motile behaviors (movement, grooming, standing), and a greater percentage of the sham-exposed showing the righting reflex at 14 days of age. The results in the two groups were indistinguishable upon retesting at 21 days of age. These differences have now been analyzed and found to be statistically significant at the 0.05 level by an analysis of variance using an arc sine transformation.

Organ weights were obtained at sacrifice from the rats not subjected to audiogenic seizure testing (Table XII-1), and showed no remarkable differences attributable to the experimental treatment.

Experiment 3. The only birth parameter which differed between the two groups was the number of stillbirths (Table XII-2). This also differed from the results of Experiment 2, which are shown for comparison. The larger number in the sham-exposed group was partially due to one litter of 20, in which 8 were stillborn. A total of 12 sham-exposed litters included stillborn pups, as compared to a single litter in which there were three stillborns in the exposed group. Only a few offspring died throughout the remainder of the experiment; these also occurred primarily in sham-exposed litters (Table XII-2). Body weight at 1 day of age, and at subsequent ages, was similar in the two groups. The exposed females were slightly heavier than the sham-exposed at each interval, but the differences were not statistically significant (Table XII-3).

TABLE XII-1. Organ Weights of Rats Exposed to a 60-Hz, 100-kV/m Electric Field Throughout Prenatal Development and the First 8 Postnatal Days (Mean % Body Weight $\pm$ S.E.)

Age (Sex):	28 Days (Males)		53-56 Days (Males)		74-77 Days (Females)	
Organ	Exposed	Sham-Exposed	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Liver	5.1 $\pm$ 0.1	5.1 $\pm$ 0.1	4.4 $\pm$ 0.1	4.4 $\pm$ 0.2	4.2 $\pm$ 0.1	4.1 $\pm$ 0.1
Spleen	0.43 $\pm$ 0.01	0.44 $\pm$ 0.04	0.30 $\pm$ 0.02	0.31 $\pm$ 0.01	---	---
Adrenal	0.02 $\pm$ 0.001	0.02 $\pm$ 0.001	0.02 $\pm$ 0.001	0.02 $\pm$ 0.002	0.03 $\pm$ 0.001	0.03 $\pm$.001
Testes	0.61 $\pm$ 0.02	0.59 $\pm$ 0.02	0.95 $\pm$ 0.02	0.94 $\pm$ 0.01	---	---
Ovaries	---	---	---	---	0.05 $\pm$ 0.002	0.05 $\pm$ 0.002
Epididymides	0.071 $\pm$ 0.003	0.071 $\pm$ 0.003	0.14 $\pm$ 0.010	0.15 $\pm$ 0.01	---	---
Seminal Vesicles	---	---	0.14 $\pm$ 0.01	0.11 $\pm$ 0.01	---	---
Uterus	---	---	---	---	0.17 $\pm$ 0.01	0.18 $\pm$ 0.01
Kidney (Left)	---	---	---	---	0.36 $\pm$ 0.01	0.37 $\pm$ 0.01
Thymus	---	---	---	---	0.17 $\pm$ 0.01	0.17 $\pm$ 0.01
Bladder & Prostate	0.18 $\pm$ 0.01	0.17 $\pm$ 0.01	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	---	---

TABLE XII-2. Litter Size and Survival of Offspring of Pregnant Rats Exposed to a 60-Hz Electric Field at 100 kV/m

	Experiment 2**		Experiment 3***	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
Total Females in Group	24	20	29	29
Mean Live Births/Litter $\pm$ S.E.	9.9 $\pm$ 0.8	11.2 $\pm$ 0.8	10.2 $\pm$ 0.9	10.8 $\pm$ 0.7
Number of Stillbirths	8	3	3	24
Number Discarded*	5	2	7	5
Number of Litters Evaluated	19	18	22	24
Number of Postnatal Deaths	3	4	2	8

*Less than 6 offspring for continuing postnatal evaluation

**Exposure: 0 d.g. through 8 days postnatally

***Exposure: 17 d.g. through 25 days postnatally

TABLE XII-3. Body Weights of Rats Exposed to a 60-Hz Electric Field at 100 kV/m from 17 Days of Gestation Through 25 Days Postnatally

Age (Days)	Mean Weight* $\pm$ S.E. (g)			
	Males		Females	
	Exposed	Sham-Exposed	Exposed	Sham-Exposed
1	7.5 $\pm$ 0.1	7.3 $\pm$ 0.2	7.1 $\pm$ 0.1	6.8 $\pm$ 0.2
7	16.4 $\pm$ 0.3	16.0 $\pm$ 0.3	15.9 $\pm$ 0.3	15.1 $\pm$ 0.4
14	32.6 $\pm$ 1.0	32.9 $\pm$ 0.9	32.0 $\pm$ 0.9	31.6 $\pm$ 0.8
21	51.8 $\pm$ 1.5	50.8 $\pm$ 1.6	50.4 $\pm$ 1.4	48.8 $\pm$ 1.5
28	90.2 $\pm$ 1.5	89.0 $\pm$ 2.1	83.8 $\pm$ 1.2	81.6 $\pm$ 2.2
35	150.6 $\pm$ 1.6	147.8 $\pm$ 1.6	128.6 $\pm$ 2.3	126.8 $\pm$ 1.3
42	214.4 $\pm$ 2.7	210.6 $\pm$ 3.2	170.0 $\pm$ 1.8	169.8 $\pm$ 1.9

*Calculated as means of individual litter means

The various measures of physical maturation were obtained at the same times as in Experiment 2, and again showed no consistent differences between the two experimental groups. The data obtained from a number of the measures of neuromuscular development, pooled across replicates (Table XII-4), suggested differences between groups, but they were not statistically significant at $p < 0.05$. These included the grasp reflex at 1 day ($p = 0.08$), head lift at 7 days ($p = 0.09$) and movement at 1 and 7 days ($p = 0.2$). The effects of electric-field exposure on "grooming" "standing alone," and "righting" that were seen in Experiment 2 were not observed in Experiment 3. The only measure which was significantly affected was negative geotropism ($p = 0.03$).

Mouse Multigeneration Experiment. The number of pregnancies among F_0 , F_1 and F_2 mice resulting from 1-wk breeding periods are shown in Table XII-5. On an overall basis, 83 of 96 (86.5%) exposed mice and 88.5% of sham-exposed females became pregnant, with little difference between generations or replicates. There was no consistent pattern of difference between the two groups, and it is unlikely that the 2% difference will be statistically significant.

The total number of mice born, average litter size and mortality data are shown in Table XII-6. Although there were slightly fewer births in the exposed group, there appear to be no real differences between the two groups in average litter size or total number of mice born. The number of litters born was identical for each group.

TABLE XII-4. Percent of Rat Offspring Showing Criterion or Normal Response to Several Measures of Neuromuscular Development (Approximately 180 Offspring per Group). Offspring were exposed from 17 d.g. through 25 days of age to a 60-Hz electric field at 100 kV/m.

<u>Measure of Development</u>	Age:	Percent of Offspring Showing Normal Response							
		<u>Exposed</u>				<u>Sham-Exposed</u>			
		<u>1</u>	<u>7</u>	<u>14</u>	<u>21</u>	<u>1</u>	<u>7</u>	<u>14</u>	<u>21</u>
Grasp		76	65	99	92	91	65	97	90
Righting		--	21	64	95	--	17	61	95
Movement		32	27	79	92	18	16	74	94
Head Lift		67	34	79	95	70	26	63	93
Grooming		--	--	44	87	--	--	45	89
Standing Alone		--	--	25	82	--	--	22	86
Negative Geotropism		--	--	32	59	--	--	22	66

TABLE XII-5. Experimental Design and Pregnancy Data from Multigeneration Mouse Experiment

Replicate	Generation	Rack 1 (Exposed)					Rack 2 (Sham-Exposed)				
		Number Females Bred	Number Males Used	Pregnancies		Number Females Kept	Number Females Bred	Number Males Used	Pregnancies		Number Females Kept
				Number Females					Number Females		
A	F ₀	16	8	13	81.2	8	16	8	13	81.2	8
B	F ₀	16	8	13	81.2	8	16	8	15	93.8	8
A	F ₁	16	8	14	87.5	8	16	8	16	100	8
B	F ₁	16	8	14	87.5	8	16	8	12	75	8
A	F ₂	16	8	14	87.5	8	16	8	14	87.5	8
B	F ₂	16	8	15	93.8	8	16	8	15	93.8	9
Total		96	48	83	86.5		96	48	85	88.5	

TABLE XII-6. Summary of Mice Born, Litter Size and Mortality Data in Multigeneration Mouse Experiment

Replicate	Generation	Total Pups Born*	Average Litter Size ($\bar{x} \pm SD$)	Day 1 Mortality		Mortality of Pups Selected**				
				Number Dead	%	Day 7		Day 70		
						(N)	Number Dead	%	Number Dead	%
Rack 1 (Exposed)										
A	F ₁	83	10.4 + 1.7	0	0	(64)	0	0	3	4.7
B	F ₁	97	12.1 + 1.0	0	0	(64)	7	10.9	18	28.1
A	F ₂	89	11.1 + 2.8	16	18.0	(53)	1	1.9	9	17.0
B	F ₂	90	11.2 + 1.9	1	1.1	(64)	0	0	0	0
A	F ₃	83	10.4 + 2.8	8	9.6	(57)	0	0	1	1.8
B	F ₃	84	10.5 + 1.4	0	0	(64)	0	0	4	6.2
Total A		255	10.6 + 2.4	24	9.4	(174)	1	0.6	13	7.5
Total B		271	11.3 + 1.6	1	0.4	(192)	7	3.6	22	11.5
Total A&B		526	11.0 + 2.0	25	4.8	(366)	8	2.2	35	9.6
Rack 2 (Sham-Exposed)										
A	F ₁	88	11.0 + 2.2	0	0	(64)	8	12.5	11	17.2
B	F ₁	99	12.4 + 1.8	7	7.1	(64)	1	1.6	11	17.2
A	F ₂	96	12.0 + 2.1	1	1.0	(64)	0	0	7	10.9
B	F ₂	82	10.2 + 2.4	3	3.7	(61)	9	14.8	13	21.3
A	F ₃	98	12.2 + 2.0	1	1.0	(64)	0	0	2	3.1
B	F ₃	87	10.9 + 2.0	0	0	(64)	0	0	0	0
Total A		282	11.8 + 2.0	2	0.7	(192)	8	4.2	20	10.4
Total B		268	11.2 + 2.2	10	3.7	(189)	10	5.3	24	12.7
Total A&B		550	11.5 + 2.1	12	2.2	(381)	18	4.7	44	11.6

*The total number of pups born is probably an underestimate because of frequent cannibalism of stillborn or weak offspring by dams.

**All litters were reduced to 8 pups on day 1 to reduce the influence of litter size on the growth rate.

Evidently, the presence of the electric field had no influence on mortality in the three generations of mice. No real differences in mortality between the two groups were observed, nor were any trends indicated. There were no deaths among the dams, and causes for most of the deaths among the young mice appeared to be unrelated to treatment.

In the sham-exposed groups, two litters were lost because of malfunctions in the watering system, with subsequent dehydration and agalactia in the dams. Two other litters were lost because the dams escaped from their cages and could not return to their young. A few mice died accidentally.

Of the 48 litters in the exposed groups, five were lost. Lactation apparently became insufficient between 7 and 14 days postpartum in three dams, with subsequent loss of those litters. A temporary malfunction of the water system is suspected in these cases. Two other litters were lost because the dams exhibited very little maternal instinct and failed to take care of their young.

Irrespective of treatment, animals from several litters failed to gain weight properly between 7 and 14 days, or between 14 and 21 days of age; these mice later gained weight rapidly. Most of the problems and litter losses noted above occurred early in the experiment; the incidence declined as the investigators recognized and corrected the problems. The only deaths without apparent cause were four offspring (1.1%) from the exposed group, and seven (1.8%) from the sham-exposed group.

A comparison of weights between exposed and sham-exposed female mice on selected days of age may be seen in Table XII-7; corresponding

TABLE XII-7. Average Weights of Three Generations of Female Mice Exposed to a 60-Hz Electric Field at 100 kV/m

		Weights (grams)									
Age:		Day 1		Day 14		Day 28		Day 35		Day 70	
Replicate	Generation	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$
RACK 1 EXPOSED											
A	F ₁	(34)	1.8 $\pm$ 0.2	(34)	7.1 $\pm$ 0.6	(34)	16.4 $\pm$ 1.9	(34)	20.3 $\pm$ 1.6	(34)	28.9 $\pm$ 1.4
A	F ₂	(22)	1.8 $\pm$ 0.2	(22)	7.6 $\pm$ 0.4	(22)	19.0 $\pm$ 1.7	(11)	23.6 $\pm$ 1.6	(22)	29.2 $\pm$ 1.8
A	F ₃	(24)	1.8 $\pm$ 0.1	(24)	7.0 $\pm$ 0.8	(24)	16.7 $\pm$ 2.4	(24)	22.1 $\pm$ 1.8	(23)	28.6 $\pm$ 1.1
Total A	F ₁ - F ₃	(80)	1.8 $\pm$ 0.2	(80)	7.2 $\pm$ 0.6	(80)	17.2 $\pm$ 2.0	(69)	21.4 $\pm$ 1.7	(79)	28.9 $\pm$ 1.4
B	F ₁	(23)	1.8 $\pm$ 0.2	(23)	7.3 $\pm$ 1.0	(23)	16.5 $\pm$ 2.1	(23)	22.8 $\pm$ 1.5	(23)	29.5 $\pm$ 2.5
B	F ₂	(36)	1.9 $\pm$ 0.2	(36)	7.1 $\pm$ 1.1	(36)	18.1 $\pm$ 2.3	(36)	23.2 $\pm$ 1.9	(36)	29.4 $\pm$ 1.3
B	F ₃	(29)	2.0 $\pm$ 0.1	(29)	7.8 $\pm$ 0.6	(27)	18.0 $\pm$ 2.8	(27)	22.3 $\pm$ 1.9	(27)	29.9 $\pm$ 1.8
Total B	F ₁ - F ₃	(88)	1.9 $\pm$ 0.2	(88)	7.4 $\pm$ 0.9	(86)	17.6 $\pm$ 2.4	(86)	22.8 $\pm$ 1.8	(86)	29.6 $\pm$ 1.8
A & B	F ₁ - F ₃	(168)	1.9 $\pm$ 0.2	(68)	7.3 $\pm$ 0.8	(166)	17.4 $\pm$ 2.2	(155)	22.2 $\pm$ 1.8	(165)	29.3 $\pm$ 1.6
RACK 2 SHAM-EXPOSED											
A	F ₁	(28)	1.9 $\pm$ 0.2	(28)	7.4 $\pm$ 0.6	(27)	17.1 $\pm$ 1.8	(27)	21.3 $\pm$ 1.5	(26)	29.9 $\pm$ 1.6
A	F ₂	(28)	1.9 $\pm$ 0.1	(28)	7.2 $\pm$ 0.9	(27)	17.2 $\pm$ 2.8	(23)	22.9 $\pm$ 2.0	(27)	30.7 $\pm$ 1.9
A	F ₃	(30)	1.8 $\pm$ 0.1	(30)	7.2 $\pm$ 0.8	(29)	17.5 $\pm$ 1.9	(29)	23.0 $\pm$ 1.4	(28)	29.9 $\pm$ 1.9
Total A	F ₁ - F ₃	(86)	1.9 $\pm$ 0.1	(86)	7.3 $\pm$ 0.8	(83)	17.3 $\pm$ 2.2	(79)	22.4 $\pm$ 1.6	(81)	30.2 $\pm$ 1.8
B	F ₁	(27)	1.9 $\pm$ 0.2	(27)	7.6 $\pm$ 0.6	(25)	18.8 $\pm$ 1.8	(25)	23.7 $\pm$ 1.0	(24)	29.0 $\pm$ 1.6
B	F ₂	(30)	2.0 $\pm$ 0.1	(19)	7.5 $\pm$ 0.9	(29)	17.9 $\pm$ 1.7	(29)	23.2 $\pm$ 1.9	(28)	29.3 $\pm$ 1.9
B	F ₃	(34)	1.8 $\pm$ 0.2	(34)	7.3 $\pm$ 0.5	(34)	18.0 $\pm$ 1.5	(34)	23.3 $\pm$ 1.3	(34)	28.5 $\pm$ 1.8
Total B	F ₁ - F ₃	(91)	1.9 $\pm$ 0.2	(80)	7.4 $\pm$ 0.6	(88)	18.2 $\pm$ 1.6	(88)	23.4 $\pm$ 1.4	(86)	28.9 $\pm$ 1.8
A & B	F ₁ - F ₃	(177)	1.9 $\pm$ 0.2	(66)	7.4 $\pm$ 0.7	(171)	17.8 $\pm$ 1.9	(167)	22.9 $\pm$ 1.5	(167)	29.5 $\pm$ 1.8

data for the males are presented in Table XII-8. No weight differences were observed from one generation to the next; weights from all three generations within replicates were pooled and are presented graphically in Figure XII-1. There appear to be no weight differences between exposed and sham-exposed groups. Gender had the only obvious effect on weight, with males consistently outgaining females after 21 days of age.

This experiment is still in progress and the data have not yet been subjected to statistical analyses. However, there appear to be no differences in weights between the exposed and sham-exposed groups. Apparently, under the conditions of this experiment, the presence of a 60-Hz (100-kV/m) vertical electric field had no effect on mice conceived, born, and raised in the field for three successive generations with regard to body weight, breeding capacity, litter size, or mortality.

TABLE XII-8. Average Weights of Three Generations of Male Mice
Exposed to a 60-Hz Electric Field at 100 kV/m

		Weights (grams)									
Age:		Day 1		Day 14		Day 28		Day 35		Day 70	
Replicate	Generation	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$	(N)	$\bar{x} \pm SD$
RACK 1 EXPOSED											
A	F ₁	(30)	1.8 $\pm$ 0.2	(30)	7.0 $\pm$ 0.8	(27)	16.4 $\pm$ 2.4	(27)	23.8 $\pm$ 2.4	(27)	34.6 $\pm$ 2.1
A	F ₂	(23)	1.8 $\pm$ 0.2	(22)	7.5 $\pm$ 0.6	(22)	20.4 $\pm$ 2.2	(11)	26.2 $\pm$ 1.6	(22)	35.9 $\pm$ 1.6
A	F ₃	(33)	1.9 $\pm$ 0.2	(33)	7.2 $\pm$ 0.7	(33)	19.0 $\pm$ 2.2	(31)	25.0 $\pm$ 2.1	(32)	34.2 $\pm$ 1.8
Total A	F ₁ - F ₃	(86)	1.8 $\pm$ 0.2	(85)	7.2 $\pm$ 0.7	(82)	19.2 $\pm$ 2.3	(69)	24.7 $\pm$ 2.1	(81)	34.8 $\pm$ 1.8
B	F ₁	(17)	1.9 $\pm$ 0.2	(17)	7.4 $\pm$ 0.9	(17)	20.3 $\pm$ 1.8	(17)	27.3 $\pm$ 1.4	(17)	37.0 $\pm$ 2.1
B	F ₂	(28)	2.0 $\pm$ 0.2	(28)	7.2 $\pm$ 1.2	(28)	19.3 $\pm$ 3.7	(28)	26.2 $\pm$ 2.8	(28)	37.0 $\pm$ 2.3
B	F ₃	(35)	2.0 $\pm$ 0.1	(35)	8.0 $\pm$ 0.7	(34)	19.8 $\pm$ 3.2	(34)	26.8 $\pm$ 2.6	(33)	38.9 $\pm$ 2.3
Total B	F ₁ - F ₃	(80)	2.0 $\pm$ 0.2	(80)	7.6 $\pm$ 0.9	(79)	19.7 $\pm$ 3.1	(79)	26.7 $\pm$ 2.4	(78)	37.8 $\pm$ 2.3
A & B	F ₁ - F ₃	(166)	1.9 $\pm$ 0.2	(165)	7.4 $\pm$ 0.8	(161)	19.4 $\pm$ 2.7	(148)	25.8 $\pm$ 2.3	(159)	36.3 $\pm$ 2.0
RACK 2 SHAM-EXPOSED											
A	F ₁	(28)	2.0 $\pm$ 0.2	(28)	7.5 $\pm$ 0.7	(27)	19.7 $\pm$ 1.9	(27)	24.8 $\pm$ 2.4	(26)	36.9 $\pm$ 2.1
A	F ₂	(28)	2.0 $\pm$ 0.2	(28)	7.3 $\pm$ 0.8	(28)	18.6 $\pm$ 3.0	(24)	25.8 $\pm$ 2.4	(28)	36.0 $\pm$ 2.1
A	F ₃	(34)	1.9 $\pm$ 0.2	(32)	7.4 $\pm$ 0.8	(34)	18.4 $\pm$ 2.6	(32)	26.2 $\pm$ 2.5	(32)	36.9 $\pm$ 2.7
Total A	F ₁ - F ₃	(90)	2.0 $\pm$ 0.2	(88)	7.4 $\pm$ 0.8	(87)	18.9 $\pm$ 2.5	(83)	25.6 $\pm$ 2.4	(86)	36.6 $\pm$ 2.3
B	F ₁	(28)	1.9 $\pm$ 0.2	(28)	7.6 $\pm$ 0.7	(28)	20.4 $\pm$ 2.4	(28)	27.5 $\pm$ 1.7	(28)	36.7 $\pm$ 2.1
B	F ₂	(23)	2.1 $\pm$ 0.2	(18)	7.1 $\pm$ 0.6	(21)	19.5 $\pm$ 1.9	(21)	26.4 $\pm$ 2.0	(20)	35.4 $\pm$ 2.4
B	F ₃	(30)	2.0 $\pm$ 0.2	(30)	7.5 $\pm$ 0.6	(30)	19.6 $\pm$ 1.9	(30)	26.5 $\pm$ 1.6	(30)	36.4 $\pm$ 2.3
Total B	F ₁ - F ₃	(81)	2.0 $\pm$ 0.2	(76)	7.4 $\pm$ 0.6	(79)	19.9 $\pm$ 2.1	(79)	26.8 $\pm$ 1.7	(78)	36.2 $\pm$ 2.2
A & B	F ₁ - F ₃	(171)	2.0 $\pm$ 0.2	(164)	7.4 $\pm$ 0.8	(166)	19.4 $\pm$ 2.3	(162)	26.2 $\pm$ 2.1	(164)	36.4 $\pm$ 2.3

FIGURE XII-1. Comparison of Growth in Mice Exposed or Sham Exposed to 100 kV/m Electric Field over Three Generations. Weights pooled for three generations; data for two independent replicates are shown.

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XIII. ANIMAL BEHAVIOR

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ABSTRACT

Behavioral effects of high-strength, 60-Hz electric fields were studied in several experiments. Using a shuttlebox in which one end was shielded from a variety of electric-field strengths while the other was exposed, we determined that during short-term (45-min) tests rats avoided exposure at ≥ 90 kV/m; this response was not significantly attenuated or enhanced over four test sessions made at weekly intervals. Over a longer-duration test, rats avoided exposure at ≥ 75 kV/m, although they preferred exposure to fields less than 50 kV/m, but only during the light part of the daily cycle. In both the short- and longer-duration exposures, traverses from one end of the shuttlebox to the other end were transiently increased among exposed rats. Thirty days of exposure to a 100-kV/m electric field significantly attenuated subsequent avoidance performance in the shuttlebox, indicating a possible adaptation to the electric field.

Mice were tested to determine the effects of electric-field exposure on sound-induced seizure sensitivity. Results indicated no effect of electric-field exposure at 100 kV/m for 14 or 30 days.

A. INTRODUCTION

The perception of chronic stimulation by an animal can result in activation of the pituitary-adrenocortical axis, causing numerous changes in systemic function, such as increased blood pressure and heart rate, and alterations in blood chemistry (Selye, 1956). Perception of 32-kV/m, 60-Hz electric fields by pigeons has been reported (Graves, et al., 1977) using the presence of the field as a cue in a conditioned suppression paradigm. Avoidance of 100-kV/m, 50-Hz electric fields by rats has

been reported by Bayer et al. (1977). The last Annual Report (Phillips, et al., 1978) described research we have conducted which shows that rats avoid electric fields of 90 kV/m and greater during a single 45-min exposure session.

This report describes three perception and avoidance experiments which substantiate and extend the earlier findings. In the first experiment, we studied avoidance of 60-Hz electric fields by rats during four sequential 45-min tests, spaced 1 wk apart. In the second experiment (and the subsequent replicate), perception and avoidance of electric fields by rats were examined over a 23.5-hr session. In the third set of experiments, we investigated the effects of chronic exposure of rats to a 100-kV/m field prior to the perception and avoidance tests.

Experiments were also conducted to assess the possible effect of electric-field exposure for 14 or 28 days on the sensitivity of mice to sound-induced seizures.

Additionally, several engineering and dosimetric tests were performed to make sure that the behavioral results were not the result of factors other than the electric field per se. These tests measured corona and ozone formation, electric-field strength, and vibration within the shuttlebox.

B. METHODS

1. Shuttlebox Experiments. The Special Studies Exposure System described in the Second Interim Progress Report (Phillips and Kaune, 1977) was used to generate the electric fields for the behavioral experiments.

Apparatus. Rats were tested for electric field avoidance in a Lexan* shuttlebox (Figure XIII-1) described in the Second Interim Progress Report (Phillips and Kaune, 1977). Briefly, this shuttlebox, 103 cm long by 11 cm wide by 10 cm deep, had 44.5 cm of one end shielded from the electric field by covering the surface with aluminum window screen. The opposite end was covered with visually identical plastic mesh screen which did not attenuate the electric field. In a center (transition) region, two light beams allowed us to monitor the position and traverse activity of the rat.

In all shuttlebox experiments water was supplied from a manifold located in the field-free region below the shuttlebox. Seven demand-activated valves, 12.7 cm apart, protruded approximately 0.5 cm above the mesh floor, three on either end and one in the center of the shuttlebox. These valves were at ground potential, as was the water. This system has been described in detail elsewhere (Phillips et al., 1976a).

Food was supplied to the rats during exposure sessions that exceeded 45 min. Approximately 25 g of pelleted food was available, ad libitum, at either end of the shuttlebox from Lexan* hoppers that had a low profile to minimize any perturbation of the electric field.

The shuttlebox was ventilated at the top (136 holes; 0.7-cm-diameter, 2.5 cm apart).

Electric Field Measurements. Measurements inside the shuttlebox at floor level were made with a flat, nonperturbing 5-cm-diameter circular probe (Kaune, 1978).

*General Electric type MR 4000, polycarbonate plastic

FIGURE XIII-1. Diagram of Shuttlebox System Used to Test Perception and Avoidance of Electric Fields

Corona and Ozone Measurements. We tested for corona discharge by audible noise, emission of visible light, and ozone generation. We checked audible noise by listening, which experience has shown to be a sensitive method. Visible light emission was monitored using extended-exposure photography (Beamer, 1978). Ozone levels were measured with a chemiluminescent ozone analyzer, a series of potassium-iodide impingers and an ozone reaction tube-type sampler.* The O_3 sensitivity level of the analyzer is 1 ppb, while the impinger and sampler methods are sensitive to O_3 concentrations of 5 ppb.** The techniques used, and many of the results, have been described by Phillips et al. (1978).

Vibration Measurements. The amplitude and frequency of floor vibrations in the shuttlebox were measured with two piezoelectric accelerometers.*** Electrically isolated magnetic mounts were used to attach the two accelerometers to the underside of the floor of the shuttlebox. Typically, one accelerometer was placed in the shielded end of the shuttlebox and one in the unshielded end. The sensitivity of these accelerometers is about 1 m/sec^2 ; their frequency is from 2 Hz to 12 kHz.

Procedures for Behavioral Experiments. Two experiments were conducted to investigate the behavioral response of rats to a range of electric field strengths. The parameters evaluated were avoidance behavior, traverse activity, food consumption and serum corticosterone levels. Avoidance of electric fields was defined as a statistically significant increase in

*Bendix Gas/Tec pump with Dräger Detection Tubes Drägerwerk, A. G. Lubek, F. R. of Germany

**Bendix Corp., Model 8002, Lewisburg, WV

***BBN Instruments Co., Model 508, Cambridge, MA

the time that exposed rats spent in the shielded end of the shuttlebox apparatus. Traverse activity is defined as the number of traverses made between the two ends of the shuttlebox during test sessions.

Forty naive male Sprague-Dawley rats (Hilltop Labs), approximately 90 days old, were distributed into five groups that had equivalent body weight distributions. In the first experiment, the groups ($N = 8/\text{group}$) were randomly designated for behavioral tests at field strengths of 0, 60, 75, 90 and 105 kV/m, with a single field-strength value used for animals within each group throughout the experiment. These particular field strengths were selected on the basis of preliminary experiments, which suggested that rats avoid electric fields of 100 and 133 kV/m but not 75-kV/m fields (Phillips and Kaune, 1977). Rats were subjected to one test (45 min) per week for 4 wk. The amount of time spent in exposed, center and shielded regions, the number of traverses to each region, and the duration (time on each side of the box per traverse) were recorded.

Prior to and between sessions, rats were housed individually in standard stainless steel cages where they were given free access to food and water. The light:dark cycle was 12 hr:12 hr (lights on at 7 a.m. and off at 7 p.m.). The order of testing experimental and control groups was balanced across time of day to avoid potential diurnal cycle effects, and to insure that a minimum of one subject from each group was tested on a given day. Prior to each session, the wire-mesh screen and shuttlebox were sponged and dried, and the shuttlebox and cover were rotated 180° to alternate the side of exposure, thus balancing side of exposure within and across groups. During a given day, half the rats were tested

with the right side of the box exposed to the field, while the remainder were exposed on the opposite side. This procedure was followed to avoid possible confounding due to side bias or spatial association.

In the second experiment, the groups ($N = 8/\text{group}$) were tested at field strengths of 0, 25, 50, 75, and 100 kV/m for 23.5 hr. (Because of an electric power failure, it was necessary to eliminate one rat in the 0-kV/m group from the experiment.) The 25- and 50-kV/m field strengths were used to test the possibility that a behavioral response may occur at a lower field strength with a longer duration of exposure.

The longer exposure duration of the second experiment allowed a comparison of avoidance and traverse activity over the light and dark periods of a 24-hr cycle. In addition to avoidance and traverse activity measures, food consumption at each end of the shuttlebox was measured. Within 2 min after the end of each session, a blood sample was taken by cardiac puncture while the rat was anesthetized with fluothane gas (Halothane). Serum samples were analyzed fluorometrically for corticosterone concentration.

In the 23.5-hr tests, the shuttlebox was rotated 180° daily to reduce possible side bias. Both food containers were filled with 25 g of fresh food before each session. Sessions began at 11:00 a.m. and ended at 10:30 a.m. the following morning. The two light periods (8 hr and 3.5 hr), with one dark period (12 hr) between them, allowed us to examine changes of side preference during the light and dark cycles under the influence of electric fields.

2. Chronic Exposure Experiments. A series of experiments were conducted in which rats were either exposed or sham exposed to a 100-kV/m, 60-Hz electric field for 30 days prior to testing in the shuttlebox at field strengths of 0, 25, 50, 75 and 100 kV/m (N = 12 exposed and 12 sham-exposed per field strength/group).

Due to space limitations within the exposure system, three replicate experiments were conducted and the results were combined. In each experiment, 40 male Sprague-Dawley rats (55-60 days of age) were exposed (~ 20 hr/day) for 30 days prior to behavioral testing. The shuttlebox apparatus (Figure XIII-1) was the same as that used in previous experiments. In the first of the three experiments the emphasis was on those field strengths where effects had been seen in other avoidance studies, i.e., 50, 75, and 100 kV/m. After the first experiment we decided that information from a 25-kV/m exposure was necessary; the designs of experiments 2 and 3 were therefore altered to include exposure of rats at 25 kV/m.

Each test session lasted 45 min. The order in which rats were tested was balanced to avoid possible bias by diurnal effects. No rat was tested more than once. Records were kept of the traverse activity and the amount of time rats spent in the exposed, center and shielded regions of the shuttlebox.

3. Audiogenic Seizure Study

Electric-Field Exposures. Mice in these experiments were exposed to a 100-kV/m field for 14 or 28 days. The exposures were conducted in the Mouse Exposure System (Phillips et al., 1978).

Apparatus. Mice were tested for audiogenic seizure susceptibility and latency in a 30-gal. galvanized steel garbage can. Two alarm bells that produced a "white" noise output of 110 db were mounted inside the can. The can was located inside a sound-deadened room. A mirror, angled over the can, allowed investigators to time latency to seizures from outside the room through a window in the door of the sound-deadened enclosure.

Procedure. Sixty male SJL/J mice, received at 14 days of age, were housed in groups with ad libitum access to food and water until they were 21 days of age. The SJL/J mouse (Jackson Laboratories) is genetically susceptible to sound-induced central nervous system seizures; however, for maximum sensitivity, the mice must be "primed" at 21 days of age. This priming consisted of a 60-sec exposure to the 110-db noise. No mice exhibited seizures during this priming. At 28 days of age all mice were pretested for seizure latency. The SJL/J mice demonstrate two sequential forms of seizures when exposed to loud noise: first, they go into a rapid running behavior, followed by a clonic-tonic seizure. The susceptibility of mice to such seizures is dependent upon several factors, including the general excitability state of the central nervous system.

Based on mean latencies to both types of seizure at 28 days of age, two homogeneous groups were selected for subsequent exposure to the electric field. These mice were exposed or sham exposed for 14 or 28 days (from day 29 of age to day 42, or from day 29 to day 57), then retested for seizure latency. All seizure testing was conducted between 10:30 a.m. and 12:00 p.m. to avoid possible effects of circadian rhythm on seizure sensitivity.

C. EXPERIMENTAL RESULTS

1. Environmental Measurements

Shuttlebox Electric Field Measurements. Field-strength measurements made inside the outer box which incorporated the metal and plastic screens indicated a very small field beneath the metal end, and 95% of the applied field beneath the plastic end. However, when measurements were made with the inner box in place, an erratic instability in field strength was observed in the unshielded end of the system. Sudden random shifts of as much as 25% in field strength were observed, but only with both boxes in place. The nature of this instability is not well understood but may be related to charge migration through the air between the tops of the two boxes.

In replicate experiments of our early work, and for the subsequent behavioral experiments, a new shuttlebox system was built incorporating only one Lexan box (Figure XIII-1) in which field strength measurements were stable. The measured, floor-level field strength along the length of the box is shown in Figure XIII-2. Field-strength scans across the width of the box in the unshielded region showed the field to be quite uniform ($\pm 1\%$) in the exposed end of the box and fairly uniform ($\pm 10\%$) in the transition region between the shielded and unshielded ends.

Shuttlebox Vibration Measurements. The ambient levels of acceleration of the floor of the shuttlebox without the electric field were typically in the range of 0.05 m/sec^2 , peak-to-peak, over a wide frequency range. Occasionally, much stronger vibrations ($\sim 0.5 \text{ m/sec}^2$, peak-to-

THIS FIGURE INDICATES THE % OF THE APPLIED ELECTRIC FIELD ATTENUATED BY THE METAL AND PLASTIC COVERINGS OVER THE SHUTTLEBOX.

FIGURE XIII-2. Field Strength (kV/m) Along Long Axis of the Perception and Avoidance Shuttlebox

peak) with frequencies in the range 30-100 Hz were observed, evidently associated with the operation of electric motors in the building's heating and ventilation system.

With the shuttlebox removed and the system energized to a field strength of 130 kV/m, the level of vibration was unchanged from the 0-field case. With the shuttlebox in place, however, a 120-Hz vibration of 0.15 m/sec^2 , peak-to-peak, was observed in the unshielded end of the shuttlebox. When the shuttlebox was reversed, vibration was observed in the shielded end of the shuttlebox. The two accelerometers were also interchanged, and the same results were obtained. Rotating the entire lower electrode unit 180° resulted in the vibration also moving to the other side of the exposure system. The conclusion was that one end of the electrode was vibrating while the other was not. The amplitude of this vibration, measured as a function of field strength, was found to depend on the square of the field strength. These data, and the observation that the vibration frequency is 120 Hz, indicate that this vibration is the result of electrical attraction between the two exposure electrodes (Reitz and Milford, 1960). A 120-Hz vibration with a peak-to-peak acceleration of 0.15 m/sec^2 corresponds to a peak-to-peak physical displacement of the shuttlebox floor of $0.25 \text{ } \mu\text{m}$.

Corona and Ozone Testing. The results of photographic room mapping for visible light emissions were negative, as were tests for audible noise. The results of ozone testing were also negative at numerous locations within the shuttlebox and exposure room. Ozone concentrations did not exceed ambient levels present without the electric field.

2. Behavioral Tests

Shuttlebox Side Preference Studies. The results of the repeated, short (45-min) sessions (Figure XIII-3, Table XIII-1) indicate a significant avoidance response by rats to electric-field exposure. In the first 45-min session, rats exposed to field strengths of 90 or 105 kV/m spent significantly less time in the exposed end of the shuttlebox than did 0-kV/m controls (Student's t test = 3.24 and 4.28, respectively, both, $p < 0.01$; 15 df). When data were analyzed by a repeated measures analysis of variance (Winer, 1962; Satterthwaite, 1946), it was found that exposed rats spent significantly more of the 45-min session in the unexposed region of the shuttlebox, both across sessions [$F(4,35) = 3.64$, $p < 0.05$] and within three of the individual sessions:

Session 1 - $F(4,35) = 3.29$, $p < 0.05$
Session 2 - $F(4,35) = 0.63$, NS
Session 3 - $F(4,35) = 3.70$, $p < 0.05$
Session 4 - $F(4,35) = 7.28$, $p < 0.01$

The source of the variance differences among groups is primarily from the 90 and 105 kV/m groups [Dunnett's t test (Winer, 1972)]. The t values were computed on the means of the four sessions for each group with the following results:

0 kV/m vs. 60 kV/m: $F(4,35) = 1.95$, NS
0 kV/m vs. 75 kV/m: $F(4,35) = 0.78$, NS
0 kV/m vs. 90 kV/m: $F(4,35) = 2.56$, $p < 0.05$
0 kV/m vs. 105 kV/m: $F(4,35) = 3.70$, $p < 0.01$

FIGURE XIII-3. Mean Percent of Time ($\pm$ S.E.) Rats Spent on the Unexposed Side of the Shuttlebox as a Function of Field Strength and Test Session. Data are normalized to eliminate time spent in the center.

TABLE XIII-1. Mean ($\pm$ S.E.*) Percentage of Time Rats Spent in the Exposed, Center and Unexposed (Shielded) Regions of a Shuttlebox During Four 45-Min Sessions of Exposure to 60-Hz Electric Fields

Field Strength (kV/m)	N	Mean % of Residence Time ($\pm$ S.E.)			Significance**
		Exposed	Center	Unexposed	
<u>SESSION 1</u>					
0	8	51.1 $\pm$ 7.9	10.3 $\pm$ 1.7	38.5 $\pm$ 7.8	NS
60	8	35.6 $\pm$ 2.3	13.5 $\pm$ 2.3	50.8 $\pm$ 9.6	NS
75	8	53.9 $\pm$ 10.2	9.4 $\pm$ 1.6	36.9 $\pm$ 10.0	NS
90	8	24.3 $\pm$ 3.9	9.9 $\pm$ 2.4	66.0 $\pm$ 5.8	<0.01
105	8	17.4 $\pm$ 2.8	10.1 $\pm$ 1.8	72.4 $\pm$ 4.3	<0.01
<u>SESSION 2</u>					
0	8	39.1 $\pm$ 9.8	6.7 $\pm$ 1.8	53.1 $\pm$ 10.7	NS
60	8	33.0 $\pm$ 8.4	6.2 $\pm$ 1.0	60.7 $\pm$ 8.7	NS
75	8	40.0 $\pm$ 5.9	5.9 $\pm$ 1.9	54.1 $\pm$ 11.2	NS
90	8	43.6 $\pm$ 9.9	9.5 $\pm$ 1.5	46.9 $\pm$ 10.2	NS
105	8	25.6 $\pm$ 9.7	5.6 $\pm$ 1.4	67.5 $\pm$ 10.6	NS
<u>SESSION 3</u>					
0	8	45.7 $\pm$ 10.2	6.7 $\pm$ 1.7	47.5 $\pm$ 9.8	NS
60	8	36.6 $\pm$ 11.9	4.7 $\pm$ 1.3	58.6 $\pm$ 12.0	NS
75	8	38.5 $\pm$ 11.1	5.5 $\pm$ 1.3	56.0 $\pm$ 10.6	NS
90	8	38.6 $\pm$ 10.7	6.1 $\pm$ 1.5	55.2 $\pm$ 11.1	NS
105	8	22.5 $\pm$ 10.5	5.7 $\pm$ 1.1	71.5 $\pm$ 10.1	<0.05
<u>SESSION 4</u>					
0	8	52.7 $\pm$ 10.8	5.1 $\pm$ 0.8	42.1 $\pm$ 10.4	NS
60	8	31.5 $\pm$ 10.0	5.5 $\pm$ 0.8	63.0 $\pm$ 9.7	NS
75	8	39.5 $\pm$ 9.4	5.2 $\pm$ 0.8	55.1 $\pm$ 9.5	NS
90	8	23.0 $\pm$ 7.2	8.5 $\pm$ 1.3	68.7 $\pm$ 7.3	NS
105	8	26.0 $\pm$ 10.2	5.9 $\pm$ 1.1	68.1 $\pm$ 10.2	NS

* Values have been rounded off.

** Two-tailed Mann-Whitney U Test, comparing values of 0-kV/m group with those of experimental group

Traversal activity, as determined by the number of end-to-end traverses in the shuttlebox during repeated 45-min sessions, was significantly affected by the electric field [$F(4,35) = 8.36, p < 0.05$]; (Table XIII-2). However, while these data indicate a trend toward higher traversal activity among exposed animals over repeated sessions, analysis within sessions does not reveal a statistically significant difference between groups.

Subsequent to the experiment, two possible problems were identified: 1) the electric field was erratic when the inner/outer shuttlebox was used, as already discussed; 2) a 4% fifth harmonic and 3% seventh harmonic were observed in the electric field (Phillips et al., 1978). Both of these problems were eliminated, as discussed earlier. To test whether the observed behavioral effects could have resulted from either of these problems, a replicate experiment (40 naive rats tested in a single 45-min session) was performed. The results of the first study were substantiated (Figure XIII-4). Rats exposed to fields of 90 or 105 kV/m spent significantly more time in the shielded end of the shuttlebox than sham-exposed controls [Student's t test = 2.54 (13 df) and 1.82 (14 df), respectively; $p < 0.05$]. Neither the electric-field instability nor the harmonic distortion present in the first experiment was apparently the factor mediating the avoidance response.

In 23.5-hr tests, rats exposed to 75 or 100 kV/m spent significantly more time [$F(4,34) = 10.06, p < 0.001$] in the region shielded from the electric field than 0-kV/m controls (Figure XIII-5). Rats tested at 25 or 50 kV/m spent significantly more time [$F(4,34) = 9.58,$

TABLE XIII-2. Number of Traverses ($\pm$ S.E.) Through the Center Region of the Shuttlebox Made by Rats Exposed to Various Electric Field Strengths During Four 45-Min Exposure Sessions

kV/m	N	Number of Traverses							
		Week 1		Week 2		Week 3		Week 4	
0	8	51.38 $\pm$	9.01	41.50 $\pm$	10.37	34.25 $\pm$	5.52	25.75 $\pm$	3.28
60	8	58.63 $\pm$	13.43	41.25 $\pm$	8.66	38.13 $\pm$	8.87	36.13 $\pm$	4.05
75	8	57.63 $\pm$	9.61	37.75 $\pm$	12.00	33.38 $\pm$	10.53	42.13 $\pm$	9.79
90	8	47.25 $\pm$	8.67	49.75 $\pm$	7.96	39.25 $\pm$	11.25	44.13 $\pm$	6.68
105	8	56.38 $\pm$	9.50	34.38 $\pm$	6.01	35.13 $\pm$	6.78	40.38 $\pm$	9.25

FIGURE XIII-4. Mean Percent of Time ($\pm$ S.E.) Rats Spent in Exposed, Center and Unexposed Regions of the Shuttlebox During a Single, 45-min Test Session. Data are presented in hourly increments during the light and dark periods of the session.

UNEXPOSED SIDE PREFERENCE OF 40 NAIVE RATS TESTED FOR ELECTRIC FIELD AVOIDANCE DURING A SINGLE 23.5 HOUR SESSION. NOTE THE EXPOSED SIDE PREFERENCE AT 25 AND 50 kV/m.

FIGURE XIII-5. Mean Percent of Time Rats Spent in the Unexposed Region During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

$p < 0.05$] in the exposed region of the shuttlebox than controls during the light portion of the daily cycle, but not during the dark portion. Avoidance of the exposed side among rats tested at 75 or 100 kV/m decreased steadily during the dark period, then increased at the onset of the second light period. The opposite was the case for rats tested at 25 or 50 kV/m.

Traverse activity (Figure XIII-6) over the 23.5-hr sessions was significantly affected [$F(4,34) = 79.28, p < 0.001$] by the light cycle, as expected. All rats were initially active in the novel shuttlebox environment. This traverse activity decreased to low levels within 2 hr after the start of the session. During the first hour of exposure there was a significant [$F(4,34) = 7.16, p < 0.05$] effect of exposure on traverse activity. Control rats made fewer traverses than exposed rats, while the group exposed at 100 kV/m made the greatest number of traverses during the first hour. Traverse activity in all groups increased slightly with the start of the dark period, continued to rise during the period, then decreased at the onset of the next light period (Figure XIII-6).

Over the 23.5-hr sessions, rats exposed at 75 or 100 kV/m spent significantly [$F(4,34) = 10.06, p < 0.05$] less time on the exposed side per traverse than rats tested at 0 kV/m. During these sessions, rats exposed at 25 or 50 kV/m did not differ significantly from 0-kV/m rats in residence time per traverse on the exposed side.

There were no differences among groups in the amount of total food consumed on the exposed or shielded side of the shuttlebox, nor were there any differences in total food consumption among groups.

FIGURE XIII-6. Mean Number of Traverses Through the Center Region of the Shuttlebox by Rats During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

The results of fluorometric analysis of serum corticosterone levels indicated no statistically significant differences among groups (Table XIII-3).

The results of a subsequent replication of the second (23.5-hr) experiment did not differ significantly from the results reported here, with one exception: rats tested at 25 kV/m did not differ significantly from control (0-kV/m) rats. Rats tested at 75 or 100 kV/m avoided the electric field, while rats tested at 50 kV/m spent significantly more time exposed to the field during the light period than 0-kV/m controls. There were no significant differences among groups in traverse activity after the first hour of exposure. The results of the initial experiment and the replicate are compared in Tables XIII-4 to -8 by light and dark periods within the 23.5-hr session.

Chronic Exposure Experiments. The results indicate that rats exposed to 100 kV/m for 20 hr/day for 30 days prior to the behavioral test spent a significantly higher percentage of the 45-min session in the exposed region when tested at 25 kV/m, than did previously sham-exposed controls (Student's t test = 2.10 $p < 0.05$, 22 df). Within the pre-exposed group, there was significantly greater exposed-side residence time across field strengths [$F(4,55) = 6.44$, $p < 0.05$], while within the previously sham-exposed group there was significantly greater unexposed-side residence across field strengths [$F(4,54) = 6.84$, $p < 0.05$].

The behavior of rats pre-exposed to 100 kV/m for 30 days differed from that of previously sham-exposed rats at two field strengths: 25 and 100 kV/m (Figure XIII-7). Preference for the exposed side of the .

TABLE XIII-3. Plasma Corticosterone Levels (Fluorometric Assay) in Rats at the End of 24-Hr Shuttlebox Test

<u>Field Strength (kV/m)</u>	<u>N</u>	<u>Corticosterone Levels (Mean $\pm$ S.E.)</u>
0	6	155 $\pm$ 17
25	6	140 $\pm$ 16
50	6	129 $\pm$ 30
75	6	140 $\pm$ 32
100	5	171 $\pm$ 40

TABLE XIII-4. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of the Shuttlebox During a 23.5-hr Exposure Session to Electric Fields at 0-100 kV/m. Values shown are means $\pm$ S.E.

Field Strength (kV/m)	Initial Experiment (N = 39)			Replicate Experiment (N = 40)		
	Exposed	Center	Unexposed	Exposed	Center	Unexposed
0	47.2 $\pm$ 1.7	4.5 $\pm$ 0.8	48.3 $\pm$ 1.8	49 $\pm$ 2.6	9 $\pm$ 1.1	42 $\pm$ 2.5
25	51.6 $\pm$ 7.9	8.6 $\pm$ 3.8	39.0 $\pm$ 6.7	43 $\pm$ 2.7	9 $\pm$ 0.9	48 $\pm$ 2.6
50	59.3 $\pm$ 3.5	5.9 $\pm$ 1.2	34.6 $\pm$ 3.4	55 $\pm$ 2.7	15 $\pm$ 1.7	30 $\pm$ 2.6
75	27.6 $\pm$ 2.6	3.1 $\pm$ 0.5	69.0 $\pm$ 2.7	32 $\pm$ 2.7	11 $\pm$ 1.5	57 $\pm$ 2.8
100	19.8 $\pm$ 2.4	5.6 $\pm$ 1.1	76.1 $\pm$ 1.0	29 $\pm$ 2.5	5 $\pm$ 0.6	66 $\pm$ 2.5

TABLE XIII-5. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of the Shuttlebox During First Light Period (Hr 1-8) With Electric Field Exposure at 0-100 kV/m. Values shown are means $\pm$ S.E.

Field Strength (kV/m)	Initial Experiment (N = 39)			Replicate Experiment (N = 40)		
	Exposed	Center	Unexposed	Exposed	Center	Unexposed
0	47.3 $\pm$ 2.5	1.4 $\pm$ 0.6	51.3 $\pm$ 2.4	50.1 $\pm$ 4.8	9.4 $\pm$ 2.1	39.1 $\pm$ 4.7
25	53.7 $\pm$ 4.6	3.3 $\pm$ 1.5	42.9 $\pm$ 4.1	42.9 $\pm$ 5.1	9.1 $\pm$ 2.0	46.1 $\pm$ 4.8
50	67.0 $\pm$ 6.6	1.6 $\pm$ 0.4	31.5 $\pm$ 6.3	67.8 $\pm$ 4.7	9.2 $\pm$ 2.5	23.1 $\pm$ 4.2
75	24.0 $\pm$ 4.4	2.1 $\pm$ 0.5	72.6 $\pm$ 4.8	43.5 $\pm$ 5.3	9.2 $\pm$ 2.7	47.9 $\pm$ 5.2
100	10.9 $\pm$ 4.5	1.7 $\pm$ 0.6	87.4 $\pm$ 5.0	41.9 $\pm$ 5.2	4.2 $\pm$ 1.4	54.0 $\pm$ 5.2

TABLE XIII-6. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of a Shuttlebox During the Second Light Period with Electric Field Exposure at 0-100 kV/m. Values shown are means $\pm$ S.E.

Field Strength (kV/m)	Initial Experiment (N = 39)			Replicate Experiment (N = 40)		
	Exposed	Center	Unexposed	Exposed	Center	Unexposed
0	36.2 $\pm$ 4.6	6.3 $\pm$ 3.2	57.4 $\pm$ 2.9	64.2 $\pm$ 7.3	1.4 $\pm$ 0.4	31.6 $\pm$ 7.1
25	71.7 $\pm$ 3.2	8.9 $\pm$ 3.5	19.1 $\pm$ 4.0	34.0 $\pm$ 6.9	3.6 $\pm$ 1.3	62.6 $\pm$ 6.6
50	77.5 $\pm$ 6.3	6.6 $\pm$ 4.4	15.8 $\pm$ 1.9	58.7 $\pm$ 7.7	7.8 $\pm$ 3.7	33.6 $\pm$ 7.7
75	20.2 $\pm$ 5.4	0.7 $\pm$ 0.7	79.0 $\pm$ 4.7	33.9 $\pm$ 7.6	10.7 $\pm$ 4.4	55.5 $\pm$ 7.9
100	13.6 $\pm$ 4.3	6.2 $\pm$ 3.4	80.1 $\pm$ 6.3	24.5 $\pm$ 7.1	2.6 $\pm$ 1.1	73.3 $\pm$ 7.0

TABLE XIII-7. Percent of Time Rats Spent in the Exposed, Center and Unexposed Regions of a Shuttlebox During Light Periods (Hr 1-8 and 23.5) with Electric Field Exposure at 0-100 kV/m. Values shown are means $\pm$ S.E.

Field Strength (kV/m)	Initial Experiment (N = 39)			Replicate Experiment (N = 40)		
	Exposed	Center	Unexposed	Exposed	Center	Unexposed
0	43.6 $\pm$ 2.6	3.0 $\pm$ 1.2	53.3 $\pm$ 1.9	55.5 $\pm$ 4.1	7.6 $\pm$ 1.7	37.0 $\pm$ 3.9
25	59.7 $\pm$ 4.0	5.2 $\pm$ 1.6	35.0 $\pm$ 4.5	41.4 $\pm$ 4.1	7.2 $\pm$ 1.4	51.6 $\pm$ 4.0
50	70.5 $\pm$ 4.8	3.3 $\pm$ 1.4	26.3 $\pm$ 4.7	64.8 $\pm$ 4.0	8.7 $\pm$ 2.1	26.6 $\pm$ 3.8
75	22.8 $\pm$ 3.2	1.6 $\pm$ 0.5	74.8 $\pm$ 3.4	40.0 $\pm$ 4.3	9.7 $\pm$ 2.3	50.4 $\pm$ 4.3
100	11.8 $\pm$ 3.1	3.2 $\pm$ 1.2	85.0 $\pm$ 3.8	36.1 $\pm$ 4.2	3.7 $\pm$ 1.0	60.4 $\pm$ 4.2

TABLE XIII-8. Percent of Time Rats Spent in the Exposed, Center and Shielded Regions of a Shuttlebox During the 12-Hr Dark Period with Electric Field Exposure at 0-100 kV/m. Values shown are means $\pm$ S.E.

Field Strength (kV/m)	Initial Experiment (N = 39)			Replicate Experiment (N = 40)		
	Exposed	Center	Unexposed	Exposed	Center	Unexposed
0	50.8 $\pm$ 2.0	5.9 $\pm$ 1.1	43.3 $\pm$ 2.4	42.1 $\pm$ 3.0	11.2 $\pm$ 1.3	46.9 $\pm$ 3.1
25	44.3 $\pm$ 3.4	11.9 $\pm$ 1.6	44.5 $\pm$ 3.3	43.9 $\pm$ 3.5	11.0 $\pm$ 1.2	45.3 $\pm$ 3.3
50	48.1 $\pm$ 2.6	8.6 $\pm$ 1.7	43.0 $\pm$ 3.8	44.7 $\pm$ 3.3	21.1 $\pm$ 2.5	34.3 $\pm$ 3.4
75	32.4 $\pm$ 3.7	4.6 $\pm$ 0.7	63.3 $\pm$ 3.7	23.7 $\pm$ 2.9	12.3 $\pm$ 1.9	64.2 $\pm$ 3.5
100	27.8 $\pm$ 2.0	8.0 $\pm$ 1.6	64.3 $\pm$ 2.9	22.8 $\pm$ 2.6	6.6 $\pm$ 0.7	70.6 $\pm$ 2.7

UNEXPOSED SIDE PREFERENCE BY FIELD STRENGTH OVER THE 45 MIN. SESSION.

FIGURE XIII-7. Mean Number of Traverses Through the Center Region of the Shuttlebox by Rats During a 23.5-hr Test Session. Data are presented in hourly increments during the light and dark periods of the session.

shuttlebox by the 25-kV/m group was significantly increased over controls during the last 15 min of the session (Student's t test = 2.18, $p < 0.05$, 22 df). The apparent preference, among pre-exposed rats, for the exposed side at 100 kV/m is not statistically significant.

During the 45-min behavioral tests there were no significant differences between groups (pre-exposed or sham-exposed) in the number of traverses made to any region at any of the field strengths tested. At 0 kV/m, the sham-exposed rats made more traverses to the center and unexposed regions of the shuttlebox than exposed rats during the 15-min to 30-min period of the session (Student's t test = 2.24 and 2.49, respectively, $p < 0.05$, 22 df), but overall (0-45 min), there was no significant difference between groups.

3. Audiogenic Seizure Experiments. The latencies to running fits and clonic-tonic seizures did not differ significantly between control and exposed mice (Table XIII-9 and -10).

D. DISCUSSION

1. Shuttlebox Side-Preference Experiments. These experiments demonstrate behavioral effects of 60-Hz electric fields and substantiate earlier reports of avoidance (Bayer et al., 1977) and increased activity (Moos, 1964) during exposure. The results do not support previous findings of lowered serum corticosterone levels (Marino et al., 1976) due to exposure to a 60-Hz electric field, although this may be due to the different exposure durations in the two experiments.

TABLE XIII-9. Audiogenic Seizure Sensitivity of Male SJL/J Mice
After 14 Days Exposure to 60-Hz Electric Fields

<u>BEFORE EXPOSURE</u>			
<u>Group</u>	<u>Number Seized/ Number Tested</u>	<u>Latency to Running Fit Mean $\pm$ S.E.</u>	<u>Latency to Clonic-Tonic Seizure Mean $\pm$ S.E.</u>
Exposed	13/13	4.66 $\pm$ 0.23	7.98 $\pm$ 0.25
Sham- Exposed	13/13	4.65 $\pm$ 0.24	7.77 $\pm$ 0.24
<u>AFTER EXPOSURE</u>			
<u>Group</u>	<u>Number Seized/ Number Tested</u>	<u>Latency to Running Fit Mean $\pm$ S.E.</u>	<u>Latency to Clonic-Tonic Seizure Mean $\pm$ S.E.</u>
Exposed	11/11	4.02 $\pm$ 0.22	8.05 $\pm$ 0.30
Sham- Exposed	9/11	4.13 $\pm$ 0.17	8.78 $\pm$ 0.37

TABLE XIII-10. Audiogenic Seizure Sensitivity of Male SJL/J Mice
After 28 Days Exposure to 60-Hz Electric Fields

<u>BEFORE EXPOSURE</u>			
<u>Group</u>	<u>Number Seized/ Number Tested</u>	<u>Latency to Running Fit Mean $\pm$ S.E.</u>	<u>Latency to Clonic-Tonic Seizure Mean $\pm$ S.E.</u>
Exposed	16/16	4.77 $\pm$ 0.19	7.87 $\pm$ 0.23
Sham- Exposed	16/16	4.98 $\pm$ 0.21	7.88 $\pm$ 0.24
<u>AFTER EXPOSURE</u>			
<u>Group</u>	<u>Number Seized/ Number Tested</u>	<u>Latency to Running Fit Mean $\pm$ S.E.</u>	<u>Latency to Clonic-Tonic Seizure Mean $\pm$ S.E.</u>
Exposed	12/16	5.08 $\pm$ 0.27	7.57 $\pm$ 0.19
Sham- Exposed	9/16	5.09 $\pm$ 0.25	7.60 $\pm$ 0.28

It is important to note that the results in this study were obtained after secondary field factors (e.g., corona, ozone and noise) were eliminated as possible sources of biological effects or psychological cues. Preference and avoidance experiments were conducted with exposed and shielded regions of the shuttlebox each oriented toward the vibrating end of the exposure system 50% of the time. Orientation of the shuttlebox made no difference in results, and responses cannot be ascribed to field-induced cage vibration.

The avoidance response demonstrates that rats can discriminate between exposed and shielded regions of the shuttlebox, and that the threshold for this avoidance behavior is related to the duration of exposure. In the short-duration test (45 min), statistically significant avoidance of electric fields of 90 kV/m or higher was observed, while rats exposed at 60 or 75 kV/m had a similar but not statistically significant response. In a longer test (23.5 hr), the minimum field strength for avoidance was 75 kV/m, while rats exposed at 25 or 50 kV/m had an apparent preference for exposure during the light period. While bimodal behavioral responses mediated by different stimulus intensities have been noted previously (Schneirla, 1959), these results provide the first evidence of such a bimodal response to a 60-Hz electric field.

Our experiments demonstrate an increased traverse activity in exposed animals. In 45-min tests, both exposed and control rats showed equal traverse activity during the first test session. However, in subsequent sessions, exposed rats had more traverses than control rats, the latter showing a greater decline in traverses over sessions. Over

longer exposure durations, exposed rats initially had more traverses than controls, but this higher traverse activity was not maintained.

Analysis of serum corticosterone levels does not indicate sustained arousal due to field exposure. However, since blood samples were drawn at the end of a 23.5-hr exposure session, our analysis would not reflect early, transitory effects of electric fields on corticosterone levels such as those described by Graves and Poznaniak (in press).

We conclude that rats perceive and respond to electric fields due to their direct interaction with the electric field. One possible explanation for the observed findings is perception of field-induced movement of the skin or hair of the rat. The threshold for electric-field-induced hair oscillation has been determined in swine (Phillips, et al., 1976b; Kaune et al., 1977); we plan to conduct similar research with rats.

2. Audiogenic Seizure Experiment. Exposure of mice to a 100-kV/m electric field for 14 or 30 days did not affect their sensitivity to sound-induced seizures. These results suggest that exposure to electric fields does not affect the intrinsic level of the central nervous system excitability.

E. FUTURE PLANS

Two studies of avoidance learning in rats exposed to various electric-field strengths are currently in progress. One experiment is examining effects of acute exposure (24 hr) on learning. The other is

studying avoidance learning of rats exposed in utero and raised in the field; groups of exposed and control rats are being tested at 30, 60, and 90 days of age. All electric-field exposures are at 100 kV/m.

Our plans for the coming year include a study on the perception threshold of rats in an electric field. This study will use a conditioned suppression paradigm. These experiments will provide a basis for other studies to investigate the modality of electric-field perception. An experiment has also been planned to investigate whether exposure to a 60-Hz electric field will produce malaise/illness in rats.

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XIV. MUTAGENESIS

Principal Investigators: F. P. Hungate and S. C. Causey

ABSTRACT

Studies of mutagenic effects of high-strength dc fields using a wide variety of microbial test systems has failed to provide evidence of increased frequency of induction of either forward or reverse mutation events. Because of this, earlier studies with Salmonella strain TA100, which had indicated that dc fields are mutagenic, are being repeated.

Tests of mutagenicity of dc fields are continuing with Drosophila, using a repair-deficient strain.

A. INTRODUCTION

Little is known about the mutagenic effects of electric fields. Studies from the U.S. Navy Project Sanguine suggest that small electromagnetic fields do not produce significantly increased frequencies of recessive lethals in Drosophila. However, the fields tested were smaller than those encountered under high-voltage transmission lines, and the data provide no basis for confidence that larger electric fields are not mutagenic. The present study is directed toward testing for mutagenic effects of very large fields. It is assumed that negative data will provide confidence that lesser fields to which people are exposed are nonmutagenic. If the data indicate a mutagenic potential, a test system will then be available to examine the nature of dose-effect relationships and to study the mechanisms involved. Data reported earlier (Hungate,

et al., in press) indicated significantly increased mutation frequencies following exposure of the Ames Salmonella strain TA100 and Photobacterium fisheri, MAV to dc electric fields of 800 kV/m. Inconclusive data were obtained from tests for sex-linked recessive lethal mutation in Drosophila. Major effort was devoted this year to identify alternative biological test systems useful for establishing the nature of the dose-response relationship and the possible mechanisms involved in mutation induction.

B. MATERIALS AND METHODS

The exposure apparatus was designed to provide large uniform fields suitable for exposing organisms contained in a petri dish. The paired electrodes are 30-cm-diameter copper plates with their edges rolled to avoid corona. The electrodes are separated by a Lucite spacer, 25 cm in diameter, 3 mm thick and, typically, 2 cm high, although heights of 1.5 to 6 cm have also been used. A small hole in the Lucite spacer permits insertion of a polyethylene tube, which delivers moisture-saturated air into the chambers during exposures. This air leaks from the chambers through the small interface between the Lucite spacer and the electrodes. An Associated Research DC HYPOT, Model 5270, is used to energize the upper copper plate to obtain the desired field strengths for bacterial exposures. The lower plate is connected to ground. A battery pack applying 3.7 kV dc has been used for the Drosophila tests, and a second, similar pack is now available to provide 7.4 kV dc for the bacterial studies. The applied voltage is measured at the start and end of each

test with a static voltmeter. Simultaneous control specimens are maintained in an identical system. The controls are supplied with the same air source as exposed specimens, but with no applied voltage.

Bacteria are cultured in 125-ml flasks, with shaking, in a water bath held at 37°C. When a predetermined cell density (in the exponential growth phase) is achieved, the culture is diluted with fresh medium and a 20-ml aliquot is placed in each 10 x 1.5-cm plastic petri dish, to be exposed or used as a control culture. During the exposure period, the lids of the petri dishes are removed to prevent shielding by the lid. Following exposure, typically for 16-24 hr, the bacteria are resuspended in the medium, transferred to capped centrifuge tubes, pelleted, the medium removed, and the cells resuspended in a small volume of fresh medium. Approximately 10^8 cells are surface-plated on test medium to identify the frequency of mutants appearing on medium permitting growth of only mutated cells. The total cell numbers are determined by serial dilution and plating on nonselective medium.

Drosophila Canton-S strain pupae (0-24 hr postpupation) were exposed for 3 days to the dc field. A control was run simultaneously and was handled comparably. It received the same washed air as supplied to the exposed pupae. Following removal from the field and hatching of the flies, the males were individually mated to Muller-5 strain virgin females to test for the presence of sex-linked recessive lethals (Abramson and Lewis, 1971).

C. RESULTS AND DISCUSSION

Biological Tests. Three E. coli strains carrying plasmids (extra-chromosomal DNA) which code for resistance to specific drugs or antibiotics were studied for evidence of changes induced in the plasmids by electric fields. Plasmid RP4, in E. coli strain J53, confers resistance to ampicillin, kanamycin and tetracycline. In three tests, these cells were exposed for 21 hr and evaluated for expression of drug resistance. An average of 29% reduction of resistant cells was observed following exposure to a 500-kV/m dc field, compared to an average of 5.5% reduction in the unexposed controls. In all exposures, the air within the exposure chamber, as well as in the control chamber, was continually flushed with washed sterile air. In one similar test of E. coli strain C600, containing the plasmid R6K (which codes for resistance to ampicillin and streptomycin), there was a reduction of 17.8% in colonies resistant to the combined antibiotics, while unexposed controls showed no change. E. coli J53, containing the plasmid R388 (which codes for resistance to trimethoprim and sulfonamides), has been tested twice, with an average reduction of 25%, while controls were unchanged. While factors associated with resistance to ampicillin appear to be the most sensitive to the dc fields, we have not been able to isolate stable mutants, nor is there evidence of plasmid loss (curing).

Three tests of E. coli CSH23, carrying an F'lac pro A,B plasmid, which enables the host to utilize lactose as a carbon source, showed a slight (0.1%) loss of ability to grow on lactose in one test, and no

change in the other two tests. While it was determined that plasmid curing had occurred in one test, this system appears to be relatively unresponsive to dc fields.

It was well known that a variety of physical agents induce lysogeny in lysogenic strains of λ -phage. Two strains have been tested for response to a 500-kV/m dc field. E. coli CSH49, which contains the ϕ 80hD type virus, showed a 35-40% greater yield of virus particles in two 21-hr exposures and no increased yield in a third, compared to controls. A 4-hr exposure of this same system gave a yield 20% over the control. Similarly, E. coli CSH45, containing λ -phage, yielded a 37% increase of virus particles following a 21-hr exposure to the same field strength. Thus, at this time, there is a slight indication that lysogeny is induced by exposure to a dc field. This effect could be due to an inactivation of the genes that maintain the lysogenic state or to a change in their product, a repressor protein.

E. coli CSH23 has been tested for mutation of the Nal-A gene. Mutation at this locus enables cells to grow on otherwise restrictive concentrations of nalidixic acid. In four tests we observed no enhancement of mutation frequency over that in controls, following growth overnight in a 500-kV/m dc field.

One additional test was completed for induction of sex-linked lethal recessive mutants in Drosophila, giving 5 mutants per 2140 tested from male pupae exposed for 3 days to 185 kV/m, as compared to 6 mutants per 1919 tested in controls. This is the first test of males that gave fewer mutants in exposed pupae than in controls. Combining these results

with the data previously reported indicates 27 mutants per 7929 (0.34%) exposed male pupae and 17 per 6934 (0.25%) in controls. Female pupae similarly exposed gave 3 mutants per 2751 exposed and 1 per 2792 in the controls. Combined with previous data, the total is 12 mutants per 11,889 exposed (0.10%) and 7 mutants per 9347 controls (0.07%). While suggestive, these data fail to show a significant difference between the control and experimental groups.

We are currently testing a repair-deficient strain of Drosophila obtained from Dr. Melvin Green, University of California, Davis to determine if its response to electric fields is greater than that observed using the Canton-S strain.

Physical Tests. In the absence of recognized models to account for direct effects of dc fields, we have examined possible indirect causes of the observed biological effects. Since ozone is a well-recognized byproduct of electric fields, particularly if corona is present, we examined the exposure system photographically, using sensitive film and darkroom conditions. With fields up to 900 kV/m there was no evidence of corona. In addition, a Bendix Ozone Analyzer (Model 8002), capable of measuring ozone in concentrations down to 1 ppb, was used to test for ozone production. This unit detected no ozone with field strengths up to 500 kV/m, and barely detectable concentrations (1 ppb) with fields through 800 kV/m. When the washed air supply was disconnected, the unit immediately indicated ozone concentrations typical of the room air, around 15-30 ppb. These data indicate that the exposure system is

unusually free of ozone due to bubbling the incoming air through water, and that ozone is therefore not a causative factor for the biological effects observed.

The possibility of X-ray production in the field was investigated by placing three lithium fluoride dosimeter chips (Harshaw Chemical Co., TLD-700), wrapped in a single layer of paper, in a 1000-kV/m field for up to 3 days. Tests involved both flowing wet air and normal room air (60-70% RH). The initial test indicated some dose accumulation (27 ± 3 mR, compared to 14 ± 0 on control chips), but in two replicates there was no evidence of X-ray production (20 ± 3 mR in exposed vs. 19 ± 4 in controls; 19 ± 0 in exposed vs. 17 ± 0 in controls).

Attempts to evaluate air ions as possible factors are in a formative state. Since available equipment for measuring air ion concentrations requires much more air than is available from our chambers, we must now resort to calculated levels based upon measurement of current flow. Other techniques for direct measurement are being studied but none have yet been tested.

D. FUTURE PLANS

The experiments with Salmonella TA100 will be repeated. If positive effects are again found, several sources of possible artifacts can be evaluated: 1) replace polystyrene petri dishes with glass dishes to eliminate the possibility that high dc fields interacted with the plastic to produce toxic byproducts; 2) replace the dc power supply (transformer)

with a better power supply to eliminate ac ripple; 3) add a second control group, one which is kept in an energized exposure system but shielded from the dc field by a metal screen (Faraday) cage. No further experiments on dc electric fields are contemplated in FY 1980. Experiments will be initiated to assess possible mutagenetic effects of ac electric fields using test systems developed in the course of the dc experiments.

The analysis of recessive sex-linked lethal induction in Drosophila will continue until it is clear that such mutational events are, or are not, induced by the electric field. In this effort, we are now using the repair-deficient strain of Drosophila obtained from Dr. M. Green, and are also testing 370-kV, as well as 185-kV, dc fields. These tests will also be done in ac fields as soon as a continuous ozone-monitoring system is in place.

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XV. DOSIMETRY

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ABSTRACT

Interactive effects in electric-field exposure systems can significantly alter the fields experienced by an experimental subject. We have made measurements with a hemi-ellipsoidal rat model which show: (1) interactions between grounded rats and overhead electrodes in exposure systems can enhance exposure to electric fields, with the enhancement being considerably greater in multiple-animal than in single-animal systems; (2) plastic rat cages will decrease slightly the overall exposure of grounded rats to electric fields, but will increase by as much as (or more than) 20% the electric fields at the surface of those parts of the subject's body close to the walls of the cage; (3) animals will be significantly shielded from electric fields in systems where several animals are simultaneously exposed in close proximity to one another.

The short-circuit current, I_{sc} (μA), induced in a rat of weight $W(g)$ by an unperturbed electric field, E_0 (kV/m), is $I_{sc} = \alpha W^n E_0$, where $\alpha = 0.0033$ and $n = 0.63$.

Measurements with a prone rat model show that 15% of the short-circuit current originates in the head, 16% in the portion of the body between transverse sections through the neck and chest, 23% between transverse sections through the chest and abdomen, and 43% in the body posterior to a section through the abdomen. When the rat rears, these values change to 41%, 30%, 18%, and 11%, respectively, and the short-circuit current is doubled.

A. HEMI-ELLIPSOID MEASUREMENTS

1. Introduction. Electric-field exposure is conventionally specified in terms of the unperturbed field strength; that is, the field strength measured or calculated before a living organism is introduced into the field. Living tissues are conductors (Geddes and Baker, 1967) and the applied

field will be perturbed by the body of an exposed organism. Therefore, the actual fields experienced by the organism--the fields at the outer surface and inside the body--will be different from the unperturbed electric field.

Estimates show that the fields induced inside the body of a living organism exposed to a 60-Hz electric field are very small (Barnes et al., 1967; Phillips et al., 1976a; Sheppard and Eisenbud, 1977). Therefore, to a good approximation, the perturbation in the applied field outside the body depends only on the nature of the applied field, the body shape, and the location and orientation of the body relative to ground. It therefore follows that the field outside and at the outer surface of the body of a living organism can be explored using a conducting model of the same shape.

The currents induced inside the body of an exposed organism arise as the result of a time-varying, induced electric charge on the surface of the body. Since the induced field inside the body is very small, the surface-charge density will be almost exactly the same as that induced on the surface of a conducting model (Reitz and Milford, 1960).

These considerations show that the electric field at the outer surface of the body or, alternatively, the induced charge on the surface of the body, is a key parameter in evaluating the interaction of the body with an applied 60-Hz electric field.

A previous progress report (Phillips et al., 1978) described a conducting hemi-ellipsoid model which was instrumented for the measurement of short-circuit currents and electric fields at 11 points on the surface

of the model. The hemi-ellipsoidal shape was selected because: (1) the fields at the surface can be calculated for uniform field exposure, and can thus be used as a check on the measurement technique; and (2) this shape is a rough approximation to the shape of a prone (resting) rat.

Using this model, we have studied the dependence of the surface electric fields on the following parameters of typical parallel-plate exposure system: electrode spacing; presence or absence of animal cages; and presence or absence of other animals.

2. Experimental Apparatus and Methods

Hemi-Ellipsoid Model. The hemi-ellipsoid model was made from balsa wood and was painted with a silver-base conducting paint. The principal axes of the model measure 8.13, 6.30, and 3.18 cm, respectively. Eleven 1.3-cm x 1.3-cm copper patches were set flush in the surface of the ellipsoid along the $x = 0$ and $z = 0$ planes, as shown in Figure XV-1. Each patch was electrically isolated from the remainder of the conductive surface of the model and was equipped with a wire for direct readout of its induced current. These wires were routed through holes drilled from the base of the ellipsoid to the patch location.

A total of 12 wires were attached to the ellipsoid, 11 to the patches, and one to the conducting surface of the body. A switch was constructed which either connected one selected input to the output while grounding all the other inputs, or connected all inputs to the output. The output lead from the switch was connected to a low-impedance, current-to-voltage converter, which has been described elsewhere (Kaune, in press).

FIGURE XV-1. Hemi-Ellipsoid Model Instrumented for Measurement of Electric Fields at 11 Places on Its Conducting Surface.

Exposure System. The Special Studies Exposure System was used to generate vertical, uniform, 60-Hz electric fields for use during the tests described here. This system consisted of two planar 1.0-m x 1.8-m parallel metal electrodes, separated by a gap of 0.55 m (Phillips et al., 1976a; Kaune, in press). The upper electrode was energized and the lower one was grounded.

Some of the tests required that the electrode separation be varied. Rather than move the relatively bulky electrodes described above, a separate 0.76-m x 1.68-m electrode was fabricated from thin aluminum sheet attached to a frame made of 2.5-cm-diameter steel tubing. This electrode was inserted between the two permanent electrodes of the Special Studies System, and positioned at a given height above the lower electrode, using cylindrical plexiglass spacers at the four corners. A wire was connected to the upper electrode to complete the high-voltage circuit. Considerable care was taken to always place the spacers and the movable electrode in the same horizontal position relative to the permanent electrodes.

Rat Cage Modules. Lexan modules for rat housing during electric-field exposure (Phillips and Kaune, 1977) were used in these measurements. A cage module has overall dimensions of 52 cm wide x 51 cm long x 10.7 cm high, and is subdivided into eight individual rat cages (Figure XV-2). Drinking water is supplied from four nozzles whose tips protrude slightly above the floor into notches cut into the central wall of the module. The inside dimensions of the rat cage are 12.4 cm wide x 25.1 cm long x 10.0 cm

FIGURE XV-2. Rat Cage Module is Made of Lexan and Houses Eight Rats for Exposure to 60-Hz Electric Fields.

high. Each cage has two 0.32-cm-thick side walls and two 0.64-cm-thick side walls, as shown in Figure XV-2; the roof thickness is 0.47 cm. Food pellets are supplied from an inclined holder outside the outer wall (Figure XV-2).

Unperturbed Electric-Field Measurements. The unperturbed electric field between the exposure electrodes was measured on the surface of the lower electrode using a flat disc probe, which has been described elsewhere (Phillips et al., 1976b; Kaune, in press). This probe provided a measurement of the average surface field over a 5-cm-diameter circle.

3. Results

Comparison Between Measurement and Theory. In a previous progress report (Phillips et al., 1978), patch and short-circuit currents of the hemi-ellipsoid model were measured during exposure to a uniform electric field and were found to agree well with theoretical predictions.

Effect of Electrode Separation: Single Animal System. An important consideration in the design of electric-field exposure systems is the spacing between electrodes. For parallel-plate systems meant to generate unperturbed, uniform electric fields, larger electrode spacings require (1) that larger voltages be used for a given electric field strength, and (2) that larger electrodes be used to minimize fringing

effects on field uniformity. Smaller electrode spacings are, therefore, desirable, but are limited by possible interactions between the exposed animal and the upper electrode. Such interactions would alter the charge distribution on the upper electrode and would enhance the electric field at the surface of the body of the exposed subject.

To investigate the effect of electrode spacing, the hemi-ellipsoid was placed at the center of the lower electrode (for these tests, the surface of the lower electrode was aluminum plate). The long axis (i.e., the x-axis) of the ellipsoid was lined up with the long axis of the exposure system. Measurements of the patch currents and the model short-circuit currents were made for electrode spacings of 8.7, 4.9, 3.6, 2.4, 2.0, 1.6, and 1.2, where the spacings are expressed as numbers of hemi-ellipsoid heights (6.30 cm). As the electrode spacing was varied, the high voltage was adjusted to maintain a constant, unperturbed field strength, as determined using the flat probe located on the x-axis 27 cm from the hemi-ellipsoid model (the field perturbation from the hemi-ellipsoid perturbed the field probe reading by only 1.2% [Phillips et al., 1978]).

To detect any changes as the electrode spacing was decreased, all measured currents were divided by values for an electrode spacing of 8.7. The results are given in Table XV-1; in this table, the patch locations are identified using the same notations as in Figure XV-1. The estimated uncertainty in any ratio is 0.01, resulting from round-off errors in the meters used to monitor the field probe and patch currents.

TABLE XV-1. Surface Electric Fields and Short-Circuit Currents for a Grounded Hemi-Ellipsoidal Model Exposed to a Vertical, 60-Hz, Electric Field. Data are presented for various electrode separations; at each separation, the voltage was adjusted to give a constant unperturbed electric field. Measured values are expressed as fractions of the values measured for an electrode separation of 8.7 hemi-ellipsoid heights.

Location of Measurement	Electrode Separation in Units of Hemi-Ellipsoid Height					
	4.9	3.6	2.4	2.0	1.6	1.2
6X	1.01	1.01	1.03	1.04	1.08	1.14
5X	1.01	1.01	1.03	1.04	1.08	1.17
4X	1.01	1.01	1.03	1.05	1.10	1.24
3X	1.00	1.00	1.02	1.05	1.12	1.37
2X	1.00	1.00	1.03	1.06	1.15	1.58
1X	1.00	1.00	1.03	1.06	1.18	1.80
Top	1.00	1.00	1.03	1.07	1.19	1.92
1Z	1.00	1.00	1.03	1.06	1.17	1.66
2Z	1.00	1.00	1.02	1.05	1.12	1.38
3Z	1.00	1.00	1.02	1.04	1.10	1.26
4Z	1.00	1.00	1.02	1.03	1.08	1.20
Short-Circuit Current	1.00	1.00	1.02	1.05	1.13	1.39

These data show field enhancements for the smaller electrode spacings. It is remarkable, however, how small these enhancements are; for example, at the very top of the body, the field is only increased by 7% when the electrode spacing is reduced from 8.7 to just 2.0 hemi-ellipsoid heights.

Effect of Electrode Separation: Multiple-Animal Systems. The Special Studies Exposure System was fitted with two rat cage modules and the hemi-ellipsoid model was placed in the cage shown in Figure XV-3. Measurements of the patch and short-circuit currents of the model were taken for various electrode spacings, and with the cages surrounding the model either empty or loaded with rats.

High-voltage settings were determined for the various electrode spacings by replacing the model with the flat field probe and requiring that the unperturbed electric field be constant. This procedure was performed before and after a series of measurements with the hemi-ellipsoid model, and the resulting high-voltage settings were found to vary by not more than 1%.

Preliminary tests showed that the variability resulting from movement by live animals was sufficient to confuse effects associated with electrode spacing. Dead rats were therefore used to test the effects of electrode spacing in multiple animal systems. The bodies of nine animals were placed in an approximately normal position, lined up with the long axes of the nine cages surrounding the hemi-ellipsoid; needle electrodes were inserted in the abdomens of the animals and connected to ground.

Measurements were taken for electrode separations of 8.7, 4.9, 3.6, 3.0, 2.4, and 1.8 hemi-ellipsoid heights. The hemi-ellipsoidal model was oriented both with the patches that are labeled 1x-6x in Figure XV-1 toward the center of the cage, and away from the center of the cage (in this latter orientation, the patches will be referred to as 1x'-6x'). The measurements for patches 1z-4z, the top patch, and the short-circuit current of the model were averaged for the two orientations of the model, since by symmetry considerations, they should be (and were, within experimental error) unchanged by a 180° rotation. Measurements with the cages surrounding the model either empty or loaded with nine rats are presented in Table XV-2; as in Table XV-1, the data are presented in the form of ratios to the values measured with an electrode spacing of 8.7. Uncertainties in the ratios given in Table XV-2 are 0.02, resulting from uncertainties in high-voltage normalization and from round-off errors in the digital meters used to measure the field probe and model currents.

Comparison of the "empty-cage" data in Table XV-2 to the data in Table XV-1 (with the cages removed) suggests that interaction with overhead electrodes is slightly enhanced by cages; however, differences are slight, and may result from experimental errors. Of more significance is the empty/full cage comparison (Table XV-2), which shows that interaction with overhead electrodes is significantly enhanced by the proximity of other animals. For example, to limit enhancement effects to less than 5% requires that the electrode spacing be at least 2.4 animal heights for single subjects and at least 3.6 animal heights for multiple subjects.

TABLE XV-2. Surface Electric Fields and Short-Circuit Currents in a Hemi-Ellipsoidal Model Placed in a Rat Cage Module and Exposed to a Vertical 60-Hz Electric Field. Data are presented for various electrode separations and with the other cages in the module empty or full of rats. Electric field and current data are expressed as ratios relative to the values with an electrode separation of 8.9 hemi-ellipsoid heights. The model was oriented so that patch 6x was toward the center of the cage, and 6x' was away from the center of the cage.

Location of Measurement	Electrode Separation in Units of Hemi-Ellipsoid Height									
	4.8		3.6		3.0		2.4		1.8	
	Empty	Full	Empty	Full	Empty	Full	Empty	Full	Empty	Full
6x	1.00	1.02	1.00	1.05	1.01	1.07	1.02	1.13	1.07	1.24
5x	1.00	1.02	1.01	1.05	1.01	1.08	1.03	1.13	1.08	1.24
4x	1.00	1.02	1.01	1.05	1.02	1.07	1.03	1.13	1.09	1.24
3x	1.00	1.03	1.01	1.05	1.01	1.08	1.03	1.13	1.10	1.25
2x	1.01	1.02	1.01	1.05	1.02	1.08	1.04	1.13	1.11	1.25
1x	1.01	1.02	1.01	1.05	1.02	1.08	1.04	1.13	1.12	1.25
Top	1.00	1.02	1.01	1.04	1.02	1.07	1.04	1.12	1.13	1.25
1x'	1.01	1.02	1.02	1.05	1.02	1.07	1.05	1.11	1.12	1.23
2x'	1.01	1.02	1.02	1.05	1.02	1.07	1.05	1.11	1.11	1.22
3x'	1.01	1.02	1.02	1.04	1.02	1.06	1.05	1.09	1.10	1.19
4x'	1.01	1.02	1.02	1.05	1.02	1.06	1.05	1.09	1.08	1.17
5x'	1.01	1.01	1.02	1.04	1.02	1.05	1.04	1.09	1.07	1.15
5x'	1.01	1.02	1.02	1.04	1.02	1.05	1.04	1.08	1.07	1.15
1z	1.00	1.03	1.02	1.05	1.02	1.08	1.04	1.13	1.12	1.25
2z	1.00	1.02	1.01	1.06	1.02	1.08	1.04	1.12	1.10	1.24
3z	1.01	1.03	1.02	1.05	1.02	1.07	1.04	1.12	1.09	1.24
4z	1.00	1.03	1.02	1.05	1.02	1.07	1.04	1.12	1.09	1.23
Short-Circuit Current	1.01	1.03	1.02	1.05	1.02	1.07	1.04	1.12	1.10	1.23

Effects of Plastic Animal Cages. The Special Studies Exposure System was arranged as shown in Figure XV-3, except that the plastic cage modules were removed for some tests. Patch and short-circuit currents of the hemi-ellipsoid model were measured both with the animal cages (including roofs) in place, and removed; ratios between these two sets of data were calculated. These data are given in Figures XV-4, XV-5 and XV-6 for three positions of the model within a rat cage.

Figure XV-4 shows the effects of the cage module on the surface electric fields and on the short-circuit current of the hemi-ellipsoid model placed in the center of a rat cage; reductions of 1-6% in all values were observed. With the roof of the cage removed, additional reductions of 1-2% were observed.

Figure XV-5 presents data with the model against the edge of the cage. A significant enhancement in the surface field was observed near the cage wall. Figure XV-6 presents data with the model in the corner of the cage; again, enhancements were observed in surface field strength near the walls.

Effect of Rats in Other Cages. The Special Studies Exposure System was arranged as shown in Figure XV-3. Measurements of the patch and short-circuit currents of the model were made with the nine cages closest to the model filled with live rats (weight range, 400-500 g), and empty. Multiple observations were made, covering: (1) 15 min of the exploratory period, immediately after placing the animals in the cages;

FIGURE XV-3. Two Rat Cage Modules Mounted on Lower Electrode of Special Studies Exposure System. Hemi-ellipsoid rat model is shown in cage used for most of measurements reported in text.

FIGURE XV-4. Top View of Hemi-Ellipsoid Model in Center of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

FIGURE XV-5. Top View of Hemi-Ellipsoid Model Nest to Edge of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

FIGURE XV-6. Top View of Hemi-Ellipsoid Model in Corner of Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Cage in Place to Those Measured with Cage Removed.

and (2) 1 hr during which the rats were asleep. Ratios between data taken during the active period and empty-cage data are given in Figure XV-7. These ratios show clearly that interactions in multiple-animal systems have the effect of significantly reducing surface electric fields and short-circuit currents. The measured 31% reduction in short-circuit current is consistent with earlier reported measurements, which showed a 35% reduction (Phillips and Kaune, 1977).

Since multiple measurements were made, estimates of variability were obtained. During the active period, variabilities in individual patch currents were relatively large (standard deviations up to 35% of means) for those patches close to the floor (and, thus, most strongly shielded by the bodies of neighboring rats); variabilities were small (standard deviations less than 10% of means) for those patches close to the top of the body, and for the short-circuit current.

Comparison of active and sleeping-rat data showed systematic differences: active rats more strongly shielded the model than did sleeping rats. The short-circuit current of the model was about 10% lower when the neighboring rats were active than when they were sleeping.

4. Conclusions. Electric fields to which humans, animals, or other living organisms are exposed are typically specified in terms of the field strengths present before introduction of the subjects. In parallel-plate exposure systems, the exposure field strength is often calculated by dividing the interelectrode voltage by the separation

FIGURE XV-7. Top View of Hemi-Ellipsoidal Model in Rat Cage, Showing Ratios of Patch and Short-Circuit Currents Measured with Nine Surrounding Cages Filled with Live, Active Rats to Those Measured with Surrounding Cages Empty.

between electrodes, thus neglecting any effects on field strength due to the presence of the animal cages. Measurements have been presented here which prove that interactions among exposed subjects, electrodes, and animal cages can significantly alter the fields the subjects actually experience. These are, specifically: (1) interactions between grounded rats and overhead electrodes in exposure systems can enhance exposure to electric fields, with the enhancement being considerably greater in multiple-animal than in single-animal systems; (2) plastic rat cages will decrease slightly the overall exposure of grounded rats to electric fields, but will increase by as much as (or more than) 20% of the electric fields at the surface of those parts of the subject's body close to the walls of the cage; (3) animals will be significantly shielded from electric fields in systems where several animals are simultaneously exposed in close proximity to one another.

B. RAT SHORT-CIRCUIT CURRENTS

1. Introduction. The current which passes between a grounded animal exposed to an electric field and ground is called the short-circuit current. To complement published short-circuit currents for humans (EPRI, 1975; Deno, 1977) and swine (Phillips et al., 1976a; Kaune et al., 1978), measurements with rats were undertaken.

2. Methods. Seven Sprague-Dawley rats (weight range, 143-500 g) were killed and individually exposed to a 60-Hz, 44-kV/m electric field produced by the Special Studies Exposure System. Short-circuit current measurements were made as follows: (1) Each rat was placed in an approximately normal resting posture, tail outstretched, on the surface of the lower exposure electrode. (2) The body was insulated from the electrode with a sheet of Mylar. (3) A needle electrode was inserted in the abdomen and connected to a current-to-voltage converter [sensitivity, 1 μ A = 0.1 V; input impedance, < 100 Ω at 60 Hz (Kaune, in press)].

3. Results. The data are shown in Figure XV-8. The estimated reliability of these measurements is $\pm 6\%$, based on measurements of the effect of minor changes in body shape ($\pm 3\%$) and measurements of the variability in short-circuit current among rats of the same weight ($\pm 5\%$). The data are described by the formula,

$$I_{sc} = \alpha W^n E_0, \quad (1)$$

where I_{sc} is short-circuit current in μ A, W is weight in g, E_0 is the unperturbed field strength in kV/m, $\alpha = 0.0033 \pm 0.0013$, and $n = 0.63 \pm 0.06$.

C. INDUCED CURRENT DISTRIBUTION IN RATS

1. Introduction. The measurements reported here were made to determine the distribution of short-circuit current within the grounded bodies of prone and rearing rats exposed to a 60-Hz electric field.

FIGURE XV-8. Short-Circuit Current Induced in Resting Rats of Various Weights Exposed to Vertical, Uniform, 60-Hz, 44-kV/m Electric Field. Solid curve is theoretical fit to data described in text.

2. Methods. A dead, 500-g, Sprague-Dawley rat was frozen and sectioned into five pieces through the neck, chest, abdomen, and base of the tail (Figure XV-9). Wires were soldered to thumbtacks, which were then coated with ECG electrode paste and inserted in the interior faces of the five body sections. The frozen body pieces were sewn back together, with Mylar sheets inserted between the various segments for electrical insulation.

For induced-current measurements, each of the leads from the five body sections was, in turn, connected by coaxial cable to the current-to-voltage converter described earlier, while the other four leads were directly grounded.

3. Results. Measurements were made with the partially frozen rat in the normal resting posture, and also propped up at angles of 70° and 90° to the horizontal, to simulate a rearing rat. The data, expressed as fractions of short-circuit current, are presented in Figure XV-9. For the rearing posture, the 70° and 90° measurements (which differed by no more than 15%) were averaged. The short-circuit current in the normal resting posture (obtained by adding the induced currents for the five body sections) was compared to the prediction of Eq. (1), with good agreement. We thereby proved, experimentally, that a partially frozen rat is a conducting model (this was also verified by direct 60-Hz impedance measurements, which showed sufficient conduction between two needle electrodes placed in the neck and rump of a partially frozen rat).

FIGURE XV-9. Grounded Rat Model Exposed to Vertical, 60-Hz, 10-kV/m Electric Field. Conducting body of model is divided into five segments by planes through neck, chest, abdomen, and tail. Fraction of total short-circuit current originating within each of five sections are shown as percentages.

4. Conclusions. These data can be used to compute total currents crossing sections through the necks, chests, abdomens and tails of prone and rearing rats. By using similar data for other species, it should be possible to compare the current densities and electric fields induced within the bodies, and derive approximate scaling relationships between species.

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XVI. SUMMARY OF BIOLOGICAL INVESTIGATIONS

The purpose of this summary is to provide a list of biological experiments that have been completed on this project, and a quick reference to the location of detailed descriptions of the experiments in the 1978 and 1979 Annual Reports.

Table XVI-1 gives a summary of experiments completed within each major area of research, identifies each as an initial or replicate experiment, and cites the Annual Report in which the experiment is described in detail. Only experiments that have been completed are included in this list. Experiments that are in progress, and those completed since this report was prepared, will be discussed in monthly reports throughout FY 1980.

Table XVI-2 briefly summarizes those experiments in which confirmed effects have been observed in mice or rats exposed to 60-Hz electric fields. Experiments where we have failed to confirm effects in replicate experiments (i.e., those cases where effects were observed in one experiment but not replicated in a second experiment) are not included in this summary. To identify the latter studies, see Sections IV (Hematology and Serum Chemistry), IX (Endocrinology) and XI (Neurophysiology), in which effects were observed in a first experiment but not in the second (nor, in some cases, in the third) replicate experiments. In addition, some studies where effects were found in the first experiment are currently being replicated to confirm the earlier findings (e.g., Section IX, Endocrinology).

TABLE XVI-1. Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Behavior - D. L. Hjeresen and R. L. Sheldon

<u>Experiment</u>	<u>Initial (I) or Replicate (R)</u>	<u>Annual Report</u>
Avoidance and traverse activity (rats, exposed 45 min)	I,R	1978-1979
Avoidance and traverse activity (rats, exposed 24 hr)	I,R	1978-1979
Serum corticosterone levels (rats, exposed 24 hr)	I	1979
Avoidance and traverse activity (rats, exposed 30 days before 45-min test)	I,R	1979
Audiogenic seizure sensitivity (rats, exposed 14 days)	I	1979
Audiogenic seizure sensitivity (rats, exposed 28 days)	I	1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Bone Growth and Structure - B. J. McClanahan

<u>Experiment</u>	<u>Initial (I) or Replicate (R)</u>	<u>Annual Report</u>
Bone growth and structure (rats, exposed 30 days)	I,R	1978-1979
Body weight (rats, exposed 30 days)	I,R	1978-1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Cardiovascular Function - D. I. Hilton and R. D. Phillips

Experiment	Initial (I) or Replicate (R)	Annual Report
Heart rate, ECG analysis (male rats, exposed 8 hr, 40 hr, 30 days)	I	1978
Heart rate, ECG (female rats, exposed 30 days)	I	1979
Heart rate, ECG (female rats, exposed 120 days)	I	1979
Heart rate, blood pressure, vascular reactivity (male rats, exposed 35 days)	I	1979
Heart rate, blood pressure, vascular reactivity (female rats, exposed 120 days)	I	1979
Response to cold stress (male rats, exposed 30 days)		
Heart rate	I	1979
Thermoregulation	I	1979
Thyroid stimulating hormone	I	1979
Serum corticosterone level	I	1979
Heart rate and blood pressure <u>during</u> exposure (rats, exposed 1 hr)	I	1979
Serum corticosterone (rats, exposed 30 days)	R	1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Central Nervous System - J. C. Hampton

<u>Experiment</u>	<u>Initial (I) or Replicate (R)</u>	<u>Annual Report</u>
Histopathology of CNS (mice, exposed 30 days)	I	1978

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Endocrinology - M. J. Free

Experiment	Initial (I) or Replicate (R)	Annual Report
Endocrine organ weights (rats, exposed 30 days)	I,R	1978
Hormone levels in plasma: FSH,* LH,** testosterone, TSH,*** growth hormone, thyroxine (rats, exposed 30 days)	I,R	1978
Testis blood flow and androgen secretion rates (rats, exposed 30 days)	I	1978
Corticosterone levels (rats, exposed 30 days)	I,R	1979
Hormone levels in plasma (rats, exposed 120 days)	I	1979
Hormone levels in developing rats (exposed from weanling to adult, 21-56 days of age)	I	1979
Endocrine organ weights in developing rats (exposed from weanling to adult (21-56 days of age)	I	1979
Testosterone and corticosterone levels in plasma (rats, exposed 120 days)	I	1979

*Follicle stimulating hormone
 **Luteinizing hormone
 ***Thyroid stimulating hormone

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Hematology and Serum Chemistry - H. A. Ragan

Experiment	Initial (I) or Replicate (R)	Annual Report
Hematology and serum chemistry (rats, exposed 15 days)	I,R	1978
Hematology and serum chemistry (rats, exposed 30 days)	I,R	1978
Hematology and serum chemistry (rats, exposed 60 days)	I,R	1978
Hematology and serum chemistry (rats, exposed 120 days)	I,R	1979
Hematology and serum chemistry (mice, exposed 30 days)	I,R	1978
Hematology and serum chemistry (mice, exposed 60 days)	I,R	1978
Hematology and serum chemistry (mice, exposed 120 days)	I,R	1979
Hematology and serum chemistry (mice, F ₁ , exposed 77 days)	I,R	1979
Hematology and serum chemistry (mice, F ₂ , exposed 77 days)	I,R	1979
Hematology and serum chemistry (mice, F ₃ , exposed 77 days)	I,R	1979
Iron kinetics (rats, exposed 60 days)	I,R	1979
Iron kinetics (mice, exposed 60 days)	I,R	1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Immunology - J. E. Morris

Experiment	Initial (I) or Replicate (R)	Annual Report
Levels of serum immunoglobulins (rats, exposed 15 and 30 days)	I	1978
Levels of serum immunoglobulins (mice, exposed 30 and 60 days)	I	1978
Serum levels of complement (mice, exposed 30 and 60 days)	I	1978
Distribution of T and B lymphocytes (mice, exposed 30 and 60 days)	I	1978
Cell-mediated immunity (mice, exposed 30 days)	I,R,R	1979
Antibody response to KLH* (mice, exposed 30 and 60 days)	I	1979
Lymphocyte mitogen stimulation (mice, exposed 90 and 140 days)	I	1979

*Keyhole limpet hemocyanin

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Metabolic Status and Growth - R. D. Phillips

Experiment	Initial (I) or Replicate (R)	Annual Report
Growth, food and water consumption (rats, exposed 30 days)	I,R	1978
Organ weights (rats, exposed 30 days)	I,R	1978
Liver composition (juvenile male and female rats, exposed 30 days)	I,R	1978
O ₂ consumption and CO ₂ production (rats, exposed 30 days)	I	1978
331 O ₂ consumption and CO ₂ production (rats, <u>during</u> exposure)	I	1979
Growth, food and water consumption (rats, exposed 120 days)	I,R	1979
Organ weights (rats, exposed 120 days)	I,R	1979
Growth and food consumption (mice, exposed 120 days)	I	1979
Organ weights (mice, exposed 120 days)	I	1979
Liver composition (rats, exposed 30 days)	I,R	1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Mutagenesis* - F. P. Hungate

Experiment	Initial (I) or Replicate (R)	Annual Report
<u>Drosophila</u> , recessive lethals (185 and 370 kV/m, dc)	I,R	1979
<u>Salmonella</u> , TA-100. Histidine reversion to phototrophy, base exchange (Ames Assay; 800 kV/m, dc)	I	1979
<u>Salmonella</u> , TA-98. Reversion to histidine phototrophy, frame shift (800 kV/m, dc)	I,R	1979
<u>Photobacterium fisheri</u> . Forward mutation to tetracycline resistance (800 kV/m, dc)	I,R	1979
<u>E. coli</u> , plasmid-borne drug resistance (500 kV/m, dc)	I,R	1979
<u>E. coli</u> , lysogen induction (500 kV/m, dc)	I,R	1979
<u>E. coli</u> , forward mutation: (500 and 750 kV/m, dc)		
To nalidixic acid or rifampicin resistance	I,R	1979
To ampicillin, tetracycline, valine, or chloramphenicol resistance	I	1979
Phage, reversion (nonreplicating)	I,R	1979

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TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Neurophysiology - R. A. Jaffe

<u>Experiment</u>	<u>Initial (I) or Replicate (R)</u>	<u>Annual Report</u>
Synaptic transmission (rats, exposed 30 days)	I,R	1978-1979
Peripheral nerve function (rats, exposed 30 days)	I,R,R	1979

TABLE XVI-1 (Cont'd.). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Pathology - R. A. Renne

Experiment	Initial (I) or Replicate (R)	Annual Report
Histopathology (rats, exposed 30 days)	I,R	1978
Histopathology (mice, exposed 30 days)	I,R	1978
Body and organ weights (rats, exposed 30 days)	I,R	1978
Body and organ weights (mice, exposed 30 days)	I,R	1978
Histopathology (rats, exposed 120 days)	I,R	1979
Histopathology (mice, exposed 120 days)	I	1979
Body and organ weights (rats, exposed 120 days)	I,R	1979
Body and organ weights (mice, exposed 120 days)	I	1979
Virology (rats, exposed 30 days)	I	1979

TABLE XVI-1 (Cont'd). Results Through September, 1979 (Included in 1978 and 1979 Annual Reports)

Reproduction, Growth and Development - M. R. Sikov

<u>Experiment</u>	<u>Initial (I) or Replicate (R)</u>	<u>Annual Report</u>
Reproduction and fertility (rats, exposed 30 days)	I,R	1978
Teratology (rats, exposed in utero to 20 days of gestation)	I,R	1978
Postnatal growth and development (rats, exposed from conception to 8 days of age)	I,R	1978-1979
Growth and neuromuscular development (rats, exposed from 17 days of gestation to 25 days of age)	I,R	1979
Three-generation mouse study (exposed in utero and raised in the field through three generations)	I,R	1979

TABLE XVI-2. Summary of Experiments Showing Confirmed Effects

- Behavior: Rats spent significantly more time out of a 60-Hz electric field than in it at ≥ 90 kV/m during a 45-min test. Also, exposed rats made more traverses between the exposed and unexposed regions of a shuttlebox than sham-exposed controls.
- Behavior: Rats exposed to 75 or 100 kV/m spent more time out of the electric field than sham-exposed controls during the 12-hr light period of a 24-hr test (12 hr light:12 hr dark). Rats exposed to 25 and 50 kV/m spent more time in the field than sham-exposed controls during the 12-hr light period. During the 12-hr dark period the only significant effect was a preference by rats exposed to 100 kV/m to stay out of the field.
- Cell-Mediated Immunity: Mice exposed to 100 kV/m for 30 days showed a statistically significant decrease in immune response to an antigen protein (KLH*) applied to the skin when compared to the response of sham-exposed controls.
- Growth and Development: A smaller percentage of rats exposed to 100 kV/m from conception to 8 days after birth exhibited the righting reflex and a higher percentage showed motile behaviors than did sham-exposed rats at 14 days of age.
- Synaptic Transmission: Sympathetic ganglia of rats exposed to 100 kV/m for 30 days were more excitable than those of sham-exposed rats.
- Hematology: Male and female mice exposed to 100 kV/m from conception to 77 days after birth had higher leukocyte counts (all white-cell types) than sham-exposed controls.
- Red-blood-cell parameters of the third generation of mice exposed in utero and for 77 days after birth had higher values than those of sham-exposed controls.

* Keyhole limpet hemocyanin