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

**ANNUAL REPORT
APRIL 1977 TO MARCH 1978**

APRIL 1978

**PREPARED FOR
U.S. DEPARTMENT OF ENERGY
ASSISTANT SECRETARY FOR ENERGY TECHNOLOGY
DIVISION OF ELECTRIC ENERGY SYSTEMS**

UNDER CONTRACT EY-76-C-06-1830

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EXECUTIVE SUMMARY

This report describes progress made from April 1, 1977 to February 28, 1978 in assessing the biological effects of exposing laboratory rodents to 60-Hz electric fields.

Exposure Systems: Systems have been built for exposing small laboratory animals (rats and mice) to uniform, vertical, 60-Hz electric fields. The Special Studies Exposure System, used for dosimetry and behavioral experiments, was described in the First Interim Report (September, 1976). The Rat Exposure System, capable of simultaneously exposing 288 rats housed in individual plastic (lexan) cages, was described in the Second Interim Report (May, 1977). This report describes the Mouse Exposure System and the final checkout of both the Rat and Mouse Exposure Systems.

In the Mouse Exposure System, 288 mice can be exposed to uniform, vertical, 60-Hz electric fields with field strengths as high as 150 kV/m; the same number of mice can be sham exposed under identical environmental and housing conditions.

Significant harmonic distortions were found in all three exposure systems and were traced to enhancements of relatively small harmonics in the building supply voltage by a resonance between the high-voltage transformers and the exposure systems. The problem was solved by filtering the input power and damping the resonance.

Extensive tests have been made and document that the exposure systems are free of corona discharge and ozone formation and that the animals do not receive spark discharges or other shocks in the housing system during exposure to electric fields. The systems are free of significant levels of harmonic distortion and vibration.

Electric field measurements showed that the electric field strength within a rat cage varies by about 7%, due to the effects of the plastic (lexan) cage material, and that the differences among fields in different cages are less than 3%.

Dosimetry: Studies were conducted to investigate the electrical interaction between the rat and electric field with the watering system (nozzles in the cages). Impedance between the nozzle and ground is about 60 kohm; impedance between a rat and ground is variable and ranges between 10 to 20 kohm. The fraction of short-circuit current flowing through the mouth of a rat when it drinks from the nozzle is less than 25% of the total current flow through the rat.

A new hemi-ellipsoidal model was built to study the distribution of electric fields at the surface of the rat's body. Measurement of the electric field at 11 locations on the surface of this model are in good agreement with theory.

Biological Effects: Experiments in 11 major areas were initiated during this reporting period to screen for biological effects over a wide range

of parameters in rats and mice exposed to 60-Hz electric fields at 100 kV/m for up to 60 days. Most of the initial screening experiments with 30- and 60-day exposures have been completed.

Several features of experimental design were common to all biological experiments. All animals received from the supplier (Hilltop Laboratories) were carefully 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 virology 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. Except for several cardiovascular and behavioral experiments, the field strength used for all exposures was 100 kV/m. Neither animal caretakers nor investigators were informed which animals were being or had been 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. Data for all studies are expressed as sample means and standard errors. The differences between means were analyzed with the Student's t test ($p \leq 0.05$ indicated that means were significantly different).

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

- Hematology and Serum Chemistry: This study measured 18 different parameters concerning blood cells and 11 parameters of serum

chemistry. The assays were performed on rats exposed for 15, 30, and 60 days, and on mice exposed for 30 and 60 days. All experiments were replicated in order to differentiate effects induced by exposure to electric fields from false positive findings intrinsic in multiparameter evaluations (e.g., approximately 5% of the comparisons between exposed and sham-exposed animals will be significantly different at $p < 0.05$ by chance). In these studies, 21 significant effects were observed out of 275 comparisons, or approximately 7.6%. Since the apparent effects were not consistent across exposure durations or across species, nor observed in replicate experiments, the observed differences between exposed and sham-exposed animals were probably the result of random variability and not effects of the electric field per se, and should not be considered biologically significant.

- Immunology: No differences in the blood (serum) concentrations of two kinds of immunoglobulins (antibodies) or in the relative concentrations of the functionally distinct kinds of lymphocytes were observed in rats (15 and 30-day exposure) or mice (30 and 60-day exposure). In an in vivo cell-mediated immunity experiment, mice exposed for 30 days showed a statistically significant decrease in immune response to an antigen protein (KLH) applied to the skin. This experiment was replicated and similar results were obtained. Although the reduced immune response

by the exposed group was not statistically significant in the replicate experiment, the direction of the response was consistent with that of the first experiment. To assess this potential effect, an experiment has been initiated to assay mitogenic stimulation that correlates with cell-mediated immunity.

- Pathology: No significant differences were observed between exposed and sham-exposed rats and mice (30-day exposure) in an extensive histopathological survey. An apparent higher incidence of prostatitis in exposed rats as compared to sham-exposed rats was not statistically significant ($p < 0.2$). However, additional studies are in progress to investigate any possible relationship between exposure to electric fields and susceptibility to viral infections.
- Metabolic Status and Growth: Exposure of rats to the electric field for 30 days had no measurable 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 metabolic rate.
- Bone Growth and Structure: Bone growth and structure of juvenile rats was not altered by exposure to a 60-Hz electric field at 100 kV/m for 30 days.

- Endocrinology and Male Reproduction: Exposure of male rats for 30 days did not affect the following parameters: body weight; the weights of various endocrine glands including those associated with reproduction; the circulating blood levels of testosterone, follicle stimulating hormone, corticosterone, luteinizing hormone and thyroxine; blood flow in several regions of the reproductive system; concentrations of androgens in testicular venous blood; testosterone secretion rate from the testis.
- Cardiovascular Function: Exposure of rats for 8 hr (80 kV/m), 40 hr (8 hr/day for 5 days at 80 kV/m), or 30 days (100 kV/m) had no detectable effect on ECGs or heart rates.
- Central Nervous System: Histopathological examination of the brains of mice exposed to 100 kV/m for 30 days revealed no differences from those of sham-exposed mice.
- Neurophysiology: Transmission of impulses between nerves (synapses) was studied in ganglia from rats which had been exposed or sham-exposed for 30 days to 100 kV/m. There were no statistically significant differences between the two groups. However, there is a trend in the data that suggests increased excitability of the synapses in exposed animals.

- Reproduction, Growth and Development. A 6-day exposure did not affect the ability of rats to mate, and subsequent exposure of the pregnant females through 20 days of gestation had no effect on size or morphology of the fetuses. A 30-day exposure of males and unmated females had no effect on mating or fertility. Similarly, exposure of pregnant females during the gestation period and subsequent exposure of the newborn for 8 days produced no effect on growth, survival or neuromuscular development.
- Animal Behavior: Rats were tested for side preference for 45 min in a shuttle box, one end of which is shielded and the other end unshielded from a 60-Hz electric field. The animals exhibited a statistically significant preference for the shielded (no field) side at field strengths of 90 kV/m and higher. The modality of perception of the electric field by rats is presently under investigation.

In summary, exposure of rats and mice to 60-Hz electric fields at 100 kV/m for up to 60 days had no statistically significant, reproducible effects except in the behavioral experiments. Rats, given a choice, spent more time out of the electric field than in it at ≥ 90 kV/m. The results of three experiments showed trends which suggest the possibility of effects: decreased cell-mediated immunity, increased prostatitis and

increased excitability of synapses. In-depth studies are in progress to evaluate these potential effects. Also, it is possible that longer exposure durations (e.g., 4 months) may enhance subtle effects not evident with 30- and 60-day exposures.

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I. INTRODUCTION

Research was initiated on this project on March 9, 1976. The first two interim reports described progress made during the first 12 months, through March 31, 1977. This interim progress report covers the period April 1 to February 28, 1978.

A. 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 provide an improved data base for establishing safe exposure limits.

The major objective of this project is to determine the biological effects on small laboratory animals of exposure to 60-Hz electric fields. To achieve this goal a number of procedural and experimental objectives must be satisfied:

- Design, fabricate and test a prototype exposure system for exposing rats and mice to well-defined and relatively uniform 60-Hz electric fields (COMPLETED).
- Design, fabricate and test a system for housing rats and mice during exposure to 60-Hz electric fields. The caging, watering and feeding systems must not alter electric field uniformity significantly and must adhere to good animal care practices (COMPLETED).
- Conduct biological experiments in the prototype exposure system to determine the maximum field strength that does not

produce corona discharge and ozone formation, behavioral aversion, hair stimulation, or shocks when the animal eats or drinks (COMPLETED).

- Design and fabricate systems for exposing large numbers of rats and mice to well-defined and relatively uniform 60-Hz electric fields (COMPLETED).
- Conduct a broad and comprehensive series of screening experiments to investigate biological effects of exposure to 60-Hz electric fields (IN PROGRESS).
- Conduct dosimetry investigations to complement the animal studies (IN PROGRESS).
- Establish the relationships between field strength and duration of exposure for any effects observed in the screening experiments (PLANNED).

B. SCHEDULE AND MILESTONES

During the first 24 months of this project all of the tasks required for exposure of the animals (described above) have been completed. Most of the effort for the last year has been directed toward assessing effects of 30- and 60-day exposures of rats and mice to 100-kV/m, 60-Hz electric fields.

- The harmonic distortions and line transients which influenced the electric field in preliminary experiments were eliminated before the biological screening experiments were started.

- Eleven individual studies on rats were initiated on April 18, 1977 to screen for biological effects of exposure to 60-Hz electric fields.
- The mouse exposure system was completed and has been in use since June 20, 1977 for the biological studies.
- Experiments have been completed in nine of the eleven initial studies. The two remaining studies are in progress, and a number of replication experiments were made where the possibility of effects was observed in the initial experiments.

Future milestones for the project are:

- Quantitative measurements of possible electrode vibration induced by the electric field will be completed in March 1978.
- Neurophysiology experiments of rats exposed for 30 days will be completed by June, 1978.
- The first in a series of exposures of mice for 120 days will be completed by May, 1978, and these will be used for evaluation of effects in the Metabolic Status, Immunology and Hematology studies.
- The first in a series of exposures of rats for 120 days will be completed in June, 1978. The experiments will be similar to those made with 30- and 60-day exposures.
- Screening studies on animals exposed for 120 days will be completed early in FY 1979.

-

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. William T. Kaune will continue as Associate Project Manager and as Task Leader on the Dosimetry portion of the project. Dr. Richard P. Schneider will serve as Task Leader for the Biological Studies as well as principal investigator for the cell and membrane physiology subtask.

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

TASK A: Administration - R. D. Phillips
Subtask: Management - R. D. Phillips
Subtask: Consultants
A. W. Guy - Dosimetry
W. A. Lewis - Engineering
C. C. Diamond - Engineering
R. H. Lovely - Physiological-Psychology

Subtask: Travel

Subtask: Services (photographs, illustrations, duplication, stenography and clerical assistance on reports and other publications)

TASK B: Biological Studies - R. P. Schneider

Subtask: Growth and Development - M. R. Sikov

Subtask: Hematology and Serum Chemistry - H. A. Ragan

Subtask: Immunology - J. E. Morris

Subtask: Physiology - D. I. Hilton

Subtask: Behavior - D. L. Hjeresen

Subtask: Neurophysiology - R. A. Jaffe

Subtask: Biochemistry - B. J. McClanahan

Subtask: Endocrinology and Male Reproduction - M. J. Free

Subtask: Pathology - G. M. Zwicker

Subtask: Cell and Membrane Physiology - R. P. Schneider

Subtask: Cell-Mediated Immunity - H. A. Ragan

Subtask: Biometrics - R. L. Buschbom

Subtask: Animal Care - V. G. Horstman

TASK C: Exposure Systems - W. T. Kaune

Subtask: Operation - W. T. Kaune

Subtask: Dosimetry - W. T. Kaune

TASK D: Transmission Line Survey - J. G. Droppo

Subtask: Chemical Environment - J. G. Droppo

II. EXPOSURE SYSTEMS

Principal Investigator: W. T. Kaune

ABSTRACT

This report (as well as two earlier progress reports) presents our work on the development of systems for exposure of small laboratory animals (rats and mice) to uniform, vertical, 60-Hz electric fields. Final checkout of the rat exposure system was completed. Appreciable levels of harmonic distortion (third, fifth, and seventh harmonic) were found and traced to enhancements of relatively small harmonics in the building supply voltage by a resonance between the high-voltage transformer and the exposure system. This problem was solved by filtering the input power and damping the resonance. Ozone measurements revealed no levels above background due to exposure system energization. Electric field measurements showed that the electric field within a rat cage varied by about 7%, due to the effects of the plastic cage material, and that differences among fields in different cages are less than 3%.

The mouse exposure system is described. Several hundred mice can be exposed to uniform, vertical, 60-Hz electric fields with field strengths as high as 150 kV/m; a similar number can be sham exposed. This system is designed to prevent the occurrence of spark discharges and other shocks, significant levels of harmonic distortion, corona discharge and ozone generation, or significant levels of hum and vibration.

A. SPECIAL STUDIES EXPOSURE SYSTEM

This system has been described in detail in our first two reports (Phillips et al., 1976; Phillips and Kaune, 1977). The following section describes: 1) the observation and elimination of harmonic distortion in the special studies exposure system, and 2) the calibration of our electric field probes in a distortion-free environment.

1. Harmonic Distortion

The high-voltage waveform applied to the Special Studies Exposure System was observed using a voltage divider manufactured by Ross Engineering Corp. (Model VD-60-6.2-B-K-A); the frequency range of this device is dc to 2 MHz. A conducting field probe simulating a small animal was used in the Special Studies Exposures System to obtain the waveform of the probe short circuit current. Figure II-1A shows the observed waveforms at an exposure system voltage of 25 kV. The shape of these waveforms depended only weakly on the exposure system high voltage. The body current waveform shown in Figure II-1A is actually the negative of the probe current waveform because of an inversion in the electronics used to convert current to voltage.

More distortion is seen in the body current waveform than in the high-voltage waveform. This is because the probe is capacitively coupled to the exposure system high voltage. The amplitude of the n^{th} harmonic of the body current will be n -times larger than the corresponding high voltage harmonic.

A Tektronix 564 oscilloscope equipped with a Tektronix 3L5 spectrum analyzer was used to measure the amplitude of the individual harmonics in the body current waveforms. An analysis of a square wave by this unit was in good agreement ($\pm 5\%$) with theory. The second column of Table II-1 gives the observed amplitudes of the odd harmonics expressed as a fraction of the fundamental amplitude. The amplitudes of the even

WITH DAMPING
RESISTOR

WITH INPUT POWER
FILTERING

FIGURE II-1. Electric field and body current waveforms in the Special Studies Exposure System: A) shows the original waveforms; B) shows the waveforms with a 10- Ω damping resistor in series with the transformer primary; C) shows the waveforms with the damping resistor and input power to the high-voltage supplies filtered.

harmonics were small, as expected, for a waveform symmetrical around the abscissa. Table II-1 also gives the total harmonic distortion, THD, calculated as follows:

$$\text{THD} = \frac{\sqrt{\sum V_n^2}}{V_1}$$

where V_n is the amplitude of the n^{th} odd harmonic and V_1 is the amplitude of the 60-Hz fundamental. Table II-2 given calculated values of the harmonic amplitudes and the THD for the high voltage waveform.

TABLE II-1. Measured Harmonic Distortion in the Short Circuit Current of a Probe Exposed in the Special Studies Exposure System

Harmonic to Fundamental Ratio	Amplitude Ratio (%)		
	Basic System	With Damping Resistor	Input Power Filtered and Damping Resistor
V_3/V_1	1.9	2.8	2.5
V_5/V_1	23.0	6.5	0
V_7/V_1	22.0	5.3	0
V_9/V_1	2.0	0.9	0
V_{11}/V_1	0	0	0
Total Harmonic Distortion	32.0	8.8	2.5

TABLE II-2. Calculated Harmonic Distortion in the Special Studies Exposure System Electric Field

Harmonic to Fundamental Ratio	Amplitude Ratio (%)		
	Basic System	With Damping Resistor	Input Power Filtered and Damping Resistor
V_3/V_1	0.6	0.9	0.8
V_5/V_1	4.6	1.3	0
V_7/V_1	3.1	0.8	0
V_9/V_1	0.2	0.1	0
V_{11}/V_1	0	0	0
Total Harmonic Distortion	5.5	1.8	0.8

While electric fields generated by power lines contain harmonic components, they are not nearly as large as the ones shown in Figure II-1A. As will be demonstrated, the high level of distortion is due to a resonant interaction between the high-voltage transformer (which, due to flux leakage, has an inductive component in its output impedance) and the exposure system (which is capacitive).

The high-voltage transformer (Hipotronics Model 7100-5, Brewster, NY) has a stated output impedance of about 8% at 100 kV, 5 kVA. The secondary winding wire resistance is 17 k Ω , and the primary wire resistance transformed into the secondary (turns ratio = 440) will be about the same. The resistive eddy current losses in the transformer iron are not known. The estimated output inductance of the transformer is around 400 H.

Based on measurement, the load capacitance is 290 pF. Of this, 120 pF is the exposure system capacitance; the remainder is the capacitance of the coaxial cable connection to the transformer. The system resonant frequency is, therefore, approximately 450 Hz and the quality factor $Q \approx 5$.

If this model has relevance, then a reduction in Q should substantially lower the harmonic contamination in the vicinity of the resonant frequency. A 10-ohm, 400-watt resistor, chosen for critical damping, was placed in series with the primary of the transformer. The resulting waveforms, shown in Figure II-1B, are significantly less distorted. The third columns of Tables II-1 and II-2 show the amplitudes of the harmonic components. The major effect is a reduction of the fifth and seventh harmonics by about 75%.

The 220 volts supplying the high-voltage system was examined and waveforms similar to those shown in Figure II-1B were observed. This fact, and the relative independence of the waveform shapes with transformer high voltage, prompted us to install two Dynamic AC Line Conditioners (Models LC-1201B, California Instruments, San Diego, CA) to supply undistorted power to the high-voltage supplies. Figure II-1C and the fourth columns of Tables II-1 and II-2 show data taken in this configuration. All harmonics above the third have been reduced to unmeasurable levels. It is interesting that the third harmonic is present in all tests at the 2-3% level, evidently a result of distortion in the high-voltage transformer itself.

2. Field Measurements

The Second Interim Report (Phillips and Kaune, 1977) outlined our program of field measurements and discussed the uncertainty in calibrating electric field probes in the presence of substantial amounts of harmonic distortion. This section presents our most recent probe calibration data, taken after the elimination of harmonic distortion in the Special Studies Exposure System, as described in the previous section.

The field probe was described in the First Interim Report (Phillips et al., 1976). The basic equation governing the operation of this probe is

$$E = C_h \frac{I}{\omega \epsilon_0 A}, \text{ where}$$

E = applied electric field

I = short circuit current of the probe

$\omega = 2 \pi \times 60 \text{ Hz}$

ϵ_0 = permittivity of space

A = probe area

C_h = probe calibration factor.

In theory, the calibration factor, C_h , should be 1.0. In practice it will be slightly different and is determined by calibrating the probe in a parallel plate field. The plate voltage is measured and the true field calculated and compared to the measured field. This procedure is described in more detail in the Second Interim Report. Two probes were

calibrated, one 2 in. in diameter (previously described), and a new 3/4-in.-diameter unit. A plate separation of 0.15 m was used, with the smaller of the two parallel plates measuring 0.76 m x 1.68 m. The calibration data for the two probes are given in Table II-3. The calibration factor at 25 kV for the 2-in. probe is quite close to that obtained in the Second Interim Report, $C_h = 0.977$, which indicates that the harmonic distortion did not produce as large an error as expected.

TABLE II-3. Probe Calibration Factors Obtained Between Parallel Plates

Parallel Plate High Voltage (kV)	C_h	
	2-in. Probe	3/4-in. Probe
10.7 $\pm$ 0.1	0.958 $\pm$ 0.007	0.913 $\pm$ 0.007
21.3 $\pm$ 0.1	0.968 $\pm$ 0.007	0.930 $\pm$ 0.007
31.5 $\pm$ 0.1	0.968 $\pm$ 0.007	0.928 $\pm$ 0.007
41.8 $\pm$ 0.1	0.972 $\pm$ 0.007	

Errors shown are statistical. The calibration factors have an additional estimated systematic uncertainty of ± 0.010 .

B. RAT EXPOSURE SYSTEM

This system was described in detail in the Second Interim Report. The harmonic distortion present in this system has been eliminated, and ozone and field measurements have been performed.

1. Harmonic Distortion

The techniques developed to eliminate harmonic distortion in the Special Studies Exposure System, described previously in this report, were applied also to the rat exposure system. Power from the two AC Line Conditioners, free of harmonic distortion, was applied to the inputs of the high-voltage transformers of the two rat exposure systems and 10-ohm, 250-watt damping resistors were placed in series with the two high-voltage transformer primaries. These two steps reduced the harmonic distortion in these two system to very low levels, as determined by direct observation of the waveforms with an oscilloscope. Spectrum analyzer measurements of the rat exposure system waveforms will be reported in the next interim report.

2. Ozone Measurements

As reported in the First Interim Report, the exposure systems were designed to be corona-free. The electrodes are built with an outer edge of 8.3 cm diameter tube to prevent excessive field concentration. The remainder of the electrodes are flat metal surfaces. All protruding objects on the room walls, (for example, fluorescent lights and fire-sprinkler heads), were covered with grounded wire screen, smoothly shaped, to prevent corona. All high-voltage connections were terminated in grading rings or balls to minimize field concentrations. The water system was built in the field-free, interior of the electrodes to prevent any field concentration around protruding nozzles. Biological waste materials are collected in the field-free, interior of the electrodes. However,

even with these steps, the sensitivity of animals to ozone (a consequence of corona discharge) dictated that experimental work be performed to detect any corona-produced ozone concentrations above room backgrounds.

A Monitor Labs Ozone Analyzer was used to survey the rat exposure system. Before presenting the results, one difficulty in using ozone analyzers to measure ozone concentrations in small systems must be discussed. The sample flow rate for the Monitor Labs unit is 0.75 l/min. When sampling small systems, such as one of our rat cages with a volume of 3.3 l, the ventilation due to the sample flow is sufficiently large to prevent an accumulation of ozone which would otherwise occur over long periods of time.

This problem was solved by adopting a procedure in which the end of the teflon sample line was placed at the point to be sampled, the exposure system then energized for a long period with zero sample flow, and then a quick sample taken. If the ozone sampler can respond quickly to a change in concentration, an accurate measurement can be made before the volume in the small cage volume is depleted by the sampling process.

The response time of the analyzer was stated as being only a few seconds by the manufacturer. However, to verify this with the fairly long teflon tube used, a 3-cm-high metal point was placed on an electrode with the point extended upward and the sampler tube placed about 2 cm from the tip. With the ozone monitor running, the exposure system was quickly energized to a field strength of 100 kV/m. The top graph in Figure II-2 shows the observed concentration of ozone in the air passing

WITH METAL POINT IN CAGE **WITH METAL POINT REMOVED**

FIGURE II-2. Ozone concentration measurements in the Rat Exposure System. In the top graph, a 3-cm-high needle point was used to produce ozone. With the needle point removed, the lower graph shows no change from background as the high voltage is turned on.

through the ozone monitor. This result shows that the response time is indeed short, around 15 seconds, even with the long teflon tube used in the experiment. The sharp increase in ozone concentration immediately after the high voltage was turned off was reproducible. The lower graph in Figure II-2 gives the same data with the metal point removed.

The background concentration of ozone in the rat exposure room varied in the range 0.02-0.05 ppm. The result of many tests with the rat exposure system energized for periods up to 4 hr was that no evidence was found of any ozone concentration above background. Based on these measurements, we have concluded that there are no significant levels of ozone produced by corona discharge in the rat exposure system.

3. Voltage and Field Measurements

In the rat exposure system, all the electrodes are energized at a voltage $V_{pp}/2$ relative to ground. Adjacent electrodes are electrically 180° out of phase, so that the electrode-to-electrode voltage is V_{pp} . To accomplish this, the high-voltage transformer has two secondary windings on its core.

The voltage relative to ground of each side of this transformer was measured using the Ross Voltage Divider. The two outputs were found to be equal to better than 0.5%. The two meters of the high-voltage supplies (one for each high-voltage output) were then calibrated at 20 kV using the Ross voltage divider. Table II-4 gives calibration data for one of the high-voltage supplies of the rat exposure system. The other supply is essentially identical in performance.

TABLE II-4. High-Voltage Calibration Data for One of the Rat Exposure System High Voltage Supplies. The Interelectrode Voltage is the sum of the two output voltages.

<u>Panel Meter (kV)</u>		<u>Voltage Divider (kV)</u>		<u>Interelectrode Voltage (kV)</u>
<u>Output 1</u>	<u>Output 2</u>	<u>Output 1</u>	<u>Output 2</u>	
5.0	5.1	5.03	5.00	10.03
10.0	10.0	10.0	10.0	20.0
15.0	15.0	15.0	15.1	30.1
20.0	19.9	20.0	19.9	39.9
25.0	25.0	24.9	24.8	49.7
30.0	30.0	29.8	29.6	59.4

To measure the interelectrode electric fields, the probes discussed earlier in this report and in previous reports were used. These probes measure the field at the surface of an electrode and must be operated, along with the electronics connected to the probe, at the electrode potential which, for the rat exposure system, can be as high as 30 kV. To do this, special battery-driven electronics were placed in the field-free interior volume of the electrode and read visually at a safe distance.

In the Second Interim Report, a G-factor was introduced to determine the deviation of a parallel plate exposure system from an ideal parallel plate system. This factor is defined by the equation

$$E = G(V_{pp}/d),$$

where E = measured electric field at the center of the lower electrode

V_{pp} = interelectrode voltage

d = distance between parallel electrodes

(V_{pp}/d) = ideal parallel plate field.

For the rat exposure system, with a bare lower electrode (i.e. no animal cages) we found

$$G = 0.997 \pm 0.016.$$

Three rat cage modules (24 cages in all) were then placed on an electrode, and floor-level electric field measurements taken at the center of most of the cages. Figure II-3 shows a plan view of an electrode equipped with 24 rat cages. The measured field strengths are shown at the center of each cage. The nominal parallel plate field is 99.9 kV/m. Comparison between measurements taken before and after insertion of the rat cages shows that the cages themselves reduce the floor-level field at their center by about 3-4%.

The 3/4-in. diameter probe was used to measure the floor-level field strength at various points within a rat cage. This data is shown in Figure II-4, which is a plan view of one cage with each point of field measurement marked with an x. This data shows slight decreases in floor-level field strength near walls and small increases near corners. The maximum variation in field strength is about 7%.

FIGURE II-3. Floor-level electric field strengths measured inside the rat exposure cages.

FIGURE II-4. Floor-level electric field strength measured within a single rat exposure cage.

Measurements close to the cage walls are fairly difficult to make and the data given in Figure II-4 have uncertainties of about 5%, as judged by repeated measurement variability. In the next reporting period these measurements will be repeated and extended to a complete survey of the whole cage area.

C. MOUSE EXPOSURE SYSTEM

The goal was to build a system suitable for the exposure of large numbers of mice to uniform, vertical, 60-Hz electric fields. This system is similar to the rat exposure system described in our last interim report.

1. Exposure Room

Figure II-5 is a plan view of the mouse exposure system. A 20 ft x 20 ft room was divided into three compartments. Two exposure racks, each containing 96 mouse cages, are located in the two larger compartments. These two compartments are separated from one another by wire screening. The third compartment, separated from the animal exposure compartments by an interior wall constructed of sheet rock (floor to ceiling), holds the high-voltage transformer. Connection between the transformer and either exposure rack is made with metal tubular bus. The door to the transformer room is locked and access restricted to only the project manager and engineer. From inside the exposure rooms it is not possible to visually determine which exposure rack is energized. This allows us

FIGURE II-5. Plan view of the Mouse Exposure System.

to implement a "blind" design, where the project investigators are not aware of which animals are exposed and which are sham-exposed until they have completed their measurements and the code is broken.

2. Exposure Rack

An exposure rack is shown in Figure II-6. Five 1.0 m x 2.2 m electrodes are arranged vertically and supported by plexiglass stands at four corners. The vertical separation between electrodes is 0.20 m. Each of the lower four electrodes has positions for three animal modules, with each module having eight cages. Water is supplied from beneath the cages through a manifold. The animal housing system will be discussed later in this report.

Each of the lower four electrodes has two layers. The upper layer consists of three rectangular sections of 1/2-in. x 1/2-in. stainless steel mesh; a cage module is attached to each section with nylon screws. The lower layer of each electrode forms a stainless steel door. Paper (cageboard) is placed on this layer to absorb excreta from mice standing on the upper, screen layer.

Each electrode is equipped with a water bottle placed adjacent to the electrode on a separate plexiglass stand. Connection is made to the water manifold of each shelf with plastic tubing. Each bottle is placed 0.15 m higher than the electrode to provide a small pressure head.

FIGURE II-6. Electrode rack of Mouse Exposure System.

3. High-Voltage System

All five electrodes of each rack are energized, as shown in Figure II-7. Alternate electrodes are connected with 1/2-in. diameter stainless steel tubing. The high voltage is supplied to each of these distribution buses by long horizontal sections of 3/8-in. copper tubing connected to the high-voltage transformer.

A custom, high-voltage power supply was purchased from Hipotronics, Inc., of Brewster, New York. This transformer, shown in Figure II-8, has two outputs of equal voltage (15 kV maximum, 250 VA) but of opposite phase.

4. Safety

The safety aspects of the exposure system are based on experience gained from the prototype exposure system, as described in the First Interim Report. The main door to the exposure room is interlocked with a limit switch. The door to the transformer compartment is locked with a key that is kept in a lockbox; only the project manager and engineer have access to this lockbox. A lock and tag procedure is used when entry to the transformer room is required.

The hall door to the mouse exposure system is also equipped with a key interlock. The key cannot be removed until the door is closed and locked, and the high-voltage system cannot be energized until this key is inserted into an interlock panel.

FIGURE II-7. View of the Mouse Exposure System showing the high-voltage bus work connecting alternate electrodes and the safety grounding rods.

FIGURE II-8. The high-voltage transformer for the Mouse Exposure System.

While work is in progress inside the exposure system, both racks have protective grounds applied (Figure II-7). Designated people have been trained as operators of this system and are responsible for the installation and removal of the protective grounds as well as the operation of the high-voltage controls. They are required to search the exposure compartments when they remove protective grounds and prior to energization of the high-voltage supplies.

5. Animal Housing

The system developed for housing mice during exposure was modeled after the rat housing system. The main differences reflect the smaller size of the mouse. The basic compartment measures 12.7 cm by 25.4 cm; there are eight such compartments in each module; typically, three mice are placed in each compartment. A roof height of 5.1 cm is used to prevent the mice from standing up on their hindlimbs and altering their orientation with respect to the electric fields. This roof has 21 1/4-in-diameter holes drilled over each mouse compartment for ventilation. The temperature rise in an exposure compartment containing three mice is less than 1°C.

The feeding trays are of the same design as used in the rat cage module and are designed to accommodate dry pellet feed in a relatively low profile. The watering system is identical to that used in the rat exposure system and is placed entirely under the floor of the cage, thus preventing any perturbation of the exposure field. A photograph of this

system is given in the Second Interim Report. The watering system and an animal's interaction with it will be discussed further in the next chapter on dosimetry.

To provide mice access to the watering nozzles, notches were cut in the cage walls. These notches were originally 1.3 cm x 1.3 cm, but considerable difficulty was encountered with mice getting their heads caught in this hole and, often, suffocating. The hole size was reduced to 0.64 cm x 1.3 cm high. Mice chewing on the edges of these notches, and on edges in general, is a problem and occasional patching of the mouse cages has been found necessary. No such problem has developed with rats.

6. Field Measurements

Preliminary field measurements have shown that the floor-level field strengths are very uniform, even more uniform than in the rat exposure system (reflecting the smaller plate spacing). More extensive measurements will be given in the next interim report.

7. Ozone Measurements

A program of ozone measurements essentially identical to that described earlier for the rat exposure system has been performed, with the same conclusion: the level of corona discharge in the mouse exposure system is too small to produce measurable ozone concentrations.

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III. DOSIMETRY

Principal Investigator: W. T. Kaune

ABSTRACT

We have studied the electrical interaction between rat, electric fields, and the exposure system watering nozzle in an electric field. Impedance between the nozzle and ground is about 60 k Ω ; impedance between a rat and ground is variable in the range 10-20 k Ω . The fraction of short-circuit current flowing through the mouth of a rat when it drinks from the nozzle is less than 25% of the total current flow through the rat.

A new hemi-ellipsoidal model was built to study the distribution of electric fields at the surface of the rat's body. Measurements of the electric field at 11 points on the surface of this model are in good agreement with theory.

The goals of this part of the overall research effort have been discussed in our first two interim reports (Phillips et al., 1976a; Phillips and Kaune, 1977). During this reporting period, measurements were made to quantitate more precisely the exposure of rats in the rat exposure system, and a probe has been constructed to study the distribution of the normal electric field over the surface of rat models.

A. INTERACTION OF A RAT WITH THE WATERING SYSTEM

As has been discussed in our previous reports, the exposure systems were designed to minimize, as much as practical, interactions between the animals and their watering systems. This section presents our initial experimental steps to document this fact.

Figure III-1A shows a drinking rat; in Figure III-1B the important electrical parameters are displayed. The electric field induces a current, I , in the body. Unless the rat is poorly grounded, virtually all this current passes through the rat's feet to the floor; the current to ground is called the short circuit current. The voltage of the rat's body is IZ_f , where Z_f is the impedance through the feet between the rat's body and the floor; the small voltages between the various internal points of the rat's body have been neglected (Phillips et al., 1975b).

However, a second current path to the floor exists through the mouth when the rat is drinking. There are two series impedances associated with this path (Figure III-1): Z_m is the contact impedance between the rat's mouth and the drinking nozzle; R_n is the resistance between the watering nozzle and the floor. This resistance occurs because the drinking nozzle is inserted into the tubular-metal water manifold through a rubber grommet, and electrical contact between the two is through distilled water. Once Z_m , R_n , and Z_f , are known, the current through the mouth can be calculated.

The following sections present our initial measurements of R_n and Z_f .

1. Measurement of R_n

This quantity was measured by connecting a 60-Hz source in series with a resistance, R , between the nozzle and the water manifold. The current in the circuit was determined by measuring the voltage across R .

a)

b)

FIGURE III-1. The lower drawing (b) shows an electrical equivalent circuit of a rat drinking from the nozzle (a) in the housing system during electric field exposure.

The voltage between the nozzle and manifold was measured and, when divided by the current, yielded the nozzle impedance. In addition, a measurement of the phase of the nozzle impedance was obtained by direct observation of both waveforms on an oscilloscope.

Six separate nozzles in the rat exposure system were measured with observed impedances of 55 k Ω , 64 k Ω , 75 k Ω , 73 k Ω , 50 k Ω , and 60 k Ω . The average is 63 k Ω with a standard deviation of 10 k Ω . The phases of all these values were $0 \pm 5^\circ$ and, in subsequent calculations, will be treated as pure resistance.

2. Measurement of Z_f

A rat was anesthetized with 4 ml of 20% Nembutal and placed on the floor of an exposure cage. A 60-Hz voltage source in series with a resistance, R , was connected between the rat and the cage floor. Connection to the rat was made via a needle electrode inserted intra-dermally in the abdomen just anterior to the rear legs. Measurements of the circuit voltages provide a direct calculation of $|Z_f + Z_e|$, where Z_e is the contact impedance between the rat and the needle electrode, and Z_f is the impedance between the rat and the floor. The rat's tissue impedance is small and has been neglected here (Phillips et al., 1976b). The phase of $Z_f + Z_e$ was calculated by comparing $|V_R|$ the voltage across the series resistance and $|V_A|$ the voltage between the needle electrode and the cage floor (i.e. across the animal) to the source voltage, V_0 . Formally,

$$\cos \phi = \frac{V_0^2 - |V_R|^2 - |V_Z|^2}{2 |V_R| |V_Z|}.$$

In the four cases checked, this formula agreed (within 5°) with the phase determined by direct observation with an oscilloscope.

The impedance between two needle electrodes placed in the rat was measured. Assuming Z_e is the impedance of one needle-rat interface and that the two needles have equal impedances, we found at 60-Hz:

$$Z_e = 1.2 \text{ k}\Omega \angle -55^\circ;$$

that is, the impedance was 1.2 k Ω with the current leading voltage by 55°.

The first measurement between the rat (with all four feet on the floor) and the cage floor yielded an impedance of 5.6 k Ω ; the current for this measurement was 65 μ A. Shortly thereafter, this value rose to 7.4 k Ω . After some additional experimenting, this value rose to 38 k Ω . This last value was relatively independent of how hard the feet were pressed on the floor. We could find no way, by the use of pressure or reorientation of the body to the floor, to reduce the impedance between the rat and the cage floor back to the former values of 5-10 k Ω . The phase through this series of measurements was about -20°. We finally tried wetting the feet, which reduced the impedance to 10 k $\Omega \angle -40^\circ$, after 15 minutes the rat-floor impedance had risen to 19 k $\Omega \angle -40^\circ$.

The variation in the measured impedances before wetting the rat's feet presumably reflects a physiological change in the animal, possibly restricted blood flow to the feet or a decrease in skin moisture. The fact that the phase angles were low (-11 to -16° after subtraction of the needle electrode impedance) suggests that the conduction interface

between the feet and the cage floor did not involve moisture, since phase angles of -40° were observed when the feet were dampened.

Additional measurements are required on conscious, unrestrained animals since it appears likely that the observed impedances may be different for anesthetized animals. However, a reasonable estimate from the measurements reported here on anesthetized animals is that the feet-nozzle impedance, Z_f , will be approximately in the range of 10-20 $k\Omega$.

The results of the measurements of Z_f and R_n can be used to estimate the maximum fraction of the rat short circuit current that will pass through the mouth, I_m , while drinking. Based on values of $R_n = 63 k\Omega$, $Z_f = 10-20 k\Omega$, we get

$$\frac{I_m}{I_{sc}} \lesssim 15-25\%,$$

assuming the mouth to nozzle impedance, Z_m , is zero. Nonzero values will reduce I_m/I_{sc} .

In the next reporting period we will measure this current directly, as well as obtain additional data on the circuit parameters R_n , Z_f , and Z_m .

B. SURFACE ELECTRIC FIELD MEASUREMENTS WITH MODELS

In our Second Interim Report we described a simple hemi-ellipsoidal animal model that is being used to perform basic measurements of rat short circuit currents and the effects on this parameter of animal

cages, other animals in close proximity, and electrode separation. This section describes a second ellipsoidal model instrumented for more detailed measurements.

A drawing of the model is shown in Figure III-2. It was made from balsa wood and painted with a conducting paint. The coordinate system used to describe the model is also shown. The principal axes of the hemi-ellipsoid measure 8.13, 6.30, and 3.18 cm. Eleven 1.3 cm x 1.3 cm copper patches are set flush in the surface of the ellipsoid. The patches are labeled for reference as shown in Figure III-2.

Each patch is electrically isolated from the rest of the ellipsoid and is equipped with a wire for a direct readout of its current. This current can be directly related to the average normal electric field over the patch area by the equation:

$$E_n = \frac{I}{\omega \epsilon_0 A}, \text{ where}$$

E_n = average normal electric field

I = short circuit current of the patch

ω = $2\pi \times 60$ Hz

ϵ_0 = permittivity of air

A = patch area.

The model was made in the form of a hemi-ellipsoid for two reasons: 1) it approximates the shape of a resting rat, and 2) its interaction with the applied uniform field can be analyzed mathematically and provide a check on empirical measurements made with the model.

FIGURE III-2. A hemi-ellipsoidal rat model instrumented for the measurement of surface electric fields at 11 places.

To test the model, it was exposed in the Special Studies Exposure System at a field strength of 92 kV/m (i.e., uniform field strength before insertion of the model). The short circuit current of the total ellipsoid and of each of the 11 patches were then measured. The short circuit currents are shown in Table III-1 and the normal electric field data in Table III-2.

TABLE III-1. Measured and Calculated Short Circuit Currents for an Ellipsoid Exposed to a Vertical Uniform Field of 92 kV/m

<u>Measured Short Circuit Current (μA)</u>	<u>Calculated Short Circuit Current (μA)</u>	<u>Difference</u>
9.51	9.61	1%

TABLE III-2. Measured and Calculated Normal Electric Fields for an Ellipsoid Exposed to a Vertical 92-kV/m Electric Field. The Positions are Defined in Figure III-2.

<u>Position</u>	<u>Measured Normal Electric Field (kV/m)</u>	<u>Calculated Normal Electric Field (kV/m)</u>	<u>Difference</u>
T	350	356	1.4%
1X	335	349	4%
2X	349	335	4%
3X	306	306	0
4X	268	265	1%
5X	198	197	1%
6X	111	100	11%
1Z	306	288	6%
2Z	179	169	6%
3Z	110	101	9%
4Z	52	53	2%

These tables also contain theoretical calculations for comparison to the measured values. The theoretical calculations, while relatively straightforward, are rather complicated and are presented in Appendix A to this report. As these tables show, however, the agreement between measurement and theory is good. This gives us confidence that we can build nonperturbing patches of known area for the measurement of the surface electric field.

This model will be used to investigate the effect on the surface electric field distribution of animal housing systems, electrode separation, and the proximity of other rats.

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IV. BIOLOGICAL STUDIES: EXPERIMENTAL DESIGN AND RATIONALE

Task Leader: R. P. Schneider

A broad and comprehensive series of biological screening experiments are being conducted to assess for possible effects of exposure on rats and mice to 60-Hz electric fields. The biological studies consist of eleven major areas of investigation which cover a wide spectrum of biological functions, emphasizing processes reported to be affected and others which seem mostly likely to be sensitive to electric fields. Despite the differences in the parameters measured in the biological studies, many features of experimental design were common to all of them. The rat and mouse exposure systems were designed to eliminate secondary phenomena caused by electric fields, such as corona discharge and ozone formation, spark discharges and shocks. The rat exposure system is described in detail in the last Progress Report; the mouse system is described in this report.

Unexposed controls (sham-exposed) are kept in the exposure room in an environment identical to that of exposed animals in order to compensate for possible variables other than the electric fields. In fact, the systems have been designed so that it is not possible to visually ascertain which are exposed and which are sham-exposed racks of animals. This arrangement precludes animal caretakers and investigators inadvertently treating the two groups differently.

All rats used in this program were of the Sprague-Dawley strain and were received at 41-42 days of age, except those for the "Bone Growth" and "Reproduction and Development" studies. Upon arrival they were ear-coded and about 5% of the animals were necropsied and subjected to a detailed virology and histopathology examination to ascertain their health. The remaining rats were then randomly sorted into exposed, sham-exposed and cage-control groups. Exposed and sham-exposed groups were then acclimated for 14 days in Lexan cages identical to ones used for exposure. The rats were housed individually. The cage controls were held in individual metal cages in another room. In order to permit feeding, watering and cage cleaning, the exposure systems were shut down for about 3.5 hr per day, i.e. exposure was for 20.5 hr per day. At weekly intervals the housing modules containing exposed and sham-exposed animals were interchanged between racks in conjunction with interchanging the power supply to the racks in order to minimize possible local environmental effects other than the electric field. In order to eliminate inadvertent bias, investigators were unaware of which animals were exposed or sham-exposed until after all measurements had been performed.

Mice (all Swiss-Webster) were handled in the same manner as rats, except that they were 60 days old upon arrival and each cage compartment contained three mice.

When possible, the data were expressed as means with standard error and the differences between the means were tested for significance with Student's *t*-test. The means were considered not to be different if the level of significance (*p*) was greater than 0.05.

V. 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 rats and mice to unperturbed field strengths of 100 kV/m for 15, 30 or 60 days. Statistically significant differences between exposed and sham-exposed rats and mice were seen, but failed to replicate in subsequent experiments.

A. INTRODUCTION

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 in 138- and 345-kV transmission systems, in which 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 only 3 hr under controlled conditions. He felt these changes were the result of nonspecific excitation effects, and other blood and serum parameters remained within normal limits. Asanova and Rakov (1962) reported increased reticulocyte and neutrophil counts in Russian 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 of secondary electric field effects (e.g., shocks, ozone, noise, etc.) causing some alterations in the exposed animals. The results of electric field studies on blood and serum chemistry of exposed animals are summarized in Table V-I. In brief, at field strengths of 10-100 kV/m there are indications of increased concentrations of neutrophils and eosinophils, depression of lymphocytes, increases in some serum constituents, i.e. glutamic oxaloacetic transaminase, lactic dehydrogenase, glucose, calcium, blood urea nitrogen, and alterations in concentrations of serum protein fractions.

The present study was undertaken to evaluate clinical pathologic effects of electric field exposures of rats and mice under exactly defined conditions designed to eliminate secondary influences of the field.

B. METHODS

For these studies, female Swiss-Webster mice and female Sprague-Dawley rats were used. The mice were ~ 74 days of age at the start of exposure and the rats ~ 56 days of age. There were three groups in each experiment: exposed, sham-exposed, and cage controls. Each group of mice consisted of 15 animals, killed after 30 or 60 days. Each group of rats consisted of 10-20 animals exposed for 15, 30 or 60 days.

TABLE V-1. 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)	rat	15	30 d						SGOT ↑ Albumin ↑ α globulins ↓
Luransky et al. (1976)	rat	0.1-5	4 m			eos ↓			glucose ↑ BUN ↑ α & β globulins ↑ LDH ↑
Meda et al. (1972)	rat	10	6 h			eos ↑	↑	↓	
Bayer et al. (1976)	rat	100	1 y			↓			
Le Bars and Andre (1976)	rat	50	30-100 d	=	=	=	=	=	BUN ↑
Blanchi et al. (1973)	mouse	10	55 d			eos ↑	↑	↓	
Poznaniak et al. (1977)	mouse	25-50	6 w	.		↓			Corticosteroids ↑
Knickerbocker et al. (1967)	mouse	157	1500 h				=	=	Marrow =
Le Bars and Andre (1976)	rabbit	50	30-100 d			↓	↓	↓	BUN ↑ Ca ↑ glucose ↓

- no change

↑ increase

↓ decrease

Exposures: Exposure conditions and dosimetry have been detailed earlier (cf. Exposure Systems and Dosimetry Sections). Briefly, the animals were exposed about 21 hr daily to an unperturbed field strength of 100 kV/m. At the end of the exposure period, animals were transported to the clinical pathology laboratory and blood samples were obtained within 3 hr. All hematologic and clinical chemistry evaluations were performed without prior knowledge of the treatment group to which the animals had been assigned.

Hematology: From rats, duplicate blood samples were obtained from free-flowing blood following incision of a tail vein. Mice were lightly anesthetized with ether, and blood collected from the supraorbital sinus into siliconized Pasteur pipettes wet with EDTA.

The Coulter Counter Model S¹ was used to determine the following parameters: white blood cell count, red blood cell count, hemoglobin concentration, volume of packed red cells, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Smears for leukocyte differential counts were prepared in duplicate on coverslips, stained with Wright-Giemsa stain and a minimum of 100 leukocytes classified by each of two medical technologists.

Reticulocytes were stained using new methylene blue, and the reticulocytes enumerated in a minimum of 1000 erythrocytes. Reticulocyte counts were always performed by the same technician in order to reduce variability between technicians' counting values.

¹ Coulter Electronics, Hialeah, FL.

Blood was diluted in ammonium oxalate and platelets were counted using standard hemocytometer methods under phase microscopy. These counts also were always performed by the same technician.

For bone marrow cellularity, the left femur was removed, debrided, and flushed with Hank's balanced salt solution. The nucleated cell count was then determined in duplicate, using a high-resolution Coulter Model Z_H particle counter.

Red cell osmotic fragility was determined using commercially available saline dilutions.¹ The results were graphed and the mean corpuscular fragility determined for each animal.

Prothrombin times were performed in triplicate using the Fibrometer system.² Quality control was maintained using lyophilized human reference serum.³

Serum Chemistry: Blood from both species was collected, without anticoagulants, via cardiac puncture from anesthetized animals. Serum was removed and quick-frozen within 1 hour after collection of the blood.

Blood urea nitrogen (BUN), serum alkaline phosphatase (SAP), serum glutamic oxaloacetic transaminase (SGOT), glucose and serum proteins were assayed using AutoAnalyzer II methodologies.⁴

Serum iron values were determined by a modification of the method of Jung and Parekh (1970), and triglycerides by the enzymatic method of Hycel.⁵ Serum proteins were fractionated by electrophoresis using cellulose acetate strips and the fractions quantitated densitometrically.

¹ Unopette Osmotic Fragility Test, Becton-Dickson Co., NJ.

² Fibrometer, BBL Corp.

³ Prothrombin Control, Hyland

⁴ Technicon Instrument Co., Tarrytown, NY

⁵ Triglyceride Set, Hycel Inc., Houston, TX.

C. RESULTS

Mouse Studies: Mice were exposed in two replicate studies for 30 and two for 60 days. The results of hematologic evaluations are shown for the 30-day exposures in Tables V-2, and V-3, and clinical chemistry results are shown in Table V-4. Mean values are included from animals of the original lot, randomly assigned as cage controls. The latter served as a reference standard for the general health status of the colony, and as quality control for methods.

After 30 days of exposure, statistically significant increases in erythroid parameters were found in the first replicate in electric field-exposed mice when compared with the sham-exposed group (Table V-17). At that time, no differences were observed between the two groups in regard to leukocyte parameters. In the second 30-day exposure replication there were no significant differences between the exposed and sham-exposed groups.

In the first replicate study after 60 days of exposure (Tables V-5,-6,-7), red cell measurements were comparable between the two groups, but total leukocyte count, and neutrophil and lymphocyte concentrations in blood were significantly lower in the electric-field-exposed mice (Table V-17). In addition, serum alpha globulin concentrations and spleen weights of the exposed mice were lower than those of sham-exposed animals (Table V-17).

In the repeat study after 60 days of exposure, the total leukocyte count of the exposed animals was significantly higher ($p < 0.01$) than the mean value from the sham-exposed mice. This effect is opposite to that observed in the first 60-day replicate.

TARIE V.2. Hematologic Values in Mice Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value + 1 standard error). Each group consisted of 15 mice.

	Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
Volume Packed Red Cells (ml/100 ml)	40.5 ± 0.3	39.1 ± 0.5	40.1 ± 0.4	39.2 ± 0.4	39.1 ± 0.4	39.6 ± 0.5	39.9 ± 0.3	39.1 ± 0.3	39.9 ± 0.3
Red Blood Cells (X10 ⁶ /mm ³)	8.71 ± 0.10	8.43 ± 0.09	8.60 ± 0.13	8.66 ± 0.09	8.58 ± 0.15	8.50 ± 0.13	8.69 ± 0.06	8.51 ± 0.09	8.55 ± 0.09
Hemoglobin (g/dl)	14.8 ± 0.1	14.1 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.8 ± 0.1	14.4 ± 0.2	14.7 ± 0.1
Mean Corpuscular Volume (μ ³)	47.0 ± 0.3	47.0 ± 0.3	47.0 ± 0.3	46.1 ± 0.4	46.3 ± 0.4	47.3 ± 0.4	46.6 ± 0.2	46.7 ± 0.3	47.2 ± 0.2
Mean Corpuscular Hemoglobin (μg)	17.2 ± 0.1	16.9 ± 0.1	17.3 ± 0.2	17.1 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.4 ± 0.1
Mean Corpuscular Hemoglobin Concentration (%)	36.9 ± 0.2	36.6 ± 0.2	37.0 ± 0.2	37.8 ± 0.3	37.9 ± 0.2	37.6 ± 0.2	37.4 ± 0.2	37.3 ± 0.2	37.3 ± 0.2
Reticulocytes (X10 ³ /mm ³)	177 ± 15	148 ± 14	194 ± 17	174 ± 18	176 ± 14	172 ± 19	176 ± 12	162 ± 10	183 ± 12
Platelets (X10 ³ /mm ³)	1212 ± 78	1302 ± 70	974 ± 83	1116 ± 44	1127 ± 42	1002 ± 52	1164 ± 45	1215 ± 43	988 ± 48
Bone Marrow (X10 ³ /femur)	10.6 ± 0.3	10.7 ± 0.4	9.8 ± 0.5	12.9 ± 0.5	12.7 ± 0.6	11.7 ± 0.4	11.8 ± 0.4	11.7 ± 0.5	10.8 ± 0.4

TABLE V-3. Total Leukocyte Count and Cell Distributions in Mice Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error). Each group consisted of 15 mice.

	Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
White Blood Cells ($\times 10^3/\text{mm}^3$)	5.4 $\pm$ 0.4	6.3 $\pm$ 0.5	6.0 $\pm$ 0.4	7.9 $\pm$ 0.7	6.9 $\pm$ 0.8	5.3 $\pm$ 0.4	6.7 $\pm$ 0.5	6.6 $\pm$ 0.5	5.7 $\pm$ 0.3
Neutrophils (%)	11.4 $\pm$ 0.7	10.7 $\pm$ 0.7	11.2 $\pm$ 0.8	13.6 $\pm$ 1.0	15.0 $\pm$ 1.5	14.5 $\pm$ 1.2	12.5 $\pm$ 0.6	12.9 $\pm$ 0.9	12.9 $\pm$ 0.8
Neutrophils ($\times 10^3/\text{mm}^3$)	0.61 $\pm$ 0.05	0.67 $\pm$ 0.07	0.65 $\pm$ 0.05	1.08 $\pm$ 0.16	1.03 $\pm$ 0.14	0.75 $\pm$ 0.07	0.85 $\pm$ 0.09	0.85 $\pm$ 0.08	0.70 $\pm$ 0.04
Lymphocytes (%)	86.5 $\pm$ 0.9	87.6 $\pm$ 0.9	86.3 $\pm$ 0.8	85.8 $\pm$ 1.1	84.5 $\pm$ 1.4	84.4 $\pm$ 1.2	86.2 $\pm$ 0.7	86.1 $\pm$ 0.9	85.4 $\pm$ 0.7
Lymphocytes ($\times 10^3/\text{mm}^3$)	4.64 $\pm$ 0.32	5.50 $\pm$ 0.46	5.17 $\pm$ 0.33	6.71 $\pm$ 0.59	5.84 $\pm$ 0.70	4.46 $\pm$ 0.34	5.68 $\pm$ 0.38	5.67 $\pm$ 0.42	4.82 $\pm$ 0.24
Monocytes (%)	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.6 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.03 $\pm$ 0.02	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	0.03 $\pm$ 0.01	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01
Eosinophils (%)	1.8 $\pm$ 0.4	1.2 $\pm$ 0.3	1.7 $\pm$ 0.4	0.4 $\pm$ 0.2	0.40 $\pm$ 0.2	0.8 $\pm$ 0.3	1.1 $\pm$ 0.3	0.8 $\pm$ 0.2	1.3 $\pm$ 0.2
Eosinophils ($\times 10^3/\text{mm}^3$)	0.10 $\pm$ 0.03	0.07 $\pm$ 0.02	0.10 $\pm$ 0.02	0.03 $\pm$ 0.01	0.04 $\pm$ 0.02	0.10 $\pm$ 0.07	0.07 $\pm$ 0.02	0.06 $\pm$ 0.02	0.10 $\pm$ 0.04

TABLE V-4. Some Serum Chemistry and Organ Weight Values from Mice Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error). Each group consisted of 15 mice.

	Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
Serum Glutamic Oxaloacetic Transaminase (RF Units)	---	---	---	316 $\pm$ 11	332 $\pm$ 15	316 $\pm$ 16	---	---	---
Triglycerides (mg/dl)	115 $\pm$ 7	128 $\pm$ 10	140 $\pm$ 16	121 $\pm$ 5	123 $\pm$ 5	122 $\pm$ 5	118 $\pm$ 4	126 $\pm$ 5	123 $\pm$ 4
Protein (g/dl)	5.0 $\pm$ 0.1	4.9 $\pm$ 0.1	4.9 $\pm$ 0.1	5.8 $\pm$ 0.07	5.9 $\pm$ 0.1	5.7 $\pm$ 0.1	5.4 $\pm$ 0.1	5.40 $\pm$ 0.1	5.30 $\pm$ 0.1
Albumin (g/dl)	2.56 $\pm$ 0.07	2.60 $\pm$ 0.06	2.60 $\pm$ 0.03	3.28 $\pm$ 0.08	3.43 $\pm$ 0.06	3.12 $\pm$ 0.06	2.92 $\pm$ 0.09	3.02 $\pm$ 0.08	2.86 $\pm$ 0.06
<u>Globulins</u>									
Alpha (g/dl)	0.87 $\pm$ 0.03	0.84 $\pm$ 0.03	0.77 $\pm$ 0.02	1.11 $\pm$ 0.03	1.09 $\pm$ 0.02	1.07 $\pm$ 0.02	0.99 $\pm$ 0.03	0.97 $\pm$ 0.03	0.92 $\pm$ 0.03
Beta (g/dl)	1.05 $\pm$ 0.04	1.01 $\pm$ 0.06	1.02 $\pm$ 0.04	0.92 $\pm$ 0.02	0.89 $\pm$ 0.03	0.92 $\pm$ 0.03	0.99 $\pm$ 0.02	0.95 $\pm$ 0.04	0.97 $\pm$ 0.03
Gamma (g/dl)	0.48 $\pm$ 0.03	0.43 $\pm$ 0.03	0.48 $\pm$ 0.02	0.50 $\pm$ 0.03	0.48 $\pm$ 0.02	0.58 $\pm$ 0.02	0.49 $\pm$ 0.02	0.46 $\pm$ 0.02	0.53 $\pm$ 0.02
<u>Weights</u>									
Body (g)	29.2 $\pm$ 0.4	28.4 $\pm$ 0.4	30.0 $\pm$ 0.5	27.7 $\pm$ 0.3	27.9 $\pm$ 0.3	29.7 $\pm$ 0.3	28.5 $\pm$ 0.3	28.2 $\pm$ 0.3	29.9 $\pm$ 0.3
Liver (g)	1.30 $\pm$ 0.03	1.35 $\pm$ 0.04	1.39 $\pm$ 0.05	1.3 $\pm$ 0.03	1.4 $\pm$ 0.05	1.3 $\pm$ 0.05	1.3 $\pm$ 0.02	1.4 $\pm$ 0.04	1.3 $\pm$ 0.04
Spleen (mg)	141.5 $\pm$ 8.1	120.1 $\pm$ 5.8	160.3 $\pm$ 11.3	109.1 $\pm$ 4.2	121.9 $\pm$ 5.1	115.5 $\pm$ 5.6	125.3 $\pm$ 5.4	121.0 $\pm$ 3.8	137.9 $\pm$ 7.4
Femur (mg)	72.4 $\pm$ 2.2	65.4 $\pm$ 1.8	69.5 $\pm$ 2.3	70.6 $\pm$ 1.8	69.4 $\pm$ 1.7	74.6 $\pm$ 2.1	71.5 $\pm$ 1.4	67.4 $\pm$ 1.2	72.1 $\pm$ 1.6

TABLE V-5. Hematologic Values in Mice Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error). Each group consisted of 15 mice.

	Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
Volume Packed Red Cells (ml/100 ml)	37.3 $\pm$ 0.5	36.9 $\pm$ 0.5	37.9 $\pm$ 0.3	38.9 $\pm$ 0.3	38.1 $\pm$ 0.6	39.5 $\pm$ 0.8	38.1 $\pm$ 0.3	37.5 $\pm$ 0.4	38.7 $\pm$ 0.4
Red Blood Cells ($\times 10^6/\text{mm}^3$)	7.90 $\pm$ 0.10	7.89 $\pm$ 0.15	8.09 $\pm$ 0.07	8.28 $\pm$ 0.11	8.04 $\pm$ 0.15	8.32 $\pm$ 0.18	8.09 $\pm$ 0.08	7.97 $\pm$ 0.11	8.21 $\pm$ 0.10
Hemoglobin (g/dl)	13.9 $\pm$ 0.2	13.4 $\pm$ 0.2	13.9 $\pm$ 0.1	14.2 $\pm$ 0.2	13.8 $\pm$ 0.3	14.6 $\pm$ 0.3	14.1 $\pm$ 0.1	13.6 $\pm$ 0.2	14.3 $\pm$ 0.2
Mean Corpuscular Volume (μ^3)	48.0 $\pm$ 0.4	48.0 $\pm$ 0.4	48.0 $\pm$ 0.4	47.9 $\pm$ 0.4	48.1 $\pm$ 0.4	48.5 $\pm$ 0.4	48.0 $\pm$ 0.3	48.1 $\pm$ 0.3	48.3 $\pm$ 0.3
Mean Corpuscular Hemoglobin (μg)	17.8 $\pm$ 0.2	17.2 $\pm$ 0.2	17.3 $\pm$ 0.1	17.3 $\pm$ 0.2	17.3 $\pm$ 0.2	17.7 $\pm$ 0.2	17.6 $\pm$ 0.1	17.3 $\pm$ 0.1	17.5 $\pm$ 0.1
Mean Corpuscular Hemoglobin Concentration (%)	37.7 $\pm$ 0.3	36.9 $\pm$ 0.3	37.0 $\pm$ 0.2	36.4 $\pm$ 0.2	36.1 $\pm$ 0.3	36.8 $\pm$ 0.5	37.1 $\pm$ 0.2	36.5 $\pm$ 0.2	36.9 $\pm$ 0.3
Reticulocytes ($\times 10^3/\text{mm}^3$)	151 $\pm$ 20	164 $\pm$ 21	135 $\pm$ 19	168 $\pm$ 20	159 $\pm$ 11	169 $\pm$ 20	160 $\pm$ 14	162 $\pm$ 12	152 $\pm$ 14
Platelets ($\times 10^3/\text{mm}^3$)	1076 $\pm$ 72	1110 $\pm$ 74	968 $\pm$ 52	1028 $\pm$ 52	1054 $\pm$ 57	940 $\pm$ 56	1052 $\pm$ 44	1082 $\pm$ 46	954 $\pm$ 37
Bone Marrow ($\times 10^3/\text{femur}$)	11.5 $\pm$ 0.4	11.3 $\pm$ 0.5	10.6 $\pm$ 0.5	11.5 $\pm$ 0.6	12.7 $\pm$ 0.7	11.2 $\pm$ 0.5	11.5 $\pm$ 0.4	12.0 $\pm$ 0.4	10.9 $\pm$ 0.4

TABLE V-6. Total Leukocyte Count and Cell Distributions in Mice Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value + 1 standard error). Each group consisted of 15 mice.

	Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
White Blood Cells ($\times 10^3/\text{mm}^3$)	5.2 $\pm$ 0.4	7.7 $\pm$ 0.9	4.7 $\pm$ 0.3	6.2 $\pm$ 0.5	3.9 $\pm$ 0.4	4.7 $\pm$ 0.3	5.7 $\pm$ 0.3	5.8 $\pm$ 0.6	4.7 $\pm$ 0.2
Neutrophils (%)	14.0 $\pm$ 1.3	14.3 $\pm$ 1.3	15.0 $\pm$ 1.3	21.5 $\pm$ 3.3	15.3 $\pm$ 1.5	11.8 $\pm$ 0.9	17.8 $\pm$ 1.9	14.8 $\pm$ 1.0	13.4 $\pm$ 0.8
Neutrophils ($\times 10^3/\text{mm}^3$)	0.73 $\pm$ 0.10	1.12 $\pm$ 0.12	0.77 $\pm$ 0.11	1.31 $\pm$ 0.21	0.59 $\pm$ 0.07	0.55 $\pm$ 0.05	1.02 $\pm$ 0.13	0.86 $\pm$ 0.09	0.66 $\pm$ 0.06
Lymphocytes (%)	85.1 $\pm$ 1.4	84.0 $\pm$ 1.4	83.8 $\pm$ 1.3	74.3 $\pm$ 3.8	83.8 $\pm$ 1.5	87.2 $\pm$ 0.8	79.7 $\pm$ 2.2	83.9 $\pm$ 1.0	85.5 $\pm$ 0.8
Lymphocytes ($\times 10^3/\text{mm}^3$)	4.42 $\pm$ 0.33	6.73 $\pm$ 0.76	4.07 $\pm$ 0.23	4.74 $\pm$ 0.46	3.31 $\pm$ 0.38	4.12 $\pm$ 0.30	4.58 $\pm$ 0.27	5.02 $\pm$ 0.53	4.10 $\pm$ 0.18
Monocytes (%)	0.4 $\pm$ 0.1	1.1 $\pm$ 0.3	0.8 $\pm$ 0.2	0 $\pm$ 0	0.07 $\pm$ 0.05	0.10 $\pm$ 0.05	0.2 $\pm$ 0.1	0.9 $\pm$ 0.1	0.5 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.02 $\pm$ 0.01	0.08 $\pm$ 0.02	0.04 $\pm$ 0.01	0 $\pm$ 0	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.04 $\pm$ 0.01	0.02 $\pm$ 0.01
Eosinophils (%)	0.6 $\pm$ 0.1	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	2.2 $\pm$ 0.4	0.9 $\pm$ 0.2	1.0 $\pm$ 0.2	1.4 $\pm$ 0.2	0.8 $\pm$ 0.1	0.8 $\pm$ 0.1
Eosinophils ($\times 10^3/\text{mm}^3$)	0.03 $\pm$ 0.01	0.05 $\pm$ 0.02	0.02 $\pm$ 0.01	0.14 $\pm$ 0.03	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	0.09 $\pm$ 0.02	0.04 $\pm$ 0.01	0.04 $\pm$ 0.01

TABLE V-7. Some Serum Chemistry and Organ Weight Values from Mice Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error). Each group consisted of 15 mice.

	-Replicate 1			Replicate 2			Composite		
	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control	Exposed	Sham-Exposed	Cage Control
Serum Glutamic Oxaloacetic Transaminase (RF Units)	212 $\pm$ 11	199 $\pm$ 21	217 $\pm$ 20	---	---	---	---	---	---
Triglycerides (mg/dl)	107 $\pm$ 4	110 $\pm$ 6	100 $\pm$ 2	122 $\pm$ 6	103 $\pm$ 6	115 $\pm$ 8	115 $\pm$ 4	107 $\pm$ 5	104 $\pm$ 4
Protein (g/dl)	5.2 $\pm$ 0.3	5.0 $\pm$ 0.2	5.1 $\pm$ 0.1	5.7 $\pm$ 0.1	6.0 $\pm$ 0.1	6.3 $\pm$ 0.1	5.6 $\pm$ 0.1	5.4 $\pm$ 0.2	5.4 $\pm$ 0.1
Albumin (g/dl)	2.89 $\pm$ 0.06	2.76 $\pm$ 0.06	3.02 $\pm$ 0.05	3.04 $\pm$ 0.07	3.19 $\pm$ 0.12	3.38 $\pm$ 0.05	2.97 $\pm$ 0.05	2.92 $\pm$ 0.07	3.11 $\pm$ 0.06
<u>Globulins</u>									
Alpha (g/dl)	0.85 $\pm$ 0.04	0.95 $\pm$ 0.02	0.80 $\pm$ 0.03	1.09 $\pm$ 0.03	1.18 $\pm$ 0.06	1.17 $\pm$ 0.04	0.98 $\pm$ 0.03	1.04 $\pm$ 0.04	0.90 $\pm$ 0.05
Beta (g/dl)	0.94 $\pm$ 0.03	0.99 $\pm$ 0.02	0.88 $\pm$ 0.03	1.10 $\pm$ 0.04	1.15 $\pm$ 0.05	1.10 $\pm$ 0.09	1.03 $\pm$ 0.03	1.05 $\pm$ 0.03	0.94 $\pm$ 0.04
Gamma (g/dl)	0.47 $\pm$ 0.02	0.51 $\pm$ 0.03	0.43 $\pm$ 0.02	0.44 $\pm$ 0.03	0.43 $\pm$ 0.03	0.60 $\pm$ 0.04	0.45 $\pm$ 0.02	0.48 $\pm$ 0.02	0.48 $\pm$ 0.03
<u>Weights</u>									
Body (g)	27.4 $\pm$ 0.5	28.1 $\pm$ 0.7	28.7 $\pm$ 0.6	30.2 $\pm$ 0.5	28.0 $\pm$ 0.7	30.6 $\pm$ 0.7	28.9 $\pm$ 0.5	28.1 $\pm$ 0.6	29.2 $\pm$ 0.6
Liver (g)	1.06 $\pm$ 0.07	1.25 $\pm$ 0.08	1.29 $\pm$ 0.05	---	---	---	---	---	---
Spleen (mg)	107.3 $\pm$ 5.4	128.5 $\pm$ 8.3	124.2 $\pm$ 6.2	---	---	---	---	---	---
Femur (mg)	66.2 $\pm$ 2.0	66.9 $\pm$ 1.1	69.5 $\pm$ 2.1	---	---	---	---	---	---

When the replicate data were combined for each exposure period there are no statistically significant differences between the exposed and sham-exposed groups of mice.

Rat Studies: Rats were exposed for 15 days in two replicate studies. Results are shown in Tables V-8, V-9, and V-10. Following the first 15-day exposure, there was a significant reduction in leukocytes due to an absolute decrease in lymphocytes (Table V-18). In the replicate study, with a larger number of rats, the only statistically significant differences noted were an increase in beta globulins and a decrease in triglycerides in the exposed rats as compared with the sham group (Table V-18). When the two replications were combined there were no statistically significant differences between the groups.

For the 30-day exposures there were, again, two replications with groups of 10 rats each (Tables V-11, V-12, and V-13). In the first replication, the only difference noted was a decrease in alpha globulins in exposed rats (Table V-18). In the second replication, mean bone marrow cellularity of electric-field-exposed rats was increased over that of the sham-exposed group (Table V-18). When the results of the two 30-day replications were combined there were no statistical differences between the treatment groups.

Two replications for the 60-day exposure of rats have been completed at this time (Tables V-14, V-15, and V-16). In the first, a statistically significant increase in platelet concentration and a decrease in beta

TABLE V-8. Hematologic Values in Rats Exposed for 15 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=10)	Exposed (n=30)	Sham-Exposed (n=30)	Cage Control (n=19)
Volume Packed Red Cells (ml/100 ml)	40.6 $\pm$ 0.6	41.5 $\pm$ 0.3	42.2 $\pm$ 0.5	42.7 $\pm$ 0.3	42.9 $\pm$ 0.3	42.3 $\pm$ 0.8	42.0 $\pm$ 0.3	42.4 $\pm$ 0.2	42.2 $\pm$ 0.5
Red Blood Cells ($\times 10^6/\text{mm}^3$)	7.35 $\pm$ 0.11	7.44 $\pm$ 0.07	7.50 $\pm$ 0.11	7.79 $\pm$ 0.06	7.91 $\pm$ 0.08	7.40 $\pm$ 0.15	7.64 $\pm$ 0.07	7.75 $\pm$ 0.07	7.45 $\pm$ 0.09
Hemoglobin (g/dl)	16.1 $\pm$ 0.2	16.6 $\pm$ 0.1	16.8 $\pm$ 0.2	17.2 $\pm$ 0.2	16.9 $\pm$ 0.1	16.6 $\pm$ 0.3	16.8 $\pm$ 0.2	16.8 $\pm$ 0.1	16.7 $\pm$ 0.2
Mean Corpuscular Volume (μ^3)	56.8 $\pm$ 0.4	57.2 $\pm$ 0.4	57.8 $\pm$ 0.4	55.5 $\pm$ 0.4	54.9 $\pm$ 0.3	57.7 $\pm$ 0.7	55.9 $\pm$ 0.3	55.7 $\pm$ 0.3	57.7 $\pm$ 0.4
Mean Corpuscular Hemoglobin (pg)	21.8 $\pm$ 0.1	22.1 $\pm$ 0.2	22.2 $\pm$ 0.2	22.2 $\pm$ 0.2	21.6 $\pm$ 0.2	22.7 $\pm$ 0.3	22.1 $\pm$ 0.1	21.8 $\pm$ 0.1	22.4 $\pm$ 0.2
Mean Corpuscular Hemoglobin Concentration (%)	39.5 $\pm$ 0.1	39.7 $\pm$ 0.1	39.6 $\pm$ 0.1	40.8 $\pm$ 0.3	40.0 $\pm$ 0.1	39.9 $\pm$ 0.3	40.4 $\pm$ 0.3	39.9 $\pm$ 0.1	39.8 $\pm$ 0.2
Reticulocytes ($\times 10^3/\text{mm}^3$)	153 $\pm$ 13	118 $\pm$ 10	103 $\pm$ 9	127 $\pm$ 9	123 $\pm$ 9	134 $\pm$ 21	136 $\pm$ 8	122 $\pm$ 7	118 $\pm$ 12
Platelets ($\times 10^3/\text{mm}^3$)	1049 $\pm$ 75	1018 $\pm$ 47	944 $\pm$ 48	909 $\pm$ 39	956 $\pm$ 39	924 $\pm$ 85	955 $\pm$ 37	977 $\pm$ 30	935 $\pm$ 48
Bone Marrow ($\times 10^3/\text{femur}$)	41.9 $\pm$ 2.7	45.8 $\pm$ 3.2	43.9 $\pm$ 3.3	48.6 $\pm$ 1.2	48.8 $\pm$ 1.7	44.6 $\pm$ 1.2	46.4 $\pm$ 1.3	47.8 $\pm$ 1.5	44.2 $\pm$ 1.8

TABLE V-9. Total Leukocyte Count and Cell Distributions in Rats Exposed for 15 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=10)	Exposed (n=30)	Sham-Exposed (n=30)	Cage Control (n=19)
White Blood Cells ($\times 10^3/\text{mm}^3$)	7.5 $\pm$ 0.5	9.3 $\pm$ 0.3	6.5 $\pm$ 0.3	6.9 $\pm$ 0.2	6.5 $\pm$ 0.2	6.9 $\pm$ 0.4	7.1 $\pm$ 0.2	7.4 $\pm$ 0.3	6.7 $\pm$ 0.2
Neutrophils (%)	8.0 $\pm$ 1.0	8.3 $\pm$ 1.3	11.5 $\pm$ 1.1	9.4 $\pm$ 0.9	10.4 $\pm$ 1.3	9.3 $\pm$ 1.4	8.9 $\pm$ 0.7	9.7 $\pm$ 0.9	10.5 $\pm$ 0.9
Neutrophils ($\times 10^3/\text{mm}^3$)	0.60 $\pm$ 0.06	0.77 $\pm$ 0.09	0.75 $\pm$ 0.09	0.65 $\pm$ 0.07	0.68 $\pm$ 0.08	0.64 $\pm$ 0.10	0.63 $\pm$ 0.07	0.7 $\pm$ 0.08	0.7 $\pm$ 0.09
Lymphocytes (%)	89.9 $\pm$ 1.2	89.9 $\pm$ 1.5	87.1 $\pm$ 1.2	89.0 $\pm$ 1.1	88.7 $\pm$ 1.2	88.8 $\pm$ 1.5	89.3 $\pm$ 0.8	89.1 $\pm$ 0.9	87.9 $\pm$ 0.9
Lymphocytes ($\times 10^3/\text{mm}^3$)	6.74 $\pm$ 0.47	8.36 $\pm$ 0.35	5.66 $\pm$ 0.22	6.14 $\pm$ 0.22	5.76 $\pm$ 0.25	6.13 $\pm$ 0.36	6.34 $\pm$ 0.35	6.59 $\pm$ 0.30	5.89 $\pm$ 0.30
Monocytes (%)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.2	0.5 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.09 $\pm$ 0.01	0.08 $\pm$ 0.02	0.04 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01
Eosinophils (%)	1.2 $\pm$ 0.3	1.0 $\pm$ 0.2	0.9 $\pm$ 0.3	1.6 $\pm$ 0.2	1.4 $\pm$ 0.2	1.9 $\pm$ 0.5	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2	1.4 $\pm$ 0.3
Eosinophils ($\times 10^3/\text{mm}^3$)	0.11 $\pm$ 0.02	0.10 $\pm$ 0.02	0.08 $\pm$ 0.02	0.12 $\pm$ 0.02	0.09 $\pm$ 0.02	0.13 $\pm$ 0.03	0.11 $\pm$ 0.01	0.09 $\pm$ 0.02	0.10 $\pm$ 0.02

TABLE V-10. Serum Chemistry and Organ Weight Values from Rats Exposed for 15 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=10)	Exposed (n=30)	Sham-Exposed (n=30)	Cage Control (n=19)
Blood Urea Nitrogen (mg/dl)	23.2 $\pm$ 1.0	22.5 $\pm$ 1.0	21.2 $\pm$ 0.8	26.2 $\pm$ 0.9	25.1 $\pm$ 0.9	24.3 $\pm$ 1.1	25.2 $\pm$ 0.8	24.2 $\pm$ 0.7	22.7 $\pm$ 0.7
Alkaline Phosphatase (BLB Units)	9.6 $\pm$ 0.8	8.1 $\pm$ 0.6	7.3 $\pm$ 0.5	5.7 $\pm$ 0.3	5.5 $\pm$ 0.4	5.1 $\pm$ 0.5	7.0 $\pm$ 0.5	6.3 $\pm$ 0.3	6.2 $\pm$ 0.4
Serum Glutamic Oxaloacetic Transaminase (RF Units)	222 $\pm$ 19	208 $\pm$ 20	191 $\pm$ 10	441 $\pm$ 35	372 $\pm$ 13	366 $\pm$ 41	368 $\pm$ 31	321 $\pm$ 17	278 $\pm$ 29
Serum Iron (μ g/dl)	279 $\pm$ 24	297 $\pm$ 12	292 $\pm$ 12	---	---	---	---	---	---
Glucose (mg/dl)	---	---	---	134 $\pm$ 5	135 $\pm$ 4	145 $\pm$ 5	---	---	---
Triglycerides (mg/dl)	---	---	---	96 $\pm$ 3	107 $\pm$ 3	89 $\pm$ 4	---	---	---
Serum Proteins (g/dl)	6.1 $\pm$ 0.1	6.1 $\pm$ 0.1	6.0 $\pm$ 0.1	6.4 $\pm$ 0.1	6.4 $\pm$ 0.1	6.4 $\pm$ 0.1	6.3 $\pm$ 0.1	6.3 $\pm$ 0.1	6.2 $\pm$ 0.1
Albumin (g/dl)	3.57 $\pm$ 0.06	3.51 $\pm$ 0.06	3.36 $\pm$ 0.05	3.38 $\pm$ 0.08	3.49 $\pm$ 0.05	3.54 $\pm$ 0.07	3.44 $\pm$ 0.06	3.49 $\pm$ 0.04	3.45 $\pm$ 0.05
<u>Globulins</u>									
Alpha (g/dl)	1.11 $\pm$ 0.04	1.03 $\pm$ 0.09	1.16 $\pm$ 0.04	1.23 $\pm$ 0.03	1.17 $\pm$ 0.03	1.23 $\pm$ 0.05	1.19 $\pm$ 0.03	1.16 $\pm$ 0.02	1.19 $\pm$ 0.03
Beta (g/dl)	0.90 $\pm$ 0.02	0.95 $\pm$ 0.02	0.93 $\pm$ 0.03	1.15 $\pm$ 0.03	1.06 $\pm$ 0.02	1.05 $\pm$ 0.02	1.07 $\pm$ 0.03	1.02 $\pm$ 0.02	0.99 $\pm$ 0.02
Gamma (g/dl)	0.50 $\pm$ 0.04	0.52 $\pm$ 0.03	0.53 $\pm$ 0.04	0.68 $\pm$ 0.05	0.66 $\pm$ 0.03	0.60 $\pm$ 0.03	0.62 $\pm$ 0.04	0.61 $\pm$ 0.02	0.56 $\pm$ 0.03

TABLE V-11. Hematologic Values in Rats Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=20)
Volume Packed Red Cells (ml/100 ml)	41.7 $\pm$ 0.3	42.0 $\pm$ 0.3	40.6 $\pm$ 0.6	42.0 $\pm$ 0.4	42.6 $\pm$ 0.3	42.6 $\pm$ 0.4	41.9 $\pm$ 0.3	42.3 $\pm$ 0.3	41.6 $\pm$ 0.4
Red Blood Cells ($\times 10^6/\text{mm}^3$)	7.63 $\pm$ 0.09	7.78 $\pm$ 0.05	7.57 $\pm$ 0.15	7.68 $\pm$ 0.09	7.89 $\pm$ 0.08	7.77 $\pm$ 0.06	7.67 $\pm$ 0.07	7.84 $\pm$ 0.05	7.67 $\pm$ 0.10
Hemoglobin (g/dl)	16.5 $\pm$ 0.1	16.6 $\pm$ 0.1	16.1 $\pm$ 0.3	16.7 $\pm$ 0.2	17.0 $\pm$ 0.1	17.0 $\pm$ 0.1	16.6 $\pm$ 0.1	16.8 $\pm$ 0.1	16.6 $\pm$ 0.1
Mean Corpuscular Volume (μ^3)	56.2 $\pm$ 0.4	55.5 $\pm$ 0.2	55.2 $\pm$ 0.5	55.2 $\pm$ 0.4	54.7 $\pm$ 0.5	55.5 $\pm$ 0.4	55.7 $\pm$ 0.3	55.1 $\pm$ 0.3	55.4 $\pm$ 0.4
Mean Corpuscular Hemoglobin (μg)	21.5 $\pm$ 0.2	21.2 $\pm$ 0.1	21.2 $\pm$ 0.2	21.9 $\pm$ 0.1	21.7 $\pm$ 0.2	21.9 $\pm$ 0.1	21.7 $\pm$ 0.1	21.4 $\pm$ 0.1	21.6 $\pm$ 0.1
Mean Corpuscular Hemoglobin Con- centration (%)	39.5 $\pm$ 0.2	39.3 $\pm$ 0.1	39.6 $\pm$ 0.1	40.3 $\pm$ 0.2	40.3 $\pm$ 0.1	40.2 $\pm$ 0.2	39.9 $\pm$ 0.2	39.8 $\pm$ 0.1	39.9 $\pm$ 0.2
Reticulocytes ($\times 10^3/\text{mm}^3$)	126 $\pm$ 16	132 $\pm$ 10	106 $\pm$ 12	126 $\pm$ 12	122 $\pm$ 12	138 $\pm$ 10	126 $\pm$ 10	127 $\pm$ 8	122 $\pm$ 8
Platelets ($\times 10^3/\text{mm}^3$)	1010 $\pm$ 78	847 $\pm$ 65	949 $\pm$ 58	912 $\pm$ 72	1041 $\pm$ 71	853 $\pm$ 44	961 $\pm$ 53	944 $\pm$ 52	901 $\pm$ 37
Bone Marrow ($\times 10^3/\text{femur}$)	39.2 $\pm$ 3.3	36.6 $\pm$ 3.2	41.0 $\pm$ 2.7	48.9 $\pm$ 1.3	43.4 $\pm$ 2.2	42.3 $\pm$ 3.4	44.1 $\pm$ 1.9	40.0 $\pm$ 2.1	41.7 $\pm$ 2.7
Mean Corpuscular Fragility (% saline)	0.465 $\pm$ 0.003	0.462 $\pm$ 0.003	0.470 $\pm$ 0.003	---	---	---	---	---	---
Prothrombin Time (n=5) (sec)	---	---	---	11.2 $\pm$ 0.1	11.8 $\pm$ 0.1	10.9 $\pm$ 0.3	---	---	---

TABLE V-12. Total Leukocyte Count and Cell Distributions in Rats Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=20)
White Blood Cells ($\times 10^3/\text{mm}^3$)	7.4 $\pm$ 0.6	7.3 $\pm$ 0.4	7.9 $\pm$ 0.3	9.1 $\pm$ 0.3	9.4 $\pm$ 0.5	6.6 $\pm$ 0.3	8.2 $\pm$ 0.4	8.4 $\pm$ 0.4	7.3 $\pm$ 0.3
Neutrophils (%)	14.4 $\pm$ 1.9	12.4 $\pm$ 1.3	11.6 $\pm$ 2.4	10.0 $\pm$ 1.0	10.0 $\pm$ 1.7	11.4 $\pm$ 1.8	12.2 $\pm$ 1.2	11.2 $\pm$ 1.1	11.5 $\pm$ 1.5
Neutrophils ($\times 10^3/\text{mm}^3$)	1.07 $\pm$ 0.16	0.91 $\pm$ 0.09	0.92 $\pm$ 0.19	0.91 $\pm$ 0.09	0.94 $\pm$ 0.22	0.75 $\pm$ 0.15	0.96 $\pm$ 0.09	0.94 $\pm$ 0.11	0.84 $\pm$ 0.12
Lymphocytes (%)	82.9 $\pm$ 2.2	84.3 $\pm$ 1.2	85.9 $\pm$ 2.8	87.9 $\pm$ 1.2	88.2 $\pm$ 2.1	86.1 $\pm$ 2.0	85.5 $\pm$ 1.6	86.3 $\pm$ 1.3	86.0 $\pm$ 1.7
Lymphocytes ($\times 10^3/\text{mm}^3$)	6.13 $\pm$ 0.50	6.15 $\pm$ 0.38	6.79 $\pm$ 0.35	8.00 $\pm$ 0.34	8.29 $\pm$ 0.39	5.68 $\pm$ 0.27	7.08 $\pm$ 0.37	7.22 $\pm$ 0.35	6.24 $\pm$ 0.25
Monocytes (%)	0.7 $\pm$ 0.2	1.2 $\pm$ 0.4	3.4 $\pm$ 0.9	0.4 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.50 $\pm$ 0.12	0.65 $\pm$ 0.22	0.40 $\pm$ 0.12
Monocytes ($\times 10^3/\text{mm}^3$)	0.05 $\pm$ 0.01	0.09 $\pm$ 0.04	0.09 $\pm$ 0.02	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.04 $\pm$ 0.01	0.06 $\pm$ 0.02	0.05 $\pm$ 0.01
Eosinophils (%)	2.1 $\pm$ 0.4	1.9 $\pm$ 0.4	1.5 $\pm$ 0.4	1.8 $\pm$ 0.3	1.8 $\pm$ 0.4	2.5 $\pm$ 0.6	1.9 $\pm$ 0.4	1.9 $\pm$ 0.2	2.0 $\pm$ 0.4
Eosinophils ($\times 10^3/\text{mm}^3$)	0.22 $\pm$ 0.04	0.21 $\pm$ 0.02	0.14 $\pm$ 0.03	0.16 $\pm$ 0.03	0.17 $\pm$ 0.04	0.16 $\pm$ 0.05	0.20 $\pm$ 0.04	0.19 $\pm$ 0.03	0.15 $\pm$ 0.03

TABLE V-13. Some Serum Chemistry Values from Rats Exposed for 30 Days to Unperturbed Field Strengths of 100 kV/m (mean value + 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=10)	Sham-Exposed (n=10)	Cage Control (n=10)	Exposed (n=20)	Sham-Exposed (n=20)	Cage Control (n=20)
Blood Urea Nitrogen (mg/dl)	25.9 ± 1.0	28.6 ± 1.4	27.0 ± 1.6	27.2 ± 1.2	27.6 ± 1.2	27.5 ± 1.0	26.5 ± 0.8	28.1 ± 0.9	27.3 ± 0.9
Alkaline Phosphatase (BLB Units)	4.8 ± 0.4	5.2 ± 0.3	5.4 ± 0.3	4.0 ± 0.3	4.8 ± 0.4	4.4 ± 0.3	4.5 ± 0.3	5.0 ± 0.3	4.9 ± 0.3
Serum Glutamic Oxaloacetic Transaminase (RF Units)	259 ± 14	275 ± 14	220 ± 18	483 ± 55	406 ± 17	333 ± 29	365 ± 37	337 ± 18	280 ± 23
Serum Iron (µg/dl)	179 ± 17	217 ± 20	220 ± 18	195 ± 10	233 ± 21	230 ± 21	187 ± 10	225 ± 14	225 ± 13
Glucose (mg/dl)	134 ± 8	128 ± 4	151 ± 7	137 ± 10	164 ± 10	153 ± 16	135 ± 6	145 ± 7	157 ± 7
Triglycerides (mg/dl)	---	---	---	115 ± 13	117 ± 9	86 ± 5	---	---	---
Serum Proteins (g/dl)	6.52 ± 0.15	6.92 ± 0.22	6.13 ± 0.21	6.81 ± 0.08	6.83 ± 0.08	6.91 ± 0.09	6.66 ± 0.09	6.87 ± 0.12	6.52 ± 0.14
Albumin (g/dl)	3.89 ± 0.10	3.89 ± 0.10	3.70 ± 0.10	3.91 ± 0.07	3.92 ± 0.05	3.92 ± 0.06	3.86 ± 0.06	3.91 ± 0.05	3.81 ± 0.06
<u>Globulins</u>									
Alpha (g/dl)	1.07 ± 0.05	1.24 ± 0.05	1.02 ± 0.08	1.17 ± 0.04	1.16 ± 0.03	1.29 ± 0.04	1.12 ± 0.03	1.20 ± 0.03	1.15 ± 0.05
Beta (g/dl)	1.00 ± 0.03	1.13 ± 0.07	0.94 ± 0.04	1.10 ± 0.03	1.06 ± 0.04	1.07 ± 0.02	1.05 ± 0.02	1.10 ± 0.04	1.00 ± 0.03
Gamma (g/dl)	0.64 ± 0.04	0.66 ± 0.05	0.48 ± 0.03	0.63 ± 0.02	0.68 ± 0.03	0.67 ± 0.03	0.64 ± 0.03	0.67 ± 0.03	0.57 ± 0.03

TABLE V-14. Some Hematologic Values in Rats Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	-Replicate 1			Replicate 2			Composite		
	Exposed n=10	Sham-Exposed n=10	Cage Control n=10	Exposed n=20	Sham-Exposed n=20	Cage Control n=20	Exposed n=30	Sham-Exposed n=30	Cage Control n=30
Volume Packed Red Cells (ml/100 ml)	44.0 $\pm$ 0.4	42.9 $\pm$ 0.5	42.6 $\pm$ 0.6	44.3 $\pm$ 0.4	44.5 $\pm$ 0.4	41.0 $\pm$ 0.7	44.2 $\pm$ 0.3	44.0 $\pm$ 0.3	41.5 $\pm$ 0.5
Red Blood Cells ($\times 10^6/\text{mm}^3$)	8.18 $\pm$ 0.11	8.09 $\pm$ 0.13	7.85 $\pm$ 0.09	8.33 $\pm$ 0.08	8.45 $\pm$ 0.10	7.53 $\pm$ 0.14	8.28 $\pm$ 0.07	8.33 $\pm$ 0.09	7.64 $\pm$ 0.10
Hemoglobin (g/dl)	17.4 $\pm$ 0.1	17.2 $\pm$ 0.2	17.0 $\pm$ 0.2	17.0 $\pm$ 0.1	17.1 $\pm$ 0.1	16.3 $\pm$ 0.1	17.1 $\pm$ 0.1	17.1 $\pm$ 0.1	16.5 $\pm$ 0.1
Mean Corpuscular Volume (μ^3)	54.5 $\pm$ 0.5	53.6 $\pm$ 0.5	55.0 $\pm$ 0.5	54.2 $\pm$ 0.4	53.7 $\pm$ 0.5	55.5 $\pm$ 0.5	54.3 $\pm$ 0.3	53.7 $\pm$ 0.3	55.3 $\pm$ 0.3
Mean Corpuscular Hemoglobin (μg)	21.4 $\pm$ 0.2	21.4 $\pm$ 0.2	21.7 $\pm$ 0.2	20.8 $\pm$ 0.1	20.7 $\pm$ 0.2	22.2 $\pm$ 0.5	21.0 $\pm$ 0.1	20.9 $\pm$ 0.2	22.0 $\pm$ 0.3
Mean Corpuscular Hemoglobin Concentration (%)	40.0 $\pm$ 0.2	40.5 $\pm$ 0.2	40.2 $\pm$ 0.2	38.4 $\pm$ 0.2	38.5 $\pm$ 0.2	40.0 $\pm$ 0.7	38.9 $\pm$ 0.2	39.2 $\pm$ 0.2	40.1 $\pm$ 0.5
Reticulocytes ($\times 10^3/\text{mm}^3$)	102 $\pm$ 9	121 $\pm$ 7	102 $\pm$ 10	118 $\pm$ 9	126 $\pm$ 8	140 $\pm$ 9	113 $\pm$ 7	124 $\pm$ 5	127 $\pm$ 7
Platelets ($\times 10^3/\text{mm}^3$)	972 $\pm$ 68	762 $\pm$ 32	842 $\pm$ 42	997 $\pm$ 48	844 $\pm$ 52	924 $\pm$ 38	989 $\pm$ 39	817 $\pm$ 37	897 $\pm$ 29
Bone Marrow ($\times 10^3/\text{femur}$)	39.8 $\pm$ 2.5	39.7 $\pm$ 2.1	36.4 $\pm$ 1.8	41.6 $\pm$ 1.7	40.8 $\pm$ 1.5	41.4 $\pm$ 2.1	41.0 $\pm$ 1.4	40.4 $\pm$ 1.2	39.7 $\pm$ 1.5
Prothrombin Time (n=5) (sec)	11.6 $\pm$ 0.2	11.6 $\pm$ 0.2	11.6 $\pm$ 0.2	---	---	---	---	---	---

TABLE V-15. Total Leukocyte Count and Cell Distributions in Rats Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed n=10	Sham-Exposed n=10	Cage Control n=10	Exposed n=20	Sham-Exposed n=20	Cage Control n=20	Exposed n=30	Sham-Exposed n=30	Cage Control n=30
White Blood Cells ($\times 10^3/\text{mm}^3$)	6.2 $\pm$ 0.5	6.4 $\pm$ 0.3	5.9 $\pm$ 0.3	7.6 $\pm$ 0.4	6.1 $\pm$ 0.3	7.2 $\pm$ 0.3	7.1 $\pm$ 0.3	6.2 $\pm$ 0.2	6.8 $\pm$ 0.2
Neutrophils (%)	10.0 $\pm$ 1.0	12.6 $\pm$ 1.5	12.7 $\pm$ 1.1	12.7 $\pm$ 2.3	12.2 $\pm$ 1.1	14.3 $\pm$ 1.6	11.8 $\pm$ 1.6	12.3 $\pm$ 0.9	13.8 $\pm$ 1.1
Neutrophils ($\times 10^3/\text{mm}^3$)	0.62 $\pm$ 0.08	0.81 $\pm$ 0.07	0.74 $\pm$ 0.07	1.12 $\pm$ 0.38	0.75 $\pm$ 0.09	1.04 $\pm$ 0.11	0.95 $\pm$ 0.25	0.77 $\pm$ 0.06	0.96 $\pm$ 0.08
Lymphocytes (%)	87.2 $\pm$ 1.4	84.7 $\pm$ 1.5	84.8 $\pm$ 0.9	84.0 $\pm$ 2.5	84.4 $\pm$ 1.1	82.2 $\pm$ 1.7	85.1 $\pm$ 1.8	84.5 $\pm$ 0.9	83.1 $\pm$ 1.2
Lymphocytes ($\times 10^3/\text{mm}^3$)	5.41 $\pm$ 0.42	5.42 $\pm$ 0.30	5.00 $\pm$ 0.22	6.25 $\pm$ 0.23	5.09 $\pm$ 0.20	5.91 $\pm$ 0.26	5.97 $\pm$ 0.21	5.23 $\pm$ 0.17	5.61 $\pm$ 0.20
Monocytes (%)	0.5 $\pm$ 0.4	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	1.0 $\pm$ 0.2	0.8 $\pm$ 0.2	0.6 $\pm$ 0.1	0.8 $\pm$ 0.2	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1
Monocytes ($\times 10^3/\text{mm}^3$)	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.07 $\pm$ 0.01	0.06 $\pm$ 0.02	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.04 $\pm$ 0.01
Eosinophils (%)	2.8 $\pm$ 0.5	2.5 $\pm$ 0.3	2.3 $\pm$ 0.5	2.4 $\pm$ 0.4	2.7 $\pm$ 0.3	2.8 $\pm$ 0.4	2.5 $\pm$ 0.3	2.6 $\pm$ 0.2	2.6 $\pm$ 0.3
Eosinophils ($\times 10^3/\text{mm}^3$)	0.16 $\pm$ 0.03	0.16 $\pm$ 0.02	0.14 $\pm$ 0.03	0.19 $\pm$ 0.04	0.17 $\pm$ 0.02	0.21 $\pm$ 0.03	0.18 $\pm$ 0.03	0.17 $\pm$ 0.01	0.19 $\pm$ 0.02

TABLE V-16. Some Serum Chemistry and Organ Weight Values from Rats Exposed for 60 Days to Unperturbed Field Strengths of 100 kV/m (mean value $\pm$ 1 standard error).

	Replicate 1			Replicate 2			Composite		
	Exposed n=10	Sham-Exposed n=10	Cage Control n=10	Exposed n=20	Sham-Exposed n=20	Cage Control n=20	Exposed n=30	Sham-Exposed n=30	Cage Control n=30
Blood Urea Nitrogen (mg/dl)	23.9 $\pm$ 1.0	25.4 $\pm$ 2.2	25.4 $\pm$ 1.2	23.8 $\pm$ 0.8	25.1 $\pm$ 1.0	21.1 $\pm$ 0.6	23.8 $\pm$ 0.6	25.2 $\pm$ 1.2	22.5 $\pm$ 0.7
Alkaline Phosphatase (BLB Units)	2.5 $\pm$ 0.2	3.0 $\pm$ 0.2	2.9 $\pm$ 0.2	3.7 $\pm$ 0.3	3.3 $\pm$ 0.2	6.0 $\pm$ 0.7	3.3 $\pm$ 0.2	3.2 $\pm$ 0.2	4.9 $\pm$ 0.5
Serum Glutamic Oxaloacetic Transaminase (RF Units)	392 $\pm$ 47	338 $\pm$ 16	416 $\pm$ 43	522 $\pm$ 42	589 $\pm$ 40	559 $\pm$ 38	479 $\pm$ 33	505 $\pm$ 35	511 $\pm$ 31
Triglycerides (mg/dl)	101 $\pm$ 5	106 $\pm$ 6	98 $\pm$ 9	138 $\pm$ 5	130 $\pm$ 8	127 $\pm$ 16	126 $\pm$ 5	121 $\pm$ 6	117 $\pm$ 11
Serum Proteins (g/dl)	6.6 $\pm$ 0.1	6.8 $\pm$ 0.1	6.6 $\pm$ 0.1	6.6 $\pm$ 0.1	6.6 $\pm$ 0.1	6.6 $\pm$ 0.1	6.6 $\pm$ 0.1	6.7 $\pm$ 0.1	6.6 $\pm$ 0.1
Albumin (g/dl)	3.47 $\pm$ 0.13	3.36 $\pm$ 0.07	3.46 $\pm$ 0.11	3.51 $\pm$ 0.10	3.55 $\pm$ 0.07	3.55 $\pm$ 0.04	3.50 $\pm$ 0.08	3.49 $\pm$ 0.06	3.52 $\pm$ 0.05
<u>Globulins</u>									
Alpha (g/dl)	1.32 $\pm$ 0.04	1.41 $\pm$ 0.05	1.27 $\pm$ 0.06	1.31 $\pm$ 0.06	1.32 $\pm$ 0.04	1.31 $\pm$ 0.05	1.31 $\pm$ 0.04	1.35 $\pm$ 0.04	1.30 $\pm$ 0.04
Beta (g/dl)	1.11 $\pm$ 0.02	1.23 $\pm$ 0.03	1.17 $\pm$ 0.04	1.05 $\pm$ 0.03	1.05 $\pm$ 0.02	1.04 $\pm$ 0.03	1.07 $\pm$ 0.02	1.11 $\pm$ 0.02	1.08 $\pm$ 0.03
Gamma (g/dl)	0.72 $\pm$ 0.04	0.76 $\pm$ 0.03	0.65 $\pm$ 0.03	0.70 $\pm$ 0.02	0.74 $\pm$ 0.03	0.70 $\pm$ 0.02	0.71 $\pm$ 0.02	0.75 $\pm$ 0.02	0.68 $\pm$ 0.02

TABLE V-17. Statistically Significant Differences in Clinical Pathology Parameters in Mice Exposed to 60 Hz, 100 kV/m (Student's *t* test)

Exposure (Days)	Replication	N	Parameter	Mean $\pm$ SE		P<
				Exposed	Sham-Exposed	
30	1	15	Erythrocytes	8.71 $\pm$ 0.10	8.43 $\pm$ 0.09	0.05
			Hemoglobin	14.8 $\pm$ 0.1	14.1 $\pm$ 0.2	0.01
			VPRC	40.5 $\pm$ 0.3	39.1 $\pm$ 0.5	0.05
	2	15	None	---	---	---
60	1	15	Leukocytes	5.2 $\pm$ 0.4	7.7 $\pm$ 0.9	0.02
			Neutrophils	0.73 $\pm$ 0.10	1.12 $\pm$ 0.12	0.05
			Lymphocytes	4.4 $\pm$ 0.3	6.73 $\pm$ 0.8	0.01
			α -globulins	0.85 $\pm$ 0.04	0.95 $\pm$ 0.02	0.05
			Spleen Weight	107.3 $\pm$ 5.4	128.5 $\pm$ 8.3	0.05
	2	15	Leukocytes	6.2 $\pm$ 0.5	3.9 $\pm$ 0.4	0.01
			Neutrophils	1.31 $\pm$ 0.21	0.59 $\pm$ 0.07	0.01
			Lymphocytes	4.7 $\pm$ 0.5	3.3 $\pm$ 0.4	0.05
			Eosinophils	0.14 $\pm$ 0.03	0.03 $\pm$ 0.01	0.01
			Body Weight	30.2 $\pm$ 0.5	28.0 $\pm$ 0.7	0.01

TABLE V-18. Statistically Significant Differences in Clinical Pathology Parameters in Rats Exposed to 60 Hz, 100 kV/m (Student's *t* test)

Exposure (Days)	Replication	N	Parameter	Mean $\pm$ SE		P<
				Exposed	Sham-Exposed	
15	1	10	Leukocytes	7.5 $\pm$ 0.5	9.3 $\pm$ 0.3	0.01
			Lymphocytes	6.7 $\pm$ 0.5	8.3 $\pm$ 0.4	0.02
	2	20	β -globulins	1.15 $\pm$ 0.03	1.06 $\pm$ 0.02	0.02
			Triglycerides	96 $\pm$ 3	107 $\pm$ 3	0.05
30	1	10	α -globulins	1.07 $\pm$ 0.05	1.24 $\pm$ 0.05	0.05
	2	10	Marrow Cellularity	48.9 $\pm$ 1.3	43.4 $\pm$ 2.2	0.05
60	1	10	Platelets	972 $\pm$ 68	762 $\pm$ 32	0.02
			β -globulins	1.11 $\pm$ 0.02	1.23 $\pm$ 0.03	0.01
	2	20	Leukocytes	7.6 $\pm$ 0.5	6.1 $\pm$ 0.3	0.01
			Lymphocytes	6.3 $\pm$ 0.2	5.1 $\pm$ 0.2	0.01

globulin level was found in exposed rats compared with sham-exposed animals (Table V-18). The decrease in beta globulins is opposite to the effect noted in the second replication at 30 days of exposure. A significant increase in leukocytes was evident in the second replicate at 60 days due to an absolute increase in lymphocytes. As with beta globulins, this lymphocyte effect was opposite to that observed in the first replicate after 30 days exposure.

D. DISCUSSION AND CONCLUSIONS

Under the conditions of this study, the results of exposing mice do 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 between replications or between exposure periods.

In the rat portion of this study no reproducible changes were observed in either the 15-, 30-, or 60-day exposures, or among these exposures. However, there is a statistically significant increase ($p < 0.01$) in the replicate composites of platelet concentrations of rats exposed for 60 days. The greatest difference in platelet count between the two groups was in the first 60-day replicate, although a nonsignificant difference was also evident after the second replicate. The composite leukocyte concentrations are also significantly greater ($p < 0.05$) in the exposed rats as compared to the sham-exposed group after 60 days exposure;

this apparent leukocytosis is due to an absolute increase in lymphocytes. However, this difference is due entirely to the marked difference in total leukocytes from the second replicate, since values of both groups were almost identical in the first replicate. In addition, leukocyte concentrations in the sham-exposed group were significantly lower than those from either the exposed or cage-control groups.

In summary, with the possible exception of elevated platelet concentrations at the 60-day exposure period, there have been no consistent effects observed on clinical pathologic parameters in either mice or rats exposed to 100 kV/meter electric fields for periods as long as 60 days.

E. FUTURE PLANS

Clinical pathologic parameters will be examined in mice and rats presently being exposed to 100 kV/m fields for ~4 months. These evaluations will be screening-type tests similar to those used in this report. If effects on the hematopoietic or other organ systems are observed, the next series of studies will be in greater depth to define the mechanisms involved.

To evaluate possible subtle effects on the hematopoietic system, iron kinetics and red cell life spans will be determined in rodents exposed to the 100 kV/m field for 60-120 days. In addition, the effects on bone marrow precursor cells will be quantitated using in vivo and in vitro assays. Finally, as a sensitive test system, a mouse strain

with a high incidence of leukemia will be exposed to the field for 4-6 months, then held for lifetime observation to determine the incidence and onset of leukemia as compared to the sham-exposed control group.

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VI. IMMUNOLOGY

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

ABSTRACT

No effect of 15- and 30-day exposures to 100-kV/m electric fields was evident on the levels of serum immunoglobins (IgG and IgM) in rats when compared to sham-exposed controls. Exposure of mice for 30 and 60 days had no significant effect on IgG or IgM levels, complement levels or on relative concentrations of T- and B-lymphocytes in their blood.

Exposure of mice to the field for 30 days caused a statistically significant decrease in delayed hypersensitivity response to keyhole limpet hemocyanin antigen based on skin thickness in one experiment. In a replicate experiment there was an apparent decrease (22%), which was not statistically significant. The diameters of the responses were the same for exposed and control animals, however, and no change in contact hypersensitivity to dinitrofluorobenzene was noted.

A. INTRODUCTION

The effect of exposure to electric fields on the immune system of animals has not been studied directly. Meda (1972) reported decreases in the concentration of lymphocytes in rats exposed to 100 kV/m for 6 hr; Bianchi et al. (1973) reported similar effects in mice after 55 days at 100 kV/m. Rats exposed to 15 kV/m had reduced levels of serum gamma globulin (Marino et al., 1976), whereas Dumansky et al. reported elevated serum alpha and beta globulins in rats exposed to 0.1-5 kV/m for 4 mo. Because of these inconsistencies more work is needed to establish whether this important defense system is compromised.

The objective of this study is to survey the effects of 100-kV/m electric fields on the immune system by measuring serum immunoglobulin levels (IgG and IgM) in rats and mice complement levels in mice, as well as relative amounts of T- and B-lymphocytes, and by performing functional tests of cell-mediated immunity.

This study, then, examines both major arms of the immune system, i.e., the humoral and cellular components. The humoral component includes immunoglobins (which function as antibodies) and the B-lymphocytes which produce them. The cellular components examined include T-lymphocytes, which play an important role in the killing of foreign cells and in delayed hypersensitivity.

B. METHODS

Quantitation of Serum Immunoglobulins: Circulating immunoglobulins were measured by competitive binding in double-antibody radioimmune assays. In these assays, a known amount of the appropriate ^{125}I -labeled rat immunoglobulin was mixed with goat serum containing specific antibodies to the rat immunoglobulin. The goat antibodies formed a complex with the labeled immunoglobulins. Additions of rat serum containing unknown amounts of immunoglobulin inhibited the formation of the complex due to competition for binding with the antibody. A second antibody (horse antigoat immunoglobulin) was added to precipitate the goat-antibody rat-immunoglobulin complex, and the precipitated and unbound antigen were separated by centrifugation. One or the other of the fractions was

counted for ^{125}I gamma-radiation. The amount of ^{125}I precipitated was inversely proportional to the amount of unlabeled immunoglobulin added, and thus a measure of its amount. Sodium-22 was also added to the mixture in order to measure the quantity of solution associated with the precipitate, thus correcting for contaminating noncomplexed ^{125}I -immunoglobulin. The same system was used to assay IgG and IgM concentrations in mouse serum.

Serum Complement: Peripheral blood was collected, without anticoagulant, from anesthetized animals via suborbital plexus. Serum was removed and quick frozen within 1 hr after collection of blood. Serum complement levels were assayed using the method of Mayer (1966), adapted to a continuous-flow system as described by Nydegger (1972). This assay system employed sheep red blood cells (SRBC) as the indicator system, rabbit anti-SRBC as the standard antibody hemolysin, and guinea pig serum as a source of complement for standardizing the assay. The percent of SRBCs lysed was used as a measure of the activity of the complement in the sample of serum assayed.

Isolation of Peripheral Lymphocytes: Peripheral blood was obtained from the suborbital plexus of lightly anesthetized male Swiss-Webster mice. Ethylenediamine tetraacetate (EDTA) was used as the anticoagulant. The blood was diluted in Hanks' balanced salt solution (Ca^{++} - and Mg^{++} -free), layered over a step-density gradient of Ficoll-Isopaque and centrifuged

for 20 min at 130 x g. (This procedure separated cell types by virtue of differences in density.) The mononuclear-cell layer was removed and washed twice in Hanks' balanced salt solution (HBSS). The cells were counted, and the viability of the preparation determined with trypan blue. Differential staining of the cell preparations revealed that 95% or more of the cells were lymphocytes. These isolated cells served as the source of lymphocytes for the fluorescent-labeling studies and the rosette assays described below.

Fluorescent-Antibody Labeling of Peripheral Lymphocytes: In the fluorescent-labeling studies, the binding of goat antimouse immunoglobulin antibodies was used as a measure of B-cell populations. Approximately 2×10^6 lymphocytes, obtained from density gradient separation of peripheral blood, were mixed with goat antimouse immunoglobulin and incubated at 4°C in the presence of 3×10^{-2} M sodium azide. The cells were washed twice and incubated with fluorescent-labeled horse antigoat immunoglobulins, at 4°C in the presence of sodium azide. Following this step, the cells were washed three times and the number of labeled and unlabeled cells counted.

Spontaneous Rosette Assay: Sheep red blood cells were stored at 4°C in Alsever's solution (1:1) and used within the week of bleeding. Before use, the cells were washed three times and adjusted to a 1% suspension in HBSS. The lymphocytes, approximately 1×10^6 , were mixed

with 0.25 ml of red blood cells and incubated at 37°C for 15 min. The mixed-cell suspension was spun at 200 x g for 5 min and then incubated in ice for 2 hr. Most of the supernatant was removed and the top layer of the pellet was gently suspended by shaking. One drop of the cell suspension was mounted on a glass slide and covered with a coverslip. Two hundred lymphocytes were counted and lymphocytes which bound more than three sheep red blood cells were considered to have formed rosettes (T-cells).

In Vivo Cell-Mediated Immunity Assays: The cell-mediated arm of the immune system was evaluated in mice after 30 days of exposure by determining the delayed hypersensitivity response to keyhole limpet hemocyanin (KLH) and contact sensitivity to dinitrofluorobenzene (DNFB), using methods described elsewhere (Temple and Loewi, 1971). Mice were immunized by subcutaneous injection of 1 mg KLH in Freund's complete adjuvant 13 days prior to the termination of exposure. They were then challenged immediately after the 30-day exposure period by injection of 20 µg KLH in the shaved flank. The response was assayed as the increase in skin thickness over the pre-injection value and the size of the reaction diameter. For DNFB sensitization, 0.5% DNFB in olive oil/acetone was painted on the shaved abdomen on two successive days (Phanuphak, et al., 1974). Four days later the ears were measured with a micrometer, a challenge dose of 2% DNFB was painted on the dorsal side of the ear, and ear thickness was determined 24 hr later. Results were expressed as

mean increase in skin thickness compared with presensitized control value for each animal. The differences between means of exposed and control groups were analyzed with Student's t test, and levels of significance (p) greater than 0.05 were regarded as indicating that means were not significantly different.

C. RESULTS

Serum Immunoglobulins: Serum immunoglobulin levels in Sprague-Dawley rats exposed for 15 and 30 days at 100 kV/m were measured with a double-antibody radioimmune assay. The study included 15 rats exposed to a 60-Hz, 100-kV/m electric field and 15 sham-exposed rats. Immunoglobulin G and IgM levels in the serum of these animals are shown in Table VI-1. There were no significant differences between exposed and sham-exposed groups.

TABLE VI-1. Serum Immunoglobulin-G and Immunoglobulin-M (mg/ml) in Female Rats at the End of 15 or 30 Days of Exposure to 60-Hz Electric Fields at 100 kV/m

<u>Immunoglobulins</u>	<u>Exposure Period (Days)</u>	<u>Exposed</u>		<u>Sham-Exposed</u>	
		<u>N</u>	<u>Mean $\pm$ SE</u>	<u>N</u>	<u>Mean $\pm$ SE</u>
IgG	15	12	3.9 $\pm$ 0.6	11	4.0 $\pm$ 0.4
	30	14	4.1 $\pm$ 0.4	13	4.2 $\pm$ 0.5
IgM	15	12	1.1 $\pm$ 0.1	11	1.3 $\pm$ 0.2
	30	14	1.4 $\pm$ 0.2	13	1.2 $\pm$ 0.3

Serum immunoglobulin analysis in rats was followed by similar studies with Swiss-Webster male mice exposed for 30 or 60 days to 100 kV/m. Mice were lightly anesthetized, blood was collected from the suborbital plexus, and serum was analyzed for IgG and IgM levels (Table VI-2). The study included a group of 15 exposed, 15 sham-exposed and 15 cage-control animals for each exposure period. As with the rats, no significant differences were observed among groups.

TABLE VI-2. Serum Immunoglobulin-G and Immunoglobulin-M (mg/ml) in Male Mice Exposed to 60-Hz Electric Fields at 100 kV/m

<u>Immunoglobulins</u>	<u>Exposure Period (days)</u>	<u>Exposed</u>		<u>Sham-Exposed</u>		<u>Cage Controls</u>	
		<u>N</u>	<u>Mean $\pm$ SE</u>	<u>N</u>	<u>Mean $\pm$ SE</u>	<u>N</u>	<u>Mean $\pm$ SE</u>
IgG	30	15	5.1 $\pm$ 0.8	15	5.0 $\pm$ 0.6	15	5.2 $\pm$ 0.9
	60	15	5.9 $\pm$ 0.9	14	5.5 $\pm$ 0.5	15	5.6 $\pm$ 0.8
IgM	30	15	1.1 $\pm$ 0.2	15	1.0 $\pm$ 0.3	15	1.2 $\pm$ 0.3
	60	15	1.2 $\pm$ 0.3	14	1.2 $\pm$ 0.2	14	1.1 $\pm$ 0.3

Serum Complement Levels: Results of complement measurements in male Swiss-Webster mice are shown in Table VI-3. The animals in this study included exposed, sham-exposed and cage control mice, following 30- and 60-day exposure to 100 kV/m. No significant difference was observed among the groups.

TABLE VI-3. The Effect of Exposure to 60-Hz, 100 kV/m Electric Fields on Complement Levels in Mice ($\pm$ SE)

Exposure Period (Days)	N	Complement (% Hemolysis)		
		Exposed	Sham	Cage
		Mean $\pm$ SE	Exposed Mean $\pm$ SE	Control Mean $\pm$ SE
30	20	21.6 $\pm$ 5.4	32.8 $\pm$ 4.5	25.1 $\pm$ 2.7
60	20	18.5 $\pm$ 3.5	21.2 $\pm$ 3.8	26.9 $\pm$ 3.9

Distribution of Peripheral Blood Lymphocytes: Results of quantitative measurement of peripheral blood lymphocytes with the fluorescent-antibody labeling assay (B cells) are shown in Table VI-4. The animals in this study included exposed, sham-exposed and cage-control Swiss-Webster male mice following 30 or 60 days exposure to 100 kV/m.

TABLE VI-4. Differentiation Studies of Peripheral Blood Lymphocytes (Expressed as % of Total Lymphocytes Counted) of Male Mice Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

Assay	Exposure Period (days)	Exposed		Sham-Exposed		Cage Controls	
		N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Fluorescent Labeling (B-cells)	30	15	31.2 $\pm$ 5.2	15	32.0 $\pm$ 6.0	13	29.7 $\pm$ 4.9
	60	15	29.0 $\pm$ 5.0	14	30.0 $\pm$ 7.1	15	33.0 $\pm$ 6.5
Spontaneous Rosettes (T-Cells)	30	15	62.4 $\pm$ 9.1	15	63.3 $\pm$ 7.2	13	61.7 $\pm$ 8.4
	60	15	63.4 $\pm$ 8.2	14	61.4 $\pm$ 7.5	14	63.1 $\pm$ 9.2

In conjunction with the fluorescent-labeling studies of mouse peripheral lymphocytes, the percentages of lymphocytes which formed spontaneous rosettes (T-cells) were measured on the same groups of animals after 30 or 60 days of exposure. There was no difference between exposed and sham-exposed groups, showing that relative concentrations of T- and B-lymphocytes were not altered by the exposure (Table VI-4).

Cell-Mediated Immunity: The skin response following sensitization of mice to KLH and DNFB is shown in Table VI-5. There was a highly significant ($p < 0.001$) decreased response to KLH as measured by skin thickness at the site of injection in the electric-field-exposed mice as compared to those sham-exposed. However, the mean reaction diameters for the two groups were not significantly different. Responses to both tests in cage-control animals, which received only the challenge dose of KLH, were minor. In Experiment 2, a decreased response (22%) to KLH was observed as measured by skin thickness, but this was not statistically significant. The reaction diameters of the skin response in Experiment 2 were comparable.

The ear-thickness increase following sensitization and challenge with DNFB was comparable in the exposed and sham-exposed mice, whereas unsensitized cage controls, given only the challenge dose, had a minimal response. In Experiment 2, no significant difference in the DNFB response was observed.

TABLE VI-5. Cell-Mediated Immune Response in Mice Exposed to a 100-kV/m Electric Field for 30 Days (Mean $\pm$ SE)

	Experiment Number	N	Response to KLH Sensitization Increase in Skin Thickness (%)	Sensitization Diameter of Skin Response (mm)	Response to DNFB Sensitization Increase in Thickness of Ear (%)
Exposed	1	20	24.2 $\pm$ 3.2*	4.99 $\pm$ 0.17	115.0 $\pm$ 9.3
	2	24	37.0 $\pm$ 3.2	4.28 $\pm$ 0.16	96.7 $\pm$ 6.7
Sham-Exposed	1	20	43.2 $\pm$ 3.8	5.50 $\pm$ 0.21	124.1 $\pm$ 7.0
	2	24	47.0 $\pm$ 5.1	4.38 $\pm$ 0.22	96.5 $\pm$ 6.6
Unsensitized Controls	1	14	3.5 $\pm$ 1.6	1.08 $\pm$ 0.35	7.3 $\pm$ 2.9

* When compared to sham-exposed, $p < 0.001$.

Body Weight: No significant differences in the body weights of mice exposed and sham exposed to the electric field for 30 or 60 days were observed (Table VI-6).

TABLE VI-6. Mean ($\pm$ SE) Body Weights (g) of Male Mice Exposed to 60-Hz Electric Fields at 100 kV/m

Exposure Period (days)	Exposed		Sham-Exposed		Cage Controls	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
30	30	37.7 $\pm$ 2.6	30	36.8 $\pm$ 4.0	30	38.5 $\pm$ 2.8
60	28	39.3 $\pm$ 3.2	30	37.9 $\pm$ 3.6	30	37.8 $\pm$ 2.9

D. DISCUSSION AND CONCLUSIONS

A series of screening assays were conducted to quantitatively analyze the humoral and cellular components of the immune system as possible indicators of immunological effects of exposure to electric fields.

The data collected on the humoral arm of the immune system, as mirrored in serum immunoglobulin levels in rats and mice, indicate that there are no significant alterations as a result of exposure in our test system.

By utilizing structural surface differences between B- and T-cells, the distribution of these lymphocytes was measured in the peripheral blood of exposed and control animals. We found that there were no significant alterations in the ratio of T- and B-cells as a result of exposure.

In general, no significant changes in the major humoral and cellular elements of the immune system were observed. Preliminary studies directed at measuring the functional response of the cell-mediated component of the immune system had suggested that a reduced delayed hypersensitivity response to KLH occurred when evaluated on the basis of skin thickness. However, in replicate experiments, a statistically significant decrease was not observed, although the trend toward a decreased response was apparent. The mean reaction diameter was not significantly different in either of the two experiments. Additional studies will be necessary in order to ascertain whether or not this was an effect of the electric field. These will include in vitro mitogenic stimulation assays which correlate with cell-mediated immunity.

E. FUTURE PLANS

In the second series of screening experiments, Swiss-Webster mice will be used for functional assessment of the immune mechanism. These studies will include exposed, sham-exposed, and cage-control groups to evaluate the various parameters.

In the humoral studies, mice will be immunized with KLH (a T-cell-dependent antigen) before exposure, and after 30 and 60 days of exposure, to determine the effect of exposure on specific antibody formation. These studies will be followed by immunizations with a B-cell-dependent antigen (DNP-Ficoll). In both of these experiments the effects of exposure on the primary and secondary immune response will be measured.

The cellular studies will include analysis of peripheral lymphocytes with in vitro measurements of cell-mediated immunity. In vitro experiments will be another way to study delayed hypersensitivity response to KLH and to analyze the contradiction in the apparent decreased response observed in exposed animals. For the in vitro studies, mitogenic assays that correlate with cell-mediated immunity, we plan to use phytohemagglutinin, concanavalin-A, and lipopolysaccharide.

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VII. PATHOLOGY

Principal Investigator: G. M. Zwicker

ABSTRACT

Groups of male rats, and both male and female mice, were exposed or sham-exposed to a 60-Hz, 100-kV/m electric field for 30 days. Exposure had no significant effect on body or organ weights of rats. A histopathological survey of selected tissues revealed no significant effects. In four experiments, the total incidence of prostatitis in exposed groups was 25 of 71 (35%), compared to 11 of 50 (22%) in sham exposed and 9 of 43 (21%) in cage controls. Statistical evaluation of these data indicated that this apparent increase in prostatitis in exposed animals was not significant ($p < 0.2$). Histopathological survey of the mice revealed no apparent effect of exposure to the electric field. Body weights of exposed mice did not differ from those of the sham-exposed group, but livers were significantly larger and left kidneys significantly smaller in the exposed group.

A. INTRODUCTION

Russian investigators (Dumansky et al., 1976) have recently reported the occurrence of dystrophy and vascular changes in brain, liver, kidneys, adrenal glands and thyroid glands of male rats exposed to electric fields at 2 to 5 kV/m for 4 mo. The published description of tissue and organ changes, however, was insufficient to allow evaluation of their findings.

Except for this somewhat incomplete report, there is a lack of information on the biological effect of ac electric fields on animals. We have therefore exposed rats and mice to 60-Hz electric fields at

100 kV/m for 30 days, and have performed a histopathological survey of their tissues and organs, paying particularly close attention to the tissues in which Dumansky reported effects.

B. METHODS

Seventy-one male Sprague-Dawley rats were exposed to an unperturbed 60-Hz electric field of 100 kV/m in 4 separate but identical experiments. The numbers of exposed rats evaluated in each experiment were 10, 11, 12 and 38, respectively. The effective field strength for individual rats was approximately 65 kV/m due to the proximity of other rats (Phillips and Kaune, 1977). The rats were exposed for approximately 20.5 hr/day for 30-31 days, accumulating a total exposure of approximately 611-632 hr. Room temperature and humidity were monitored daily and averaged 22.3°C (range, 20.5-24.1°C) and 57% (range, 48-70%), respectively, during the 30-day exposure period. Fifty other rats (9,12,11 and 18), which were maintained in identical conditions except for the lack of an electric field, served as sham-exposed controls for the three experiments. All rats were ear-coded and the pathologist did not know which animals were exposed or sham exposed until all evaluations had been completed, at which time the code was broken. Forty-three cage controls were housed in shoebox cages in another room in the vivarium.

Upon completion of exposures, all rats were killed with an overdose of halothane (anesthetic) and necropsies were performed. At this time the following organs were weighed in the first experiment: heart,

kidneys, adrenal glands, liver, testis, brain, and pituitary gland. These organs and representative sections of lung, spleen, pancreas, trachea, thyroid gland, esophagus, stomach, small intestine, colon, mesenteric lymph node, submaxillary lymph node, salivary gland, thymus, skin, sternbra, urinary bladder, prostate gland, muscle, sciatic nerve, brain and spinal cord were placed in 10% neutral buffered formalin for fixation. Approximately 5- μ m-thick sections of each were stained with hematoxylin-eosin in preparation for light microscopy. For Experiments 2, 3 and 4 only prostate glands were examined.

Ten male and 10 female mice exposed to an electrical field under conditions similar to those employed for rats were killed by an overdose of ether and necropsies were performed. An equal number of sham-exposed mice of each sex were treated in the same manner. Final body weight was recorded for each mouse. Organ weights were measured for heart, kidneys, liver, brain and testes for males. These tissues and submaxillary lymph nodes, salivary gland, lung, thyroid gland, esophagus, aorta, thymus, pancreas, spleen, stomach, small intestine, large intestine, mesenteric lymph nodes, uterus, ovaries, urinary bladder, prostate gland, muscle, testis, adrenal glands, and pituitary gland were placed in 10% neutral buffered formalin for fixation. Hematoxylin-eosin-stained preparations were made for light microscopy examination.

The means of organ weight data from exposed and sham-exposed animals were compared with the Student's t test; levels of significance (p) greater than 0.05 were considered to indicate that the differences between the means were not significant.

C. RESULTS

Rats: There were no significant differences in body weights and organ weights (Table VII-1) for exposed rats compared to sham-exposed rats in the first experiment.

TABLE VII-1. Body and Organ Weights for Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	<u>Exposed</u>		<u>Sham-Exposed</u>	
	<u>N</u>	<u>Mean $\pm$ SE (g)</u>	<u>N</u>	<u>Mean $\pm$ SE (g)</u>
Final Body Weight	10	441.5 $\pm$ 25.8	10	450.8 $\pm$ 35.5
Heart	10	1.52 $\pm$ 0.16	10	1.59 $\pm$ 0.16
Left Kidney	10	1.64 $\pm$ 0.11	10	1.71 $\pm$ 0.16
Right Kidney	10	1.66 $\pm$ 0.12	10	1.67 $\pm$ 0.17
Left Adrenal Gland	10	0.035 $\pm$ 0.011	10	0.035 $\pm$ 0.005
Right Adrenal Gland	10	0.039 $\pm$ 0.009	10	0.031 $\pm$ 0.009
Liver	10	19.0 $\pm$ 1.9	10	20.0 $\pm$ 3.1
Left Testis	10	1.80 $\pm$ 0.12	10	1.82 $\pm$ 0.13
Right Testis	10	1.77 $\pm$ 0.12	10	1.82 $\pm$ 0.11
Brain	10	2.12 $\pm$ 0.14	10	2.13 $\pm$ 0.09
Pituitary	9	0.010 $\pm$ 0.002	10	0.011 $\pm$ 0.002

Histopathologic examination of organs and tissues revealed an increased incidence and severity of prostatitis among exposed rats with

25 of 71 rats affected (35%) compared to the sham-exposed control rats, where 11 of 50 (22%) had this lesion (Table VII-2). Of the 43 cage controls examined, 9 (21%) had prostatitis. The apparent higher incidence in exposed rats was not, however, statistically significant ($p < 0.2$, chi-square test).

TABLE VII-2. Incidence of Prostatitis in Rats Exposed for 30 Days in an Electric Field

<u>Experiment</u>	<u>Exposed</u>	<u>Prostatitis</u>	
		<u>Sham-Exposed Controls</u>	<u>Cage Controls</u>
I	6/10	1/9	---
II	4/11	1/12	3/5
III	4/12	4/11	3/24
IV	<u>11/38</u>	<u>5/18</u>	<u>3/14</u>
TOTALS	25/71(35%)	11/50(22%)	9/43 (21%)

There were no other lesions in any of the organs or tissues examined with an incidence or severity pattern that suggested an effect of exposure to the electric field.

Mice: There were no significant differences between final body weights of exposed and sham-exposed male and female mice (Table VII-3). For exposed male mice, the left kidney weights were slightly increased ($p < 0.05$) and the liver weights were somewhat increased ($p < 0.01$). Light microscopy examination of mouse organs and tissues revealed that there were no lesions among exposed mice with an incidence and severity pattern suggestive of an exposure effect.

TABLE VII-3. Final Body and Organ Weights for Male and Female Mice Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	Exposed		Sham-Exposed	
	N	Mean $\pm$ SE (g)	N	Mean $\pm$ SE (g)
<u>MALE</u>				
Final Body Weight	9	36.4 $\pm$ 2.5	9	34.9 $\pm$ 2.0
Heart	9	0.183 $\pm$ 0.028	9	0.204 $\pm$ 0.036
Left Kidney	9	0.289 $\pm$ 0.054*	9	0.338 $\pm$ 0.046
Right Kidney	9	0.292 $\pm$ 0.063	9	0.339 $\pm$ 0.04 ^d
Liver	9	2.40 $\pm$ 0.29 **	9	2.00 $\pm$ 0.24
Brain	9	0.469 $\pm$ 0.029	9	0.477 $\pm$ 0.027
Left Testis	9	0.116 $\pm$ 0.014	9	0.118 $\pm$ 0.026
Right Testis	9	0.118 $\pm$ 0.018	9	0.122 $\pm$ 0.024
<u>FEMALE</u>				
Final Body Weight	9	29.4 $\pm$ 0.7	9	30.4 $\pm$ 1.7
Heart	9	0.118 $\pm$ 0.013	9	0.115 $\pm$ 0.015
Left Kidney	9	0.186 $\pm$ 0.014	9	0.194 $\pm$ 0.030
Right Kidney	9	0.192 $\pm$ 0.019	9	0.186 $\pm$ 0.019
Liver	9	1.55 $\pm$ 0.107	9	1.64 $\pm$ 0.17
Brain	9	0.485 $\pm$ 0.017	9	0.496 $\pm$ 0.028

* p < 0.05

** p < 0.01

D. DISCUSSION AND CONCLUSIONS

It is not clear at this time why 35% of the exposed rats developed prostatitis. The fact that no tissue or organ changes with an incidence or severity pattern suggestive of an exposure effect were found in the brain, liver, kidneys, adrenal glands and thyroid gland by light microscopy differentiates our data from that reported by Dumansky et al. (1976). There are several possibilities for this. Our experimental animals were obtained from a source in this country and probably had a different microflora population than the rats used in the Soviet studies. The microflora population may contribute (in stressed animals) to increased disease incidence and severity in various organ systems, and could have compromised interpretation of animal data in the Soviet study. Food, water and laboratory environment in the two studies also differed, perhaps contributing to the development of dystrophic and vascular changes in animals of the Soviet study. It is also possible that longer exposure durations are required to produce more extensive effects; animal studies reported here were only exposed for 30 days, whereas rats in Dumansky's study were exposed for 4 mo.

E. FUTURE PLANS

Rats: Further studies are in progress, including serologic examination of sera from exposed and control rats with prostatitis to determine if there are antibodies present that are specific for several possible etiologic agents. Fluorescent-labeled antibody that is specific for rodent Mycoplasma

organisms will be obtained, if available, and a study to detect these suspected agents will be conducted in lung and prostate. If prostatitis in rats is associated with electric field exposure, it would be important to determine the pathogenesis of this lesion. Research involving microbiology, virology, immunology and electronmicroscopy may be needed to support this study, especially in the long-term exposures that have just been started.

Mice: Histopathology has been completed on available mouse tissues and organs, and no effects of exposure were seen. Further studies will be made on the mice being exposed for longer durations (60-120 days).

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VIII. METABOLIC STATUS AND GROWTH

Principal Investigator: J. H. Chandon

ABSTRACT

Two experiments were conducted to determine the effects of 60-Hz, 100-kV/m electric field exposure on the growth and metabolic status of male rats. In one experiment, body weight and food and water consumptions were measured daily during 31 days of exposure (21 hr/day). In another experiment using identical exposure conditions, the rates of O_2 consumption and CO_2 production, the respiratory gas exchange ratio (CO_2/O_2) and the metabolic rates of the rats were determined. No significant differences were found between sham-exposed and exposed animals in any of the metabolic parameters.

A. INTRODUCTION

Reports of the effects of 60-Hz electric fields on growth of rodents are contradictory. Bankoske et al. (1976) reported that mice exposed to electric fields of 25-50 kV/m for 6 wk exhibited increased weight gain. Marino et al. (1976), however, found that rats exposed to 15-kV/m electric fields for 1 mo had lower body weights than controls. Marino also reported that exposed animals exhibited a reduced water intake during the exposure period and that weights of adrenal and pituitary glands of exposed animals were smaller than those of controls at the end of the 30-day exposure period.

In order to ascertain whether growth is affected by electric fields we conducted experiments in which the effects of 100-kV/m, 60-Hz electric field on growth, food and water consumption, O_2 consumption, CO_2 production and metabolic rates were measured.

B. METHODS

The system used for exposing rats to electric fields is described in the last Interim Progress Report (Phillips and Kaune, 1977). Male Sprague-Dawley rats (42 days of age) were placed in the exposure facility on the same day they were received. They were held for a 12-day acclimation period prior to exposure. The experimental groups consisted of 24 rats which were exposed and an equal number of sham-exposed rats. In addition, a group of 24 rats were housed in another room in standard rat cages as cage controls.

Food consumption and body weight measurements were made daily on individual rats. Water consumption was measured daily on each experimental groups (24 rats per group), rather than individually because of limitations imposed by the special watering system on the exposure racks. These three measurements were made at the same time each day for the last 10 days of the acclimation period and for 31 days of exposure. During the exposure period, the measurements were made when the system was de-energized for animal care. At the end of the 31-day exposure period the animals were killed and major organs were weighed.

The rats used for the measurements of gas exchange and metabolic rate were identical to those used for the growth experiment. There was no cage-control group, however, and there were 10 animals each in the exposed and sham-exposed groups. These rats were acclimated for 12 days, and exposed to 60 Hz, 100 kV/m for 30 days.

The rates of O_2 consumption and CO_2 production were measured at the end of the 30-day exposure period by previously described procedures (Phillips, 1975). Metabolic rate was derived from these measurements.

The differences between the means were compared with the Student's t test. These were assumed to be not significant at p values larger than 0.05.

C. RESULTS

There were no significant differences between the means of body weights or food and water consumptions for exposed or sham-exposed groups (Figure VIII-1 and Table VIII-1). There is an apparent difference, however, when the cage-control rats are compared to either of these groups. This difference is also apparent in plots of cumulative food and water consumption over the exposure period (Figure VIII-2 and VIII-3). The cage control rats were housed in much noisier surroundings than the exposed animals. Further, they were held in larger cages, fed different food and had no visual contact with each other. In view of this, a difference between this group and the other two groups is not surprising. A leaky system and subsequent water deprivation can explain the drop in food consumption and body weight seen in sham-exposed rats (Figure VIII-1) during the first day of exposure.

There were no significant differences in the weights of heart, kidney, liver, testis, pituitary, adrenals and brain between groups which were exposed or sham exposed for 31 days (Table VIII-2). The oxygen consumption and carbon dioxide production rates, and metabolic rates calculated from these measurements, were not different between exposed and sham-exposed groups (Table VIII-1).

FIGURE VIII-1. Mean body weight, food consumption and water consumption of rats exposed to 60-Hz electric fields at 100 kV/m for 31 days (N=24/group). Shaded area denotes 95% confidence limits ($M \pm 1.96$ SE) of sham-exposed controls.

FIGURE VIII-2. Cumulative food consumption of rats during exposure to 60-Hz electric fields at 100 kV/m for 31 days (N=24/group)

FIGURE VIII-3. Cumulative water consumption of rats during exposure to 60-Hz electric fields at 100 kV/m for 31 days (N=24/group).

TABLE VIII-1. Metabolic Status of Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	N	Mean $\pm$ SE	
		Exposed	Sham-Exposed
Body Weight	10	436 $\pm$ 11	424 $\pm$ 16
O ₂ Consumption Rate (ml/min)	10	6.9 $\pm$ 0.2	7.0 $\pm$ 0.4
CO ₂ Production Rate (ml/min)	10	6.2 $\pm$ 0.2	5.9 $\pm$ 0.4
Respiratory Quotient (CO ₂ /O ₂)	10	0.89 $\pm$ 0.02	0.85 $\pm$ 0.01
Metabolic Rate (Kcal/hr)	10	2.0 $\pm$ 0.2	2.0 $\pm$ 0.3
Food Consumption (g/day)	24	33.0 $\pm$ 0.5	33.5 $\pm$ 0.7
Water Consumption (ml/day)	24	37.2 $\pm$ 0.9	37.7 $\pm$ 0.7

TABLE VIII-2. Body and Organ Weights of Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 31 days (N=24/group)

	Mean $\pm$ SE (g)		
	Exposed	Sham-Exposed	Cage Controls
Body Weight	445 $\pm$ 7	447 $\pm$ 7	429 $\pm$ 8
Heart	1.55 $\pm$ 0.05	1.54 $\pm$ 0.04	1.55 $\pm$ 0.04
L. Kidney	1.61 $\pm$ 0.04	1.62 $\pm$ 0.04	1.69 $\pm$ 0.03
R. Kidney	1.65 $\pm$ 0.03	1.60 $\pm$ 0.04	1.68 $\pm$ 0.03
Liver	18.2 $\pm$ 0.5	18.6 $\pm$ 0.4	18.9 $\pm$ 0.4
L. Testis	1.81 $\pm$ 0.03	1.84 $\pm$ 0.03	1.85 $\pm$ 0.04
R. Testis	1.80 $\pm$ 0.03	1.84 $\pm$ 0.03	1.84 $\pm$ 0.04
Pituitary	0.010 $\pm$ 0.001	0.010 $\pm$ 0.001	0.009 $\pm$ 0.001
L. Adrenal	0.033 $\pm$ 0.002	0.033 $\pm$ 0.001	0.028 $\pm$ 0.002
R. Adrenal	0.032 $\pm$ 0.002	0.030 $\pm$ 0.002	0.032 $\pm$ 0.002
Brain	2.07 $\pm$ 0.03	2.09 $\pm$ 0.02	1.96 $\pm$ 0.03

D. DISCUSSION AND CONCLUSIONS

The 60-Hz, 100-kV/m electric field had no measurable effect on growth or metabolic rate in these studies. The differences observed between growth of cage controls and the other two groups illustrate the importance of including sham-exposed groups, i.e. a group in which the only known variable is the electric field. Housing, environment, and handling procedures are important variables in experiments in which the effects of the putative insult are subtle.

The reasons for the differences between these results and those of Marino et al. (1976) and Bankoske et al. (1976) are unknown. In the experiments described by Marino et al. the water supply was at a different electrical potential than the animal. This suggests that the animals may have received shocks when drinking, and this might account for the lower water consumption and decreased body weight of exposed animals in his study.

E. FUTURE PLANS

We intend to examine the effect of 60-Hz, 100-kV/m electric fields on the response of rats to a physiological stress, such as acute cold exposure, to assess for possible damage to physiological reserve capacity. Also, we have recently developed techniques for measuring respiratory gas exchange in rats during exposure to electric fields. Measurement will be made before, during and after exposure to electric fields.

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IX. BONE GROWTH AND STRUCTURE

Principal Investigator: B. J. McClanahan

ABSTRACT

Exposure of juvenile male rats to 60-Hz electric fields at 100 kV/m for a period of 30 days did not appear to disturb the normal growth pattern and morphology of tibias and lumbar vertebrae.

A. INTRODUCTION

Artificially induced electric currents have been shown to affect the behavior of bone cells. Currents produced by implanted electrodes projecting into the medullary canal caused increased osteogenesis around the cathode and osteoclasia around the anode. Preliminary studies of various current characteristics indicate that a continuous dc, interrupted dc and an asymmetric ac all increase osteogenesis at the cathode (Bassett et al., 1964, Pawluk and Bassett, 1970). In another study employing a surgically noninvasive technique, an external static field of 100 V/cm applied at the site of a fibular fracture resulted in a healed fibula that was 25% stronger than the contralateral control fibula (Bassett et al., 1974). Stimulation from balanced ac fields has produced conflicting data: McElhaney et al., (1968) found increased bone growth from an externally applied field; Bassett (1971) reported that a symmetric ac field did not result in increased bone formation at either electrode;

while Klapper and Stallard (1974) observed increased mineralization and formation of osseous tissue equally at both electrodes.

The purpose of this investigation was to determine the influence of a uniform, externally applied 60-Hz ac electric field on the skeletal growth pattern of rats.

B. METHODS

In the first experiment, male Sprague-Dawley rats, 21 days old, were placed in the exposure cages for a 5-day acclimation period. There were ten animals in each of the exposed and sham-exposed groups. The 30-day exposure to 60-Hz electric fields at 100 kV/m was started when the animals were 26 days of age. Food consumption was measured during the first week to ensure that any disturbances in bone growth that might occur were not due to differences in food ingestion. On exposure days 21, 22, 28, and 29 each animal was injected intramuscularly with Terramycin at a dose of 20 mg/kg body weight to label bones (Frost, 1969). In a second experiment, both male and female rats (N = 12/group) were exposed. The exposure conditions were the same as in the first replicate.

The animals were killed after 30 days of exposure. Organ weights were obtained on liver, kidneys, and tibia. Samples of liver, kidneys, tibia and lumbar vertebrae were processed for histologic examination. In the replicate experiment, organ weights and specimens for histologic examination were obtained on liver and tibia at necropsy. The livers were then minced, and weighed samples were taken for glycogen, lipid and

moisture content. The samples were immediately frozen and stored until they were assayed. Because anesthetics cause rapid glycogenolysis, a guillotine was used to kill the rats. The moisture content was determined by weighing samples that had been dried for 2 hr at 135°C. Bone growth measurements were performed on cross sections of tibias cut at the tibiofibular junction. The specimens were fixed overnight in neutral buffered formalin, then dehydrated in acetone. They were then embedded (undecalcified) in ethyl butyl methacrylate, and 40 to 50- μ sections were cut with a slow-speed diamond blade saw.¹ The sections were viewed with a Zeiss epi-illumination, fluorescent microscope with a reticule scale in the eyepiece, calibrated with a stage micrometer. Differences between exposed and control means were compared by the Student's *t* test; significant levels greater than $p = 0.05$ were considered not significant.

C. RESULTS

Table IX-1 summarizes the body and organ weights for the two groups of animals. With the exception of livers, there were no significant differences between the two groups. The decrease in liver weight of the exposed rats was statistically significant ($p < 0.05$). However, histologic examination of the livers did not reveal any morphologic differences between the two groups. All livers had minor inflammatory lesions, common in these rats. A small number of mitotic figures were observed in most sections, indicating that the livers were still growing. Glycogen

¹ Model 650, South Bay Technology, Inc., El Monte, CA.

TABLE IX-1. Body and Organ Weights of Male Rats Exposed to 60 Hz Electric Fields at 100 kV/m for 30 Days. Animals were 26 Days of Age at the Start of Exposure (N=10/group)

	<u>Exposed</u> <u>Mean $\pm$ SE</u>	<u>Sham-Exposed</u> <u>Mean $\pm$ SE</u>
Body Weight (g)		
2 1/2 Wk Exposure	241 $\pm$ 6	250 $\pm$ 3
4 Wk Exposure	314 $\pm$ 9	319 $\pm$ 4
Liver (g)	11.8 $\pm$ 0.4*	13.5 $\pm$ 0.4
Kidney (both) (g)	2.40 $\pm$ 0.06	2.42 $\pm$ 0.08
Tibia (g)	0.629 $\pm$ 0.014	0.623 $\pm$ 0.014

* p < 0.05

granules were present in all sections; however, quantitation was not possible since the tissues had been fixed in an aqueous fixative in which glycogen is soluble.

When this experiment was replicated, no differences were noted in the body and organ weights or in liver moisture, protein and lipid contents of the two groups of animals (Table IX-2).

Table IX-3 lists the bone parameters which were determined on the cross sections of the tibias in the first experiment. There were no differences between the two groups of animals in the growth fronts examined. No measurements were made on Haversian systems because very few double-labeled ones were seen. No morphologic differences were noted in either the tibias or lumbar vertebrae.

TABLE IX-2. Body and Organ Weights and Liver Moisture Content of Juvenile Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days. Animals were 27 Days of Age at the Start of Exposure (N=12/group)

	<u>Exposed</u> <u>Mean $\pm$ SE</u>	<u>Sham-Exposed</u> <u>Mean $\pm$ SE</u>
<u>Male Rats</u>		
Body Weight (g)	340 $\pm$ 9	343 $\pm$ 10
Right Tibia (g) (N=6)	0.573 $\pm$ 0.012	0.582 $\pm$ 0.022
Liver Weight (g)	13.5 $\pm$ 0.5	13.7 $\pm$ 0.6
Liver Moisture (%)	70.7 $\pm$ 0.4	70.7 $\pm$ 0.3
Liver Lipid (%)	3.0 $\pm$ 0.2	3.3 $\pm$ 0.1
Liver Protein (%)	16.8 $\pm$ 0.5	15.9 $\pm$ 0.6
<u>Female Rats</u>		
Body Weight (g)	222 $\pm$ 9	229 $\pm$ 7
Right Tibia (g)	0.432 $\pm$ 0.010	0.447 $\pm$ 0.014
Liver Weight (g)	8.54 $\pm$ 0.4	9.1 $\pm$ 0.4
Liver Moisture (%)	71.3 $\pm$ 0.2	71.6 $\pm$ 0.3
Liver Lipid (%)	3.0 $\pm$ 0.2	3.1 $\pm$ 0.2
Liver Protein (%)	16.5 $\pm$ 0.5	17.4 $\pm$ 0.4

D. DISCUSSION AND CONCLUSIONS

Exposure of juvenile male rats to the 60-Hz electric fields at 100 kV/m for 30 days did not appear to cause any disturbances in the skeletal growth parameters which were measured. In another study, in

TABLE IX-3. Bone Growth Parameters of Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	<u>Exposed</u> <u>Mean $\pm$ SE</u>	<u>Sham-Exposed</u> <u>Mean $\pm$ SE</u>
Periosteal Growth, $\mu\text{m/day}$	7.3 $\pm$ 1.2	7.0 $\pm$ 0.9
Endosteal Growth, $\mu\text{m/day}$	2.3 $\pm$ 0.3	2.1 $\pm$ 0.2
Percent Single Terramycin Label		
Periosteal Surface	0	0
Endosteal Surface	---To be measured---	
Percent Double Terramycin Label		
Periosteal Surface	100	100
Endosteal Surface	---To be measured---	
Bone Area, mm^2	3.3 $\pm$ 0.1	3.4 $\pm$ 0.1
Marrow Space Area, mm^2	1.5 $\pm$ 0.2	1.4 $\pm$ 0.1

which an external ac field from a signal of 30 Hz at 200 V/cm was applied to femora encased in plaster casts for a period of 20 days, bone growth and strength were reported to be increased (McElhaney et al., 1968). The authors concluded that structural rather than compositional changes can be diminished by exposure to an electric field. In our studies, bone strength was not measured. Tetracyclines are considered to be deposited in the mineral matrix at the time of mineralization and would, therefore, be indicative only of mineralization rate and possibly, by inference, of composition. They would not, then, provide any information on alterations in molecular orientation that could influence responses to mechanical stress.

While the decrease in liver weight displayed by the animals exposed to the electric fields in the first experiment was small, it was statistically significant. Lower liver weights were not observed in either females or males when the experiment was replicated, suggesting the effect observed in the first experiment was a spurious finding. Moisture, lipid and protein fractions of the liver were not affected by exposure. In addition, no histological differences were observed between livers of exposed and sham-exposed rats.

E. FUTURE PLANS

Additional research on bone growth will be dependent, in part, on the results of the experiment in progress. If no significant effects are observed, this line of research will be terminated.

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X. ENDOCRINOLOGY AND MALE REPRODUCTION

Principal Investigator: M. J. Free

ABSTRACT

Exposure of male rats to 60-Hz electric fields at 100 kV/m for 30 days did not affect the following parameters: weight of body, thymus, thyroid, pituitary, adrenals, testes, seminal vesicles, coagulating glands, epididymides; plasma levels of adrenocortical steroids, testosterone, thyroxine, luteinizing hormone, follicle stimulating hormone; blood flow in the exteriorized and scrotal testis, caput and corpus epididymidis, cauda epididymidis, vas deferens, seminal vesicles; testicular vein concentrations of testosterone, dihydrotestosterone, androstenediols, and dehydroepiandrosterone; or testosterone secretion rate.

A. INTRODUCTION

One view of reported electric field effects on animals is a general stress-type response. In this response the "stressor" acts through the hypothalamo-pituitary-adrenal axis to bring about a variety of physiologic changes that are presumed to help the organism adapt to the "stress" condition. Since a number of putative electric-field effects reported in the literature (e.g. changes in blood proteins, steroids, body and organ weights; Marino et al., 1976) could be attributed to nonspecific stress response, and since the secondary shocks or discharges in strong electric fields may also be stressful, it is important to examine stress-responsive parameters in an experimental arrangement that minimizes or eliminates secondary stimuli.

Accordingly, rat plasma adrenocortical steroids, plasma thyroxine and endocrine gland and reproductive tract weight were examined in rats after exposure to a 60-Hz electric field at 100 kV/m for 30 days in the exposure system, which is free from secondary phenomena, such as spark discharge.

In addition to these stress studies, and in view of reports from the USSR of impaired libido among switchyard workers (Asanova and Rakov, 1966), a detailed examination of electric field effects on male reproductive tract endocrinology was carried out. This included plasma gonadotrophin levels, plasma testosterone, testicular vein androgens, volume blood flow rates through the reproductive tract and androgen secretion rates.

B. METHODS

Plasma samples (5 ml) for hormone assays were drawn by heart puncture from the halothane/N₂O/O₂-anesthetized rat within 2.5 min of removal of each rat from its individual exposure cage. This rapid procedure avoided corticosteroid secretion in response to handling (Barrett and Stockham, 1963). Key organs were excised and stored on moist filter paper in covered petri dishes prior to weighing. Adrenocortical steroids were determined by fluorometric-techniques (Guillemin et al., 1959). Thyroxine, testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed by radioimmune assay.

Rats for reproduction organ study were similarly anesthetized and maintained on closed circuit inhalation anesthesia while plasma samples were drawn from catheters located in the femoral artery and a collecting vein at the cranial pole of one testicle. After sampling, saline suspensions of 10- or 50- μ microspheres, labeled with ^{113}Sn or ^{46}Sc were injected into a left ventricular catheter while a reference blood sample was drawn at a known rate from the femoral artery. Volume flow rate of blood into testes, epididymides and vasa deferentia was calculated by comparing intraorgan radioactivity with that in the reference blood. Plasma samples were assayed for testosterone, dihydrotestosterone, androstenediols and dehydroepiandrosterone by gas chromatographic techniques, using glass capillary columns and an electron capture detector (Wohleb et al., 1976).

C. RESULTS

There were no significant effects of the electric field on body or organ weights (Table X-1) or on plasma hormone levels (Table X-2) as indicated by exposed compared with sham-exposed rats, although FSH showed a strong trend toward higher levels in the exposed group. However, these two groups housed under otherwise identical conditions differed in many respects from rats of similar age housed in conventional animal rooms with individual automatically washed metal cages. Cohort rats housed outside of the exposure room exhibited higher body, thymus, thyroid and pituitary weights, as well as higher plasma levels of FSH

TABLE X-1. Body and Organ Weights of Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	Exposed		Sham-Exposed		Cage Controls	
	N	Mean $\pm$ SE (g)	N	Mean $\pm$ SE (g)	N	Mean $\pm$ SE (g)
Body Weight	20	427 $\pm$ 9	20	440 $\pm$ 7	16	459 $\pm$ 5 ^{a,c}
Thymus	20	0.49 $\pm$ 0.03	20	0.55 $\pm$ 0.03	16	0.59 $\pm$ 0.03 ^b
Thyroid	20	0.026 $\pm$ 0.001	20	0.027 $\pm$ 0.001	16	0.031 $\pm$ 0.001 ^{a,b}
Pituitary	19	0.013 $\pm$ 0.001	19	0.014 $\pm$ 0.001	15	0.016 $\pm$ 0.001 ^c
Adrenals (both)	20	0.062 $\pm$ 0.003	20	0.062 $\pm$ 0.003	16	0.070 $\pm$ 0.003
Testes (both)	20	3.68 $\pm$ 0.08	19	3.69 $\pm$ 0.09	16	3.59 $\pm$ 0.10
Seminal Vesicles (both)	20	0.38 $\pm$ 0.02	20	0.36 $\pm$ 0.02	16	0.39 $\pm$ 0.10
Epididymides (both)	20	1.15 $\pm$ 0.03	20	1.14 $\pm$ 0.03	16	1.13 $\pm$ 0.02
Coagulating Glands (both)	20	0.129 $\pm$ 0.009	19	0.136 $\pm$ 0.007	15	0.146 $\pm$ 0.010

^a Significantly different from sham-exposed; $p < 0.05$.

^b Significantly different from exposed; $p < 0.05$.

^c Significantly different from exposed; $p < 0.01$.

TABLE X-2. Plasma Hormone Levels in Male Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

Hormone	Exposed		Sham-Exposed		Cage Controls	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Follicle Stimulating Hormone (FSH) (ng/ml)	20	114 $\pm$ 16	19	94 $\pm$ 18	18	182 $\pm$ 32 ^a
Luteinizing Hormone (LH) (ng/ml)	20	35 $\pm$ 6	19	39 $\pm$ 6	18	42 $\pm$ 7
Thyroxine (T ₄) (ng/dl)	20	6.3 $\pm$ 0.2	20	6.6 $\pm$ 0.3	18	7.1 $\pm$ 0.2 ^b
Testosterone (ng/ml)	20	1.8 $\pm$ 0.3	19	2.0 $\pm$ 0.3	18	2.1 $\pm$ 0.3
Adrenocortical Steroids (ng/ml)	20	150 $\pm$ 17	20	193 $\pm$ 32	18	124 $\pm$ 14

FSH, LH, T₄ and testosterone levels determined by radioimmune assay.
Adrenocortical steroid levels determined by fluorometric technique.

^a Significantly different from sham-exposed; p < 0.02.

^b Significantly different from exposed; p < 0.01.

and thyroxine. Adrenocortical steroids tended to be lower ($p < 0.07$) in cage controls, but levels of these hormones in all groups were higher than published "resting" levels, reflecting the known effects of prolonged isolation of individual animals from contact with other rats (Lovely et al., 1972). Apparently, the housing conditions used for exposure and sham-exposure to electric fields may have imposed some restraint on growth. However, it is worth noting that the cage controls were considerably more isolated than the other groups since they were unable to see other rats and olfactory contact was impaired by the automatic flushing of the caging system.

There were no effects of the electric field on blood flow through the male reproductive tract (Table X-3). Androgen levels in the testicular vein of exposed and sham-exposed rats are shown in Table X-4. Exposure had no effect on blood flow rates, androgen levels, or testosterone secretion rate.

D. DISCUSSION AND CONCLUSIONS

Sixty-Hz electric fields at 100 kv/M for 30 days, with secondary effects eliminated, had no effect on body weight, weights of endocrine organs or systemic plasma levels of adrenocortical steroids, thyroxine, testosterone or luteinizing hormone. A possible elevation of plasma FSH remains to be investigated further, but testicular weights were unaffected by the treatment. In-depth evaluation of the male reproductive system indicates exposure to 60-Hz electric fields has no effect on blood flow rates, androgen levels, or testosterone secretion rate.

TABLE X-3. Blood Flow in Male Reproductive Organs of Rats Exposed to 60-Hz Electric Fields at 100 kV/m for 30 Days

	Exposed		Sham-Exposed		Cage Controls	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Total Blood Flow (ml/100 g/min)						
Exteriorized testis ^a	6	25.0 $\pm$ 3.5	3	24.6 $\pm$ 1.8		
Intrascrotal testis	6	28.2 $\pm$ 4.6	3	23.2 $\pm$ 2.2		
Total testes	12	26.6 $\pm$ 2.8	6	23.9 $\pm$ 1.3		
Metabolic Blood Flow (ml/100 g/min)						
Exteriorized testis ^a	4	13.9 $\pm$ 1.2	5	16.1 $\pm$ 1.0	7	13.9 $\pm$ 0.5
Intrascrotal testis	4	14.4 $\pm$ 1.4	5	15.7 $\pm$ 1.1	23	15.1 $\pm$ 0.6
Exteriorized caput + corpus ^a	4	16.6 $\pm$ 1.6	5	17.8 $\pm$ 1.3	7	16.7 $\pm$ 2.5
Intrascrotal caput + corpus	4	10.3 $\pm$ 0.4	5	9.1 $\pm$ 1.1	22	11.2 $\pm$ 1.2
Exteriorized cauda ^a	4	12.4 $\pm$ 1.6	5	12.5 $\pm$ 1.9	6	9.9 $\pm$ 2.2
Intrascrotal cauda	4	9.9 $\pm$ 1.2	5	8.1 $\pm$ 0.8	17	8.0 $\pm$ 0.4
Exteriorized vas ^a	4	21.5 $\pm$ 3.3	5	18.2 $\pm$ 2.6	6	20.1 $\pm$ 5.3
Intrascrotal vas	4	15.0 $\pm$ 3.1	4	11.1 $\pm$ 1.9	18	20.9 $\pm$ 2.1
Seminal vesicles	4	11.3 $\pm$ 1.8	4	9.6 $\pm$ 0.9		
Body weight (g)	9	435 $\pm$ 12	8	427 $\pm$ 8	13	431 $\pm$ 12

^a Scrotum cut open and reproductive organs suspended in a saline bath at 33.5°C.

TABLE X-4. Testicular Blood Flow and Androgen Secretion
Rates of Rats Exposed to 60-Hz Electric Fields
at 100 kV/m for 30 Days

	Exposed		Sham-Exposed	
	N	Mean $\pm$ SE	N	Mean $\pm$ SE
Body Weight (g)	8	412 $\pm$ 13	8	425 $\pm$ 25
Testis Weight (decapsulized)	9	1.67 $\pm$ 0.05	8	1.73 $\pm$ 0.04
Hematocrit:				
Femoral artery	9	50.4 $\pm$ 0.9	8	50.6 $\pm$ 0.9
Testis vein	9	51.6 $\pm$ 0.9	8	51.2 $\pm$ 1.1
Testis Capillary Blood Flow (ml/min/100 g of b.w.)	4	13.9 $\pm$ 1.2	5	16.1 $\pm$ 1.0
Total Testis Blood Flow (ml/min/100 g of b.w.)	6	25.0 $\pm$ 3.5	3	24.6 $\pm$ 1.8
Total Testis Blood Flow (ml/hr/testis)	4	22.1 $\pm$ 4.8	3	26.0 $\pm$ 1.4
Testosterone (ng/ml) (Testis vein plasma conc.)	9	48.4 $\pm$ 4.2	8	49.1 $\pm$ 14.3
5 α -Androstan-3 β ,17 β -diol (ng/ml) (Testis vein plasma conc.)	9	4.2 $\pm$ 1.7	8	8.6 $\pm$ 2.6
5 α -Androstan-3 α ,17 β -diol (ng/ml) (Testis vein plasma conc.)	9	6.2 $\pm$ 4.4	8	6.5 $\pm$ 1.5
Dehydroepiandrosterone (ng/ml) (Testis vein plasma conc.)	9	38.5 $\pm$ 21.9	8	29.2 $\pm$ 13.6
Dihydrotestosterone (ng/ml) (Testis vein plasma conc.)	9	0.22 $\pm$ 0.09	8	0.25 $\pm$ 0.09
Testosterone Secretion Rate (ng/min/g testis)	9	7.0 $\pm$ 1.9	3	6.5 $\pm$ 1.2

These data are in conflict with previous studies (Marino et al., 1976 and 1977; Dumansky et al., 1976), where much lower field strengths were employed, and suggest that secondary effects (electrical discharges, magnetic fields, ozone) may have had a major impact upon the published studies. However, the possibility of a field strength "window" for biological effects cannot be ignored. Our results are in general agreement with those of the Italian (ENEL) studies summarized by Cerretelli and Malaguti (1977), where field strengths of 80-100 kV/m were used.

E. FUTURE PLANS

Since subjective observations during bone growth and structure studies suggest that prepubertal and pubertal rats were less vigorous and poorer in appearance after 30 days of 60-Hz electric field exposure (100 kV/m) (cf Chapter IX), an attempt to quantitate this effect will be made by serially sacrificing, over 30 days, groups of animals exposed or sham-exposed to similar electric fields from 28 days of age. Plasma levels of adrenocorticoids, adrenocortical stimulating hormone (ACTH), thyroxine, testosterone (in males) FSH and LH (in males and females) will be monitored, as well as liver and gonad weights.

Since time-related changes are overlooked by the treatment and sacrifice approach, and serial sacrificing at small intervals is prohibitively expensive, we will develop a chronic sampling model that will permit serial blood sampling from individual animals during exposure. Short-term (e.g. 1 day) changes, effects on endogenous secretory or metabolic rhythms and dynamics of electric-field-related changes will be examined with this system.

We will examine the effect of long-term exposure on all the parameters listed in the present study, as well as on ACTH.

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XI. CARDIOVASCULAR FUNCTION

Principal Investigator: D. I. Hilton

ABSTRACT

Electrocardiogram (ECG) and heart rate measurements were made on rats that had been exposed to 60-Hz electric fields. Electrocardiograms were recorded on animals exposed for 8 hr to an 80-kV/m field or 611 hr to a 100-kV/m field. Heart rate measurements were made on animals exposed for 8 or 40 hr to an 80-kV/m field and 611 hr to a 100-kV/m field. No significant differences in heart rates or duration of ECG characteristics were found between exposed and sham-exposed animals after 8-, 40- or 611-hr exposures.

A. INTRODUCTION

Blanchi et al. (1973) and Meda et al. (1972) have reported significant increases in the PR interval and the QRS duration of the ECG of mice exposed to 100-kV/m electric fields for 1000 hr. Chickens exposed to fields of 40 or 80 kV/m were found to have elevated heart rates 8 hr after exposure (Poznaniak, 1977). The authors were unable to replicate this finding in later experiments (personal communication). The main purpose of our experiments thus far has been to assess these reported effects and to help clarify some of the conflicting reports in the literature.

B. METHODS

Techniques for obtaining electrocardiograms and heart rate measurements have been described in detail in an earlier report (Phillips, 1970). Briefly, ECGs were obtained from transthoracic surface electrodes and recorded by a Grass Model 79D Polygraph at a paper speed of 100 mm/sec. The PR interval and QRS duration of the ECG waves were measured from peak to peak using a 10X comparator with a 0.01-mm scale. Figure XI-1 shows an illustration of an ECG recorded from a rat using transthoracic electrodes. The PQ interval of the ECG wave gives a measure of the electrical conduction time between depolarization of the atria and depolarization of the ventricles of the heart. The QRS duration gives the time required for depolarization of the ventricles. The T-wave portion is the result of repolarization of the ventricles.

Mean values between exposed and sham-exposed groups for heart rate, PR interval and QRS duration were compared using a t test. The differences are considered to be significant only when p values are less than 0.05.

C. RESULTS

Table XI-1 shows the PR interval and QRS duration from ECGs of rats exposed to an 80-kV/m electric field for 8 hr; measurements were taken at 0+, 30 and 60 minutes after the end of exposure. No significant differences were found in the PR interval or QRS duration of the exposed group compared to the sham-exposed controls.

FIGURE XI-1. Illustration of ECG recorded from a rat.

TABLE XI-1. PR Interval and QRS Duration from ECGs of Rats (N=12/group) Exposed to an 80-kV/m Electric Field for 8 Hr. Measurements were taken at 0+, 30 and 60 min postexposure.

	Time Postexposure (min)	Exposed Mean $\pm$ SE	Sham-Exposed Mean $\pm$ SE
PR Interval (msec)	0+	43.3 $\pm$ 0.9	43.1 $\pm$ 0.5
	30	41.3 $\pm$ 1.3	44.1 $\pm$ 1.2
	60	42.6 $\pm$ 1.7	44.7 $\pm$ 1.6
QRS Duration (msec)	0+	22.1 $\pm$ 0.4	22.2 $\pm$ 0.5
	30	22.7 $\pm$ 0.3	22.3 $\pm$ 0.3
	60	22.7 $\pm$ 0.2	22.7 $\pm$ 0.4

In another experiment, rats were exposed or sham-exposed to a 100-kV/m electric field for 1 month (611 hr). During the last 8 days of exposure, all animals were acclimated to restraint holders (Bollman type) for about 2 hr daily while the electric field was turned off for routine animal care. On the last day of exposure, ECG and heart-rate measurements were taken for 1 hr after termination of exposure. Table XI-2 shows the PR interval and QRS duration of the ECG wave of these rats. No significant differences were found between exposed and sham-exposed groups during the 60-min test period. Figures XI-2, XI-3, and XI-4, respectively, show the mean heart rates of rats after an 8-hr, 40-hr (8-hr/day for 5 days) or 611-hr exposure. The mean heart rates of exposed and sham-exposed groups did not differ significantly.

TABLE XI-2. PR Interval and QRS Duration from ECGs of Rats Exposed to a 100-kV/m Electric Field for 611 Hr.*

	Time (min)	Exposed (N) Mean $\pm$ SE	Sham-Exposed (N) Mean $\pm$ SE
PR Interval (msec)	0+	40.5 $\pm$ 1.4 (10)	40.3 $\pm$ 1.2 (12)
	30	40.9 $\pm$ 1.4 (9)	39.4 $\pm$ 1.3 (9)
	60	42.4 $\pm$ 1.4 (11)	39.9 $\pm$ 1.2 (12)
QRS Duration (msec)	0+	22.9 $\pm$ 0.3 (11)	23.0 $\pm$ 0.6 (11)
	30	23.3 $\pm$ 0.6 (9)	22.6 $\pm$ 0.4 (9)
	60	23.1 $\pm$ 0.6 (12)	23.2 $\pm$ 0.3 (12)

* Measurements were taken at 0+, 30 and 60 min after animals were placed in Bollman Holders. Measurements were started within 1 hour after the end of exposure.

D DISCUSSION AND CONCLUSIONS

Exposure of rats to 60-Hz electric fields at 80-100 kV/m for up to 611 hr had no significant effect on electrical conduction time of the heart nor on heart rate.

The results of these experiments cannot be compared directly to those of Blanchi et al. (1973) or Poznaniak et al. (1977), since rats were used in this study while mice were used in their studies. Also, Blanchi et al. (1973) exposed mice for 1000 hr while only 611 hr of exposure time were used in this study. Accordingly, it is not known if the failure to detect effects in our experiments was the result of differences in species and durations of exposure, or reflected the elimination of secondary electric field effects (e g., shocks, ozone, etc.).

FIGURE XI-2. Mean heart rates ($\pm SE$) of rats after 8 hr of exposure or sham exposure to 60-Hz electric fields at 80 kV/m. Measurements made for 1 hour starting immediately following exposure or sham exposure.

FIGURE XI-3. Mean heart rate ($\pm$ S.E.) of rats after 40 hr cumulative exposure (8 hr/day for 5 days) to 60-Hz electric fields at 80 kV/m.

FIGURE XI-4. Mean heart rate of rats ($\pm$ S.E., N=12/group) after 611 hr exposure to a 60-Hz electric field at 100 kV/m.

E. FUTURE PLANS

Future Plans include:

- Stress reaction experiments in which rats will be exposed to cold stress after exposure to electric fields to evaluate possible effects on physiological reserve capabilities. Various physiological parameters to be examined include: cardiovascular function, thermoregulation, metabolic reserves, TSH and corticosterone response.
- Evaluation of ECGs on rats after exposure to a 100-kV/m field for 60 days.
- Blood-pressure measurements in rats after exposure to electric fields for 30 and 60 days.
- Respiratory gas exchange (O_2 production and CO_2 production) and metabolic rate before, during and after exposure to electric fields.

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XII. CENTRAL NERVOUS SYSTEM

Principal Investigator: J. C. Hampton

ABSTRACT

Exposure of mice to 60-Hz, 100-kV/m electric fields for 30 days caused no changes in brain morphology at the light microscope level. Preliminary examination by electron microscopy indicated no effects of exposure on brain ultrastructure or on permeability of the blood-brain barrier to proteins.

A. INTRODUCTION

One would expect that if exposure to 60-Hz electric fields produces effects on animals, the nervous system would be a likely site of these effects. In spite of this expectation, there is little information available concerning morphological effects on brains at the light and electron microscope level. Dumansky et al. (1976) reported the occurrence of dystrophic and vascular changes in brain and other tissues of rats that were exposed to electric fields. However, their publication presented no data or illustrations of these changes, and the report is of limited usefulness. In this project a study was made to investigate the effects of exposure to electric fields on the brain structure of mice using both light and electron microscopy.

In addition to the structural studies, an examination was made of the permeability of horseradish peroxidase (HRP) from blood to brain. A functional barrier to diffusion exists between the central nervous

system and blood which normally prevents the penetration of proteins into the brain. The presence of HRP in the brain after its injection into the blood of mice would indicate changes in the blood-brain barrier, either degenerative or a response to the release of chemical mediators (i.e., histamine) that affect the barrier.

B. METHODS

Twenty-four C57B1/6J mice were exposed to a 60-Hz, 100-kV/m electric field for 30 days. Two additional groups served as controls, one of which was housed in the exposure room under identical conditions but without exposure to the electric field (sham-exposed), and the others in a separate room, in standard laboratory mouse cages (cage controls).

After exposure, the mice were transported to another building for sacrifice and study of tissues from the central nervous system. Five minutes prior to sacrifice, each animal received an intravenous injection of 5 mg of HRP as a tracer for detecting changes in vascular permeability. Brains were removed and specimens of cerebral cortex bordering the central sulcus and of the hypothalamic region were fixed in buffered glutaraldehyde/formaldehyde for 3 hr, then transferred to cacodylate buffer and kept at 4°C overnight. The tissues were then cut into slices approximately 0.1-mm thick and incubated in 3-3' diaminobenzidine in the presence of H_2O_2 to develop a black reaction product at brain sites penetrated by HRP. The incubated blocks were dehydrated and divided, with one-half embedded in epoxy resin for examination with the electron microscope and the other half embedded in glycolmethacrylate for study with the light microscope.

C. RESULTS

Light microscopic examination revealed no histopathologic difference among brains from the sham-exposed, cage control and exposed groups. As of this date, only one brain (cortex and hypothalamus) was examined from each of the groups (sham-exposed, cage control, and exposed) for ultrastructural changes in vascular permeability and histopathology by electron microscopy. No pathologic changes were observed and there was no evidence of HRP (MW 40,000) having crossed the blood-brain barrier. The black reaction product was clearly evident within the vessels; no extravascular deposits were seen, indicating the integrity of the barrier had not been damaged by electric field exposure. Occasionally, normal degenerative changes and lipofuscin granule accumulations were observed in all three groups, as expected.

D. DISCUSSION AND CONCLUSIONS

The lack of exposure effects observed in the histopathological screening of mouse brains is not consistent with the report of Dumansky, et al. (1976), although their exposures were at lower field strengths (0.1-5 kV/m) and of longer duration (4 mo). One would expect, however, that higher field strengths would cause increased effects.

These studies are not complete, but results to date indicate no ultrastructural changes and no increased permeability.

E. FUTURE PLANS

The tissues which have not yet been examined in the electron microscope are embedded and will be held for later evaluation.

REFERENCES

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

XIII. NEUROPHYSIOLOGY

Principal Investigator: R. A. Jaffe

ABSTRACT

The purpose of this study was to investigate the effects of chronic exposure (30 days) of rats to a 60-Hz electric field on the conduction of nerve impulses across nerve junctions (synapses). Superior cervical sympathetic ganglia were removed from rats anesthetized with urethan, placed in a chamber and continuously superfused with a modified mammalian Ringer's solution equilibrated with 95% O₂ and 5% CO₂. Several parameters and tests were used to characterize synaptic transmission through the ganglion. These included amplitude, area, and configuration of the postsynaptic compound action potential; conduction velocity and synaptic delay; conditioning-test response; frequency response; post-tetanic response; and high-frequency-induced fatigue. The results of the neurophysiologic tests and measurements indicate that synaptic transmission is not affected by chronic (30-day) exposure to a 60-Hz, high-voltage electric field.

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 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 60-Hz electric field. In this report, results from the experiments on synaptic transmissions are presented.

B. METHODS

Two groups of 15 rats each were examined. One group was exposed to a 100-kV/m, 60-Hz electric field for 30 days. The other group was maintained in identical housing without the electric field (sham-exposed). The exposure system has been described in detail in the Second Interim Report (Phillips and Kaune, 1977).

Superior cervical sympathetic ganglia, including segments of the cervical sympathetic trunk (CST) and internal carotid nerve (ICN), were removed from 15 electric-field-exposed and 15 sham-exposed rats. Rats were anesthetized with urethan and the ganglia were removed within 2 hr after the completion of the 30-day exposure period. 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. Postsynaptic compound action potentials were recorded photographically for subsequent analyses.

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

1. characteristics of the postsynaptic compound action potential (including amplitude, area and configuration).
2. conduction velocity of the compound action potential--measured between the stimulating and recording electrodes, therefore, including synaptic delay.

3. synaptic delay--calculated from conduction velocity data.
4. conditioning-test response--an adaptation of the method used by Whitcomb and Santolucito (1976), which measures 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.
5. frequency response--an adaptation of the method used by Whitcomb and Santolucito (1976), which measures the number of synapses capable of responding to a test frequency (range, 5-20 Hz) as a function of the height of the postsynaptic compound action potential.
6. post-tetanic response--the amplitude of the postsynaptic compound action potential was measured at 1-min intervals following stimulation at 20 Hz for 90 sec.
7. 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.

Differences between means of exposed and sham-exposed animals were compared by the Student's *t* test. Significance levels greater than 0.05 were considered not significant.

C. RESULTS

The results of all neurophysiologic tests and measurements performed to date suggest that synaptic transmission is not significantly affected by 30 days of exposure to a 60-Hz electric field at 100 kV/m. Specific results are presented in the following tables and figures. Table XIII-1 shows the area and maximal amplitude at the postsynaptic compound action potential that is evoked by supramaximal preganglionic nerve stimulation in exposed and sham-exposed rats. Computer-generated average postsynaptic compound action potentials are shown in Figure XIII-1. There were no significant differences between exposed and sham-exposed animals for any of these parameters.

TABLE XIII-1. Mean Values ($\pm$ SE) for the Area and Maximal Amplitude of the Postsynaptic Compound Action Potential Following Supramaximal Preganglionic Nerve Stimulation

	<u>Exposed</u>	<u>Sham-Exposed</u>
<u>Area</u>		
Mean (V•sec)	35.8	34.4
SE	2.7	2.4
N	18	26
<u>Maximum Amplitude</u>		
Mean (mV)	7.6	7.2
SE	0.6	0.5
N	18	26

FIGURE XIII-1. Computer-generated postsynaptic compound action potentials (averaged) from sympathetic ganglia of rats that have been exposed (N=16) or sham exposed (N=13) to a 60-Hz electric field at 100 kV/m for 30 days.

Table XIII-2 compares maximum conduction velocities for two components of the compound action potential and calculated values for synaptic delay. Although there appears to be a tendency for exposed animals to have a decreased conduction velocity, there are no statistically significant differences (Student's t test) between exposed and sham-exposed animals for these parameters.

TABLE XIII-2. Mean Values (+ SE) for Maximum Conduction Velocities and Calculated Synaptic Delay for Compound Action Potential Propagation Through the Rat Superior Cervical Sympathetic Ganglion

<u>Exposed Animals</u>	<u>Conduction Velocity I (msec)</u>	<u>Conduction Velocity II (msec)</u>	<u>Synaptic Delay (msec)</u>
Mean	0.78	0.61	1.82
SE	0.03	0.02	0.30
N	11	11	11
 <u>Sham-Exposed Animals</u>			
Mean	0.86	0.62	1.76
SE	0.08	0.04	0.27
N	12	11	10

Table XIII-3 compares conditioning-test responses for conditioning-test intervals between 25 msec and 800 msec. These data are graphically displayed in Figure XIII-2. A preliminary statistical evaluation indicates

TABLE XIII-3. Mean Values ($\pm$ SE) for the Test Postsynaptic Compound Action Potential Amplitude Expressed as a Function of the Amplitude of the Conditioning Postsynaptic Compound Action Potential for Specific Conditioning-Test Intervals. Conditioning Stimulus Rate = 0.5 Hz.

Conditioning-Test Interval (msec)	Exposed	Sham-Exposed
25	1.18	1.16
SE	0.05	0.05
N	14	13
50	1.04	0.99
SE	0.02	0.03
N	14	15
100	0.78	0.74
SE	0.03	0.04
N	14	15
150	0.72	0.66
SE	0.03	0.04
N	14	14
200	0.76	0.70
SE	0.03	0.03
N	14	15
300	0.90*	0.84*
SE	0.01	0.02
N	14	15
400	0.98	0.95
SE	0.01	0.01
N	14	15
500	1.00	0.98
SE	0.005	0.01
N	14	15
600	1.00	0.99
SE	0.005	0.004
N	14	15
700	1.00	1.00
SE	0.004	0.004
N	14	15
800	1.01	1.00
SE	0.003	0.003
N	14	15

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

FIGURE XIII-2. Comparison of conditioning-test response ratios of rats exposed and sham-exposed to 60-Hz electric fields at 100 kV/m.

that the data are not significantly different ($p = 0.08$). The spike height ratios (Figure XIII-2) from ganglia of exposed animals are consistently higher than those of sham-exposed animals. These data suggest that the refractory period of the ganglia from exposed animals is decreased relative to controls. More extensive statistical analyses are in progress.

Table XIII-4 presents data from the frequency response tests. Responses are expressed as the ratio of the compound action potential amplitude before test stimulation to its amplitude following 90 sec of test stimulation. The responses of exposed animals to a 90-sec test stimulation at frequencies of 5, 10, and 20 Hz were not significantly different from those of sham-exposed animals.

TABLE XIII-4. Mean Values for the Frequency Response of the Postsynaptic Compound Action Potential. Responses are Expressed as the Ratio of the Action Potential Amplitude Before Test Stimulation to Its Amplitude After 90 Sec of Test Stimulation. Control Action Potential Amplitudes were Obtained at a Stimulus Frequency of 1 Hz.

	Test Stimulation Frequencies		
	5-Hz Response Ratio	10-Hz Response Ratio	20-Hz Response Ratio
<u>Exposed</u>			
Mean	0.71	0.65	0.54
SE	0.01	0.02	0.04
N	14	14	14
<u>Sham-Exposed</u>			
Mean	0.71	0.61	0.57
SE	0.03	0.04	0.04
N	15	15	15

Table XIII-5 and Figure XIII-3 present data from the post-tetanic response experiments. The amplitude of the compound action potential was measured at 1-min intervals following 90 sec of stimulation at 20 Hz, and is expressed as a function of the action potential amplitude at 1 Hz, before tetanic stimulation. The post-tetanic response of exposed animals is not significantly different from that of sham-exposed animals, even though the ratios from exposed animals were consistently higher than those of controls from 2 through 6 min after tetanic stimulation.

TABLE XIII-3. Post-Tetanic Response of the Postsynaptic Compound Action Potential Amplitude at a Stimulus Frequency of 1 Hz, Measured at 1-Min Intervals Following 90-Sec Stimulation at 20 Hz, Relative to Control Action Potential Amplitude During 1-Hz Stimulation.

<u>Minutes</u>	<u>Post-Tetanic Response Ratio</u>					
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>7</u>
<u>Exposed</u>						
Mean	0.87	0.99	1.01	1.02	1.01	1.00
SE	0.05	0.03	0.03	0.03	0.03	0.04
N	11	11	12	11	11	12
<u>Sham-Exposed</u>						
Mean	0.88	0.97	0.97	0.96	0.93	0.99
SE	0.04	0.02	0.02	0.03	0.05	0.02
N	13	13	13	13	10	10

FIGURE XIII-3. Comparison of post-tetanic response for rats exposed and sham-exposed to 60-Hz electric fields at 100 kV/m.

Table XIII-6 and Figure XIII-4 present the results of the study on high-stimulus, frequency-induced fatigue. The amplitude of the postsynaptic compound action potential was measured at regular intervals during 60 min of continuous presynaptic stimulation at 20 Hz. The results are expressed as the ratio of the 20-Hz spike amplitude to the amplitude of the 1-Hz control. The responses of exposed and sham-exposed animals are not significantly different.

TABLE XIII-6. Fatigue Response to High-Frequency Stimulation. Superior Cervical Sympathetic Ganglia were Stimulated at 20 Hz for 60 Min. The Amplitude of the Postsynaptic Compound Action Potential was Measured 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 1 Hz Control Stimulation.

<u>Time (min)</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>E-Field-Exposed</u>											
Mean	0.55	0.47	0.44	0.45	0.43	0.34	0.33	0.35	0.33	0.30	0.27
SE	0.04	0.04	0.05	0.05	0.04	0.04	0.03	0.04	0.05	0.04	0.05
N	13	14	14	13	14	14	14	14	14	13	12
<u>Sham-Exposed</u>											
Mean	0.56	0.49	0.46	0.44	0.42	0.37	0.42	0.36	0.34	0.30	0.27
SE	0.05	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04	0.03	0.05
N	14	15	15	15	15	15	13	13	12	12	12

FIGURE XIII-4. Comparison of first ganglion fatigue data for rats exposed and sham-exposed to 60-Hz electric fields at 100 kV/m.

D. DISCUSSION AND CONCLUSIONS

The results available from the experiments on synaptic transmission indicate that a 30-day exposure to a 60-Hz electric field at 100 kV/m has no effect on synaptic function in the rat.

E. FUTURE PLANS

Peripheral Nerve Function: Jan.-Mar., 1978

Neuromuscular Function: Apr.-June, 1978

Acute Exposure Studies: 1978

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XIV. REPRODUCTION, GROWTH AND DEVELOPMENT

Principal Investigator: M. R. Sikov

ABSTRACT

Two experiments to evaluate the effects of exposure to electric fields on the reproduction, growth, and development of the rats have been performed and are nearing completion. In the first experiment it was found that a 6-day exposure prior to mating did not affect the reproductive performance of either males or females, and that continued exposure of the mated females through 20 days of gestation did not affect the size or morphology of the fetuses. Continued exposure of the males and the unmated females to a total of 30 days did not affect their mating performance or fertility on subsequent testing.

In the second experiment, it was found that exposure of the pregnant rat throughout gestation and of the resulting offspring through the first 8 days of postnatal life did not produce significant effects on growth, survival, or neuromuscular development. Tests of the reproductive integrity of the offspring are still in progress.

A. INTRODUCTION

It has been found that prenatal and neonatal mammals are more susceptible to alteration by many forms of energy, as well as by many chemicals, than are more mature individuals. This sensitivity derives from the multitude of intercellular and intracellular processes which are unique to development, as well as the variety of finely tuned regulatory processes that are susceptible to perturbation.

There have been a number of studies which suggested that sufficiently intense magnetic fields may affect reproduction and prenatal development.

There are essentially no data on the developmental effects of electromagnetic fields in the range associated with power transmission. There are indications, however, of effects on reproduction and growth at higher field strengths or very prolonged exposure times (Knickerbocker et al., 1967; Marino et al., 1976).

Accordingly, studies were undertaken to determine the effects of electric fields on reproduction and on fetal and postnatal growth and development in the rat. The first of these studies has been essentially completed and is reported here. Several aspects of the second study have also been completed, although more extensive tabulations and statistical analyses are required. A preliminary report of these findings has been included.

B. METHODS

Experiment 1 - Reproductive behavior, fecundity, and fetal development were studied in the first experiment, which was performed as three replicates. Within each replicate eight randomly assigned female and two male rats were exposed for 6 days in individual cages to the electric field (100 kV/m) or to the sham field. Groups of four females and one male were then transferred to mating cages. These were identical in design to the standard exposure cages, except that they were equivalent in size to four such cages. The rats were allowed to remain in these cages for 5 days with vaginal smears being taken each morning. Exposure or sham-exposure to the 60-Hz electric field continued during this mating period. The presence of sperm in the morning vaginal smear was

taken as an indication of mating and defined as day 0 of gestation; no further smears were taken from the sperm-positive females.

At the end of this period of cohabitation, the males and females were returned to their individual home cages for continued exposure. The males were exposed for an additional 19 days (30 days total exposure), removed from the field or sham field, allowed a 3-wk period for progression of the spermatogenic cycle, and mated to females (1 male with 4 females) that had been sterilized by ligation of the fallopian tubes. The same sterilized females were used for all three replicates. The males were sacrificed 5 days later and epididymal sperm collected for analysis of morphology and DNA properties. The female rats which mated remained in the exposure or sham-exposure field until 20 days of gestation, at which time they were killed. The females which did not mate were also maintained in the sham or exposure field for an additional 19 days, to provide a total of 30 days exposure.

The female rats that mated were killed at 20 days of gestation, the uterus was examined and the number of implantation sites and viable fetuses were noted. All living fetuses were measured, weighed, sexed, and examined for external malformations. They were then randomly assigned to two groups for examination of internal malformations. One group was fixed in Bouin's fluid, transferred to 95% ethyl alcohol and examined for visceral malformations using thin razor-blade sections. The other fetuses were fixed in ethyl alcohol, cleared and stained by the alizarin red technique and examined for skeletal defects.

The females that did not mate were placed with stock males 3 days after removal from the field and allowed a 5-day breeding period. The animals which mated were killed at 14 days of gestation to determine the reproductive integrity of the females.

Differences between exposed and control means were compared by the Student's t test, levels of significance greater than $p > 0.05$ were considered not significant.

Experiment 2 - This experiment, to evaluate the postnatal sequelae of prenatal exposure, was performed in four replicates. Within each replicate, one male and four female rats were caged together and the females checked daily for vaginal smears. The day of a sperm-positive vaginal smear was defined as 0 day of gestation. At that time each female was placed in an individual cage within the electric field or sham field, determined randomly. At 18 days of gestation females were transferred to littering cages, identical to the mating cages used in Experiment 1, containing Antron-III (an electrical conductor) for nesting. The rats were maintained there through parturition, and were removed from the field when the pups reached 8 days of age.

The time between mating and parturition was noted; offspring were examined at birth to determine the number of living and stillborn offspring. Litters were reduced to eight, evenly divided between the sexes when possible, at one day of age (litters of less than six were eliminated), when they were weighed and examined for gross abnormalities, as they were at several subsequent times (see Table XIV-3). At many of these

same times offspring were subjected to a battery of tests (see Table XIV-5) to evaluate reflex development and general neurological status. The measures used are those commonly employed in our laboratory and have been described previously (Sikov et al., 1977).

At about 2 months of age four female offspring from each litter, together with one randomly selected male, were reintroduced into the sham-exposure or exposure field to determine whether deficits in reproductive performance had been induced. This experiment is still in progress and will be reported at a later date. One-half 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 other half of the animals were handled in an identical fashion except that they were not subjected to the sound. Blood samples were obtained at 5 min after initiation of the seizure test to determine whether changes in corticosteroid levels had been produced by the stress of the seizure test and whether this differed between the sham-exposed and exposed groups. The animals which were not subjected to the seizure test were necropsied, which included weighing several organs as well as gross and histopathologic examination. Additional blood samples were taken for more extensive hematologic and hormonal analyses. Results of these determinations are not yet available.

The males of the third replicate were given Metrazol prior to testing for audiogenic seizure susceptibility, to heighten their susceptibility. 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.

C. RESULTS

Experiment 1 - The effects of exposure on reproductive performance are summarized in Table XIV-1. The number shown as having mated in the electric field includes two females in which mating was undetected but which were subsequently found with term pregnancies. The results from these animals are considered with mating and fertility assessments but are not included in the determinations of litter size or prenatal mortality. It is clear that pre-exposure to the electric field did not influence the number of animals which mated compared to those in the sham field, nor did it affect the fraction of matings which were fertile. Litter size, prenatal death, and the sex ratio were likewise unaffected by exposure to the field.

Although the number of unmated exposed or sham-exposed females available for remating was small, most of these animals remated and most of the females which mated were pregnant upon examination at 14 days of gestation. The litter size in these animals was slightly smaller than that observed in the original test period, although not significantly so, and no differences between the exposed or sham-exposed animals were seen, either in terms of litter size or in the incidence of prenatal deaths.

The 30-day exposure to the electric field produced a slight, but statistically insignificant, decrease in the number of copulations with the sterilized females. Analysis of sperm samples from these males, which were sent to Lawrence Livermore Laboratory for evaluation, has not yet been completed and data are not available.

TABLE XIV-1. Effect of Exposure to an Electric Field on Reproductive Performance of Rats

Measurement	Exposed	Sham-Exposed
<u>Initial Reproductive Performance</u>		
Number Exposed	24	24
Number Mated	20*	17
Number Fertile Matings	16*	13
Litter Size (Mean $\pm$ SE)	11.8 $\pm$ 1.1	13.0 $\pm$ 0.8
Prenatal Deaths (Mean per litter $\pm$ SE)	0.5 $\pm$ 0.2	0.8 $\pm$ 0.2
Sex Ratio (Fraction Males)	0.51	0.49
<u>Postexposure Reproductive Performance</u>		
Number of Females Remated	4	7
Number Mated	3	5
Number Fertile Matings	3	4
Litter Size (Mean $\pm$ SE)	10.7 $\pm$ 2.8	10.8 $\pm$ 3.3
Prenatal Deaths	0	0.8 $\pm$ 0.5
Number of Males Remated	6	6
Number of Sterilized Females Tested	24	24
Number Copulations	8	13
Number of Males Copulating	5	5

* Includes two females in which mating was undetected but which were subsequently found with term pregnancies.

The experimental design necessitated leaving the males with females after mating, which allowed for the possibility of an infertile mating followed by a subsequent remating, with an attendant possibility of a 1- or 2-day error in the timing of gestation. Judged by morphologic appearance and weight, this occurred in two instances; size and morphologic data from these litters have been deleted. The results obtained on the remaining litters are summarized in Table XIV-2. No measurable differences in fetal length or weight attributable to exposure were detected.

TABLE XIV-2. Effect of Exposure to an Electric Field on Fetal Size and Incidence of Morphologic Abnormalities of Rats

	<u>Visceral Abnormalities</u>	
	<u>Exposed</u>	<u>Sham-Exposed</u>
Number Examined	78	73
Minor Cardiac Variations	16	13
Cardiac Septal Defects	0	2
Levocardia	1	2
Hydronephrosis	1	1
Minor Brain Variations	1	1

	<u>Skeletal Abnormalities</u>	
	<u>Exposed</u>	<u>Sham-Exposed</u>
Number Examined	80	74
Rudimentary 14th Rib	40	40
Rib Fusion Failure	0	1
Vertebral Defects - Minor	1	1

A number of animals with minor cardiac variations, such as differences in lumen size or thickness of cardiac walls, were seen; the incidence was the same in the sham and exposed groups. Septal defects were seen in two of the sham-exposed animals but none in the exposed; the incidence in the sham-exposed group is within the range ordinarily found, as is the observation of two cases of levocardia in the sham-exposed and one in the exposed. The incidence of other developmental variations, including hydronephrosis, minor variations of the brain, and a rudimentary 14th rib, were unaffected by exposure. The incidence of more pronounced defects, such as failure of the ribs to fuse to the vertebrae and defects of the vertebrae, was unaffected by exposure.

Experiment 2 - As shown in Table XIV-3, there were no differences between exposed and sham-exposed groups in litter sizes or survival of offspring. Birth weights and subsequent growth of offspring of rats exposed to the electric and sham fields were similar (Table XIV-4). Most results obtained from tests of neuromuscular and neurological development did not differ between the sham-exposed and exposed groups (Table XIV-5). The only notable difference was the greater percentage of exposed offspring showing motile behaviors (movement, grooming, standing) and of 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. The animals of the first two replicates failed to show seizures under the testing conditions, so that no differences between groups could be detected. It is clear, however, that this

TABLE XIV-3. Effect of Exposure to an Electric Field on Litter Size and Survival

	<u>Exposed</u>	<u>Sham-Exposed</u>
Total Females in Group	24	20
Number Discarded	4*	2**
Number of Litters Studied	20	18
Mean Live Births/Litter $\pm$ SE	11.3 $\pm$ 0.5	11.6 $\pm$ 0.6
Number of Stillbirths	6	3
Number of Postnatal Deaths	3	4

**One completely resorbed litter; one dam killed the 8 remaining pups of a litter of 15.

* Three litters of 0-3 live births; one dam killed 4 pups of litter of 7

TABLE XIV-4. Effect of Exposure to an Electric Field on Body Weight* During Prenatal Development and the First Eight Postnatal Days (Mean $\pm$ SE)

Age (Days)	Males		Females	
	<u>Exposed</u>	<u>Sham-Exposed</u>	<u>Exposed</u>	<u>Sham-Exposed</u>
1	7.6 $\pm$ 0.2	7.9 $\pm$ 0.1	7.1 $\pm$ 0.1	7.3 $\pm$ 0.1
7	17.0 $\pm$ 0.4	17.6 $\pm$ 0.5	15.8 $\pm$ 0.4	16.9 $\pm$ 0.4
10	24.0 $\pm$ 0.5	24.9 $\pm$ 0.7	22.6 $\pm$ 0.5	23.9 $\pm$ 0.6
14	34.6 $\pm$ 0.7	35.4 $\pm$ 0.9	32.5 $\pm$ 0.6	34.1 $\pm$ 0.7
16	42.0 $\pm$ 1.1	42.7 $\pm$ 1.1	39.5 $\pm$ 1.0	40.9 $\pm$ 0.9
21	61.2 $\pm$ 1.3	62.4 $\pm$ 1.7	57.3 $\pm$ 1.1	58.9 $\pm$ 1.6
28	106.0 $\pm$ 2.5	109.0 $\pm$ 2.7	93.9 $\pm$ 1.1	97.2 $\pm$ 1.7
35	166.0 $\pm$ 2.6	166.0 $\pm$ 3.0	139.0 $\pm$ 1.5	142.0 $\pm$ 1.8
42	225.0 $\pm$ 4.1	225.0 $\pm$ 3.9	178.0 $\pm$ 2.2	182.0 $\pm$ 2.0

* Calculated as mean of individual litter means.

TABLE XIV-5. Effects of Prenatal and Neonatal Exposure to Electric Field on Percent of Offspring Showing Criterion or Normal Response to Several Measures of Neuromuscular Development (Approximately 150 offspring per group)

Measure of Development	Age (Days)							
	Exposed				Sham-Exposed			
	M	7	14	21	1	7	14	21
Grasp	75	35	95	89	82	46	96	91
Retraction	94	100	100	100	98	100	100	100
Turn Over	66	99	100	100	68	99	100	100
Righting	--	51	59	99	--	44	77	98
Visual Placing	--	--	--	94	--	--	--	91
Placing	--	--	--	78	--	--	--	82
Hopping	--	--	--	97	--	--	--	93
Upright	10	15	95	--	20	14	94	--
Head Lift	77	32	99	100	79	45	98	98
Movement	18	20	67	99	11	24	59	97
Vocalization	9	12	--	--	7	20	--	--
Grooming	--	--	61	97	--	--	41	91
Startle	--	--	95	98	--	--	93	93
Gait	--	--	--	99	--	--	--	97
Stand w/support	--	--	35	99	--	--	26	98
Stand Alone	--	--	56	74	--	--	32	76
Neg. Geotropism	--	--	46	82	--	--	47	78

Development Indices

Median Incisor Eruption (Days of Age)	14	14
Median Eye Opening (Days of Age)	14-15	14-15
Median Ear Unfolding (Days of Age)	<14	<14
Testes Descent (Weeks of Age)	3-4	3-4
Vaginal Opening (Weeks of Age)	5-6	5-6

strain of rat is insensitive to audiogenic seizure induction. Pretreatment with Metrazol, in replicate three, produced a number of seizures in both groups. The number showing seizures on subsequent exposure to sound was not different between the two groups.

D. DISCUSSION AND CONCLUSIONS

The results demonstrate that exposure to the electric field for 6 days prior to mating did not influence the fertility or mating performance of either males or females. Exposure continuing through 20 days of gestation likewise did not influence the morphologic integrity of the offspring, nor their size. Continued exposure of the sires of these litters, as well as of females which did not mate during the initial breeding period, did not alter their subsequent breeding performance or their ability to implant and maintain a litter to 14 days of gestation.

Only a few of the measures of postnatal neuromuscular development which were evaluated have suggested differences between the sham-exposed and the exposed groups. These results have not yet been subjected to statistical analysis, so that it is premature to speculate on their biological significance. It is obvious that in addition to routine statistics such analyses must include a determination of litter effect and of replicability, which can be established because of the replicate design. Although any effects which are subsequently established as significant are of a transient nature, they may provide a clue as to possible subtle alterations. It is clear, however, that exposure to an electric field under the conditions of Experiment 2, i.e., during gestation

and for the first 8 days of prenatal life, is without significant effect on the growth or survival of the offspring. It should be noted, however, that there are ongoing analyses of the reproductive fitness of these animals and that the organ weight and morphologic data still have not been tabulated.

E. FUTURE PLANS

The remaining analyses from Experiment 2 will be completed in the near future. An additional experiment in which the animals will be exposed during late gestation, suckling, and early postweaning life will be initiated in the near future, using measures similar to those described here.

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XV. ANIMAL BEHAVIOR

Principal Investigator: D. L. Hjerresen

ABSTRACT

Studies were conducted on the effect of exposure to 60-Hz electric fields on behavior of rats in a shuttle box in which one side was shielded from exposure and the other unshielded. Rats showed a preference for the unexposed side at field strengths of 90 and 105 kV/m, but not at 60 or 75 kV/m. Exposure to the electric field has no effect on traverse activity.

A. INTRODUCTION

During the current reporting period, behavioral experiments were conducted to determine 1) at what field strength rats prefer to remain shielded from electric field exposure; 2) the effect of exposure on activity; and 3) the effect of exposure on circulating corticosterone levels. The current experiments are relevant to literature reports of decreased activity and an unexposed side preference at 10-20 kV/m (Bayer et al., 1977), as well as decreased corticosterone levels following exposure in rats (Marino, 1976; in press).

In our Second Interim Progress Report (Phillips and Kaune, 1977), we reported the results of two side preference experiments that indicated that rats prefer to be shielded from exposure to electric fields at ≥ 90 kV/m. At and below 75 kV/m, no significant differences were seen in side preference between exposed and sham-exposed rats. The preference

was evident in a 45-min test session and did not change significantly over three additional 45-min sessions over three subsequent weeks. No evidence was seen in either experiment of an effect of exposure on activity. However, during these experiments harmonic distortion was found in the high-voltage waveform and field-strength instability of an unknown origin was noted inside the shuttle-box apparatus. Both problems were corrected as described in Section II of this report. An experiment was conducted to make sure the distortion and instability noted in the earlier experiments did not cause the rats to prefer the shielded side in the shuttle box at ≥ 90 kV/m.

B. METHODS

Forty male Sprague-Dawley rats were maintained on water and standard pellet food (Purina) ad libitum in stainless steel cages with wire mesh floors, two rats to a cage. Rats were 75 days of age at the onset of experimentation. On the basis of equivalent means and variances in body weights, the rats were assigned to five experimental exposure groups (N=8) corresponding to the field strengths tested: 0, 60, 75, 90 and 105 kV/m.

Exposures were conducted using the Special Studies Exposure System as described in detail in our Second Interim Progress Report. The rat was placed in a long, narrow shuttle box (shown in Figure XV-1 and described in the Second Interim Progress Report). One end of the shuttle box is covered with aluminum window screen to form a Faraday shield and the opposite end of the shuttle box is covered with plastic window

FIGURE XV-1. Shuttlebox system for rat behavioral experiments.

screen which has no effect on field strength or uniformity. The center 5 1/2" of the shuttle box was not covered with screen so as to obtain a 50% field strength in the center of the box and to allow the use of a photo detection system.

The operation of the photo detector system, which monitored and recorded the location of the rat in the box (right, center, left), was described in detail in the Second Interim Progress Report.

Each rat was tested for 45 min with the run order balanced across groups and time of day. Within 2 min of the end of exposure the rat was anesthetized with halothane gas and a blood sample (6-8 ml) drawn by cardiac puncture. Blood samples were fluorometrically analyzed for the concentration of circulating corticosterone.

A power failure during the experiment forced us to exclude from consideration data from one rat in the 90-kV/m exposure group. During the corticosterone analyses four blood samples were deleted due to procedural errors. Preference and corticosterone level values are presented only for the remaining rats.

C. RESULTS

The mean preference of each exposure group for the exposed, unexposed and center regions of the shuttle box are shown in Table XV-1. As observed in previous experiments, rats exposed to 90 and 105 kV/m spent significantly more time in the shielded compartment than sham-exposed controls. No significant differences were seen in traverse activity between the exposure groups.

The mean circulating corticosterone values for the exposure groups are shown in Table XV-2. No significant difference was seen between groups.

TABLE XV-1. Mean ($\pm$ SE) Percent of Time Rat Spent in Exposed, Center and Unexposed (Shielded) Regions of a Shuttle Box During Exposure to Electric Fields for 45 Min.

Field Strength (kV/m)	No. Rats	Percent Residence Time			Significance
		Exposed	Center	Unexposed	
0	8	39.88 $\pm$ 3.63	14.75 $\pm$ 3.33	45.50 $\pm$ 4.03	---
60	8	39.63 $\pm$ 2.91	10.75 $\pm$ 0.92	49.13 $\pm$ 3.45	---
75	8	37.00 $\pm$ 5.09	13.63 $\pm$ 1.37	49.50 $\pm$ 4.52	---
90	7	30.71 $\pm$ 1.82	11.43 $\pm$ 1.80	57.86 $\pm$ 2.78	*
105	8	31.50 $\pm$ 4.43	11.38 $\pm$ 1.47	57.00 $\pm$ 5.55	**

* $t = 2.57$, $p < 0.05$, 1 tailed t test with 13 df, compared to 0 kV/m controls.

** $t = 1.79$, $p < 0.05$, 1 tailed test with 14 df, compared to 0 kV/m controls.

TABLE XV-2. Mean Circulating Corticosterone Values (ng/ml) of Rats Exposed to 60-Hz Electric Fields of 0, 60, 75, 90, and 105 kV/m. Blood samples were taken within 2 minutes after the completion of the shuttlebox test.

Field Strength (kV/m)	N/Group	Corticosterone (Mean $\pm$ SE)
0	8	293.14 $\pm$ 35.61
60	7	283.57 $\pm$ 48.07
75	7	305.71 $\pm$ 37.90
90	7	204.99 $\pm$ 62.70
105	6	310.83 $\pm$ 59.89

D. DISCUSSION

The results of the side preference experiment confirm that the initial studies on side preference were not seriously affected by the harmonic distortion or waveform instability problems.

In contrast with previous reports (Marino et al., 1976; in press) no differences in plasma corticosterone levels were observed between exposed and control groups. These data are not directly comparable, however, since differences in field strengths and exposure durations exist between these studies and Marino's.

Results of all experiments to date confirm that rats prefer to remain shielded from electric field exposure, consistently at high field strengths (90-133 kV/m), but not at lower field strengths (50-75 kV/m). No significant changes in the general activity of the rats, as measured in side-to-side traverses, have been observed. The threshold for side preference seen in these experiments is much higher than that seen by Bayer et al. (1977), possibly because of the short duration of our experiments compared to the 1-yr exposure made by Bayer et al.

E. FUTURE PLANS

Two experiments are in progress to complete the side preference studies. The first is a 24-hr test using 40 rats at 0, 25, 50, 75 and 100 kV/m. The second experiment is to expose rats to 100 kV/m for 30 days and then test them at 0, 25, 50, and 75 kV/m. In both studies blood samples are being taken at the end of the test session for analysis of circulating corticosterone levels.

The next phase of behavioral studies will use a variety of methods to determine the field strength threshold of electric field perception and the modality of perception. It is presently thought that stimulation of the hair of the rat may be responsible for the perception and subsequent preference to remain shielded from the electric field. We have initiated a breeding program to establish a small colony of nude (hairless) rats (Palm and Ferguson, 1976). The perception threshold of electric fields for these rats will provide an excellent comparison to the studies conducted with normal rats in order to examine the role of hair in the perception of the electric field. Pilot studies with nude rats will begin in January, 1978, with full scale experiments scheduled to begin in April, 1978. Also, work will continue using photographic techniques to determine the threshold for hair movement.

We plan to quantitate any aversive component of the preference phenomenon. A Conditioned Emotional Response (CER) paradigm will be used where the presence of the electric field signals an impending aversive event (typically, electric footshock) and requires an avoidance response by the rat. A variation of this paradigm will use the electric field as the aversive event to be avoided.

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XVI. SUMMARY AND CONCLUSIONS

With two exceptions (Central Nervous System and Neurophysiology), the biological experiments with animals exposed to 60-Hz electric fields at 100 kV/m have been completed. Results of the completed work are summarized in Table XVI-1. The Hematology study found 21 statistically significant ($p \leq 0.05$) differences between exposed and sham-exposed rats and mice. However, no trend of effects was evident and none of the effects were reproduced in replicate experiments. The results of this study do not agree with those of Meda et al. (1972) and Bianchi et al. (1973a), who reported that rats and mice exposed to 100 kV/m had lower lymphocyte concentrations than control animals, or with those of Poznaniak et al., who (1977) reported decreased red blood cell and increased white blood cell concentrations in mice exposed to 25 and 50 kV/m.

In the Immunology experiments, no effects of exposure of rats (15 and 30 days) or mice (30 and 60 days) were observed on serum immunoglobulin concentrations, relative concentrations of T- and B-lymphocytes, or levels of complement. One measure of the delayed hypersensitivity response (skin thickness) to keyhole limpet hemocyanin was decreased, but another (reaction diameter) was unchanged in exposed mice. In a replicate experiment the effect was not statistically significant ($p > 0.05$), however the trend was consistent, i.e. the skin thickness in exposed mice was 22% less than that of sham-exposed animals. This potential effect will be studied in another experiment.

TABLE XVI-1. Summary of Effects of Exposure of Rodents to 60-Hz, 100-kV/m Electric Fields

Study	Species	Exposure Duration (days)	Parameters	Effects
Hematology	Rats	15	Hematology	Decreased leukocytes NR Decreased lymphocytes NR
		Replicate		β -globulin increased NR Triglycerides decreased NR
		30	Hematology	α -globulin decreased NR
		Replicate		Bone marrow cells decreased NR
		60	Hematology	Platelets decreased NR β -globulin decreased NR
		Replicate		Lymphocytes increased NR Leukocytes increased NR
	Mice	30	Hematology	Red blood cell concentration increased NR Hemoglobin increased NR Volume of red cells increased NR
		Replicate		NE
		60	Hematology	Leukocytes decreased NR Neutrophils decreased NR α -globulin decreased NR Lymphocytes decreased NR
		Replicate		Leukocytes increased NR Neutrophils increased NR Lymphocytes increased NR Eosinophils increased NR
Immunology	Rats	15, 30	Serum Immunoglobulins	NE
	Mice	30, 60	Serum Immunoglobulins T and B Lymphocytes Cell-Mediated Immunity Complement	NE NE Increased skin thickness to KLH NR NE
Pathology	Rats	30	Histopathology	Increased prostatitis NR
	Mice	30	Histopathology	NE
Metabolic Status and Growth	Rats	30	Daily body weight, food and water intake, O_2 consumption, CO_2 production	NE NE

TABLE XVI-1 (Continued)

Study	Species	Exposure Duration (days)	Parameters	Effects
Bone Growth	Rats	30	Bone Growth	NE
Cardiovascular Function	Rats	8 hr, 30 days	Heart Rate, ECG Analysis	NE
Endocrinology	Rats	30	Hormones, Endocrine Gland Weights	NE
Neurophysiology	Rats	30	Synaptic Transmission	NE
Central Nervous System	Mice	30	Histopathology Electron Microscopy (preliminary)	NE NE
Reproduction and Development	Rats	30	Mating and Fertility Fetal Development	NE NE
Behavior	Rats	45 min	Side Preference Corticosterone Activity	Preferred no field ≥ 90 kV/m NE NE

NOTE: NE = no effects

NR = effect not reproduced in replicate experiment

The Pathology experiment observed no reproducible effects, in contrast to Dumansky et al. (1976), who reported histopathological effects in brain, liver, kidney, adrenals, thyroid and myocardium in rats exposed to 0.1 to 5 kV/m.

There was an apparent higher incidence of prostatitis in exposed rats (35%) compared to sham-exposed controls (22%) when the data from four experiments were pooled. Although the differences are not statistically significant, a trend is apparent. The high incidence of the lesion in control animals reflects the high susceptibility of rats to this disease. If the electric field increased the susceptibility, the effect should be more visible in the histopathology survey of rats presently being exposed for 120 days.

Exposure of rats to a 60-Hz electric field at 100 kV/m for 30 days had no effect on metabolic status. In addition, the body weights of rats at the end of the exposure were measured in 4 additional experiments in this project and those of mice in three experiments (Table XVI-2). There were no significant effects of electric field exposure on the body weights of mice or rats. These data disagree with previous studies which reported that electric fields caused reduced body weight in mice (Marino et al., 1976a) and rats (Marino et al., 1976b), as well as increased body weight of mice and chickens (Poznaniak et al., 1977).

The weights of various organs of rats were measured in four studies and of mice in two studies. In one study liver weight of exposed rats was lower than sham-exposed controls; this result was not found in a

TABLE XVI-2. Results of Body and Organ Weight Measurements
From Various Experiments

Rat Body Weights

Bone Growth	NE
Endocrinology	NE
Metabolic Status and Growth	NE
Pathology	NE
Reproduction (Fetal and Neonatal)	NE

Rat Organ Weights

Bone Growth	Liver decreased, NR
Endocrinology	NE
Metabolic Status and Growth	NE
Pathology	NE

Mouse Body Weights

Pathology	NE
Hematology (30 day, 60 day)	Body weight increased (60 day), NR
Immunology (30 day, 60 day)	NE

Mouse Organ Weights

Pathology	Liver increased, left kidney decreased
Hematology (30 day, 60 day)	Spleen decreased (60 days only), NR

NE = no effect

NR = effect not reproduced in replicate experiment

replicate experiment, nor was it observed in the other three studies. Liver weights of exposed mice were higher than controls in the Pathology study while those of the left kidney were lower. However, in another experiment (Hematology), the only effect observed was reduced spleen weight in mice exposed for 60 days. Since the few observed effects on organ weights were not consistent, it seems likely that the differences are random and are not effects of exposure to electric fields.

No effect of exposure on bone growth was observed in rats.

In contrast to reports by Meda et al. (1972) and Blanchi et al. (1973b) of alterations in electrocardiograms of mice that had been exposed to 100 kV/m for 500-1000 hr, no effects were observed in this project on electrocardiograms or heart rates of rats exposed for 8, 40, or 611 hr at a similar field strength.

In the Endocrinology experiments, no effects were found on the serum levels of several hormones. In contrast, other studies have reported decreased corticosterone in rats (Marino et al., 1976b) and increased corticosterone levels in mice (Poznaniak et al., 1977).

No significant differences between exposed and sham-exposed animals were observed in any of the six measures of synaptic transmission function in the Neurophysiology experiments. The data from the experiments that measured the conditioning-test and post-tetanic responses as well as the amplitude of the postsynaptic action potential, however, show an apparent trend that suggests increased excitability of the synapses in exposed animals. Further experiments are required to ascertain the importance

of the observations since they are not statistically significant. The second and third phases of the neurophysiological experiments on rats exposed for 30 days will be completed before any experiments of the first phase are replicated.

In contrast to the report of Dumansky et al. (1976), a detailed histopathological study of the brains of mice exposed for 30 days revealed no differences between exposed and sham-exposed animals. A preliminary examination with the electron microscope (of one mouse brain in each exposure group) was also negative.

No effects of the electric field were observed on mating, fertility or on fetal and postnatal development of rats. These data disagree with those of Knickerbocker (1967), who reported that the male progeny of rats exposed to 160 kV/m were smaller than controls, and those of Marino et al. (1976a), who reported that mice exposed to 10-15 kV/m for three generations had lower body weights than controls.

Results of the behavioral experiment confirmed results of experiments reported in the last Interim Report. Rats showed a preference for the shielded side of a shuttlebox at field strengths of 90 kV/m and higher. Experiments to measure side preference of rats during a 24-hr exposure period are in progress to determine the effect of a longer exposure duration on perception and preference thresholds.

Excluding the Behavior and Neurophysiology studies, 412 t tests were used to compare parameters of exposed and sham-exposed animals in this project. One would expect that 5% of these, or 21, would appear to

be significantly different at $p \leq 0.05$ simply due to chance (e.g., if no effects were produced by exposure to electric fields). There were 29 significant differences at $p \leq 0.05$ in the series of experiments we conducted. This does not suggest that the differences observed are not real effects of electric field exposure, but it must be noted that a number of false positive results can be expected. For this reason, effects that are statistically significant must be replicated before valid conclusions can be made as to whether or not it is a true effect or simply a random event resulting from examining many parameters. Only the behavioral effect was replicated in our experiments.

When one discards effects that were inconsistent with other experiments or were not reproduced, three items remain as possible effects: the decreased cell-mediated immune response in the skin of mice (Immunology), the increased prostatitis (Pathology), and the increased excitability of synapses (Neurophysiology). The cell-mediated immunity and prostatitis effects will be examined in further experiments. These were not statistically significant but showed trends that suggest potential effects. Except for the preference to avoid the field shown in the Behavior study, no observation can be confidently identified as an effect of the electric field.

It is possible that more prolonged exposure of the rodents will produce effects that were not evident in the 30-day exposures, as suggested by Dumansky et al., 1976. Upon completion of the 30-day exposure experiments we plan to start exposing rats and mice for 4-months and conduct a series of screening experiments as was done with the 30- and 60-day exposures.

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APPENDIX ACALCULATION OF SURFACE FIELDS AND SHORT CIRCUIT CURRENTS
FOR A HEMI-ELLIPSOID RESTING ON THE GROUND

Consider a conducting hemi-ellipsoid, simulating a rat in electrical contact with the ground and exposed to a vertical electric field. While it is possible to treat this problem at 60 Hz, it is perhaps more instructive to treat it as an electrostatic problem and demonstrate how the ac case can be extracted from the static solution.

It has been shown (Phillips et al., 1976) that tissue is a good conductor at 60 Hz; so good, in fact, that the surface of an animal exposed to 60-Hz electric fields will behave nearly as a perfect Faraday shield: the internal fields will be reduced from the external fields by a factor of about 10^{-8} in most parts of the body (except the legs). We will thus begin our analysis by solving the static case, deriving equations for the external fields and charge density on the surface of the ellipsoid. At 60 Hz, these equations will not be significantly altered by the small fields now present inside the body. However, the charge density now varies with time, requiring internal currents. These currents will then be calculated, giving us a complete approximate solution at 60 Hz.

Consider a hemi-ellipsoid resting on a ground plane and exposed to a field which is uniform at infinity. This situation is shown in Figure III-2.

The surface of the ellipsoid is given by

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1, \text{ where } a > b > c.$$

For the case of a rat we take the electric field $\bar{E}$ along the Y axis. At a long distance from the ellipsoid, the uniform field potential is

$$\phi = -E_0 y.$$

Stratton (1941) gives the solution for an isolated ellipsoid in a static uniform electric field. This solution is expressed in the curvilinear coordinate system, where the coordinates of any point (q_1, q_2, q_3) are defined by the three solutions to the cubic equation:

$$\frac{x^2}{a^2 + q} + \frac{y^2}{b^2 + q} + \frac{z^2}{c^2 + q} = 1.$$

$$\begin{aligned} q_1 &> -c^2 \\ -b^2 &< q_2 < -c^2 \\ -a^2 &< q_3 < -b^2 \end{aligned}$$

Notice that $q_1 = a$ constant defines a family of ellipsoids. From this definition one can show that

$$x = \pm \left[\frac{(q_1 + a^2)(q_2 + a^2)(q_3 + a^2)}{(b^2 - a^2)(c^2 - a^2)} \right]^{1/2}$$

and

$$y = \pm \left[\frac{(q_1 + b^2)(q_2 + b^2)(q_3 + b^2)}{(a^2 - b^2)(c^2 - b^2)} \right]^{1/2}.$$

(1)

The equation is similar for z . In this coordinate system the total electrical potential is

$$\phi = \frac{-E_0 y I_2(q_1)}{I_2(\infty)},$$

where

$$I_2(q_1) = \int_0^{q_1} \frac{d\xi}{(\xi+a^2)^{1/2}(\xi+b^2)^{3/2}(\xi+c^2)^{1/2}}. \quad (2)$$

While this solution is for an isolated ellipsoid in a uniform field, we note that $\phi = 0$ for the plane $y = 0$, so that it is also the solution for a hemi-ellipsoid resting on a ground plane.

The surface of the ellipsoid is defined by $q_1 = 0$. Thus the normal electric field at the ellipsoid surface is

$$E_n = -\frac{1}{h_1} \left. \frac{\partial \phi}{\partial q_1} \right|_{q_1=0},$$

where h_1 , the q_1 metric function, is defined by

$$h_1 = \frac{\partial s}{\partial q_1} = \left[\left(\frac{\partial x}{\partial q_1} \right)^2 + \left(\frac{\partial y}{\partial q_1} \right)^2 + \left(\frac{\partial z}{\partial q_1} \right)^2 \right]^{1/2}$$

$$h_1 = \frac{1}{2} \left[\frac{(q_1 - q_2)(q_1 - q_3)}{(q_1 + a^2)(q_1 + b^2)(q_1 + c^2)} \right]^{1/2} \quad (3)$$

A direct calculation gives

$$E_n = \frac{2E_0 y}{b^2 (q_2 q_3)^{1/2}} \frac{1}{I_2(\infty)}. \quad (4)$$

Furthermore, from Stratton (1941) we have

$$(q_2 q_3)^{1/2} = abc \sqrt{\frac{x^2}{a^4} + \frac{y^2}{b^4} + \frac{z^2}{c^4}}. \quad (5)$$

Using Gauss's Law, the charge density on the surface of the ellipsoid is

$$\sigma = \frac{2\epsilon_0 E_0 y}{b^2 (q_2 q_3)^{1/2}} \frac{1}{I_2(\infty)}. \quad (6)$$

We have already argued that at 60 Hz we can still use static expressions for the normal electric field, E_n , and the surface charge density, σ . But now both E_n and σ have a $e^{j\omega t}$ time dependence. There will be a current density, $\bar{J}$, inside the ellipsoid. At the ellipsoid surface, current conservation gives the boundary condition:

$$J_n = j\omega\sigma. \quad (7)$$

Also, the current density is related to the internal electric field $\bar{E}'$ by Ohm's Law:

$$\bar{J} = \eta \bar{E}',$$

where η is the conductivity.

We must find a solution for $\bar{E}'$ satisfying Maxwell's equations and the boundary condition [eq. (7)]. Certainly the simplest choice is a uniform current density, J , along the y axis (i.e., vertical):

$$\bar{J} = J\hat{y}.$$

This satisfies Maxwell's equation. To check the boundary conditions [eq. (7)], we must first calculate J_n . If $\hat{n}$ is the outward normal to the ellipsoid

$$J_n = J\hat{n} \cdot \hat{y} = J \frac{1}{h_1} \frac{\partial y}{\partial q_1}.$$

Using equations (1), (3), (5), and (6) in the boundary condition (7), we get

$$J = \frac{2j\omega\epsilon_0 E_0}{abc I_2(\infty)}. \quad (8)$$

Notice that J is uniform, in accord with our earlier assumption. The short circuit current, I_{sc} , is the total current passing the lower face of the hemi-ellipsoid. Thus,

$$I_{sc} = \frac{2j\pi\omega\epsilon_0 E_0}{b I_2(\infty)}. \quad (9)$$

The only remaining problem is to calculate the integral $I_2(\infty)$ defined by eq. (2). This may be rewritten

$$I_2(\infty) = \frac{-2\partial}{\partial b^2} \int_0^\infty \frac{d\xi}{(\xi+a^2)^{1/2}(\xi+b^2)^{1/2}(\xi+c^2)^{1/2}}$$

The substitution $u = (\xi + a^2)^{1/2}$ brings the integral into a form which can be solved (Jahnke and Emde, 1945).

$$I_2^{(\infty)} = \frac{4}{(a^2 - c^2)^{1/2}} \frac{\partial}{\partial b^2} F(k, \phi) \text{ with}$$

$$k = \left(\frac{a^2 - b^2}{a^2 - c^2} \right)^{1/2} \text{ and } \sin \phi = \frac{(a^2 - c^2)^{1/2}}{a} \quad (10)$$

$F(k, \phi)$ is the incomplete elliptic integral of the first kind defined by

$$F(k, \phi) = \int_u^{\psi} \frac{d\psi}{\sqrt{1 - k^2 \sin^2 \psi}}$$

Only k involves b^2 and the derivative of F with respect to k is tabulated (Jahnke and Emde, 1945). By solving this equation algebraically, we get:

$$I_2^{(\infty)} = \frac{2}{(a^2 - c^2)^{1/2} (b^2 - c^2)} - \left(\frac{b^2 - c^2}{a^2 - b^2} \right) F(k, \phi) + \left(\frac{a^2 - c^2}{a^2 - b^2} \right) E(k, \phi) - \frac{c(a^2 - c^2)^{1/2}}{ab},$$

where $E(k, \phi)$ is an incomplete elliptic integral of the second kind.

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