433

IN VIVO BIOELECTROCHEMICAL CHANGES ASSOCIATED WITH EXPOSURE TO EXTREMELY LOW FREQUENCY ELECTRIC FIELDS

ANDREW A. MARINO, THOMAS J. BERGER, B. PETER AUSTIN, ROBERT 0. BECKER, and FRANCIS X. HART*

Veterans Administration Hospital and Upstate Medical Center, Syracuse, New York; and *University of the South, Sewanee, Tennessee

• *One hundred seventy-four 21- to 24-day-old Sprague-Dawley rats were continuously exposed to a 60 Hz electric field of 150 V/cm for one month in ten separate experiments. Biological effects observed included depressed body weights, serum corticoids, and water consumption. The findings are tentatively interpreted as indicating that a power frequency electric field is a biological stressor. The observed effects cannot be a consequence of Joule heating and therefore indicate that electric fields can influence biological systems either at the systemic level, or at the cellular level via electrochemical alteration of the microenvironment.*

INTRODUCTION'

While there have been many reports of biological effects resulting from exposure of organisms to electromagnetic (EM) fields,[1-4] no generally accepted coupling mechanism between organism and field has yet been elucidated. During the past decade, two new biological concepts were proposed that bear upon the problem. The first was the concept, drawing partially upon experimental results and partially upon theoretical considerations, of an analog type data transmission and control system antedating the central nervous system proper.[5,6] Evidence has been presented for this system being based upon semiconduction or other solid state physical processes.[7,8] Information is carried as small currents and voltages that produce changes in the local electrical environment of the cells. The second concept is that of electrochemical information transfer associated with the mechanisms involved in the local cellular responses to such alterations in the local electrical environment.[9,10] Both concepts imply that exposure to EM fields should have generalized biological effects; in the first case by perturbation of an operating system and, in the second, by direct cellular effects.

In view of the widespread alteration in the electrical environment produced by electrical power transmission systems, a study of the generalized effect of exposure to 60 Hz electrical fields was undertaken. This paper reports the results of that study.

METHODS

Male Sprague-Dawley rats, 21-24 days old, were continuously exposed to a 60 Hz electric field for approximately one month. The nominal electric field, computed from the plate separation and the applied voltage, was 150 rms-V/cm. It was applied across plastic cages (Fig. 1) with a variety of grounded metal tops (as shown in Fig. 2).

All rats were purchased from commercial breeders. Except where noted, they were 1-2 days in transit and were held 1-2 days after arrival prior to initiation of field exposure. All rats placed on study were free of any recognizable diseases or defects. Occasionally, respiratory infections occurred during exposure; in such cases the animal was destroyed. All rats were maintained in a single room of a government accredited, standard (i.e., not pathogen free) animal care facility and were
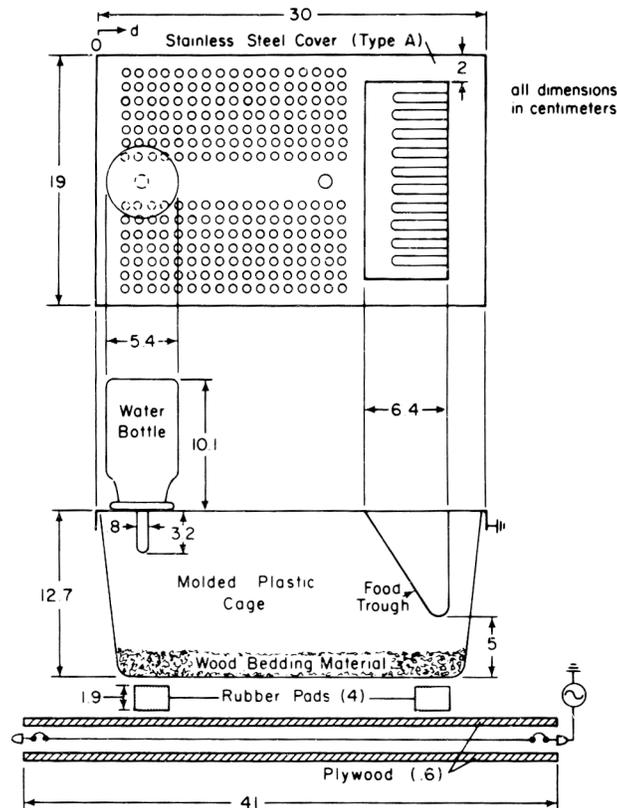

FIGURE 1. Apparatus employed to generate the power frequency electric field. A metal plate was permanently mounted between two sheets of plywood with provisions for applying and measuring the working voltage. Vibration isolation pads that supported the cage were glued to the upper wood surface.
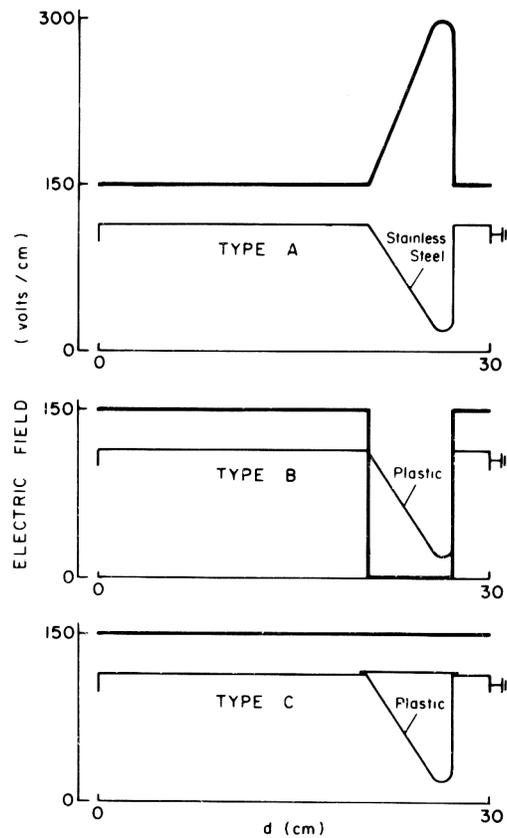
FIGURE 2. Three different designs for the grounded cage top. (1) An all stainless steel top-Type A. (2) A modification of Type A in which the metal feed trough was replaced with one of plastic-Type B. (3) A modification of Type B in which a stainless steel lid covering the plastic feed trough was added-Type C. Calculated electric field profiles corresponding to each type of cage top are shown. Perturbing effects due to the presence of the various dielectric materials and the water bottle were neglected.

fed and watered *ad libitum*. Environmental conditions were 23°C, 50% relative humidity, and light/dark cycle of 12:12.

Following exposure, the rats were weighed then sacrificed by decapitation. The serum was recovered and frozen until analyzed. In the first four experiments the experimental rats were housed in individual cages, similar to that shown in Fig. 1, with Type A cage tops (Fig. 2). Control rats were housed three per cage in larger cages with metal tops. In addition to final body weight, we measured serum hydroxycorticosterone (corticoids) and serum proteins in the pooled sera of all rats within each of the experimental and control groups. In the remaining experiments every rat was caged individually, and vibration isolation pads were added. The pads reduced the electric field induced vibration in the vicinity of the cages from 2.5×10^{-3} cm/sec to 1.0×10^{-3} cm/sec. (Normal background vibration was

2.8 X 10^{-3} cm/sec.) One of three types of cage tops was employed, depending on the particular experiment (Fig. 2). The food and water consumed by every rat were measured as were the final body weights and the final weights of the pituitary and adrenal glands. Serum corticoids were measured in sub-pools of 2-3 rats, and serum glutamic oxaloacetic transaminase (SGOT) was measured in the pooled sera of all rats within each group. Except where noted, all listed tolerances are standard deviations. All statements of statistical significance are based on the t-test (two-tail) with $p < 0.05$.

Corticoids were measured fluorometrically.[11] Total proteins were measured by the Biuret method[12] corrected for hemolysis.[13] Percent albumin was determined by electrophoresis on cellulose acetate, with planimetric integration.[14] SGOT was measured in a clinical laboratory by autoanalyzer.

RESULTS

In Experiment 1, the rats exposed to the electric field exhibited altered concentrations of serum corticoids and albumin as well as depressed body weights. In the first replicate study (Experiment 2, Table I), the exposed rats again showed depressed serum corticoids and elevated serum albumin; however, the body weights were not significantly different (at 5%). In the second replicate study (Experiment 3, Table I), results similar to Experiment 2 were observed. In the first three experiments, the experimental serum corticoids were depressed by a grand mean of 31.7% with a standard error of 2.4%. The corresponding values for the

TABLE I. Effects of Continuous Exposure to Power Frequency Electric Fields on Some Biological Parameters of Rats. Serum corticoids and albumin were measured employing the pooled sera of all rats within each group. Italic numbers in first column indicate age in days at initiation and termination of exposure.

Experiment	Number of rats	Final body weights (grams)	Serum corticoids (μg/ 100 ml)	Serum proteins (g/dl)	
				Total	Albumin
1 *(23,52)*	14 experimental	244.0[a] ± 12.6	14.6	7.4	4.4
	19 control	273.1 ± 16.7	22.0	7.1	3.8
2 *(24,57)*	14 experimental	276.5 ± 24.1	12.4	8.4	4.4
	22 control	290.8 ± 27.9	18.0	6.6	3.2
3 *(22,55)*	14 experimental	270.4 ± 14.3	10.4	8.8	4.6
	18 control	277.8 ± 15.8	14.5	7.1	3.5
4 *(21,51)*	14 experimental	228.7[a] ± 26.1	56.8	—	—
	11 control	251.0 ± 11.3	53.4	—	—

[a] $p < 0.05$

increase in albumin were 28.2% and 9.1%. The data also suggested that the average body weight was lower in the experimental groups (by a grand mean of 6.6% with a standard error of 4.3%), but a 5 % level of confidence within each experiment was achieved only in Experiment 1.

Experiment 4 was performed to determine whether the observed disturbances in the adrenal-pituitary system would prevent the exposed rats from responding to a known stress. As previously, the rats were exposed for one month and weighed. A lower average weight in the exposed group ($p < 0.05$) was observed. Immediately after weighing, the rats were subjected to a cold stress (-13°C for 1 h) and sacrificed. The serum corticoids in both groups rose markedly (Table I), indicating that the exposed rats remained capable of responding to a cold stress in the predictable fashion.

In Experiment 5, after one month of electric field exposure, the experimental rats consumed less water, had enlarged pituitaries, and showed depressed levels of serum corticoids (Table II). In Experiment 6, the experimental rats drank less water, exhibited depressed body weights, and showed enlarged adrenals and pituitaries.

In Experiment 7, the allotted period of acclimatization to the laboratory environmental conditions following arrival was increased to four days, after which time exposure was commenced. We found that water consumption was depressed as previously, but that the body and organ weights were normal. Similar results were observed in Experiment 8, wherein an acclimatization period of three days was provided.

In Experiments 9 and 10, we exposed rats obtained from a different source. The animals were purchased locally (shipment time 2 h), and acclimatized for three days prior to exposure. In Experiment 9 we found the water consumed, pituitary weights, and serum corticoids were significantly different in the exposed rats. In Experiment 10 food consumption was the only parameter significantly affected.

Values of SGOT are shown in Table II. The concentration in the experimental sera was marginally higher in some cases (Experiments 5, 7, 9), and substantially higher in others (Experiments 6, 8, 10).

The observed pattern of water consumption was consistent from experiment to experiment, thus deserving some comment. In all experiments in which it was measured, the cumulative water consumer by the experimental and control groups, when compared statistically after 1, 3, 7, and 14 days of exposure, showed no significant differences. In all cases (except Experiment 10) the comparison of water consumed during the last half of the exposure period showed significant differences, with the experimental group exhibiting depressed consumption. The differences remained significant (at 5%) even when the comparisons were extended to include the entire exposure period (Table II). These data are considered particularly important in that they indicate that microcurrents produced in the rats during the act of drinking were not significant determinants of the experimental

TABLE II. Further Effects of Continuous Exposure to Power Frequency Electric Fields on Some Biological Parameters of Rats. Serum corticoids were measured employing sub-pools of 2-3 rats within each group. Italic numbers in first column indicate age in days at initiation and termination of exposure.

Experiment	Number of rats	Cage top type	Water consumed (ml/rat)	Food consumed (grams/rat)
5 (24,57)	15 experimental	C	846[a] ± 68	587 ± 36
	18 control		940 ± 142	603 ± 40
6 (22,54)	14 experimental	A,C	749[a] ± 80	—
	20 control		891 ± 93	—
7 (25,56)	19 experimental	A	819[a]- 83	582 ± 29
	21 control		890 ± 104	588 ± 42
8 (25,55)	16 experimental	B	901a± 50	542 ± 33
	14 control		1.054 ± 84	545 ± 36
9 (24,56)	20 experimental	A	1,003a ± 82	614 ± 32
	20 control		1,099 ± 117	618 ± 43
10 (24,58)	14 experimental	A	1,143 + 157	642[a] ± 31
	16 control		1,202 ± 107	664 ± 17

[a] $p < 0.05$

TABLE II, *continued*

Experiment	Final body weight (g)	Final adrenal weight (μg/g)	Final pituitary weight (μg/g)	Serum corticoids (μg/100 ml)	SGOT (I.U.)
5	293.4 ± 17.0	180.1 ± 19.8	38.7[a] ± 3.2	6.8[a] ± 0.8	198
	286.7 ± 22.1	181.8 ± 16.0	35.2 ± 3.8	8.7± 1.2	194
6	264.8[a] ± 24.1	181.6[a]± 21.1	43.9[a] ± 4.1	7.2 ± 1.5	188
	281.0 ± 12.5	158.9 ± 18.3	40.6 ± 3.1	7.6 ± 2.1	157
7	289.5 ± 14.9	165.3 ± 18.5	32.9 ± 3.1	—	180
	287.8 ± 18.4	168.8 ± 20.3	35.2 ± 2.6	—	157
8	282.4 ± 13.3	155.2 ± 18.4	38.0 ± 2.4	6.0 ± 0.7	137
	283.0 ± 12.7	155.8 ± 30.6	39.0 ± 2.6	6.4 ± 0.6	134
9	286.8 ± 17.9	132.1 ± 15.8	31.4[a] ± 2.4	9.1[a] ± 2.0	196
	290.2 ± 13.2	125.7 ± 14.3	29.4 ± 2.9	16.3 ± 3.8	185
10	294.6 ± 15.2	173.9 ± 20.0	31.2 ± 1.8	9.5 ± 2.0	191
	300.4 ± 12.0	179.3 ± 15.8	30.6 ± 1.8	9.7 ± 4.0	133

[a] $p < 0.05$

results. If either perceptible or subliminal microcurrents were significant factors, alterations in the drinking patterns of the experimental rats would have been apparent from the start of the experiment.

No specific effects were detected in the entire series of experiments that could be ascribed to the different types of field configuration produced by the three types of grounded cage tops. Questions concerning the relative effects of uniform vs. non-uniform fields require further experimentation.

During these studies, which involved a total of 154 experimental rats and 179 control rats, an additional 11 experimental and 5 control rats died during the exposure period.

DISCUSSION

In each of the 10 experiments, one or more measured parameters were significantly different in the experimental animals as compared to the control animals. In general, these results indicate that exposure to a 150 V/cm 60 Hz electric field is productive of a physiological stress response.[15] The physiological response has been shown to be not attributable to such secondary effects as the field induced mechanical vibration or the occurrence of microcurrents produced by drinking, and we conclude that the field itself is the responsible agent.

While there are apparent inconsistencies in the data, to the extent that the same measured parameters are not always statistically significant from one experiment to the next, none of those inconsistencies would mitigate against the general conclusion reached.

It is generally agreed that stressors are additive when assayed by the physiological response.[15] This phenomenon has been manifested as the accentuation of a pre-existing, sub-clinical pathological condition by exposure to low frequency magnetic fields.[16] In the present series of experiments, as in all animal experimentation other than that involving totally germ-free animals in a rigidly controlled environment, the multitude of factors productive of minor stress responses are impossible to completely control. This is evidenced by the disparate results obtained in Experiments 9 and 10. In both experiments we attempted to mitigate the stressful effect of shipment from a distant supplier to the laboratory. The animals were purchased locally so that prolonged transit time was avoided and a period of several days acclimatization was afforded prior to the initiation of exposure. Despite these precautions, Experiment 9 demonstrated measurable differences between experimental and control animals in three parameters, while in Experiment 10 only one parameter was so influenced. We attribute this disparity to other stress-producing factors, such as disturbances in the biological rhythms and the presence of zoonoses, over which we had no control in such acute experiments.

In addition to the microcurrents described above which occurred only during eating and drinking, the exposed rats continuously experienced induced currents

because of the presence of the electric field. To establish the non-thermal nature of the effects described here, we measured the induced current in the rats and found that 0.68 μa was induced at 150 V/cm, with a corresponding current density of about 11.1 mμa/cm^2. If we assume the rat to be a uniform mass with a resistivity of 10012-cm, then the total power dissipated is about 2.3 X 10^{-12} watts, obviously too low to produce heating.

In conclusion, one month's exposure to power frequency electric fields produced quantifiable biological changes in rats. The changes produced in at least some experiments were depressed water consumption, depressed body weight, increased adrenal and pituitary weights, and altered serum concentrations of albumin, hydroxycorticosterone, and SGOT. The observed changes are consistant with, but do not categorically establish, the hypothesis that a power frequency electric field is a biological stressor. To assess the potential hazards of such exposure, further work wherein larger groups of animals might be studied at different exposure times and at different field strengths appears desirable. Additionally, the data do not permit a choice between the two postulated coupling mechanisms with respect to the observed responses. The data do establish however, that there must exist some mechanism other than Joule heating by which electric fields can alter biological function.

Address all correspondence to: Dr. Andrew A. Marino, Veterans Administration Hospital, Syracuse, New York 13210.

REFERENCES

1. A. S. Presman, *Electromagnetic Fields and Life,* Plenum Press, New York, 1970.
2. M. S. Tolgskaya and Z. V. Gordon, *Pathological Effects of Radio Waves,* Consultants Bureau, New York, 1973.
3. J. G. Llaurado, A. Sances, Jr. and J. H. Battocletti, *Biological and Clinical Effects of Low-Frequency Magnetic and Electric Fields,* Charles C. Thomas, Springfield, Illinois, 1974.
4. D. E. Beischer, J. D. Grissett and R. E. Mitchell, "Exposure of man to magnetic fields alternating at extremely low frequency," *NAMRL-1180,* Naval Aerospace Research Laboratory, Pensacola, Florida, 1973.
5. R. 0. Becker, "The significance of bioelectric potentials," *Bioelectrochem. Bioenerg., 1,* 187 (1974).
6. "The basic biological data transmission and control system influenced by elec trical forces," *Ann. NY Acad. Sci., 238* (1974).
7. The neural semiconduction control system and its interaction with applied elec trical current and magnetic fields," in *Proceedings of the XI International Congress ofRadiology,* Excerpta Medical Foundation, Amsterdam, 1966, p. 1753.
8. "Search for axial current flow in peripheral nerves of salamander," *Science, 134,* 101 (1961).

9. A. A. Pilla, "Electrochemical information transfer at living cell membranes," *Ann. NY Acad. Sci., 238,* 149 (1974).

10. R. O. Becker and A. A. Pilla, "Electrochemical mechanisms and the control of biological growth processes," in *Modern Aspects of Electrochemistry, No.* 10, J. Brockris, Ed., Plenum Press, New York, 1975, p. 289.

11. D. Mattingly, "A simple fluorimetric method for the estimation of free 11-hydroxycorti-coids in human plasma," *J. Clin. Pathol., 15,* 374 (1962).

12. T. E. Weichselbaum, "An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma," *Am. J. Clin. Pathol., 16,* 40 (1946).

13. J. B. Miale, *Laboratory Medicine: Hematology,* C. V. Mosley Company, St. Louis, 1972, p. 1214.

14. A. A. Marino, T. J. Berger, J. T. Mitchell, B. A. Duhacek and **R. O.** Becker, "Electric field effects in selected biological systems," *Ann. NY Acad. Sci., 238,* 436 (1974). *15. H.* Selye, *Stress,* Acta, Inc., Montreal, 1950.

16. H. Friedman and R. J. Carey, "The effects of magnetic fields upon rabbit brains," *Physiol. Behav., 4,* 539 (1969).