ELECTRIC FIELD EFFECTS IN SELECTED BIOLOGIC SYSTEMS*

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INTRODUCTION

A traditional idea with respect to the interaction of electromagnetic fields and biologic systems is that specific effects can occur only when the incident energy is greater than the energy associated with a temperature that is characteristic of the biologic system (kT). The functioning of the visual system and the effect of ionizing radiation are examples of these interactions. For energies less than kT, the only widely accepted effects are those due to heating. They occur at electric field strengths or incident energy densities that depend on frequency but that generally are quite high.[1] In the case of electrostatic fields (ESFs), an energistic analysis would lead to the conclusion that no biologic effects are possible, except for extremely high ESFs, because an insufficient amount of energy is coupled into the quasistatic biologic system. Indeed, the literature contains only a few reports of ESF-induced effects, which involved snails, fruit flies, moths, and worms.[2-4] There are, however, an increasing number of reports that describe biologic effects induced by electromagnetic fields at frequencies and intensities that do not induce heating.[1,5-9] Thus, in addition to energy-mediated interactions explainable by classic theory, there appears to be a second group unique to biologic systems. In this group, the electromagnetic field does not supply the energy for a given process but merely furnishes the energy to control or trigger it. The more sensitive the particular system that is affected by the electromagnetic fields, the smaller is the energy required to produce the effect. Since low-level, trigger effects induced by electromagnetic fields do occur in mammalian systems, the question arises whether such effects could be induced by ESFs.

In addition to framing the question as a parallel to the observed electromagnetic field effects, the question derives independently from the reports that consider the effect of direct electrical current on biologic systems. A variety of experiments have

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shown that dc unidirectionally applied can produce biologic effects.[10-15] Every such current is, of course, a result of an applied ESF. In no case of which we are aware, is it clear whether the observed effect is properly associated with the ESF, an electrochemical or Faradiac process, Joule heating, or some other modality. Thus, the study of the effects of "pure" ESFs, unencumbered by these secondary processes, would seem to be a reasonable step in determining mechanisms responsible for the observed effects.

It was our aim to develop convenient laboratory systems in which the existence of ESF-induced effects could be demonstrated and studied. We report here data on two systems that show considerable promise: the effect of ESFs on rats exposed continuously for 30 days and the effect of ESFs on the chromosomal patterns of Erhlich ascites tumor cells exposed *in vivo* for 14 days.

METHODS AND MATERIALS

Male Sprague-Dawley rats (21 days old) were exposed continuously for 30 days to uniform ESFs generated between the plates of a capacitor. The experimental animals were housed in plastic cages placed on wooden shelves. When ESFs parallel to the earth's surface (horizontal) were applied, the rats were lodged three per cage, and the ordinary metal cage tops were replaced by ones made of plastic. For applied ESFs perpendicular to the earth's surface (vertical), the rats were sheltered individually in smaller cages, the metal tops of which were employed as the grounded side of the capacitor. The other plate, which carried a positive potential relative to the ground, was glued between two pieces of wood for insulation purposes. It was situated beneath the cages for the vertical field exposure or beside them for horizontal field exposure, in which case a grounded plate was oppositely placed. In both cases, the resistance between the capacitor plates was greater than 10^{18} Ω. The distance between the plates was maintained constant throughout the experiments, and variations in field strength were achieved by varying the applied voltage. Dc voltages were supplied to the plates by power supplies that consisted essentially of a high-voltage transformer followed by a rectifying and filtering circuit. The control rats were housed in ordinary cages with metal tops on metal shelves. All rats were fed and watered ad lib., and the cages were cleaned according to standard animal care procedures. The rats were weighed periodically during the field exposure, and it was only then and during the times the cages were changed that the rats were removed from the field. After 30 days, the rats were sacrificed by ether overdose, and the lung, liver, and kidney were fixed in formalin and prepared for histologic examination. Peripheral blood was also obtained for electrophoretic analysis of the serum proteins. Aliquots of peripheral blood were pooled from each rat in all control and experimental groups. The whole blood was allowed to clot at room temperature for 1 hr and then cooled to 4°C for an additional 3 hr. Subsequently, the serum was recovered by centrifugation and stored at -10°C until used. Electrophoresis was conducted on cellulose acetate strips with a commercial apparatus (Gelman) and recommended procedures. The samples were electrophoresed at 2 mA per strip for 75 min, after which they were stained with ponceau S, cleared, dried, and mounted on glass to facilitate optical density measurements. Densitometer scans of the cellulose acetate strips yielded optical density curves that were integrated planimetrically to determine the relative percentage of the major serum fractions.

Mature female Swiss Ha/ICR mice were employed as hosts for Erhlich ascites tumor cells for the purpose of studying the effect of ESFs on the ascites cell chro-

mosome pattern. The host was injected with 0.2 ml of ascites fluid freshly removed from a nonexposed animal that had been innoculated 9 days previously. The host was then exposed continuously to ESFs for 14 days in the apparatus described above. On the morning of Day 14, the host was injected with Colcemid® (1 μg/g), which arrests cell division in metaphase, thus permitting direct visualization of the ascites cell chromosomes. The preparation of Colcemid is described elsewhere (Ciba). Four hours after injection, a few drops of tumor were removed and incubated at 37°C for 30 min in a 2-ml hypotonic solution of 0.075 M KC1. The swollen cells were then fixed for 5 min by adding 1 ml of a solution of glacial acetic acid and methanol (3:1) directly to the KCI solution that contained the cells. The cells were washed by centrifuging, decanting, and resuspending in fresh fixative. The process was repeated four times, and it consumed about 40 min. After the last centrifugation, the supernatant was drawn off, and a small drop of the cell suspension was placed on a microscope slide and allowed to spread and dry. The preparation was then stained with Wright's-Geimsa for study.

RESULTS

The rats exposed to vertical ESFs in the range of 6-197 V/cm and those exposed to horizontal ESFs in the range of 3-98 V/cm adapted easily to the field and exhibited no overt abnormal behavior. No differences in the curves of weight gain versus time were seen between the control and experimental groups during the 30-day exposure period for any field strength or orientation. TABLE 1 lists the average ratio of the final to the initial weights for seven experimental groups and their respective controls. No significant differences were observed, and the overall averages of the experimental and control groups were almost identical. Despite this normal development, we began to observe early in the field exposure experiments what we subsequently determined to be secondary glaucoma in some experimental animals. The condition manifested itself as a large protruding eyeball and invariably occurred in the right eye (see FIGURE 1). The glaucoma has been diagnosed as a

TABLE I

THE EFFECT OF ELECTROSTATIC FIELDS ON THE DEVELOPMENT OF RATS EXPOSED CONTINUOUSLY DURING DAYS 22-52 AFTER BIRTH

	Number of Rats		Average Ratio of Final Weight to Initial Weight	
	Experimental	Control	Experimental	Control
Vertical field (V/cm)				
197	14	12	4.33	4.41
56	14	12	5.22	5.41
28	12	17	4.56	4.53
6	20	23	4.15	4.21
Horizontal field (V/cm)				
98	16	11	4.37	4.48
28	13	8	4.92	5.25
3	14	11	4.31	4.28
Overall average			4.55	4.65

FIGURE 1. Secondary glaucoma in a rat exposed to a vertical electrostatic field of 6 V/cm for 30 days

TABLE 2

FREQUENCY OF APPEARANCE OF SECONDARY GLAUCOMA IN RATS EXPOSED TO ELECTROSTATIC FIELDS DURING DAYS 22-52 AFTER BIRTH

	Number of Rats	Number of Rats Exhibiting Secondary Glaucoma
Experimental		
Vertical field (V/cm)		
197	14	
56	14	0
28	12	2
6	20	5
Horizontal field (V/cm)		
98	16	0
28	13	0
3	14	0
Control	72	0

complication or advanced stage of uveitis by an expert in veterinary ophthalmology on the basis of ophthalmologic examinations of live animals.[16] The frequency of appearance of the secondary glaucoma is shown in TABLE 2. The effect was observed only in rats exposed to vertical ESFs and never in either rats subjected to the horizontal ESF or in the controls. Histologic examination of the lung, liver, and kidney of all experimental rats exposed to the vertical ESFs revealed no significant differences between the experimental and control animals. At this level, all the organs appeared normal, and, in general, all slides exhibited good cytoarchitectural structure.

Serum electrophoresis studies have been completed on the rats exposed to the

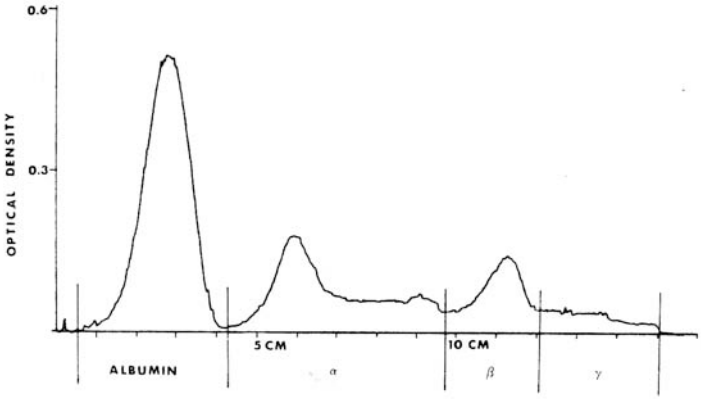

FIGURE 2. Densitometer tracing of cellulose acetate electrophoresis pattern of pooled rat serum.

TABLE 3

THE EFFECT OF VERTICAL ELECTROSTATIC FIELDS ON THE SERUM PROTEINS OF RATS EXPOSED CONTINUOUSLY DURING DAYS 22-52 AFTER BIRTH*

		Serum Proteins			
	Electric field (V/cm)	Albumin (%)	α (%)	ß (%)	γ (%)
Experimental	197	55.7† ± 1.7	30.1‡ + 1.5	12.1‡ ± 0.2	2.1† ± 0.2
Control		50.0 ± 1.9	32.9 ± 1.3	13.6 ± 0.7	3.6 ± 0.3
Experimental	56	50.3‡ ± 1.8	28.2 ± 1.6	13.7‡ ± 1.0	7.8 ± 1.6
Control		52.7 ± 1.0	28.8 ± 1.1	12.3 ± 0.7	6.3 ± 2.3
Experimental	28	54.6‡ ± 2.7	29.1 ± 0.9	12.4 ± 1.2	3.5† ± 1.2
Control		50.5 ± 1.1	29.1 ± 0.8	13.5 ± 1.0	6.6 ± 0.7

*Each set of values is an average of 12 determinations made from six scans of three cellulose acetate strips (two scans/strip).

†$p < 0.01$.

‡$p < 0.05$.

highest three vertical fields. A typical tracing of the optical density of the stained cellulose acetate strips is illustrated in FIGURE 2. Also shown is the division of the curve into the four major protein groups: the albumin fraction and the three globulin fractions. Both the experimental and control groups displayed the same qualitative curve. The relative percentage of each serum protein for the rats subjected to the three highest vertical ESFs are given in TABLE 3. As can be seen, significant differences were found at all three ESF strengths.

The chromosomes of the Ehrlich ascites tumor cells were predominantly hyperdiploid, with a modal number of 45. This tumor, unexposed to ESFs, was characterized by the presence of three marker chromosomes: two submetacentrics and one chromosome that had a prominent secondary construction. Chromosome studies were made on selected passages. In each preparation, 50 well-spread, intact metaphases were counted to determine the presence of chromosomal abnormalities, with particular emphasis on chromatid translocations, isochromatid breaks, and dicentrics. Data on abnormalities were evaluated on the basis of the percentage of cells characterized by any number of a particular type of abnormality (see TABLE 4). In addition, the average number of any type of abnormality per cell was calculated.

TABLE 4

EFFECT OF HORIZONTAL ELECTROSTATIC FIELDS IN THE RANGE 80-160 V/CM ON THE INCIDENCE OF CHROMOSOME ABERRATIONS IN EHRLICH ASCITES TUMOR CELLS EXPOSED *in Vivo* FOR 14 DAYS

	Experimental	Control
Number of mice employed	7	6
Total number of cells counted	350	300
Percentage of cells with abnormal chromosomes	20* ± 9.2	5.1 ± 5.6
Average number of abnormal chromosomes per cell	2.0* ± 0.7	0.8 ± 0.6
Percentage of cells with chromatid translocations	13.7† ± 8.8	3.1 ± 2.8
Percentage of cells with isochromatid breaks	8.3‡ ± 5.6	2.3 ± 2.7
Percentage of cells with dicentric chromosomes	1 ± 1	0

*$p < 0.005$.

†$p < 0.01$.

‡$p < 0.05$.

DISCUSSION

The secondary glaucoma observed in the rats is a complication of uveitis. Uveitis is normally present in l-2% of the laboratory rat population; however, the percentage of the group that subsequently exhibited secondary glaucoma is much lower.[16] As TABLE 2 demonstrates, secondary glaucoma has been observed in 16% of the rats exposed to vertical ESFs and has never been seen in either the rats subjected to horizontal ESFs (43 rats) or in the controls (72 rats). None of the rats in these experiments were given ophthalmologic examinations prior to exposure to the ESFs, and thus it is not possible presently to eliminate the possibility that the high incidence of secondary glaucoma in the rats subjected to the vertical fields resulted from an exacerbation of uveitis that existed before exposure. However, the small percentage of normal rats that displayed spontaneous uveitis, when compared to the observed frequency of secondary glaucoma, makes this possibility very unlikely. The more probable conclusion is that the secondary glaucoma is a complication of uveitis that itself was induced by the ESF. In either event, the existence of an ESF-induced effect is established. Also established is the absence of any proportionality between the applied ESF and the effect produced. This characteristic of biologic trigger phenomena can be seen in TABLE 2, wherein no correlation exists between the applied field strength and the number of observed instances of secondary glaucoma. The observation that the secondary glaucoma occurred only during exposure to vertical ESFs is particularly interesting. The absence of randomizing effects in the vertical exposures possibly is significant. That is, in the horizontal field, the vector that points in the direction of the rat's motion may make any angle with the ESF, whereas for vertical exposure, that angle is nearly always fixed at 90°. There apparently is no anatomic feature in the rat that could be invoked to explain why the secondary glaucoma was seen only in the right eye.

Significant differences in serum proteins between experimentals and controls were noted for all three vertical ESFs. At all three field strengths, the ratio of albumin proteins to nonalbumin proteins was altered. The ratio increased for vertical ESFs of 197 and 28 V/cm and decreased for the vertical ESF of 56 V/cm. Again, proportional effects were not detected, except in the sense that only at the higher field strengths were significant differences seen in all four protein fractions. The electrophoretic determinations were made on the pooled serum from rats from each exposure group. It may be argued that the results are related to the fact that some experimental rats exhibited secondary glaucoma. We do not attempt to evaluate this possibility here except to say that significant differences in the. serum proteins were seen at 56 V/cm, and no instances of secondary glaucoma were observed at that field strength.

Chromosomal aberrations induced by pulsed radio-frequency fields have been reported previously,[17] but we believe that the results here are the first quantitative description of field-induced effects at the chromosomal level and that they are the first description of any such effect induced by static fields. The only theoretic treatment of which we are aware predicts the possibility of an electric field-induced effect in DNA at fields of the order of 10,000 V/cm.[18] This forecast is to be compared with the fourfold increase in the percentage of abnormal chromosomes found for 14-day exposures in the 80-160-V/cm range (see TABLE 4). Although occasional cells with dicentric chromosomes were seen from the experimental animals, the most numerous abnormalities detected were isochromatid breaks and chromatid translocations. The observation that the average number of abnormal chromosomes per cell was more than twice as high in the experimental group raises the

possibility that ESFs may exist that could retard or accelerate the growth of the tumor by the induction of various chromosomal abnormalities. However, initial experiments in which the time between injection of the tumor and death was determined at different field strengths have shown no statistically significant difference between experimental and control groups. Thus, although the existence of ESF-induced chromosomal aberrations is established, both the mechanism and significance of the effect are unknown.

In conclusion, the production of secondary glaucoma and altered serum electrophoresis patterns in rats and the production of chromosomal abnormalities in tumor cells in mice upon exposure to low-strength ESFs appear to affirm unequivocally the existence of ESF-induced trigger phenomena in biologic systems. The phenomena certainly are not energetically driven by the externally applied field but instead must be initiated by information transmitted by the applied field and recognizable by the system that itself is the ultimate source of the necessary energy.

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DISCUSSION

DR. A. R. LIBOFF: Could secondary glaucoma be hereditary?

DR. MARINO: Yes, a veterinary ophthalmologist at Cornell University says that it is present in 1-2% of normal rat populations. Humans also get this disease, but I haven't researched that. We didn't find. any secondary glaucoma in the experimentals, which totaled about 100. At present, we therefore don't know whether the field exacerbates a preexisting condition or initiates it or whether another factor is responsible.

DR. LIBOFF: *Which* eye does it affect

DR. MARINO: It occurs only in the right eye.

DR. LIBOFF: Only in the right eye?

DR. MARINO: Yes, in all 10 cases, only the right eye was affected.

DR. C. A. L. BASSETT: Did you see any chromosomal changes in the normal cells?

DR. MARINO: No.

DR. A. A. KATZBERG: I have done some similar experiments at high field levels, and I found that the number of ion pairs per cubic centimeter is profoundly im\portant. How do you know that this effect is caused by an increase in the ionization level in the air, which is very humidity sensitive?

DR. MARINO: What was the highest voltage that you employed?

DR. KATZBERG: 197 V/cm.

DR. MARINO: Even if it was 10,000 V/cm, I was not concerned about that kind of an inquiry at this point.

DR. BASSETT: Did you conduct white blood cell counts, because this factor might explain the high γ-globulin?

DR. MARINO: We counted 100 leukocytes in the peripheral blood and looked for the distribution there as compared with the control, for all the animals. We found no effect, although it is possible that a very mild effect does occur.

DR. C. MINKIN: Your work is a very interesting presentation of phenomenology, but what importance does it really have?

DR. MARINO: Your question is well taken. Let me repeat the elegant remark made earlier today by Dr. Romero-Sierra: I take that question as payment for my work. I am aware of no phenomenologic effects in the literature that describe electrostatic field effects on mammalian systems.

DR. C. H. LERCHENTHAL: I suggest you read Pressman's reports.

DR. MARINO: I have read Pressman's book, and my statement still stands. Pressman refers to three reports that describe electrostatic field effects, on snails, flies, and on moths.