EFFECT OF ELECTROSTATIC FIELDS ON THE CHROMOSOMES OF EHRLICH ASCITES TUMOR CELLS EXPOSED *IN VIVO*

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• *An effect of electrostatic fields on the chromosomes of Ehrlich ascites tumor cells exposed* in vivo *has been demonstrated. Cells exposed to horizontal electrostatic fields for two weeks had almost a threefold increase in the percentage of abnormal chromosomes when compared to control cells or cells exposed to vertical electrostatic fields for the same period. Extended exposure times of 4-15 weeks resulted in the disappearance of the aberrant chromosomes. It is suggested that the affected cells were incapable of cellular replication resulting eventually in their disappearance via cell death.*

INTRODUCTION

A number of experiments have shown that electrostatic fields (ESFs) can produce biologic effects.[1-6] The effects have involved diverse biological systems including snails, fruit flies, moths, worms, and rabbits, as well as mouse fibroblasts in culture. In producing such effects, the electromagnetic field furnishes the energy to control or trigger a given process but does not supply the total energy for it.[7-9]

We have previously described convenient laboratory systems in which ESF-induced effects could be demonstrated and studied.[7] The effect of ESFs on rats exposed continuously for 30 days, and on chromosomal patterns of Ehrlich ascites tumor cells exposed to horizontal ESFs *in vivo* for 14 days, strongly suggested the existence of ESF-induced trigger phenomena in biological systems. With regard to the chromosomal data, it was observed that the average number of abnormal chromosomes per cell was more than twice as high in the experimental group when compared with the controls. Although the existence of ESF-induced chromosomal aberrations was established, numerous details remained undetermined.

In the present study we extended the exposure time of Ehrlich ascites tumor cells

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in vivo to 15 weeks, and also studied the differential effects produced by ESFs parallel to the earth's surface (horizontal) and perpendicular to the earth's surface (vertical). The data reported here indicate that a consistent and specific chromosomal effect occurred in the horizontal field group but not in the vertical field group. This differential effect disappeared when the exposure time was lengthened, indicating a return to the normal tumor baseline within the horizontal field group.

MATERIALS AND METHODS

Mature female Swiss Ha/ICR mice were employed as hosts for Ehrlich ascites tumor cells. The control mice were housed in standard plastic cages with metal tops on metal shelves. The experimental animals were housed similarly except that wooden shelves were employed and, in the case of the horizontal ESFs, plastic cage tops were utilized. Horizontal and vertical ESFs were applied to mice as described previously.[7] For vertical ESFs, the metal cage tops were used 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 placed beneath the cages for vertical field exposure or beside them for horizontal field exposure, in which case a grounded plate was oppositely placed. Distance between the plates was maintained constant throughout the experiments; variations in field strength were achieved by varying the applied voltage. Dc voltages were supplied to the plates by power supplies consisting of a high-voltage transformer followed by a rectifying and filtering circuit. All mice were fed and watered *ad libitum*, and the cages were cleaned according to standard animal care procedure.

The ascites tumor was propagated in control mice by injecting the host intraperitioneally with 0.2 ml of ascites fluid freshly removed from a control (unexposed) animal that had been inoculated 7-14 days earlier. After 14 days a few drops of tumor were removed and the tumor cells were processed for chromosomal analysis as described below. Tumor propagation in the 2-week exposed groups was identical except that the mice were exposed to the electrostatic field. Ehrlich ascites tumor cells are normally lethal to the host 2-3 weeks after injections. To propagate the tumor for longer periods, it was necessary to transplant 0.2 ml of tumor cell fluid into new hosts every 7-14 days. Consequently, tumors carried for 4-15 weeks required serial inoculations into 2-9 mice exposed continuously to the ESFs.

On the day the chromosomal analysis was to be performed, the host was injected with 1 μg/g of Colcemid solution (CIBA Pharmaceutical Co., Summit, New Jersey). This arrests cell division in metaphase and allows direct visualization of the ascites cell chromosomes. Four hours after injection, a few drops of the tumor were removed and incubated at 37°C for 30 min in a 2 ml hypotonic solution of 0.075 M KCl. 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 KCl solution containing the cells. The cells were washed by centrifuging and decanting, then were resuspended

in fresh fixative. This process was repeated four times. After the last centrifugation, the supernatant was decanted, and a small drop of the cell suspension was placed on a clean microscope slide and allowed to spread and dry. This preparation was then stained with Wright's-Geimsa for analysis.

For each preparation up to 50 well-spread, intact metaphases were counted by systematic scanning of the slide to determine the nature and incidence of chromosomal abnormalities. Particular emphasis was placed on the occurrence of chromatid exchanges and isochromatid breaks. A chromatid exchange was scored as a single abnormality for each pair of chromosomes involved. Both chromatid and isochromatid breaks were counted as a single break. In addition, other abnormalities, such as dicentrics, rings, long acrocentrics, and minutes, were noted. Data on abnormalities were evaluated on the basis of the percentage of cells characterized by any number of a particular type of abnormality, percentage of cells with abnormal chromosomes, and average number of abnormal chromosomes per abnormal cell. Finally, in a few instances numerical estimates were made on 100 additional cells to confirm the randomness of the chromosome number of the 50 cells selected for counting.

RESULTS

The chromosomes of the Ehrlich ascites tumor cells used in this study were predominantly hyperdiploid; that is, there were one or more chromosomes present in excess of the normal somatic chromosome number of the species. These cells had a modal chromosome 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 constriction. As mentioned above, chromosome studies were carried out on well-spread, intact metaphases so no overlapping of individual chromosomes occurred.

Table I summarizes the frequency of cells with abnormal chromosomes and the

TABLE I. Effect of Electrostatic Fields in the Range 80–160 volts/cm on the Incidence of Chromosome Aberrations in Erlich Ascites Tumor Cells Exposed *In Vivo*

Experiment	Weeks of exposure	Number of mice	Number of cells counted	% of cells with abnormal chromosomes	Average number of abnormal chromosomes per abnormal cell
Horizontal	2	8	400	*22.5 ± 6.6	*2.1 ± 0.6
Horizontal	4–15	11	490	13.0 ± 9.1	1.3 ± 0.6
Vertical	2	8	370	5.8 ± 5.8	1.5 ± 1.5
Vertical	6–15	12	600	9.2 ± 7.6	1.0 ± 0.4
Control	—	10	500	8.8 ± 7.1	1.1 ± 0.5

* $p < 0.005$

average number of abnormal chromosomes per abnormal cell within the control, horizontal, and vertical groups. It can be seen that cells exposed to horizontal ESFs for two weeks exhibited almost a threefold increase in the percentage of abnormal chromosomes when compared to control cells. Cells exposed to vertical ESFs for the same period, however, showed a percentage of abnormal chromosomes comparable to that of the control cells.

Extended exposure in the two ESFs appeared to produce opposite results. The percentage of cells with abnormal chromosomes tended to decrease systematically in the horizontal ESFs but increase systematically in the vertical ESFs. The number of mice analyzed prohibited a precise determination of the dependence of such cells on exposure time. In both cases, when the results were averaged over the entire extended exposure period (4-15 weeks for the horizontal ESFs, and 6-15 for the vertical ESFs), no statistically significant results were seen (Table I).

The most numerous chromosomal abnormalities detected were chromatid exchanges (translocations) and isochromatid breaks. For example, in the 2-week horizontal group, 15.5% and 9.3% of the cells showed chromatid exchanges and isochromatid breaks respectively as compared to only 1.8% and 4.0% respectively for the cells exposed for 2 weeks to vertical ESFs. In the control group, 4.7% of the cells had chromatid exchanges while 4.6% had isochromatid breaks. Comparable values were 7.5% and 6.4% in the 4-15 week horizontal group, and 3.2% and 6.2% in the 6-15 week vertical group. Figure 1A shows a typical metaphase from a control cell illustrating the three marker chromosomes. Multiple chromatid exchanges and a chromatid break in an extensively effected cell after 2-week exposure to horizontal ESFs are shown in Fig. 1B. Occasionally cells with dicentric, long acrocentric, and minute chromosomes were observed in all three groups, but no correlation was found with either field exposure. In the cells examined for chromosome number estimates, it was found that well over 75% of all cells from the control, horizontal, and vertical groups contained the hyperdiploid range of chromosomes. A minority of cells appeared to have double the normal chromosome number (tetraploid range), but there were no differences among the three groups.

DISCUSSION

Chromosomal aberrations induced by pulsed radio-frequency fields have been previously published.[10] However, we believe that the results reported here are the first qualitative and systematic description of ESF-induced biological effects at the chromosomal level. Our electrostatic fields are in the 80-160 volts/cm range and we have demonstrated significant chromosomal effects induced by this relatively narrow, low-strength range.

Chromosomal anomalies similar to those reported here have been observed in a variety of cell lines treated with diverse mutagenic and teratogenic chemicals. In those studies it was concluded that extensive, drastic structural changes in chromo-

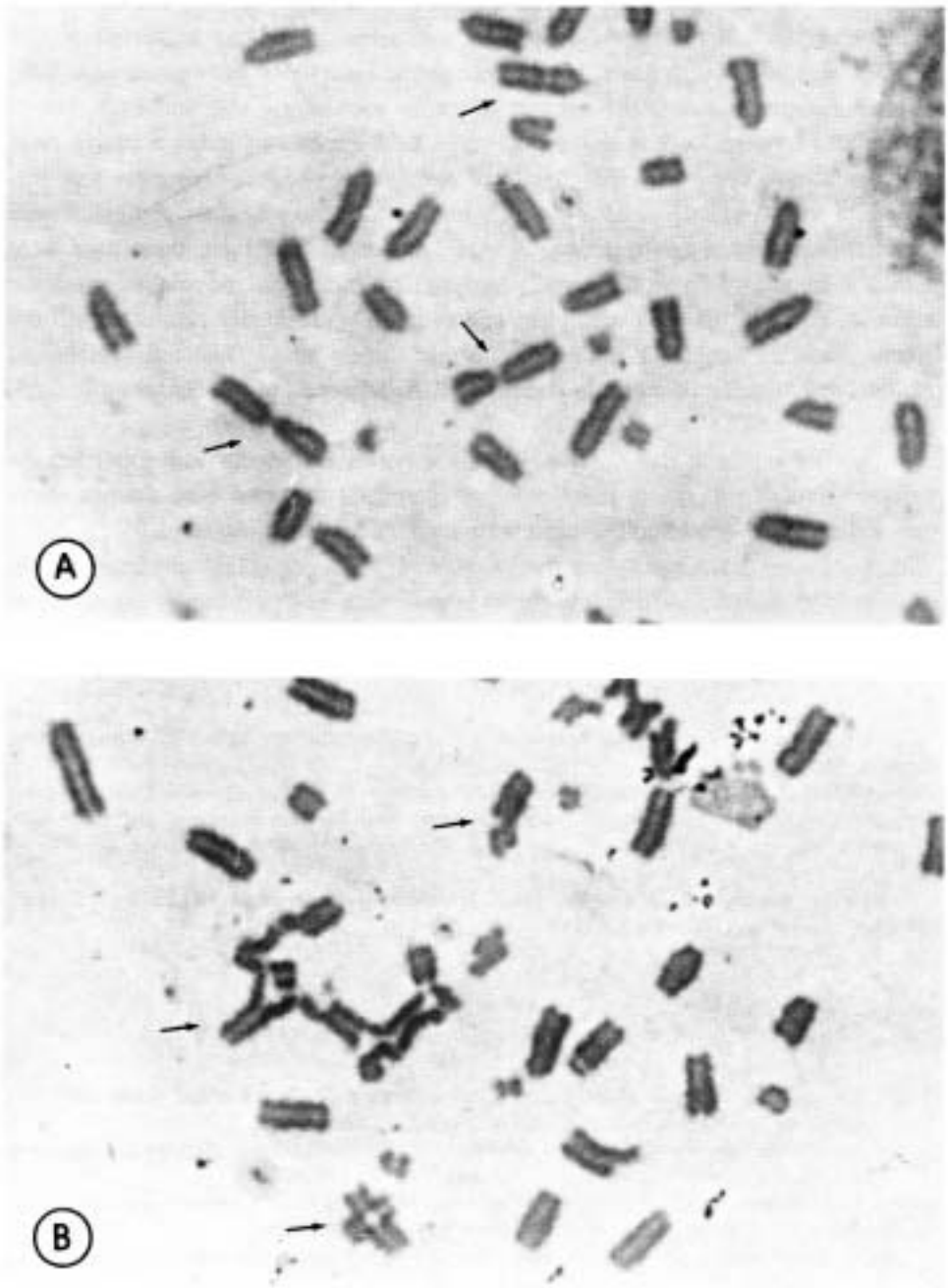

FIGURE 1. (A) Metaphase from a control cell showing the three marker chromosomes: two submetacentrics and a secondary constriction. (B) Metaphase showing multiple chromatid exchanges among various chromosomes and a chromatid break in a cell exposed to horizontal ESFs for two weeks. ×2,000.

somes usually result in cell death; however, if a small segment is deleted, duplicated, translocated, or inverted, a viable mutant could be formed.[11-13] We found that repeated transplant of the tumor over 4-15 weeks resulted in the disappearance of the ESF-induced abnormalities. It therefore seems reasonable to conclude that ESF exposure resulted in cell death rather than the creation of a viable mutant.

Chronic exposure to cells in the horizontal field appears to indicate that certain cell populations, some of which exhibited multiple chromatid exchanges and isochromatid breaks among various chromosomes within severely damaged cells, were rendered incapable of cell replication. As a function of time then, these cells were gradually eliminated from the serially propagated tumor cell population, and the surviving, non-effected cells multiplied and eventually reflected a return toward the original baseline (control) parameters of the tumor line. This could represent cellular adaptation or selection in response to the influence of the electrostatic fields applied.

The ascites tumor is free-floating within the peritioneal cavity and a cell has no preferred direction in space. It follows that the effects reported here did not result from a direct field-cell interaction but were mediated by the animal.

In conclusion, we suggest that the production of chromosomal abnormalities in tumor cells in mice following exposure to low-strength ESFs reinforces the proposal of ESF-induced trigger phenomena in biologic systems. This present work is consistent with theoretical calculations.[8]

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REFERENCES

1. D. K. Edwards, "Effects of artificially produced atmospheric electrical fields upon the activity of some adult Diptera," *Can. J. Zool., 38,* 899 (1960).
2. ———, "Influence of electrical field on pupation and oviposition in *Nepyia phantasmaria* Stkr. (*Lepidoptera: Geometridae*)," *Nature, 101,* 976 (1961).
3. C. A. L. Bassett and J. Herrmann, "The effect of electrostatic fields on macromolecular synthesis by fibroblasts *in vitro*," *J. Cell. Biol., 39,* 9a (1968).
4. H. B. Dowse and J. D. Palmer, "Entrainment of circadian activity rhythms in mice by electrostatic fields," *Nature, 222,* 564 (1969).
5. F. Brown, "Some orientational influences of nonvisual terrestrial electromagnetic fields," *Ann. NY Acad. Sci., 188,* 224 (1971).
6. W. Ng and K. Piekarski, "The effect of an electrostatic field on the mitosis of cells," *Med. Biol. Eng., 13,* 107 (1975).

7. A. A. Marino, T. J. Berger, J. T. Mitchell, B. A. Duhacek and R. O. Becker, "Electric field effects in selected biologic systems," *Ann. NY Acad. Sci., 238*, 436 (1974).
8. F. X. Hart and A. A. Marino, "Biophysics of animal response to an electrostatic field," *J. Biol. Phys.*, (in press).
9. A. Pressman, *Electromagnetic Fields and Life*, Plenum, New York, 1970.
10. J. Heller and A. Teixeria-Pinto, "A new physical method of creating chromosomal aberrations," *Nature, 183*, 905 (1959).
11. M. Joneja and S. Ungthavorn, "Chromosome aberrations in trypan blue induced teratogenesis in mice," *Can. J. Genet. Cytol., 10*, 91 (1968).
12. C. Roux, I. Emerit and J. L. Taillemite, "Chromosomal breakage and teratogenesis," *Teratology, 4*, 303 (1971).
13. M. T. Alam, M. Corbeil, A. Chagnon and S. S. Kasatiya, "Chromosomal anomalies induced by the organic phosphate pesticide guthion in Chinese hamster cells," *Chromosoma, 49*, 77 (1974).

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