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Mutagenic Potential of Alternating Current Electric Fields

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SUMMARY

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This research report entitled "Mutagenic Potential of Alternating Current Electric Fields" is presented as a competent treatment of the subject, worthy of publication. The United States Air Force Academy vouches for the quality of the research, without necessarily endorsing the opinions and conclusions of the author. Therefore, the views expressed in this article are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the US Government.

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Director of Research

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Date

ABSTRACT

Electromagnetic fields (EMF) such as those produced by power lines have become a growing source of concern to the general public. Many epidemiological studies have linked EMF to carcinogenesis implying an underlying genetic phenomenon mediated by EMF. EMF can be subdivided into either electric fields (E-field) or magnetic fields (B-fields). Our research used a reverse mutagenesis bacteriophage T4D model to quantitatively study the effects of E-fields on a molecular genetic level. Statistical analysis of the data indicated that there was no significant difference ($p=>0.05$) in the mutagenic rate of phages grown in the presence of A/C E-fields compared to the controls except at a field-strength of 1053 V/M ($p= 0.04$). This result is not consistent with the other values tested and at this time we are a loss to explain what appears to be a decrease in background spontaneous reversion rate in the phage T4 mutant.

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INTRODUCTION

Electromagnetic fields (EMF) exist as a common part of everyday life. Televisions and microwaves, basically anything that can be plugged into an electrical source, can produce EMF. Because these appliances directly affect the standard of living people enjoy, most people are willing to accept the potential health risk of EMF even if they are aware of it. However, high voltage power lines are considered a nuisance because they look bad, they're noisy and they do not directly benefit the people who live under them in a readily visible manner. Health concerns have also been raised, thus many epidemiological studies have been conducted with findings ranging from the decreased milk production in cows to possible carcinogenic effects in humans. The general consensus among scientists being a need for concern and further study of the biological effect(s) of EMF.

The focus of this research project was to examine the mutagenic effect of EMF at a molecular genetic level using a bacteriophage T4D model. By analyzing EMF in a controlled environment, the actual effects of the direct current magnetic field (DC/B) could more accurately be determined without having to make assumptions or rule out other

environmental factors influencing the study organisms. The goal of this research was to quantify the mutagenic effects of the field using a simple well-defined genetic system. A mutagenic change is a permanent change that is incorporated in an organism's DNA and will be passed on to progeny (Klug and Cummings, 1994). This paper will (1) define and explain an EMF, (2) provide some background on the bacteriophage T4, (3) review the literature concerning the biological effects of EMF, (4) state the experimental hypothesis based on the literature review, (5) present the experimental design, (6) present and analyze the data and (7) discuss the findings and state a conclusion.

EMF

EMF can be subdivided into two types of fields-an electric field (E-Field) and a magnetic field (B-Field). This is advantageous for research purposes because the fields can be separated, or mostly so, to determine the specific effect that each field has on the phenomenon being investigated.

An electric field is defined as a difference in electric potential between two objects. This can be produced in a laboratory, or in the environment, by connecting a power source to two objects with conductive properties. The strength of the electric field produced between the two objects is a function of the electrical power supplied and the distance between the objects. Thus the units used to express strength in E-Fields is V/M. The

field lines in an electric field flow from a positive charge to a negative charge (Fig 1). As distance is a factor, the intensity and magnitude of the electric field changes substantially with the proximity of an object to the field source (inversely with distance). The shape of the electric fields is easily altered by interference from practically any object. Since each object maintains a certain level of electrical conducting and insulating properties, the degree to which the field is altered varies. Electric fields also tend to produce heat proportional to the intensity of its field (Prata, 1993).

Magnetic fields exert a force on charged particles. Magnetic fields are a function of the current flowing through the wire and the distance from the wire (Fig 2). These fields, like electric fields, also dissipate rapidly with distance. Hence, the closer to the wire an object is, the greater the magnetic field. Unlike electric fields, magnetic fields are not as easily disrupted. Placing an object in a magnetic field will usually magnetize, to some extent, the object without substantially effecting the surrounding magnetic field (Prata, 1993). Some representative values reviewed in an Office of Technology Assessment report (US Congress, 1989) are a house near a 500 kV line, car contact near a 500kV line electric shaver, household background and beneath a distribution line would be between 0.001 to 0.03 kV/M for E-Fields and between 0.5 to 200 milliGauss (mG) for B-Fields.

The question usually posed is how the electric and magnetic fields join to produce an EMF. The answer lies in the concept of electric and magnetic induction, a process that causes free electric charges to move. For example, a positively charged power line would cause negative charges located in the body to line up on the body's surface. An AC (alternating) current would cause a rapid flow of charge through the body in opposite directions. Magnetic fields are linked to electric fields because alternating magnetic fields (produced by an AC current) generate electric fields (Carpenter, 1994). Direct current is when electrons flow in only one direction. DC current can also produce both E-field when a potential exists between to oppositely charged poles or B-fields when current is flowing through a conductor.

Bacteriophage T4D

Bacteriophage T4 is a large bacterial virus of *Escherichia coli* containing a single linear double-stranded DNA molecule of about 166 x 10^3 base pairs in length. Its tightly organized genome encodes close to 200 genes which choreograph a complex, well-regulated developmental process (reviewed in Guttman and Kutter, 1983). Bacteriophage T4D, henceforth referred to as phage T4 or T4, consists of a virion approximately 215 nm in total length and 80 nm in width at the head (Fig 3). The capsid or head is an icosahedron made primarily of a protein layer attached to the connector vertex of the head. A neck structure

containing a whiskered collar joins to the tail. The tail is 100 nm long and composed of 20 different species of protein and is the smallest contractile organ known to man. T4 consists of an outer sheath surrounding an inner tube through which the phage's DNA passes during the infection process. The distal portion of the tail is attached to a complex baseplate fitted with long and short tail fibers. The tail fibers are essential for infection by providing the primary host range determinants and effecting the adsorption process (Fig 3).

Phage T4 is morphologically classified as a urophage. It can be further classified as a member of the T-even-like phages along with its cousins T2 and T6 (Guttman and Kutter, 1983; see also Birge, 1981, for an overview). The T-even-like phages include numerous types; many of them sharing serological as well as morphological characteristics (Schwartz, 1980; Schwartz et al., 1980). They also share a high degree of DNA homology (Kim and Davidson, 1974). Of all of the T-even-like phages, phage T4 has been the experimental workhorse. The past 50 years of experimentation have resulted in T4 being the most well understood phage system available for studying basic molecular phenomena. One of these molecular phenomenon is genetic mutation or mutagenesis.

An amber mutation in bacteriophage T4 is a stop codon and is lethal when in essential (necessary for viability) genes. Amber mutations are a class of mutations called

nonsense or stop codon mutations that stop the translation process found in viruses, prokaryotes as well as eukaryotes such as man. These mutations can be caused by a simple, one nucleotide change in the organism's genomic DNA.

The infection process of phage T4 essentially allows the phage to inject its genome into a suitable *E. coli* host cell permitting a cycle of phage growth. Infection by phage T4 has been extensively studied (reviewed by Goldberg, 1983). If all goes well during host cell takeover and T4 gene expression. The various components of the progeny phage are constructed, the original phage DNA is multiply replicated, and then all of the components are assembled in a neatly organized particle. After about 20-30 minutes at 37 degrees Celsius about 100 new phage particles are produced, a phage encoded lysozyme subsequently lyses the membranes of the host cell, and the progeny phage are released into the environment to begin the cycle anew. A bacteriophage with the lethal amber mutation injected into the cell, will die unless it goes through the process of reverse mutagenesis to restore a DNA sequence permitting viability.

LITERATURE REVIEW-Biological Effects of EMF

A literature search unearthed considerable research in the area of biological effects of EMF. Pertinent findings were lumped into four general areas to include chromosomal aberrations, effects on protein synthesis, teratogenesis and oncogensis. In the area of chromosomal effects, Skyberg,

Hansteen, and Vistines (1993) conducted a study in Norway with workers who were occupationally exposed to high amounts of high/low level EMF. Workers in the experimental group had a statistically significant ($p=.04$ in smokers, $p=.08$ in non-smokers vs control) amount of chromosomal breakage in their lymphocytes (Skyberg and Hansteen, 1993). It is important to note that lymphocytic cells are an important part of the immune system. Regarding effects on protein synthesis, Wiesbrot and Goodman (1993) did a study in *Drosophila melanogaster* where they found a significant increase in the rate of transcription and translation in 17 chromosomal regions. Interestingly, the same 17 regions were also the same chromosomal regions that control and regulate cell growth and development (Wiesbrot and Uluc, 1993). Piera and Cobos (1992) of Spain conducted a study on how EMF effected the developmental rate of chicken embryos. The chicks were exposed to a magnetic field of 0, 181, and 361 Gauss/cm2, (the quantitative units of a B-field) A significance of $p=.0001$ was found concerning the growth rate of the exposed (361 Gs/cm2) chicks versus the control chicks. Finally, EMF have been linked to cancer in a number of epidemiological studies reviewed by Hafemeister (1996). Based on the information gathered from the literature review, we hypothesized that EMF are mutagenic since all of the above biological phenomenon point to a genetic cause. That is, an alteration of the organism's normal homeostatic

state induced by EMF would imply an alteration of the organisms genetic program, i.e. a mutation.

MATERIALS AND METHODS

Media

E. coli were grown in Hershey's broth (HB) or on enriched Hershey's agar plates. These were prepared as prescribed by Steinberg and Edgar (1962). M9 adsorption salts solution was used in diluting phage and for promoting adsorption of phage to host cells in various assays. To prepare this solution the following were added per liter of distilled deionized water: 5.8 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, and the pH adjusted to 6.8 to 7.0. All media were autoclaved and allowed to cool further to room temperature before use. Soft top agar (enriched Hershey's agar plate media with 1/2 the agar added) was used at a temperature of 45 degrees Celsius where it is liquid. Indicator cells (host *E. coli*) and phage were mixed in the soft agar before plating to titer phage and conduct experiments.

Bacteria and phage strains

There were two strains of *E. coli* used as host cells and two phage strains used to quantify the results (Table 1). The phage included an amber 42 mutant of the dCMP hydroxymethylase and an amber 44 mutant of the DNA polymerase accessory protein. The amber mutation is a stop codon or nonsense mutation. Since these are mutations in essential genetic functions for phage viability, the

mutations are lethal. Because these mutations are lethal, it was necessary that one of the *E. coli* bacterial hosts (K-704) be a permissive host. The K-704 host suppresses the mutation by inserting a glycine where the stop mutation is located thus restoring phage viability. The K-704 host allowed a stock supply of bacteriophage to be grown and titered. The *E. coli* K-12 bacterial host was a restrictive strain that was used in the experiment to assay for revertants. In order for the phages to grow in the K-12 host, the phage had to go through the process of reverse mutagenesis to regain a viable form. The phages that did grow on the control plates were a result of spontaneous reversion ("natural" reverse mutagenesis). This allowed a method to assay the mutagenic effects of the EMF on T4.

The phage and bacterial strains used, their relevant characteristics and the source or a reference are listed in Table 1.

Table 1: Bacteria an Phage Strains Used

Bacteria strain	Genotype	Source
E. coli		
K-704	F-met-gal-rK+mK+ supE(gln)rglArglB	Schlagman
K-12	rglArglB	Univ. of Rochester
Phage T4		Dept. of Biology
am42	42-	Rochester, NY 14622
am44	44-	(716) 275-3846

Titration of cells and phage plaque forming units

All titering of cell colony forming units (cfu) was carried out on enriched Hershey's agar plates with top agar overlays. Phage titers were determined after dilution by plaque formation on agar plates by the agar overlay method of Adams (1959). An aliquot of the phage-containing dilution was dispensed into soft top agar along with approximately 0.3 ml of plating indicator, and then spread evenly over the surface of an agar plate by gently tilting the plate in a circular manner. Then the soft agar was allowed to solidify on a level surface, plates were incubated for a minimum of 12 hours at the appropriate temperature followed by plaque counting.

Preparation of phage

Phage stocks were prepared by either the plate or bottle lysate method. In the plate lysate procedure 1×10^5 phage and 2×10^9 bacteria were added to 2.5 ml of soft top agar plus 3 ml of HB, and then poured over the surface of a Hershey's plate. After overnight incubation at 22 or 37 degrees Celsius several drops of chloroform were added to each plate plus 5 ml of M9 salts solution. Two hours were allowed for the cells to lyse. Then the phage containing fluid was decanted and cellular debris removed by centrifugation at 10,000 x g for 10 minutes at 4 degrees Celsius. This procedure normally yielded phage titers around 5×10^{10}. If higher titer phage stocks were desired, the phage suspensions were sedimented by centrifugation at 23,000 x g for 2 hours at 4 degrees

Celsius. The fluid decanted and the pellet was resuspended in 1-3 ml of M9 salts solution. This usually resulted in a 10-50 fold increase in titer.

Apparatus

EMF is divided into a separate electric fields(E-fields) and magnetic field(B-field) to determine precisely how each component of the EMF affected the phage. An important piece of the apparatus was the Faraday cage. The cage grounded the system and protected the experiments from any EMF existing in the lab ambient environment. AC/E-fields were generated by apply AC power for a transformer connected to two opposing, parallel, flat aluminum plates approximately 6 cm apart contained within a Faraday cage. The experimental plates were centered between the two plates before power was applied.

Phage plaque assay

The approach used to determine the mutagenic potential of DC/B EMF was based on a bacteriophage T4D genetic model using a plaque assay. This specific application of the plaque assay required bacteriophage with the amber mutation to go through the process of reverse mutagenesis to regain a viable DNA sequence, thus allowing it to titer (form a plaque) as wildtype.

The experimental assays were conducted on the restrictive bacterial host (K-12) that demands the bacteriophage go through the process of reverse mutagenesis in the first "burst" or so to result in a countable plaque.

A control was used to determine the number of spontaneous revertants. By comparing the number of revertants counted in the field to that of the control, the effect of the field could be quantified. If the number of revertants in the field increased then it would lead to the assumption that the EMF caused mutagenesis.

A simple protocol was designed to determine the effects of the EMF. Approximately 10^7 amber mutant phage and 3 x 10^8 bacteria host were mixed in soft top agar. The agar was then layered on a Hershey's plate and allowed to solidify. One half of the plates were placed in the DC/B-field at a controlled Gauss per series of experiments and half were placed in the control Faraday cage. The experiment was run for approximately 24 hours at room temperature (22+/-2 degrees Celsius). The plaque were counted with the assistance of a plate reader.

RESULTS

The graph on figure 4 depicts the effect of the AC/E-field on reverse mutagenesis of a phage T4D am42 mutation. The data are presented by plotting the number of plaque counted versus the field-strength applied. The differing relative heights of the bars indicate different populations of phage and are not a result of treatment. Although there appears to be a difference between the control values and the EMF values, they are not generally significantly different ($p=>0.05$). However, at field-strength 1053 V/M there was a significant decrease in the number of revertants

in the experimental trials ($p=0.04$) where there was less mutagenic activity than measured in the controls.

CONCLUSION

Except for the results at 1053 V/M, the experimental results reveal no significant increase in reversion rate as determined by phage T4 reverse mutagenesis; therefore, EMF of this type and at the strengths tested are not mutagenic, with the following three caveats. First, the literature review disclosed mostly epidemiological studies expressed in correlations of EMF to some biological phenomenon. This experiment took a novel approach to the problem by using a quantifiable molecular level methodology. Therefore, this methodology is new and preliminary; therefore, more assays need to be conducted to gather a larger data base to detect an subtle statistical differences. Second, this test was designed specifically for the bacteriophage T4D model and these results cannot necessarily be generalized to other genetic systems such as human cells. Although it is known that much at the molecular genetic level is universal or nearly so. Third, this experiment assayed only for the nonsense (amber) mutation. It did not take into account the other categories of nonsense mutations or the other types of mutations (frameshift, deletions, etc.) that the EMF could be causing if indeed it causes some sort of mutagenic event as the underlying explanation of the reported biological phenomenon.

The results at 1053 V/M remain an enigma. We are at a lose to explain the apparent antimutagenic effect of this particular AC/E-field strength. The most conservative explanation is that this result was a statistical aberration based on sampling error. Other explanations would invoke phenomenon, such as resonance, etc., not yet demonstrated to act on biological systems. Obviously, more corroborative work must be done to resolve the reason for this inconsistent data point. In summary, alternating current fields at the strengths tested do not demonstrate mutagenic activity in the bacteriophage T4 model.

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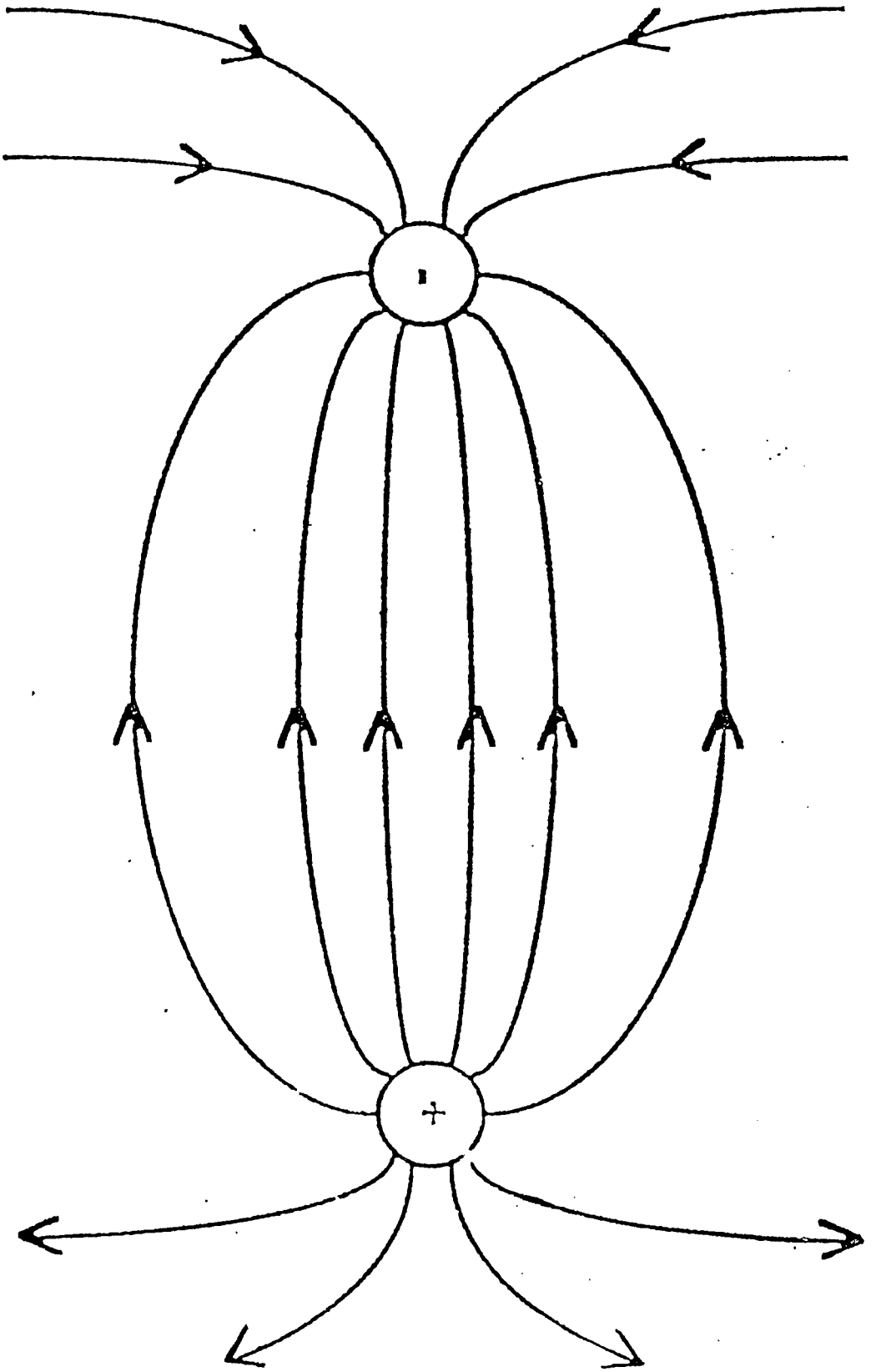
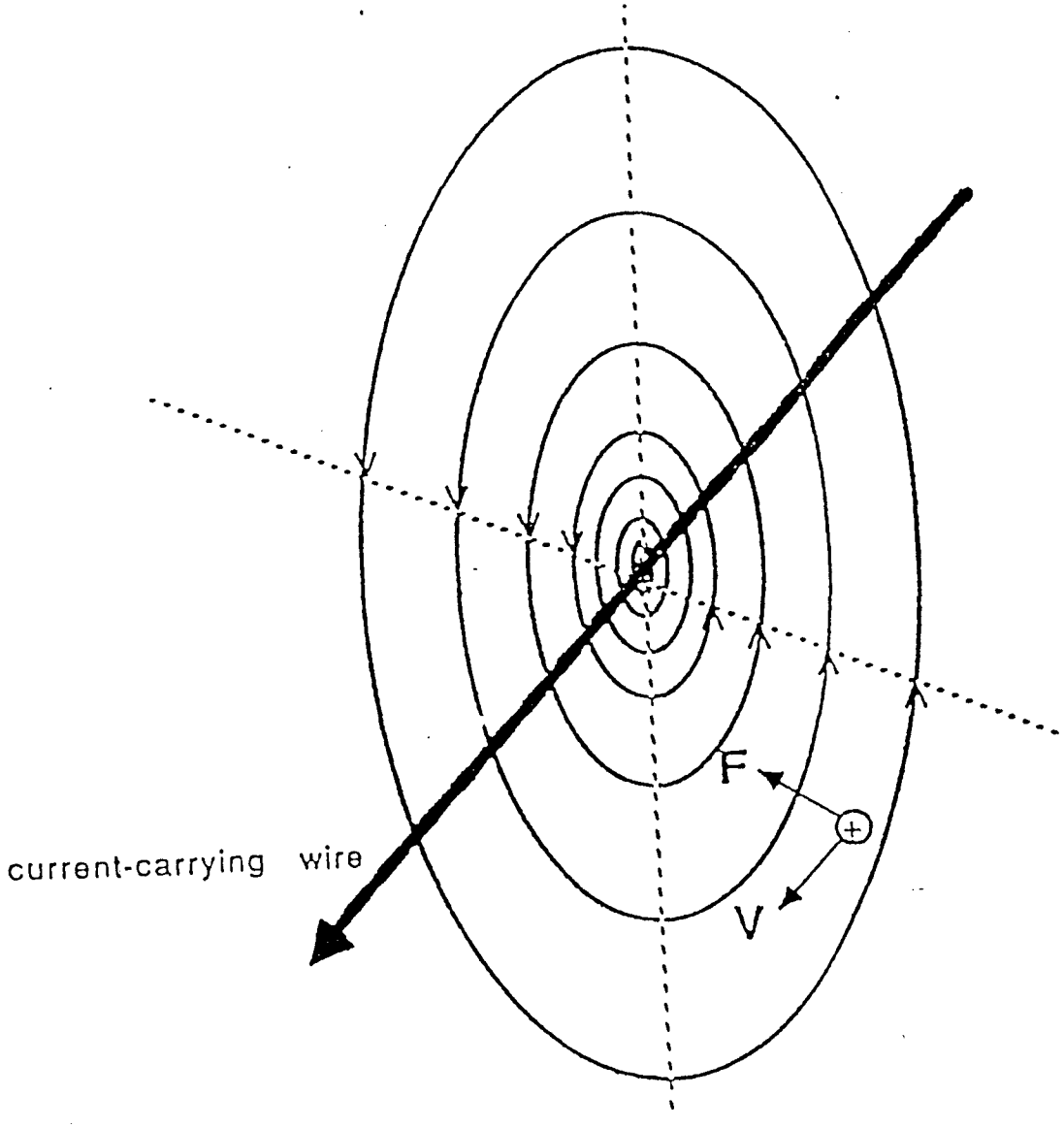
Electromagnetic Fields-Analysis of Chromosome 2R and 2L. Bioelectrochemistry and Bioenergetics 31:39-47.

FIGURE 1



The electric field of two equal but opposite charges. A small positively charged particle placed somewhere in this field will experience a force in the direction of the local field line (note arrows). The strength of the force is proportional to the spacing between the field lines (field lines closer together means a higher field and thus a larger force on small charged particle). (U. S. Congress, 1989)

FIGURE 2



The magnetic field of a long straight wire produces a force, F, on a positively charged particle that is moving nearby. The strength of the field is proportional to the spacing between the lines (closer spacing means stronger field). The direction of the magnetic force on a charged particle moving in the field is perpendicular to both the field lines and particle's direction of motion, V. (U. S. Congress, 1989).

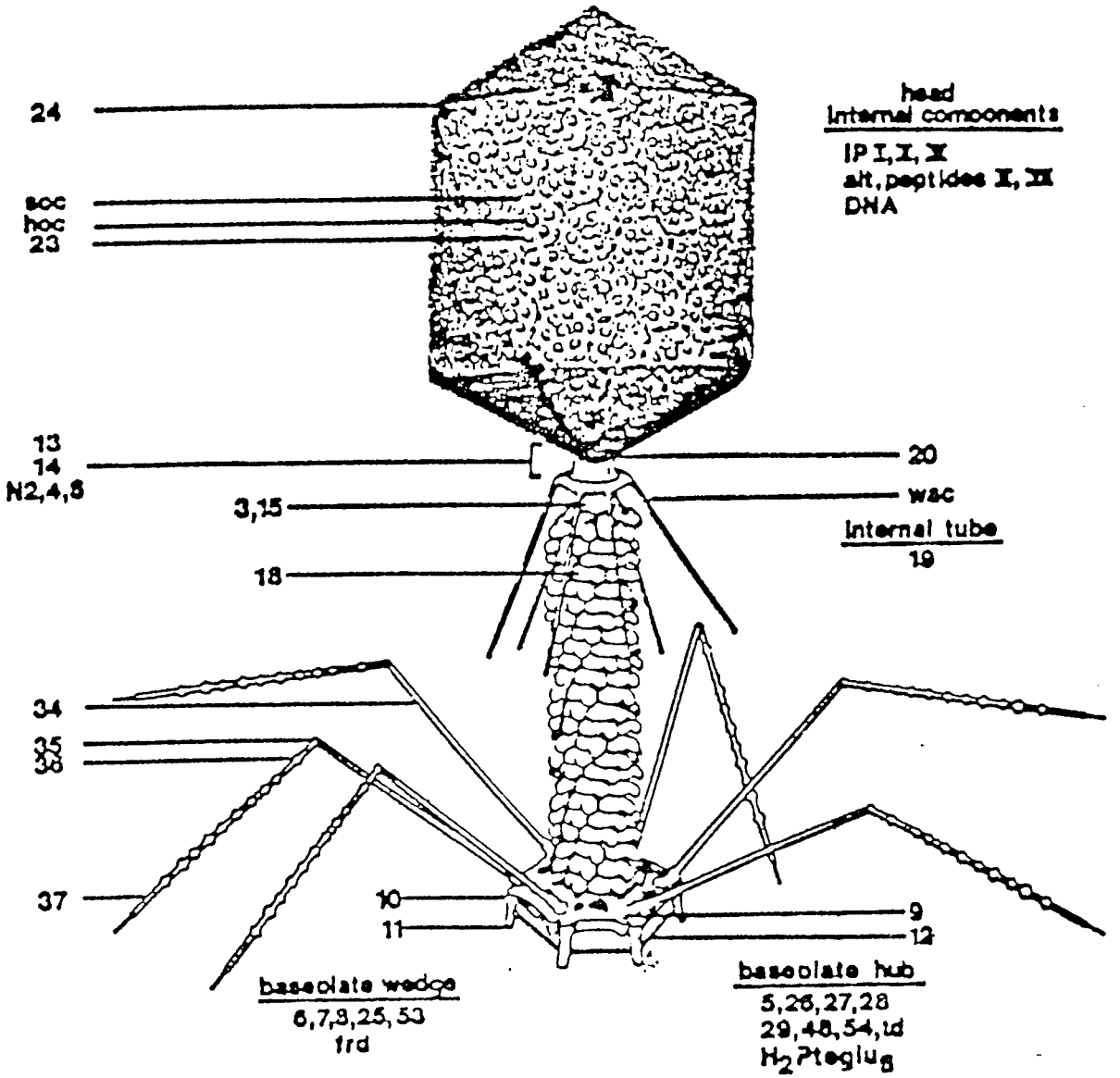


FIGURE 3 : Structure of Bacteriophage T4D+. The numbers and other designations represent gene products from the phage T4D+ genome that comprise the structure of the phage. (Guttman and Kutter, 1983)

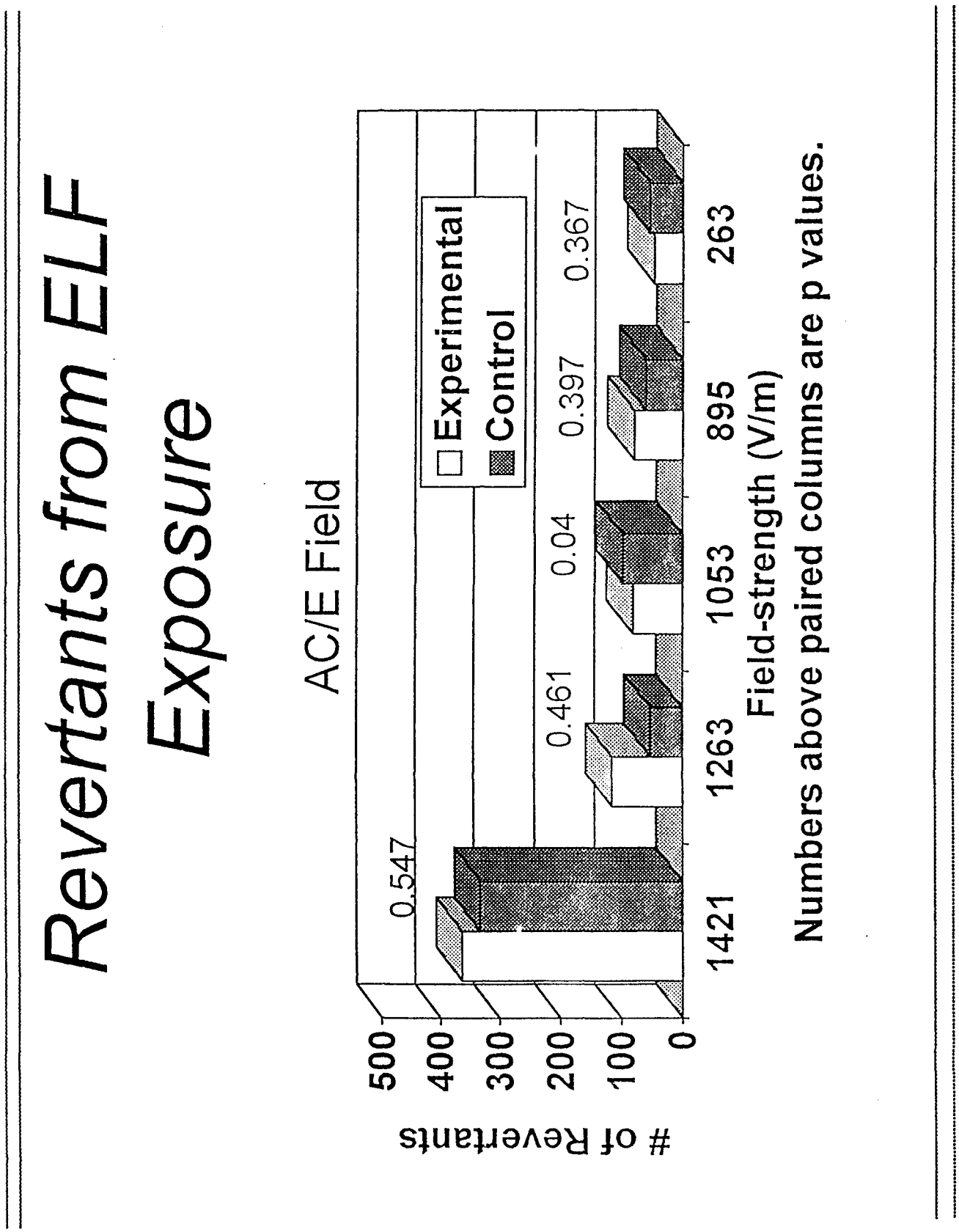

Revertants from ELF Exposure
AC/E Field
Experimental
Control
# of Revertants
500
400
300
200
100
0
0.547
0.461
0.04
0.397
0.367
1421
1263
1053
895
263
Field-strength (V/m)
Numbers above paired columns are p values.

Figure 4