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System for the Exposure of Cell Suspensions to Power-Frequency Electric Fields

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A system is described that uses an oscillating magnetic field to produce power-frequency electric fields with strengths in excess of those produced in an animal or human standing under a high-voltage electric-power transmission line. In contrast to other types of exposure systems capable of generating fields of this size, no electrodes are placed in the conducting growth media: the possibility of electrode contamination of the exposed suspension is thereby eliminated. Electric fields in the range 0.02–3.5 V/m can be produced in a cell culture with total harmonic distortions less than 1.5%. The magnetic field used to produce electric fields for exposure is largely confined within a closed ferromagnetic circuit, and experimental and control cells are exposed to leakage magnetic flux densities less than 5 μT. The temperatures of the experimental and control cell suspensions are held fixed within ± 0.1 °C by a water bath. Special chambers were developed to hold cell cultures during exposure and sham exposure. Chinese hamster ovary (CHO) cells incubated in these chambers grew for at least 48 h and had population doubling times of 16–17 h, approximately the same as for CHO cells grown under standard cell-culture conditions.

Key words: ELF, electric fields, exposure systems, biological effects

INTRODUCTION

Several systems have been described in the literature for the exposure of whole animals to power-frequency electric fields [Poznaniak et al, 1979; Kaune et al, 1980]. These, and all other whole-animal exposure systems, capacitively couple the experimental subjects, through an air dielectric, to a system of electrodes. It would appear, at first glance, that a similar approach would be suitable for exposing cell suspensions to power-frequency electric fields. However, careful analysis shows that this conclusion may not be valid.

The electric field, E_s, induced inside a cell suspension exposed through air to an external field E can be estimated [Kaune and Gillis, 1981] as $E_s \sim (\omega\epsilon_o/\sigma)E$,

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where ω is angular freuqency, ϵ_o is the permittivity of air, and σ is the conductivity of the cell suspension. The dielectric strength of air is about 2 MV/m (rms) [EPRI, 1975] and the conductivities of most cell suspensions are about 1–2 S/m. Therefore we may conclude that the maximum electric field that can be induced in a cell suspension using air coupling is $\sim$0.01 V/m, a value that is considerably less than electric-field strengths that can be induced inside human beings standing near many electric-power transmission lines [Kaune and Phillips, 1980]. (The human body, because of its elongated body shape and relatively low conductivity, couples much more strongly to an external electric field than does the typical cell suspension.) The analysis in this paragraph shows that a different approach to exposing most cell suspensions is needed to simulate maximum exposure conditions near transmission lines.

A modification that has been used recently with some success is to replace the air medium between the electrode and the cell suspension with some other dielectric material, such as silicon oxide [Greenebaum et al, 1979], glass [Price et al, 1980], or polypropylene [Lymangrover et al, 1983]. Substantially higher field strengths can be induced in conducting media with these materials because both their dielectric strengths and dielectric constants are considerably larger than those of air. The investigators noted above obtained field strengths as high as 0.2 V/m in their conducting media using insulated electrodes.

A third approach, which avoids the limitations of capacitive coupling, is to use conducting electrodes placed directly in the conducting media. Since current is injected directly into the medium, through the relatively low-impedance interface between the electrodes and media, large field strengths can be easily obtained. However, there are concerns that electrolytic processes at the electrode-medium interface could release ions into the medium which might affect living cells [NAS, 1977; Rosenberg et al, 1965; Pareilleux and Sicard, 1970].

All the above types of exposure systems have used electrodes, in capacitive or resistive contact with the experimental preparation, to generate electric fields for exposure. An alternative approach is to use an oscillating magnetic field as an electric-field source. This paper describes a system based on this principle that can generate electric fields in cell suspensions up to 3.5 V/m in strength and has no electrodes in contact with the experimental preparation.

CONCEPTUAL DESIGN

The basic exposure system consists of a transformer, an alternating voltage source connected across a multiturn primary winding, and a secondary winding formed by a conducting experimental preparation (eg, a cell suspension) held in a nonconducting toroidal chamber (Fig. 1). The alternating current I_p flowing in the primary winding produces a magnetic field that is almost entirely contained within the ferromagnetic core. This field links the secondary winding and thus induces an electromotive force (emf), ξ_s, in the preparation (ξ_s is related to the primary voltage, V_p, by $\xi_s = V_p/N_p$, where N_p is the number of primary turns). The resulting *medium* electric field, E_s, in a preparation having a uniform conductivity σ is

$$E_s = \frac{\xi_s}{\sigma A R_s} \tag{1}$$

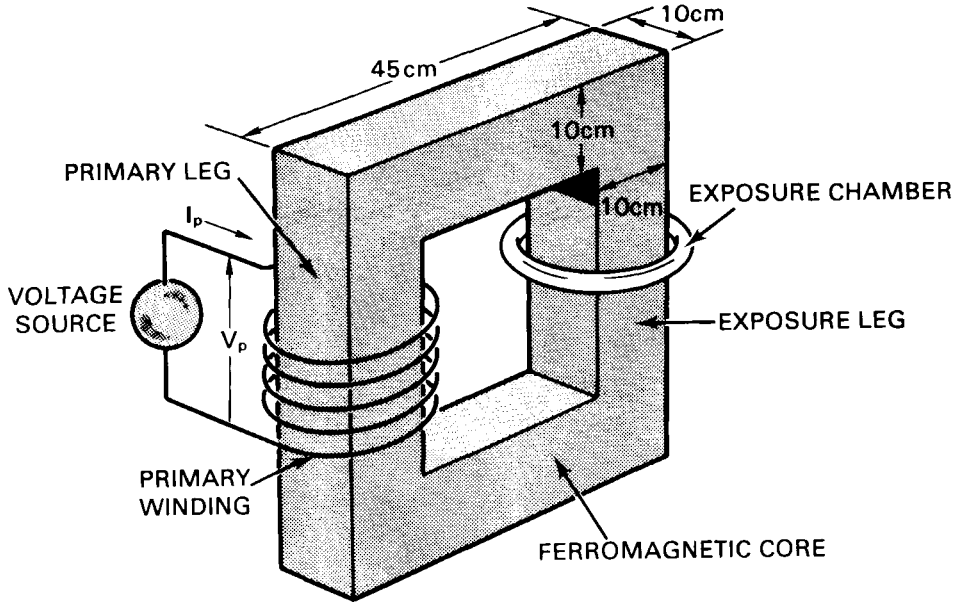

Fig. 1. Conceptual drawing of system for exposing cell suspensions to power-frequency electric fields. An alternating magnetic field, produced in a ferromagnetic core by a multiturn primary winding, induces an alternating electric field in a toroidal chamber containing growth medium and cells.

where A is the area of the current-carrying cross section at the point where E_s is being calculated and R_s is the total resistance seen by a current circulating around the toroidal chamber. The maximum medium field in a practical system is limited by the saturation magnetic flux density and size of the core.

The medium electric field, E_s, calculated using Eq 1 or measured using probes such as the one described in this paper, is an average value over a volume containing both medium and cells. The determination of the microscopic electric field at the level of a cell requires additional analysis which takes into account the cellular shape, structure, and concentration. The only way, at present, to determine microscopic field levels is by theoretical calculation. To simplify such calculations, we concluded that it was desirable to suspend the experimental cells in a homogeneous medium rather than allow them to settle on a dielectric surface. For example, a dilute suspension of spherical cells can be analyzed fairly easily [Schwan, 1972; Sheppard and Eisenbud, 1977], whereas these same cells resting on a plastic or glass surface present a very difficult and, to our knowledge, unsolved problem in field analysis.

Mechanical shaking or mixing is normally used to keep cultured cells in suspension. This was not practical with our system because of its mass and because the medium electric-field strength depended on the cross-sectional area of the suspension (Eq 1), which could be altered by shaking or mixing. As an alternative we placed agar solution on the bottom of our exposure chambers and allowed cells in the overlying medium to settle out on these beds. By preparing the agar solution with the same conductivity as the overlying medium, we thus simulated cells suspended in an electrically homogenous conducting medium.

Cells placed in the exposure chamber shown in Figure 1 will be exposed not only to an electric field but also to a small magnetic field which leaks from the ferromagnetic core. This leakage magnetic field can be minimized by 1) using a

ferromagnetic core of high magnetic permeability, 2) keeping the field in the core below saturation levels, and 3) magnetically shielding the exposure chambers containing the cells. Nevertheless, a residual field will remain, and it is important that the control cells also be exposed to this field. We accomplished this by placing a second exposure chamber around the transformer core which was identical to the first except that the conducting path around it was broken. If C is the capacitance across this break, it is easy to show that the control electric field, E_c, will be related to the medium field, E_s, by $E_c/E_s \sim \omega R_c C$, where R_c is the resistance of the control suspension. For the chambers used in the project, $R_c < 10$ kΩ and $C < 10$ pF. Therefore, $E_c/E_s < 10^{-4}$.

APPARATUS AND METHODS

Ferromagnetic Core and Primary Winding

The ferromagnetic core used in our system consisted of laminated silicon iron (Silectron 66) which had an initial relative permeability greater than 10^4 and began to saturate at a flux density of about 1.0 T. The dimensions are indicated in Figure 1.

Thirty-seven turns of insulated 16-gauge wire were wrapped on one leg of the core to form a primary winding. This winding was energized from the output of a circuit consisting of a variable transformer connected to a step-down transformer, which had a selectable turns ratio of 1:1, 19:1, or 304:1. Input power for the system was supplied from an AC line conditioner (California Instruments, model LC1201B), which was used to reduce the 3% total harmonic distortion (THD), present in the building 110-V supply, to a level <0.3%.

Exposure Chambers

The simplest type of exposure chamber would be a circular toroid such as the one shown in Figure 1. We experimented with this kind of chamber (circumference ~ 75 cm, made from 1.3-cm-diameter surgical-grade silicon tubing), but we were unable to develop a practical system because of the following problems: 1) Surface-tension effects made it very difficult to maintain the medium cross-sectional area constant around the circumference of the chamber unless the chamber was completely filled with agar and culture medium; 2) growth rates of mammalian cells were substantially reduced unless the exposure chamber contained an air phase.

Figure 2 shows the exposure and sham-exposure chambers developed for our system. Cell cultures were placed in a central 14-cm-long *sample section* made of 1.36-cm-ID (inside diameter) glass tubing. The ends of a sample section were joined to two 1.76-cm-ID pieces of glass tubing that were completely filled with agar solution to form agar plugs. The ends of the exposure chamber were connected together using a length of Tygon tubing filled with medium to form a conducting loop (Fig. 2). The ends of the control chamber were terminated with rubber stoppers which, as discussed earlier, blocked the flow of magnetically induced current. Access to the sample section of a chamber was through a centrally located port which was sealed with a rubber serum-bottle stopper. Sample sections were siliconized before each use to prevent cells from adhering to their inner surfaces, and were normally filled with 7 ml each of agar solution, culture medium, and a 95% air/5% CO_2 mixture.

A Plexiglas frame was built to hold the chambers, with the control chamber located immediately above the exposure chamber, as shown in Figure 2. The cham-

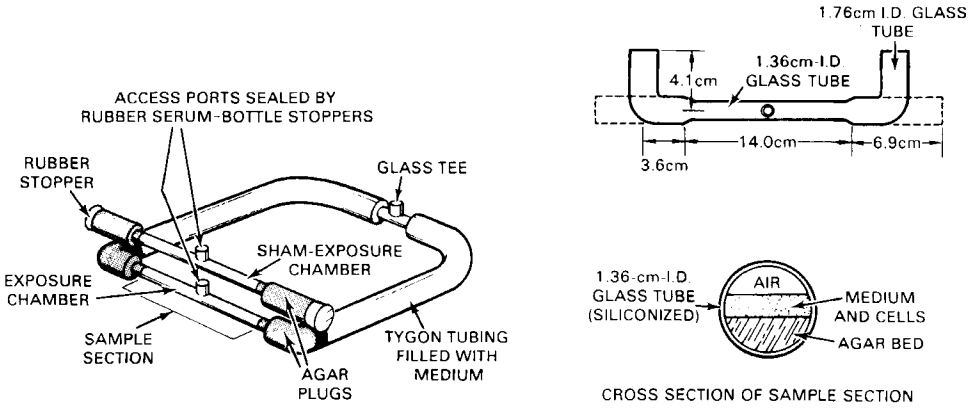

Fig. 2. Chambers to hold exposed and control cell cultures.

bers and their frame were placed in a water bath, described in the next section, and were leveled so that the elevations of the two ends of a sample section differed by less than about 1 mm.

The medium electric field inside an exposure chamber could not be easily calculated because of the chamber's relatively complex geometry. A simple probe was therefore built to measure electric fields directly in these chambers; this probe will be described in a later section of this paper.

Water Bath

The temperatures of the exposed and control chambers were regulated by immersing them in a constant-temperature water bath. This bath was built in the form of a square trough enclosing one leg of the magnetic core (Fig. 3). The trough was constructed of 6.4-mm-thick Plexiglas and held about 8.5 l of water. The outside surfaces of this bath (including a removable lid) were covered with 0.25-mm-thick μ-metal for magnetic shielding. This shielding was installed so that no electrically conducting loops through the μ-metal linked the magnetic core. Water was circulated at a rate of about 8 l/min from an external reservoir, which incorporated a pump and thermostatically controlled heater unit.

Secondary Electromotive Force Monitoring

A wire loop that encircled the exposure leg (Fig. 1) of the transformer core was installed on the bottom surface of the floor of the water bath. This loop was terminated with a 10-kΩ resistor, and a pair of banana jacks were provided so that a voltmeter could easily be connected to measure the induced voltage across the terminating resistor (the voltage was independent of the size of the resistor for resistances $>>1$ Ω). In this way we obtained a direct measurement of ξ_s, the secondary induced emf.

Electric-Field Probe

Exposure electric fields were determined by measuring the potential difference, V, between two closely spaced points in a cell suspension. The probe used for these measurements consisted of a glass handle with two parallel 1.4-cm-long, 0.5-mm-diameter stainless steel needles protruding from the end; the spacing between the needles was 3.05 mm. The differential voltage between the probe tips was measured

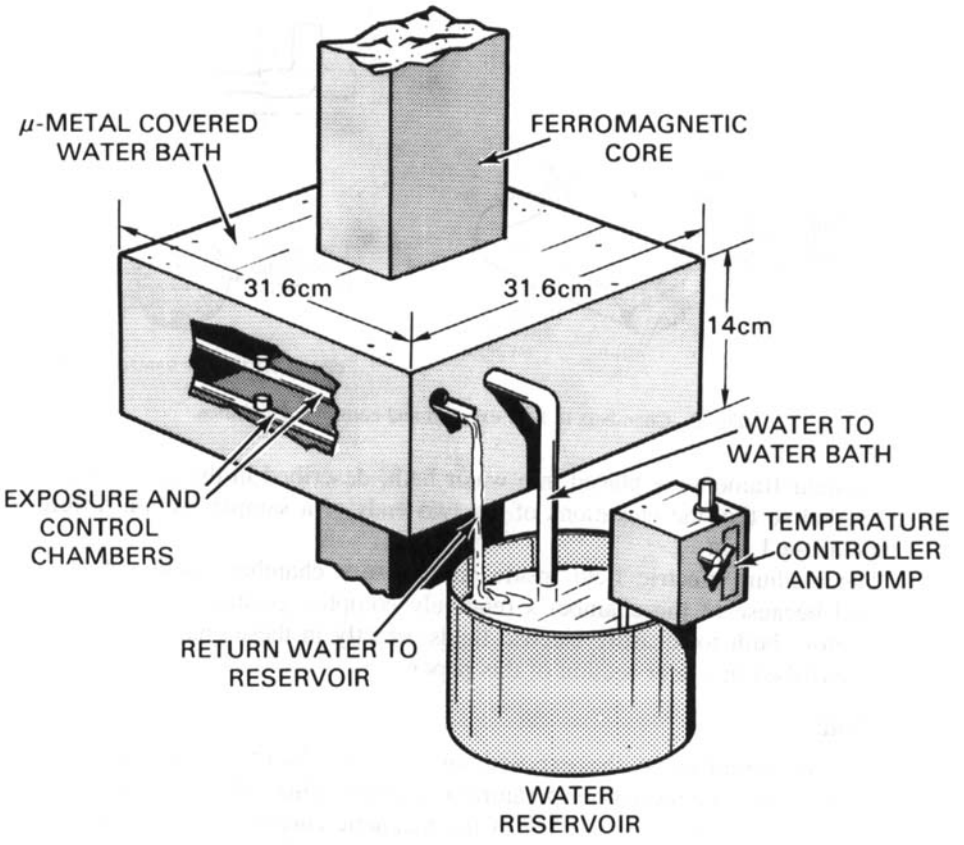

Fig. 3. Water bath used to control the temperatures of the exposed and control cell suspensions. Water from a controlled-temperature reservoir is circulated through the bath at about 8 l/min. The bath was covered with μ-metal to reduce the magnetic-field strength inside it.

using a differential amplifier (EGG Inc, model 113). In order to reduce the common-mode voltage applied to the amplifier, a single grounded hypodermic needle was inserted into the Tygon-tubing portion of the complete exposure chamber (Fig. 2).

The field probe was calibrated by measuring the known field between two large parallel stainless steel electrodes inserted in a large volume of saline. The measured calibration of the probe was $E = (327\ m^{-1})V_{probe}$, where E and V_{probe} are the average electric field and voltage between the probe tips. This number compares well with the theoretical value of 328 m^{-1} calculated using the measured probe-tip spacing.

Magnetic-Field Probe

A search-coil magnetic-field probe was built by winding 1,000 turns of No. 24 enamel-insulated wire on a Plexiglas rod to form a solenoidal coil 3.0 cm long with inner and outer radii of 2.2 cm and 3.1 cm, respectively. This coil was connected to an operational-amplifier integrator.

The field meter was calibrated using a uniform magnetic field produced inside a 74-cm-long, 10-cm-diameter solenoid wound with 353 turns of wire. Over a flux-density range of 0 to 2.4×10^{-4} T, the calibration of the meter was $B_n = (0.98 \times$

10^{-4} T/V)V_B. This value was constant to within 0.5% over the 60–420 Hz frequency range tested. (A detailed theoretical analysis indicates that it was essentially constant from below 60 Hz to about 10 kHz.)

Harmonic Distortion Measurements

A waveform was digitized using a digital oscilloscope operating with a sampling frequency of 5 kHz. One cycle of the waveform was input to a desk calculator which calculated the magnitude, V_n, and phase of the first 20 Fourier components. The total harmonic distortion (THD) was calculated using

$$\mathrm{THD} = \left[\sum_{n=2}^{20} \left(\frac{V_n}{V_1} \right)^2 \right]^{1/2} . \tag{2}$$

Conductivity Measurements

Measurements of medium and agar-solution conductivities were made using the four-electrode method [Benjamin et al, 1950]. Conductivity cells were made from 28-cm lengths of 0.93-cm-ID polyethylene tubing. Wire electrodes at each end were used to introduce a known 60-Hz current, I, into the cell. Two additional electrodes spaced a distance d = 4 cm apart were inserted into the center of the cell and the voltage, V, between these two electrodes was measured using a high-input-impedance voltmeter. The conductivity, σ, of the sample in the cell was then calculated using σ = Id/AV, where A = cross-sectional area of the cell (the cell was totally filled with the material being tested).

Cellular Testing Methods

The ultimate purpose of the work reported in this paper was to develop an exposure system for use with cultured mammalian cells. Thus, as part of the development process, we attempted to optimize cell growth rates in our exposure chambers.

The cell line used in our tests was a clone (designated CHO-K1 BH_4) of Chinese hamster ovary (CHO) cells [Kao and Puck, 1967; O'Neil et al, 1977] obtained from Dr. A. Hsie, Oak Ridge National Laboratory, Oak Ridge, TN. The growth medium used for experiments in our system was Ham's F12 medium [Ham, 1965] supplemented with 5% heat-inactivated and dialyzed fetal bovine serum (medium labeled F12-FBS 5).

Cell growth rates were determined by counting replicate samples of each treatment group in a hemocytometer. Plotting cell numbers versus length of time in culture provided an estimation of population doubling time.

EXPOSURE-SYSTEM CHARACTERIZATION

Primary Excitation Current

The primary current was measured as a function of primary voltage with no secondary load. Those quantities were approximately linearly related for primary voltages below 70 V. However, above 80 V the primary current began to rise rapidly and became increasingly distorted. As discussed earlier, this occurred because of magnetic saturation of the core material. We deemed it advisable to operate with limited saturation so we selected a maximum primary operating voltage of 90 V.

Secondary Electromotive Force Measurements

As shown by Eq 1, the exposure electric field is directly proportional to the induced secondary emf, ξ_s. ξ_s was measured using a loop of wire that encircled the transformer core in the approximate position that would be occupied by an exposure chamber. The voltage signal from this loop was amplified by a factor of 10 for emfs in the range 0.005–0.03 V and by a factor of 100 for emfs < 0.005 V. Figure 4 shows ξ_s plotted against the primary voltage, V_p; this figure also shows the results of a linear regression fit to these data using the model $\log_{10} \xi_s = \log_{10} V_p + \log_{10} N$. The fitted value for the turns ratio, N, was 37.3:1.

The measured magnitude of the first through tenth harmonic components of the secondary emf, and hence of the exposure electric field, are given in Table 1 for values of secondary emf ranging from 1.3 mV to 2.25 V. The next ten harmonic magnitudes were also measured but are not given since they were all $<0.1\%$. Total harmonic distortions, calculated using Eq 2, are also given in Table 1.

Electric-Field Measurements

The medium electric-field strength in an exposure chamber depended on the induced emf, the dimensions of the chamber, the relative conductivities of the various

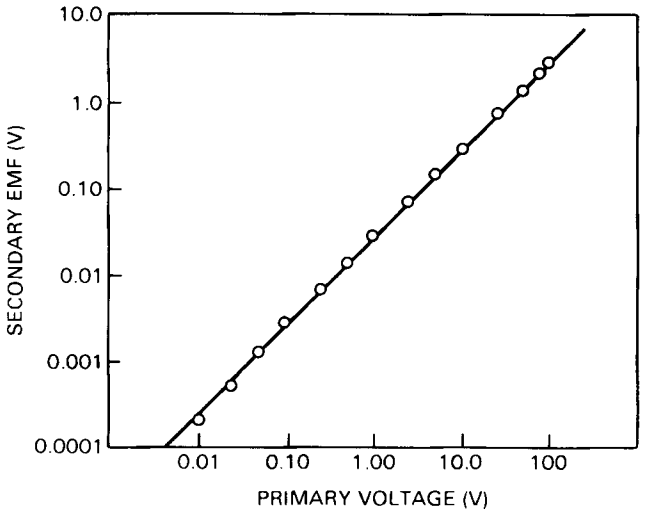

Fig. 4. Electromotive force (emf) induced in a toroidal loop as a function of primary voltage.

TABLE 1. Harmonic Spectra of the Secondary emf and Medium Electric Field*

Secondary emf (V)	Medium electric field (V/m)	Harmonic number 2	3	4	5	6	7	8	9	10	THD
0.0013	0.0018	0.4%	4.3%	0.4%	0.7%	0.4%	0.4%	0.1%	0.5%	0.1%	4.5%
0.0027	0.0038	0.2%	2.7%	0.2%	0.7%	0.2%	0.2%	0.3%	0.6%	0.1%	2.9%
0.014	0.020	0.2%	1.2%	0.2%	0.7%	0.3%	0.4%	0.2%	0.1%	0.1%	1.5%
0.028	0.039	0.2%	0.8%	0.2%	0.4%	0.1%	0.3%	0.1%	0.1%	0.0%	1.0%
0.28	0.39	0.3%	0.5%	0.3%	0.3%	0.2%	0.2%	0.1%	0.1%	0.1%	0.8%
1.13	1.59	0.4%	0.5%	0.2%	0.2%	0.1%	0.1%	0.1%	0.0%	0.0%	0.7%
2.25	3.17	0.4%	0.8%	0.3%	0.4%	0.1%	0.2%	0.0%	0.0%	0.0%	1.1%

*Harmonic magnitudes are expressed as percentages of the magnitude of the 60-Hz fundamental.

components, and the volume of medium placed in the sample section. Thus, two different preparations operated with the same induced emfs did not necessarily have exactly the same exposure field strengths. To assess this variability, we measured, through the access ports (Fig. 2), the electric fields in eight different exposure preparations at six different emf levels. The means and *standard deviations* are shown in Figure 5. Note that the standard deviations are typically 10% of the corresponding means. A linear-regression fit to the mean values using a one-parameter model yielded the result that

$$E_s = (1.41\ m^{-1})\xi_s \tag{3}$$

The measurements described in the previous paragraph were made at only one point (the center) of an exposure chamber. It cannot be concluded that the electric fields at other points in a chamber were the same as the measured value since the chambers were never perfectly level. As mentioned earlier, we estimate that the elevations of the two ends of a 14-cm-long test section differed, but by no more than about 1 mm. This means that the cross-sectional area, A, of the medium in the test section varied over its length. Assuming the test section was 67% full of agar and

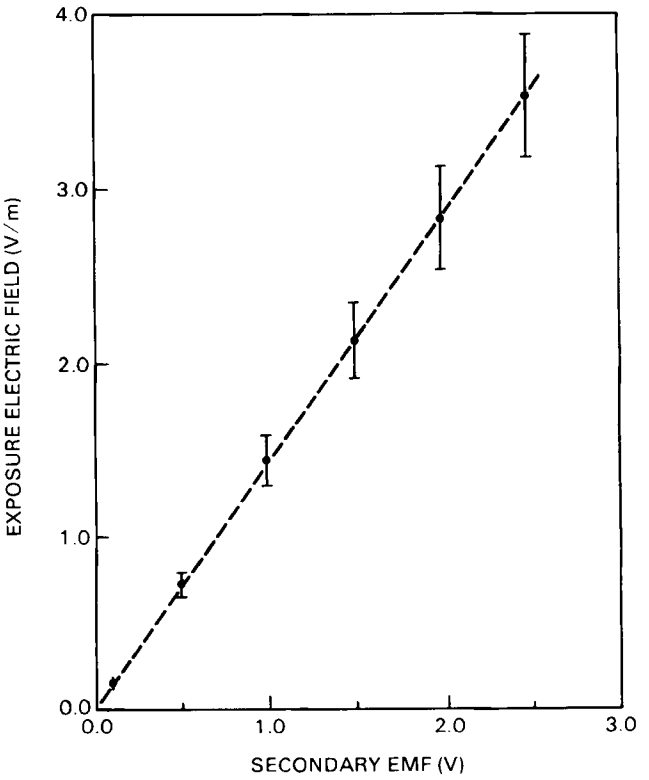

Fig. 5. Exposure electric field in an exposure chamber as a function of the secondary electromotive force (emf). Points and bars are means and standard deviations, respectively, of measurements made in eight different experimental preparations.

medium, and using Eq 1, we estimate that the electric field in the chamber could have varied by no more than $\pm 7\%$ from the value measured at its center.

The harmonic spectra of the medium electric field, E_s, was not directly measured. However, the harmonic-distortion data given in Table 1 apply also to E_s because E_s is directly proportional to ξ_s.

Magnetic-Field Measurements

The measurement of the magnitude and harmonic content of the leakage magnetic flux density was complicated by the fact that these quantities varied significantly over time for medium electric fields smaller than about 0.05 V/m. For example, at a medium electric field of 4 mV/m, the leakage flux density increased by 45% over a 24-h period. Figure 6 and Table 2 respectively give, as a function of medium electric-field strength, typical values for the magnitude and total harmonic distortion of the leakage magnetic flux density. Because of the variability mentioned above, these data only estimate actual levels that would be present at any particular instant of time.

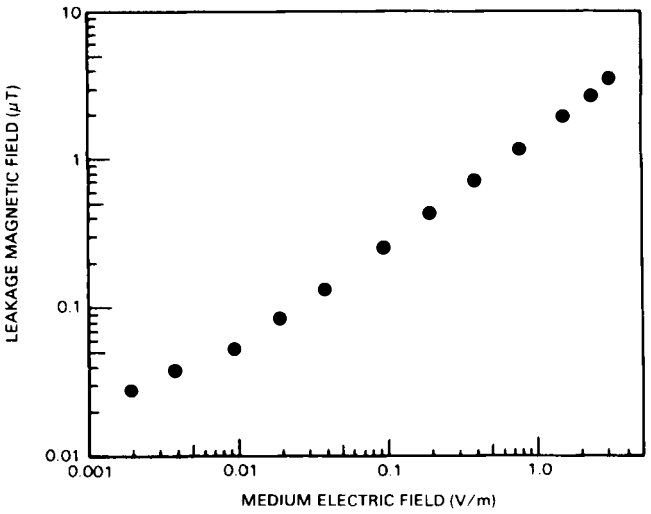

Fig. 6. Typical leakage magnetic field inside the μ-metal-lined water bath. Measurements are given for 12 values of the medium electric field.

TABLE 2. Total Harmonic Distortion of the Leakage Magnetic-Flux Density for Various Electric-Field Strengths in the Medium

Electric field (V/m) in the medium	Total harmonic distortion (%)
0.0038	10
0.018	10
0.038	6
0.38	3.4
1.6	11
3.1	29

Temperature Stability

A thermistor probe was used to determine how closely the temperature in the water bath, and that of an exposure chamber placed in the bath, could be regulated. The bath temperature was initially set to 37.1 °C and temperature data were recorded every 10 min for the ensuing 27-h period. The average and standard deviation of these data were 37.22 and 0.04 °C, respectively, showing that long-term temperature stability was very good.

Medium and Agar Conductivities

Conductivity measurements were made on culture medium (F12-FBS 5) and on agar-medium solution (0.6% Ionagar No. 2 per 100 ml of F12-FBS 5). At 35 °C, the medium conductivity was 1.9 ± 0.2 S/m. Agar conductivities within 5% of the corresponding conductivity of medium alone could be obtained if care was taken to minimize evaporation during the preparation of the agar solution.

Cell-Growth Experiments

Initial tests with several different exposure chambers led to selection of the design described in this paper. A series of experiments were then performed with the following goals: selection of a medium buffer; determination of the relationship between initial cell concentration and subsequent growth rate; selection of agar composition; and measurement of optimum growth rate. The results of these experiments are described below.

Selection of medium buffer. Two buffering systems—sodium bicarbonate and sodium bicarbonate with HEPES (N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid)—were evaluated in terms of their effects on cellular growth rates. The results indicated that the normal concentration of sodium bicarbonate in F12-FBS 5 medium provided adequate buffering capacity for continuous growth in experiments up to 48 h in duration when the sample section of the exposure chamber contained a 95% air/5% CO_2 phase whose volume was equal to about one-third of the exposure volume.

Initial cell concentration. Growth-rate measurements on experimental preparations characterized by varying initial cell concentrations yielded the following results: A significant lag period (10–12 h) was observed before cells started to assume a normal growth rate when the initial cell concentration was $< 5 \times 10^4$ cells/ml. With initial concentrations $> 3 \times 10^5$ cells/ml, cultures reached the stationary phase in elapsed times <48 h. We concluded that initial cell concentrations of about $1–2 \times 10^5$ cells/ml provided optimal growth rates for experiments up to 48 h in length.

Agar solution composition. An agar solution composition of 0.6 g Ionagar No. 2 per 100 ml of medium was found to be optimum. At Ionagar concentrations below about 0.5 g/100 ml, cells sank into the agar solution bed and were difficult to resuspend in the medium prior to sampling. Ionagar concentrations above 0.7 g/100 ml resulted in a harder agar solution which often separated from the glass surface of the exposure chamber: cells settling into the region produced by this separation were difficult to harvest.

CHO growth rates. Single-point population doubling time measured after 24 h of growth were 16–17 h when the initial cell concentration was in the range $1–2 \times 10^5$ cells/ml. This value compares well with values of 14–15 h measured for CHO cells under standard cell-culture conditions (ie, grown as monolayers in plastic flasks at 37 °C in a humidified 5% CO_2 incubator). The observed growth rates were

adequate for the kinds of experiments we planned to do—24-h exposures with the endpoints of mutation frequency, plating efficiency, and cell viability.

DISCUSSION

We have described a system for the exposure of cell suspensions to power-frequency electric fields that does not use electrodes. Thus, all questions about possible electrode contamination of the exposed cell suspension are eliminated. With this system, cells can be exposed to electric fields that are stronger than the electric fields induced in humans standing under existing or planned electric-power transmission lines.

Data presented in this paper show that electric fields ranging from < 1 mV/m to as high as 3.5 V/m can be generated in a cell suspension. However, it may be appropriate for some experiments to keep the minimum fields above about 20 mV/m because of the increase in harmonic distortion that occurs for lower field strengths (Table 1). Lower field strengths with low harmonic distortion can be obtained by using a smaller core or, perhaps, using a core made of a different material.

Electric-field strengths higher than the maximum 3.5 V/m reported in this paper can be obtained by replacing the medium in the Tygon-tubing portion of the exposure-chamber electrical circuit (Fig. 2) with a more conductive saline solution. For example, we have obtained maximum field strengths up to 10.5 V/m using 4 M NaCl.

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