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CELL GROWTH IN A LOW-INTENSITY 60 Hz MAGNETIC FIELD[1]

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ABSTRACT

The growth rate of EMT6 mouse mammary tumor cells was measured during exposure to a 2.3 mT (millitesla[2]), 60 Hz magnetic field. The length of the intermitotic interval for cultures exposed to the field was found to be identical to that of control cultures. The results of these experiments are described in the context of research that is being conducted in other laboratories on the effects of magnetic and electric fields on cell growth *in vitro*.

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INTRODUCTION

The biological impact of the fringe electromagnetic fields associated with extra-high-voltage (EHV) transmission lines (345-765 kV) has become an extremely controversial subject. At ground level directly below the EHV lines, the electric and magnetic field amplitudes are generally $\lesssim$ 10 kV/m and $\lesssim$ 0.05 mT, respectively. The potential biological effects of such fields are poorly understood, and there are currently no guidelines to define the safe limits of human exposure. As a consequence, a large number of research programs have recently been initiated in which an analysis is being made of the effects of 60 Hz electromagnetic fields on *in vivo* and *in vitro* biological test systems. The research described in this report was directed towards determining whether the magnetic component of a weak 60 Hz field could influence the growth kinetics of cultured cells. Both the exposure conditions and the experimental results are presented in detail, and a discussion is given of potentially fruitful areas for future investigations on the influence of extremely-low-frequency fields on cell growth.

MATERIALS AND METHODS

Magnetic field parameters and exposure chambers

A 60 Hz magnetic field was generated by the set of Helmholtz coils shown in Fig. 1. Current to the coils was supplied by a 110 V, 60 Hz line, and the field intensity could be regulated by controlling the current with a variac. In all of the experiments reported here, the peak field intensity was 2.33 mT (1.65 mT r.m.s.[3]). This was the maximum magnetic field strength that could be obtained using a 110 V, 60 Hz line as a source of current for the Helmholtz coils. The magnetic field was measured using a Bell Model 620 gaussmeter with a transverse Hall probe (F. W. Bell Inc., Orlando, Fla.). The magnetic field in the central 9 in. x 9 in. x 9 in. region within the set of Helmholtz coils was found by means of gaussmeter measurements to be uniform to ± 4%. This portion of the Helmholtz coils was used for exposure of cell cultures to the 60 Hz magnetic field.

During exposure, the cultures were incubated at 37 ^{o}C in a specially designed, nonmagnetic, closed-system water bath connected by means of insulated tygon tubing to the water circulator and thermoregulator shown in Fig. 1 (Haake Model FS, VWR Scientific, San Francisco, CA). Water heated by the thermoregulator was continuously pumped through the chamber by the circulator. The chamber could be opened and closed within a period of 5 min. for rapid removal of tissue culture flasks, thereby minimizing heat loss. A similar chamber was used for simultaneous incubation of control cultures in the first experiment (see Fig. 2). For reasons discussed in the Results section, a Napco CO_2 incubator (VWR Scientific, San Francisco, CA) was used in place of the control

chamber in all subsequent experiments. Temperature in the exposure and control chambers was monitored by copper-constantin thermocouple probes, and recorded at 45-60 min. intervals by an Autodata Nine data acquisition unit (Acurex, Mountain View, CA).

Cell cultures

EMT6 cells[4], originally obtained from Dr. Theodore Phillips, University of California Medical Center, San Francisco, were continuously subcultured in Dulbecco's Modified Eagle Medium (Grand Island Biological Co. (GIBCO), Grand Island, N.Y.) containing 15% fetal bovine serum (Flow Laboratories, Inglewood, CA), 50 units/ml penicillin and 50 μg/ml streptomycin (GIBCO). The cultures were grown in 25-cm^2 tissue culture flasks (Falcon Labware, Oxnard, CA), and maintained at 37°C in a 95% air/5% CO_2 incubator with a subculture interval of 3-4 days.

The subculture procedure was begun by aspirating the medium from the surface of a cell culture in exponential growth phase and gently washing the monolayer with 3 ml of a trypsin/EDTA solution (GIBCO) at 37°C. This solution was removed by aspiration, and three ml of fresh trypsin/EDTA were again added to the flask, gently swirled and incubated for 3 min. at 37°C. Three ml of 37°C culture medium was then added to the trypsinate, and the cell suspension was triturated by pipetting and transferred to a centrifuge tube. The suspension was pelleted by centrifugation at 250g for 3 min. The supernatant solution was removed by aspiration and the pellet was resuspended in 10 ml of 37°C culture medium. A 0.5 ml aliquot of the resuspended sample was diluted in 19.5 ml of 0.9% NaCl and counted on a Particle Data Inc. (Elmhurst, Ill.) electronic

cell counter. The sample was then diluted to 10^4 cells/ml in culture medium and 1 ml of the cell suspension was inoculated into each of a series of 25-cm^2 growth flasks containing 7 ml of 37^oC medium equilibrated at pH 7.4. The flasks were then placed in a 37^oC incubator continuously flushed with a 95% air/5% CO_2 mixture to maintain the pH at 7.4.

Magnetic field exposure

Exponentially growing cultures were trypsinized and a cell suspension with 2×10^4 cells/ml was obtained in the manner described above (instead of 10^4 cells/ml). One ml was inoculated into each of 45-50 flasks containing 29 ml of 37^oC culture medium equilibrated at pH 7.4. The flasks were sealed with UV-sterilized parafilm and incubated for 12 hours to allow the cells to attach to the growth surface[5]. The cultures were quickly transported by car to the magnet facility, where 25 flasks were placed in the magnetic field exposure chamber. The remainder of the flasks were used as control cultures. As shown in Fig. 3, the flasks in the exposure chamber were aligned parallel to the lines of magnetic induction. Control cultures were maintained in the same orientation. The chambers were covered with black velvet cloth to exclude light, since incandescent light is reportedly harmful to cells *in vitro* (1).

Cell growth curves

A total of 3-4 flasks were harvested from each chamber at intervals of 6-24 hours for a total of 4-5 days. The magnet and water circulator were turned off during the brief period when the flasks were removed. The harvested flasks were placed in a dark container and quickly transported to the tissue culture

laboratory. All flasks were scanned under a light microscope with 50X magnification to check for morphological and orientational changes in the cell colonies of the exposed relative to the control cultures. The culture medium was then thoroughly removed by aspiration and 2 ml of trypsin/EDTA solution were quantitatively added to each flask. The flasks were then incubated at 37°C for 5 min. For each flask, the trypsinate was triturated and transferred to a 7 dram vial with a Pasteur pipette. The residual trypsinate was quantitatively removed from each flask by washing the growth surface with 0.9% NaCl. Finally, the 2 ml of trypsinate was diluted by a factor of 7X to 370X for electronic cell counting. Each cell sample was counted 5 times in the cell counter, the population mean and standard error were calculated, and the average number of cells was plotted against time on a semilogarithmic graph. Each point of a growth curve represents the mean cell number and standard error for a total of 3-4 flasks. To determine the cell population doubling time, the data were analyzed by an exponential curve fitting routine on a Hewlett-Packard 93880A computer.

RESULTS

Experimental EMT6 cultures were continuously exposed to a 1.65 mT (r.m.s.), 60 Hz magnetic field for periods up to 100 hours duration. The exposure was begun at 12 hours after the cells were plated in the growth flasks in order to permit cell attachment to occur prior to application of the field. The growth surfaces of the flasks were oriented parallel to the lines of magnetic induction, as shown in Fig. 3. The maximum induced current density in each culture flask was 8.9 $nA \cdot cm^{-2}$, the derivation of which is given in the Appendix.

Growth curves obtained in experiment no. 1 for exposed and control cultures are shown in Fig. 4. The average intermitotic intervals during exponential growth were 11.0 and 11.6 hours for the exposed and control cultures, respectively. In addition to similar rates of growth of the exposed and control cultures, examination at 50X magnification revealed no morphological or orientational differences between these two groups of cultures.

In the experiment shown in Fig. 4, the control cultures were inadvertently exposed to a weak 0.07 mT (r.m.s.), 60 Hz field as a result of the close proximity of the control chamber and thermoregulating unit to the magnet. Eddy currents were established in the steel jacket of the thermoregulator which served as a source of the observed weak magnetic field within the vessel holding the control cultures. This problem was avoided in subsequent experiments.

Fig. 5 shows a record of the temperature in both the exposure chamber (Fig. 1) and the control chamber (Fig. 2) during the course of the experiment shown in Fig. 4. The temperature in the two chambers was continuously monitored by means of copper-constantin thermocouples, and was recorded at 45 min. inter-

vals on an Autodata Nine data acquisition unit. It is clear from this figure that the temperatures of the cell cultures in both chambers were maintained at 37.0 ± 0.2°C throughout the experiment, except for brief periods of about 5 - 10 min. duration when the chambers were opened for removal of tissue culture flasks.

Subsequent attempts to repeat the experiment shown in Fig. 4 were hindered by difficulties in maintaining the temperature of the control chamber at 37.0 ± 0.2°C. This problem arose because of extreme summer heat which frequently led to a 30-35°C ambient daytime temperature and a 20-25°C temperature at night. As a result of the large daily fluctuations in temperature, the control chamber, which was constructed of 3/8-inch-thick lucite, exhibited temperature excursions of ± 0.5°C about the 37°C growth temperature. Similar temperature variations did not occur in the magnetic field exposure chamber, which had been constructed from 3/4-inch-thick lucite. In order to proceed with the experimental program without the long delay that would accompany the construction of a new control chamber from 3/4-inch-thick lucite, it was decided to test the feasibility of using a conventional Napco cell culture incubator for maintaining the control culture flasks at 37°C.

Two experiments, shown in Figs. 6 and 7, were then conducted in which the growth characteristics of EMT6 cells were determined for cultures placed in the Napco incubator and for cultures placed in the Helmholtz coils with the magnetic field turned off. As demonstrated by Figs. 6 and 7, and by the tabulated data in Table 1, there were no significant differences in the growth rates of cells in the Napco incubator control cultures as compared with the sham-exposed cultures. The growth temperature was maintained at 37.0 ± 0.1°C for

both sets of cultures throughout the two experiments. In subsequent experiments, the Napco incubator rather than the chamber shown in Fig. 2 was therefore used for the growth of control cultures.

Two additional experiments were then carried out to assess possible effects of a 1.65 mT (r.m.s.), 60 Hz magnetic field on the growth of EMT6 cells. As shown in Figs. 8 and 9, there was no significant difference in the growth rate of exposed and control cultures in either experiment. A summary of the cell doubling times obtained from the growth curves in these two experiments is given in Table 1. As in the first experiment (Fig. 4), no morphological or orientational abnormalities were observed in the cell cultures exposed to the AC magnetic field.

The growth curves obtained in experiments 1, 4 and 5 (see Table I and Figs. 4, 8 and 9) were combined to produce composite growth curves for all of the cultures exposed to a 1.65 mT (r.m.s.), 60 Hz magnetic field and for the control, unexposed cultures. These composite curves, shown in Fig. 10, clearly demonstrate the absence of a magnetic field effect on EMT6 cell growth characteristics. This conclusion is further indicated by the similar doubling times obtained from the composite growth curves for the control and exposed groups (see Table I).

DISCUSSION

The growth characteristics observed for EMT6 cells exposed to a longitudinal 1.65 mT (r.m.s.), 60 Hz field for up to 100 hours were indistinguishable from those of control cultures, thus indicating the lack of a magnetic field effect on the length of the intermitotic cycle. A similar result has been obtained by Chandra *et al.* (3), who exposed cultured human bronchogenic carcinoma cells and Burkitt lymphoma cells to 60 Hz magnetic fields ranging in strength from 0.1 to 1 T for periods of 0.5 to 3 hours on three consecutive days. In addition, Rockwell *et al.* (4,5) observed no effect on the clonogenic capacity of cultured EMT6 cells of a 24-hour exposure to DC magnetic fields up to 2.05 T strength.

In contrast to those studies, the results of Marron, Goodman and Greenebaum (6-9) have demonstrated a retardation in the growth rate of microplasmodia from the slime mold *Physarum polycephalum* following a prolonged exposure to low frequency electric and magnetic fields. These researchers subjected microplasmodia to 45, 60 and 75 Hz electric (0.7 $V \cdot m^{-1}$) and magnetic (0.2 mT) fields, either separately or simultaneously, for periods up to 5 years duration. The time between successive mitotic divisions in cultures exposed to the electromagnetic field was observed to be 0.5 to 2 hours longer than in control cultures. This mitotic delay was reproducible, and the onset was apparently frequency dependent with approximately 14, 90 and 120 days exposure to 45, 60 and 75 Hz fields, respectively, being required before a significant effect was observed. A mitotic delay was also observed when the electric and magnetic fields were separately applied to the slime mold cultures, but the magnitude was less than with the combined fields.

The experimental observations on Physarum suggest that the effects of weak electromagnetic fields may not become manifest unless the field exposure is extended for a prolonged period of time. In contrast, however, Tobey (10) has obtained evidence that field-induced effects on cellular growth may occur shortly after the exposure is initiated and disappear at later times. In his experiment, suspensions of Chinese hamster ovary cells that were exposed to a 2 $mV \cdot m^{-1}$, 60 Hz electric field (140 $kV \cdot m^{-1}$ in air) exhibited significant 27% and 16% increases in the doubling times of the second and third cell generations, respectively. In the fourth generation, the increase in doubling time was only 6%, and in subsequent generations no significant change in doubling time was observed.

Experiments performed to date have thus shown a wide variety of responses of cultured cells to weak AC magnetic and electric fields. The underlying reason for this difference in response patterns remains obscure. It might be speculated, however, that the various cell types used in these studies may have a different innate sensitivity to the presence of weak fields and induced currents in their growth medium. In this context, it would be of interest to study the response of one cell line to weak AC fields under a variety of growth conditions that could conceivably lead to a variation in sensitivity. For example, one possible extension of the present study would be to expose EMT6 cells to 60 Hz magnetic fields under culture conditions that lead to a significantly prolonged cell cycle time, such as growth in a medium containing a reduced content of fetal calf serum. The duration of the magnetic field exposure experienced by each cell generation would thereby be lengthened, and this condition might produce field effects that were not observed in the experiments reported here for rapidly growing cells in a high-serum medium.

In a manner similar to the cellular studies discussed above, weak electromagnetic fields have been observed to produce varying degrees of response in complex systems such as experimental laboratory animals. For example, Marino et al. (11) observed a retardation in the growth of mice exposed to weak AC electric fields, whereas Phillips et al. (12) have not observed a growth rate effect using similar fields. A variety of behavioral and physiological parameters other than growth rate have similarly exhibited a highly variable response to weak fields, both with different species of test subjects and with the same species of animal studied in different laboratories. These conflicting observations have obviously made the task of deciding upon public exposure guidelines for weak electromagnetic fields a difficult problem. This is well evidenced by the continuing EHV transmission line controversy, which has wide reaching social and economic impacts in terms of both the decision on a reasonable width of the right-of-way under the power lines, and the physical and aesthetic effects on the environment of the high-voltage lines and their associated fields.

In concluding, several remarks should be made regarding possible extensions of the studies reported here. In addition to the study discussed above of AC magnetic field effects on cells placed in a low-serum medium to lengthen the intermitotic interval, other experiments that could be undertaken include:

(1) The growth rate of EMT6 cells should be studied during exposure to 60 Hz fields of higher intensity than the 1.65 mT (r.m.s.) field used in the present study.

(2) Evaluation of AC magnetic field effects on EMT6 cultures in which the cell monolayer is oriented perpendicular to the lines of magnetic induction.

(3) Cell-kinetic analysis of exposed and control cultures using the technique of flow cytofluorometry.

(4) Extension of growth rate studies to a diploid, normal tissue cell line such as human fibroblasts.

From such studies, conducted under well-controlled laboratory conditions, it is hoped that a consistent picture will emerge of the possible effects, or lack thereof, of low-strength alternating magnetic fields such as those encountered near EHV transmission lines.

FOOTNOTES

1 The research described in this report was carried out as an undergraduate thesis for partial fulfillment of the requirements for a Bachelor of Arts degree in the Health Arts and Sciences Program of the University of California, Berkeley.

2 T = tesla (10^4 gauss); mT = 10^{-3} tesla.

3 r.m.s. denotes the root-mean-square field intensity.

4 EMT6 cells are a subline of the KHJJ tumor, which was derived from a primary tumor arising in BALB/c mice after implantation of a hyperplastic alveolar nodule (2).

5 Preliminary tests showed that cells grow normally in sealed 25-cm^2 flasks containing 30 ml of CO_2-equilibrated medium for at least 5 days.

APPENDIX

Calculation of induced current in tissue cultures exposed to AC magnetic fields

In the experiments reported here, the lines of magnetic flux were parallel to the growth surface, and the maximum induced currents therefore circulated laterally through the culture medium (see Fig. 3). The following calculations are made for the current loop of maximum dimensions, namely, the perimeter of the tissue culture medium which overlays the cell monolayer. This perimeter is a rectangular loop of 4 cm length and 1.2 cm height when a total of 30 ml of culture medium is placed in the 25 cm^2 flasks, as in the present experiments.

By Faraday's law, the induced electromotive force, ε, in the current loop is given by:

$$\varepsilon = -\frac{d}{dt}(B \cdot S) \qquad (1)$$

where B is the magnetic induction and S is the area of the loop. The oscillating field B may be represented as:

$$B = B_0 \sin(2\pi\upsilon t) \qquad (2)$$

where B_0 is the amplitude of the field and υ is the frequency. Equation 1 thus becomes:

$$\varepsilon = -2\pi\upsilon S B_0 \cos(2\pi\upsilon t) \qquad (3)$$

The maximum magnitude of ε is then:

$$|\varepsilon|_{peak} = 2\pi\upsilon S b_o \tag{4}$$

The maximum current density, $|j|_{peak}$ in the loop is given by:

$$|j|_{peak} = \Lambda\ |\varepsilon|_{peak} \zeta^{-1}$$

$$= 2\pi\upsilon S b_o\ \Lambda\ \zeta^{-1} \tag{5}$$

where Λ is the conductivity of the culture medium and ζ is the length of the perimeter of the rectangular current loop.

The parameters in equation 5 appropriate to the present experiments are: $\upsilon = 60$ Hz, $S = 4.8 \times 10^{-4} m^2$, $B_o = 2.33 \times 10^{-3} T$, $\Lambda = 2.03\ S \cdot m^{-1}\ (\Omega^{-1} \cdot m^{-1})$, $\zeta = 0.104$ m. With these values, we obtain from Equation 5:

$$|j|_{peak} = 8.9\ nA \cdot cm^{-2} \tag{6}$$

REFERENCES

1. Burki, H.J. and Lam, C.K.: Comparison of the Lethal and Mutagenic Effects of Gold and White Fluorescent Lights on Cultured Mammalian Cells. Mutation Research, 54: 373-77 (1978).
2. Rockwell, S.C., Kallman, R.F. and Fajardo, L.F.: Characteristics of a Serially Transplanted Mouse Mammary Tumor and Its Tissue-Culture-Adapted Derivative. J. Nat. Cancer Inst., 49: 735-747 (1972).
3. Chandra, S. and Stefani, S.: Effects of Constant and Alternating Magnetic Fields on Tumor Cells in vitro and in vivo. In: Proceedings of the Eighteenth Annual Hanford Life Sciences Symposium. October, 1978.
4. Rockwell, S.: Influence of a 1400 Gauss Magnetic Field on the Radiosensitivity and Recovery of EMT6 cells in vitro. Int. J. Radiat. Biol., 31(2): 153-160 (1977).
5. Nath, R., Rockwell, S., Bongiorni, P. and Schulz, R.J.: Effects of a Transverse Magnetic Field on the Dose Distribution of High Energy Electrons and on the Responses of Mammalian Cells in vitro to X-Rays. In: Proceedings of the Biomagnetic Effects Workshop, pp. 48-49. Lawrence Berkeley Laboratory Report No. 7452. April, 1978.
6. Marron, M.T., Goodman, E.M., and Greenebaum, B.: Mitotic Delay in Physarum polycephalum Induced by Low Intensity 60 Hz and 75 Hz Electromagnetic Fields. Nature, 254: 66-67 (1975).
7. Goodman, E.M., Greenebaum, B. and Marron, M.T.: Effects of Extremely Low Frequency Electromagnetic Fields on Physarum polycephalum. Radiat. Res., 66: 531-540 (1976).

8. Marron, M.T., Goodman, E.M. and Greenebaum, B.: Effects of Weak Electromagnetic Fields on Physarum polycephalum: Mitotic Delay in Heterokaryons and Decreased Respiration. Experientia, 34(5): 589-590 (1978).

9. Goodman, E.M., Greenebaum, B. and Marron, M.T.: Bioeffects of Extremely Low-Frequency Electromagentic Fields. Radiat. Res., 78: 485-501 (1979).

10. Tobey, R.A.: Effects of 60-Hz Electromagnetic Fields on Growth and Survival of Suspension-Cultured Chinese Hamster Cells. In: Proceedings of Eighteenth Annual Hanford Life Sciences Symposium. October, 1978.

11. Marino, A., Becker, R. and Ullrich, B.: The Effect of Continuous Exposure to Low Frequency Electric Fields on Three Generations of Mice: A pilot Study. Experientia, 32: 565-566 (1976).

12. Phillips, R.D., Chandon, J.H., Hilton, D.I. and Sheldon, R.L.: Growth of Rats and Mice Exposed to 60-Hz Electric Fields. In: Proceedings of the National Radio Science Meeting/Bioelectromagnetic Symposium, p. 395. June, 1979.

TABLE I

GROWTH RATE PARAMETERS FOR EMT6 CELLS EXPOSED TO A LOW-INTENSITY, 60 Hz MAGNETIC FIELD*

	Cell Population Doubling Times		
Experiment No.	Control	Sham-exposed	Exposed
1	11.6 hrs.	---	11.0 hrs.
2	11.0	11.0 hrs.	—
3	11.3	11.0	—
4	12.0	--	11.9 hrs.
5	10.6	--	10.4
Composite: 1,4,5	11.2	--	11.1

* Cultures were exposed to a 1.65 mT (r.m.s.), 60 Hz field for periods up to 100 hours. Sham-exposed cultures were placed in the magnet without application of the field. Control cultures were grown either in a chamber similar to that used for cultures exposed to the magnetic field, or in a conventional CO_2 incubator. The growth curves corresponding to experiments 1 through 5 are shown in Figs. 4, 6, 7, 8, 9; the composite curves are shown in Fig. 10.

FIGURE LEGENDS

Figure 1 Photograph of the cell culture exposure chamber positioned in the homogeneous field region of a set of Helmholtz coils. The chamber is constructed of lucite and has a retainer that holds the 25-cm^2 culture flashes in the proper orientation. The water circulator with attached thermoregulator, shown in the foreground, heats and circulates 37.0^{o}C water through insulated tygon tubing into the exposure chamber.

Figure 2 Photograph of the lucite chamber for control culture flasks. Also shown are the water circulator and attached thermoregulator used to maintain the temperature at 37.0^{o}C. In the upper right corner is shown the Autodata Nine data acquisition unit that is used for recording temperatures monitored by copper-constantin thermocouples placed in both the control and the magnetic field exposure chambers.

Figure 3 Illustration of a 25-cm^2 cell culture flask as it is situated in the exposure chamber. The growth surface is oriented parallel to the lines of magnetic induction.

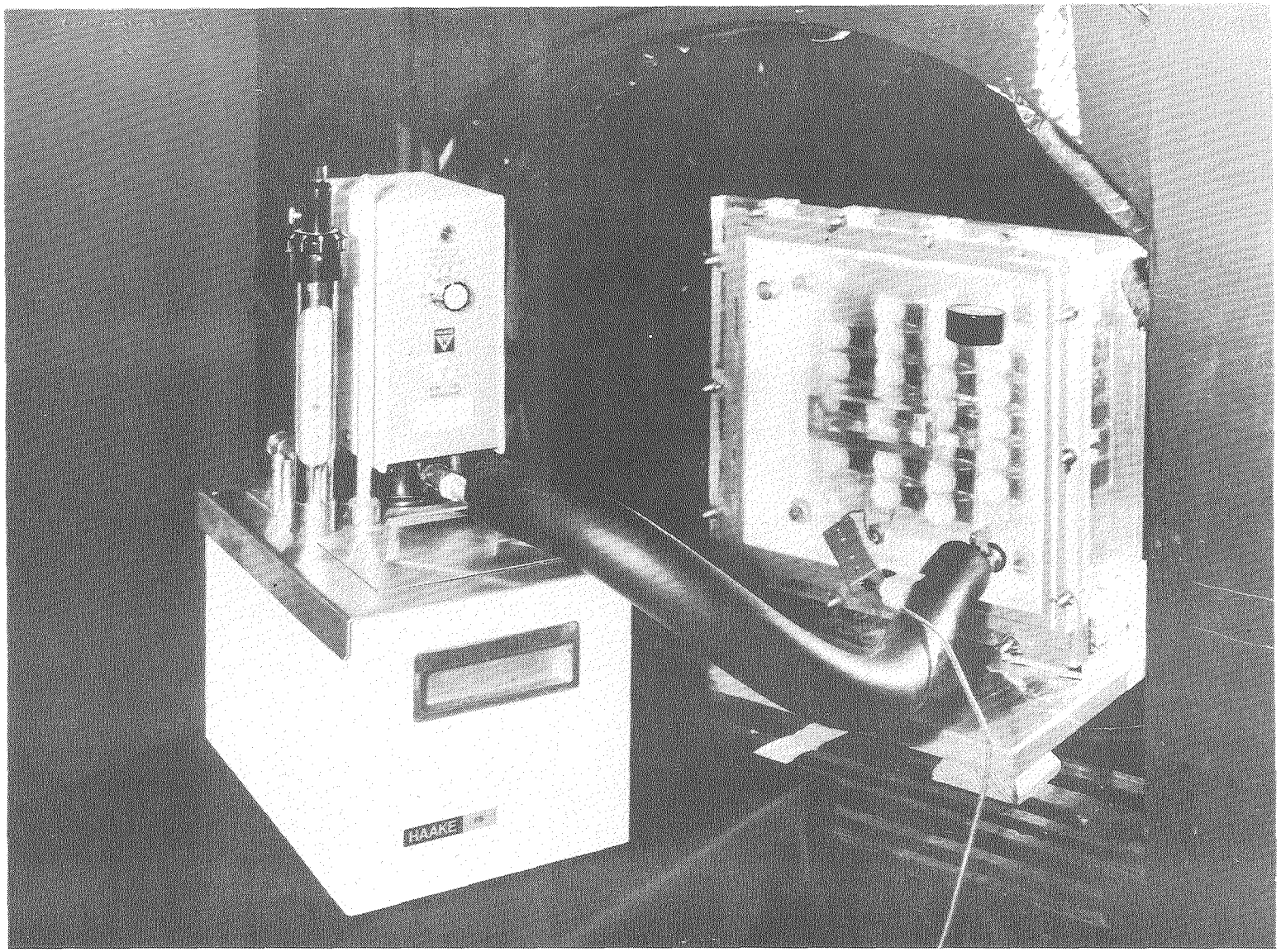
Figure 4 The graph shows *in vitro* growth curves for exposed and control EMT6 cultures. Each point represents the average cell number and the standard error of the mean for the cell populations measured in four 25-cm^2 tissue culture flasks. Doubling times are given for the two cell populations during the exponential growth phase.

Figure 5 A temperature record for the exposure chamber (solid circles) and control chamber (open circles) is shown for the 4-day period of experiment number 1 (Fig. 4). Temperature measurements were made with copper-constantin thermocouples and recorded at 45 min. intervals on an Autodata Nine data acquisition unit.

Figures 6 and 7 The graphs show two sets of growth curves for EMT6 cultures maintained in a Napco incubator (control) and in the exposure chamber with the magnetic field "off" (sham exposed). Each point represents the average cell number and the standard error of the mean for the cell populations measured in 3 to 4 culture flasks. Doubling times are given for the period of exponential growth.

Figures 8 and 9 The graphs show two sets of *in vitro* growth curves for exposed and control EMT6 cultures. Each point represents the average cell number and standard error of the mean determined for 3-4 culture flasks. Cell doubling times are given for the period of exponential growth.

Figure 10 Plots are shown of the composite EMT6 cell growth curves constructed from the data obtained in experiments number 1,4 and 5 (Figs. 4, 8 and 9) for exposed and control cultures. The plotted points represent the average cell numbers at 10-hour intervals in the three experiments. Separate sets of plotted points for the exposed and control cultures are not given since the composite growth curves for the two cell populations are nearly indistinguishable.



CBB789-11035

Fig. 1

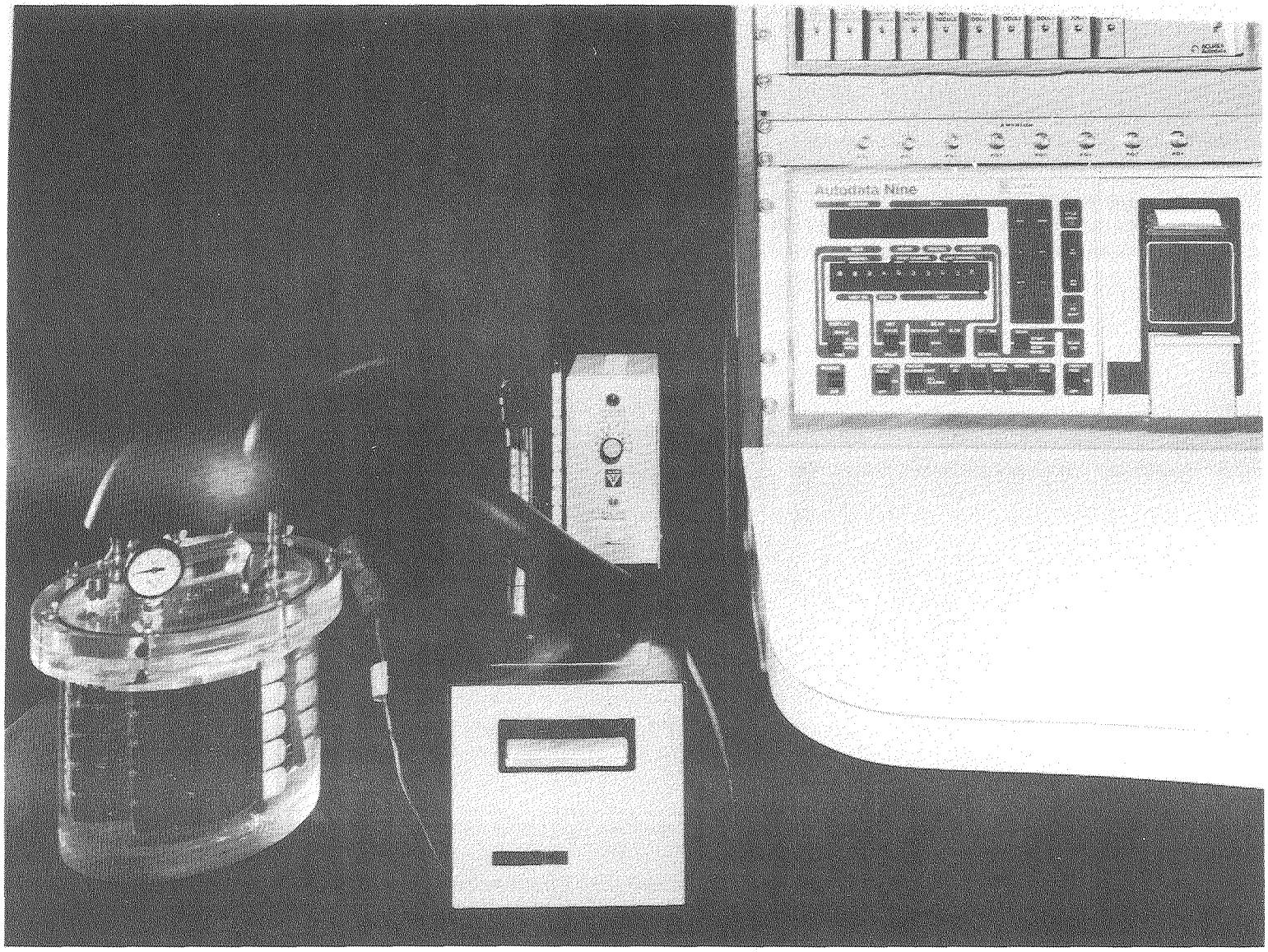


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Fig. 2

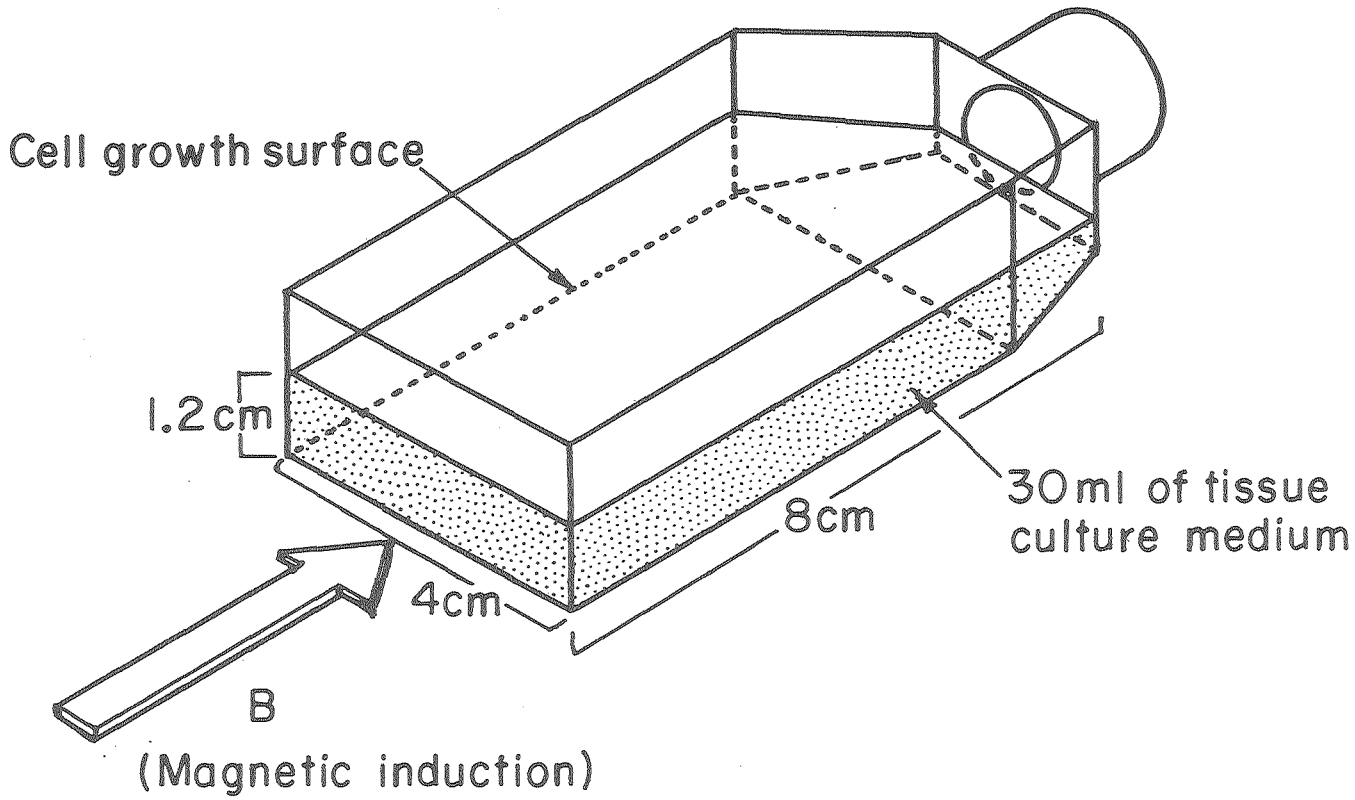

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FIGURE 3

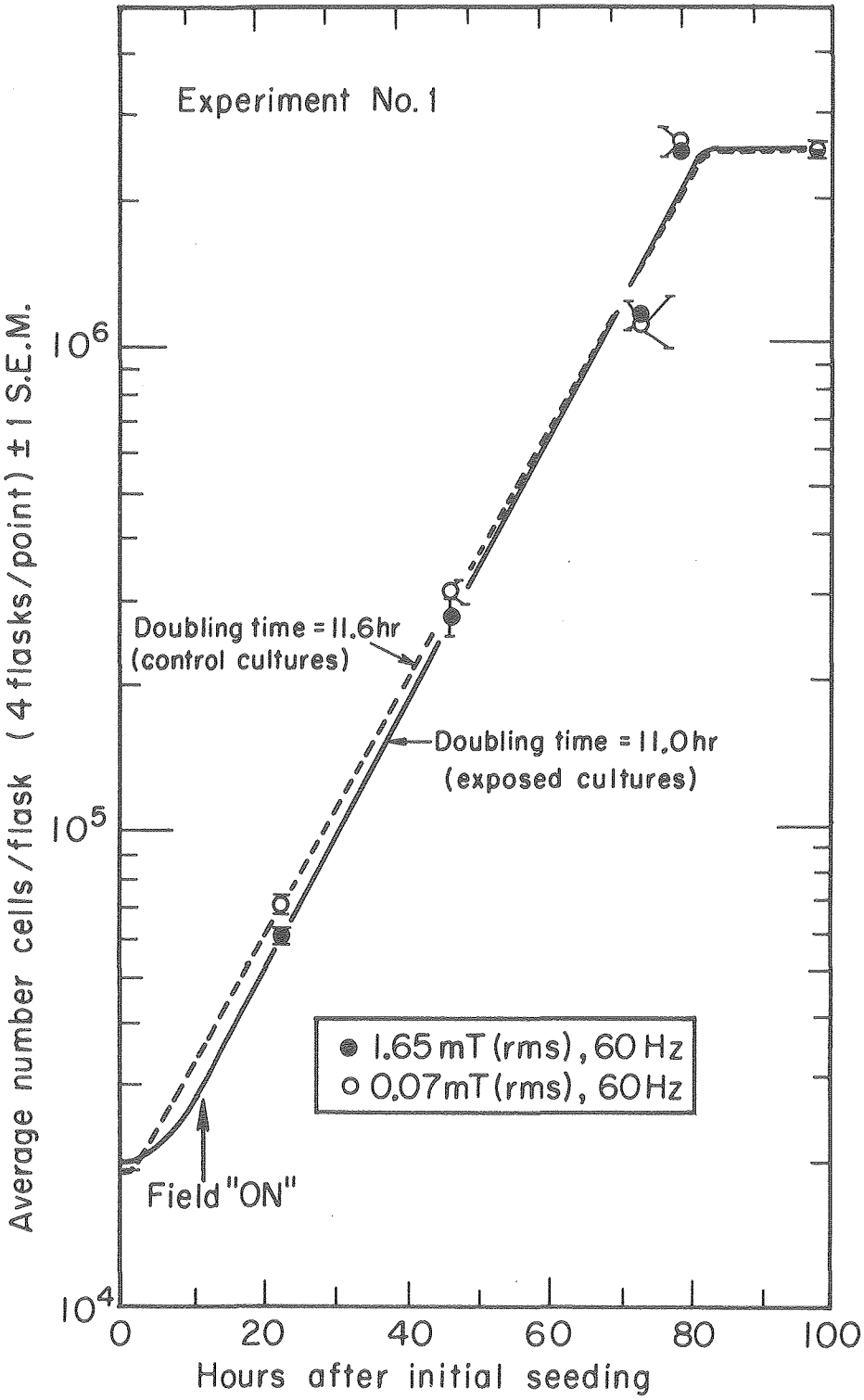

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FIGURE 4

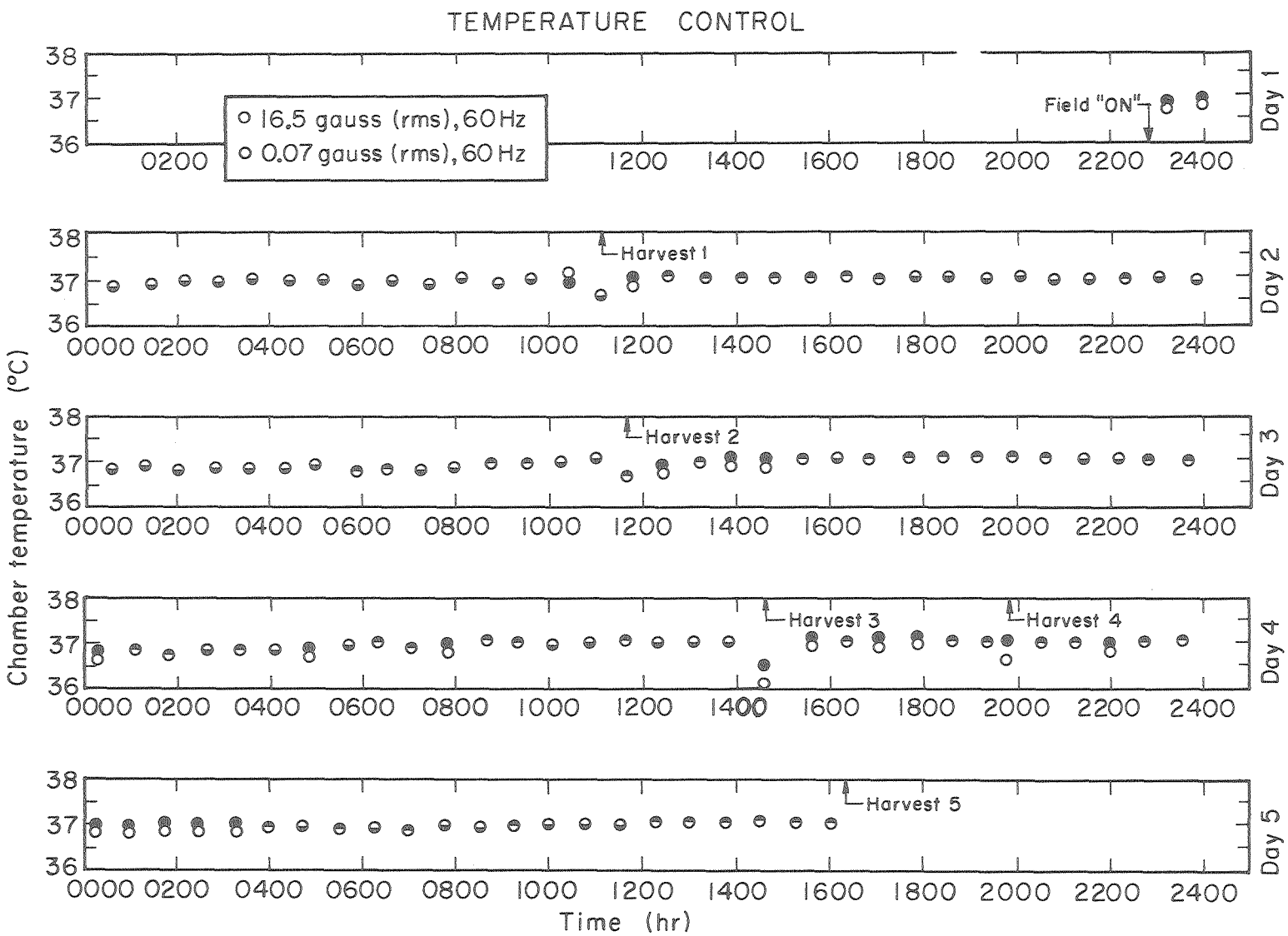

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FIGURE 5

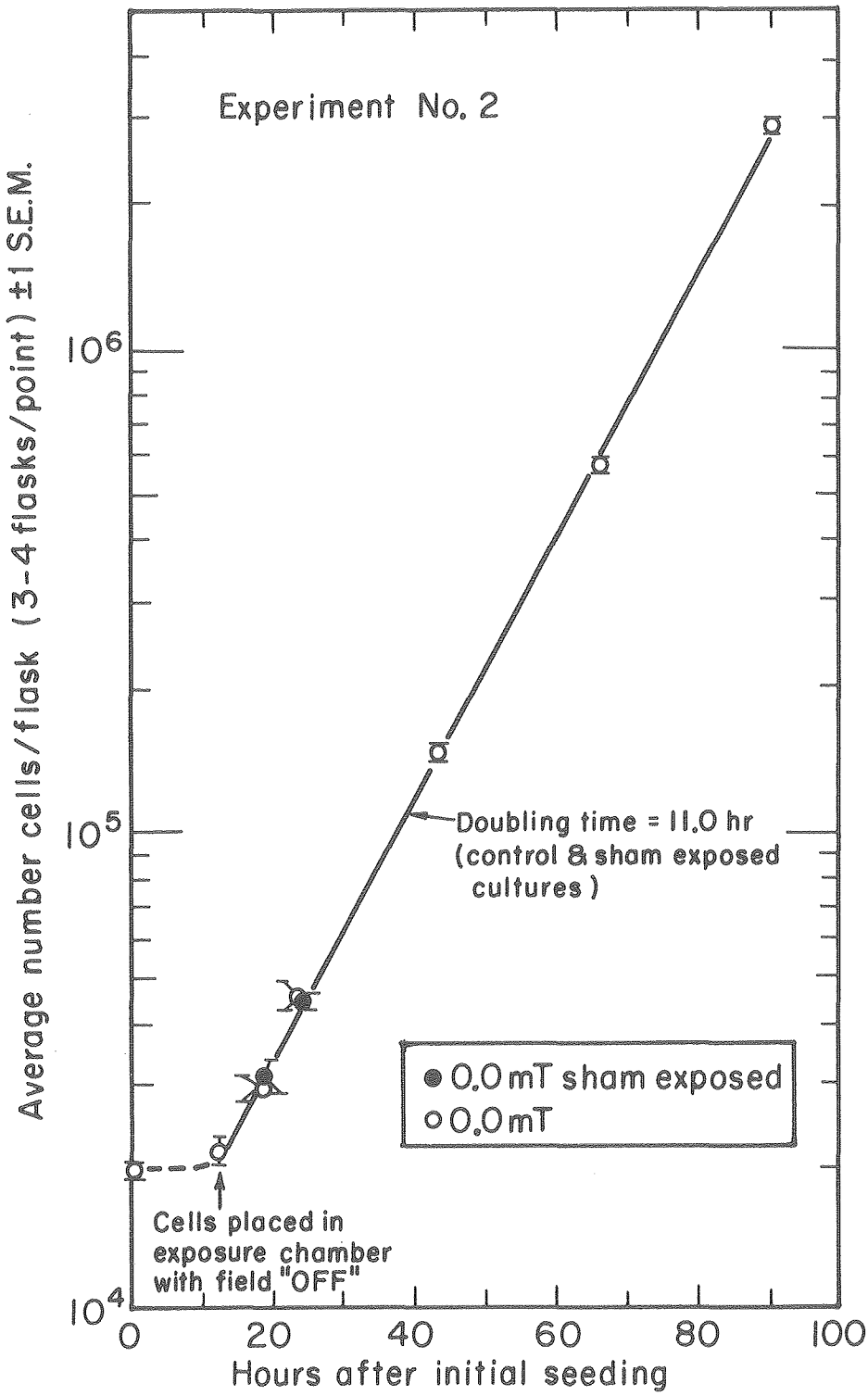

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FIGURE 6

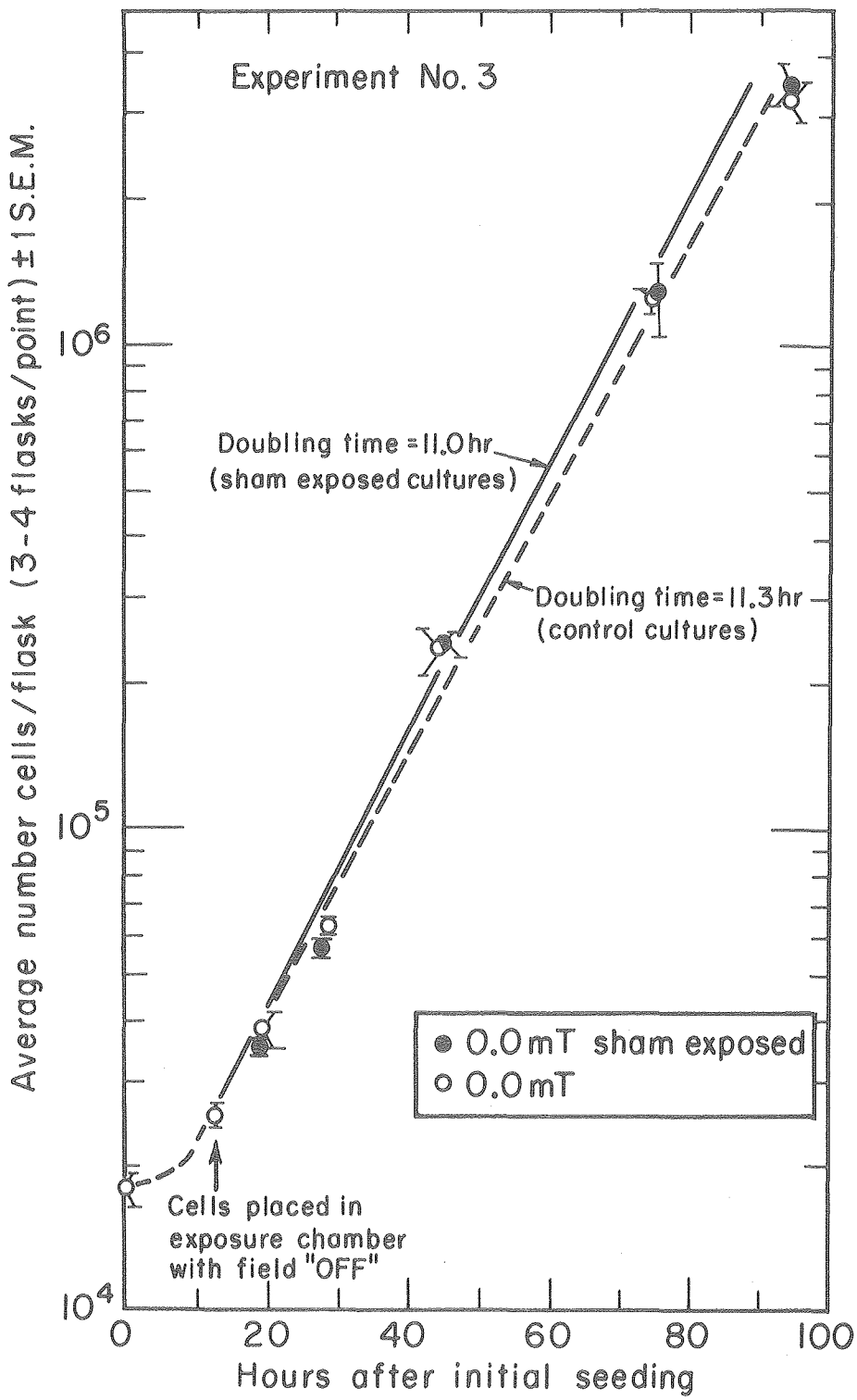

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FIGURE 7

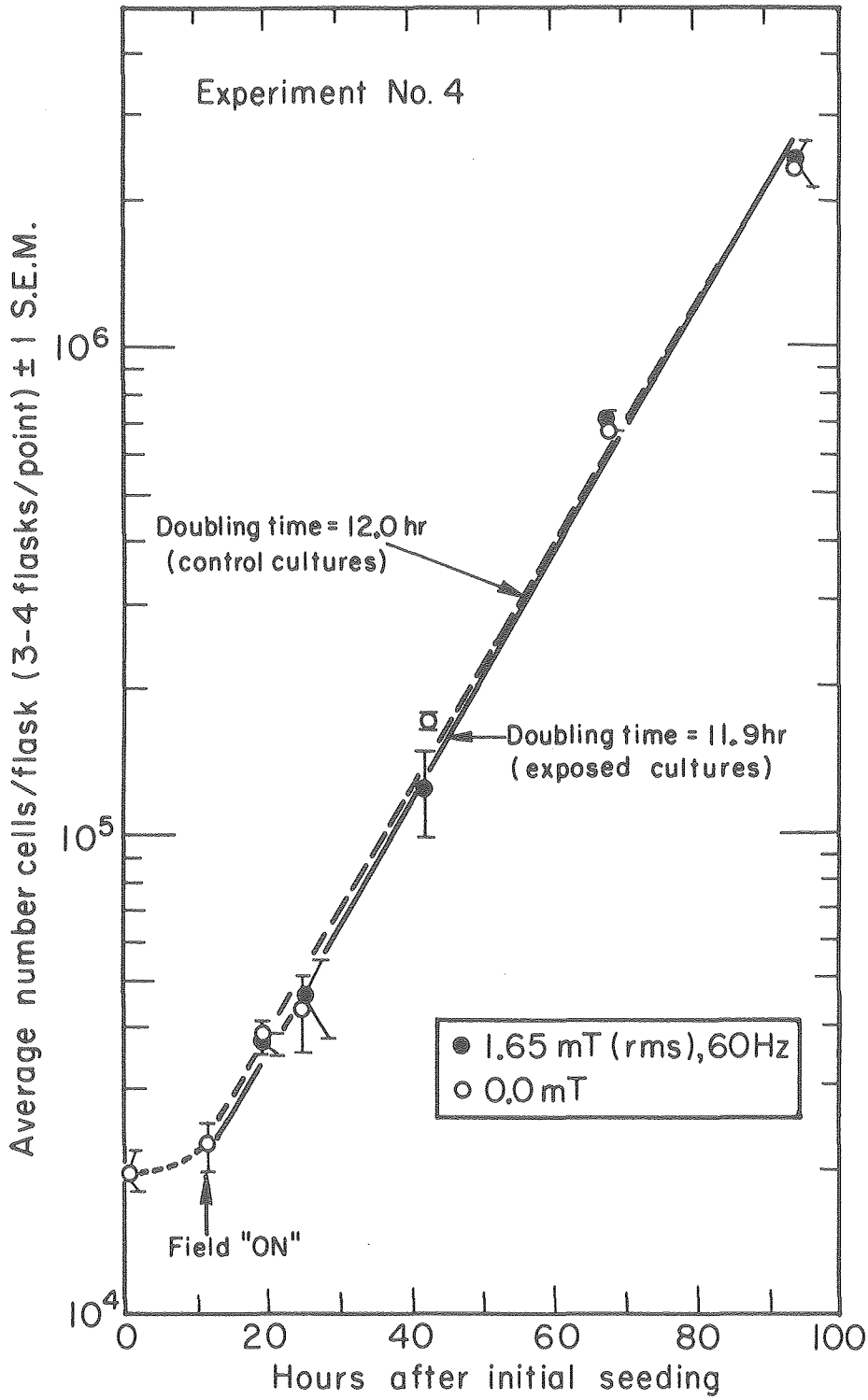

FIGURE 8

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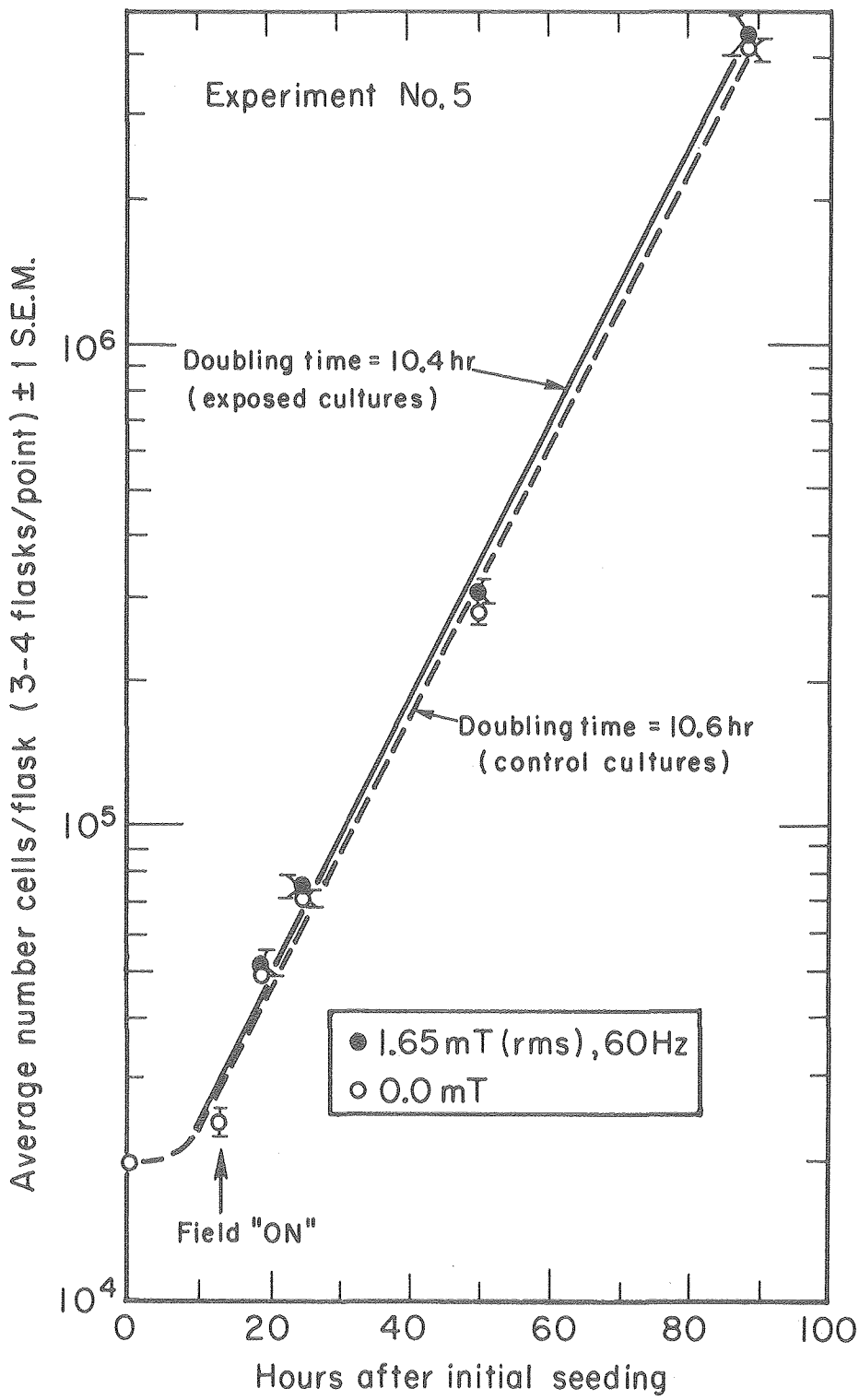

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FIGURE 9

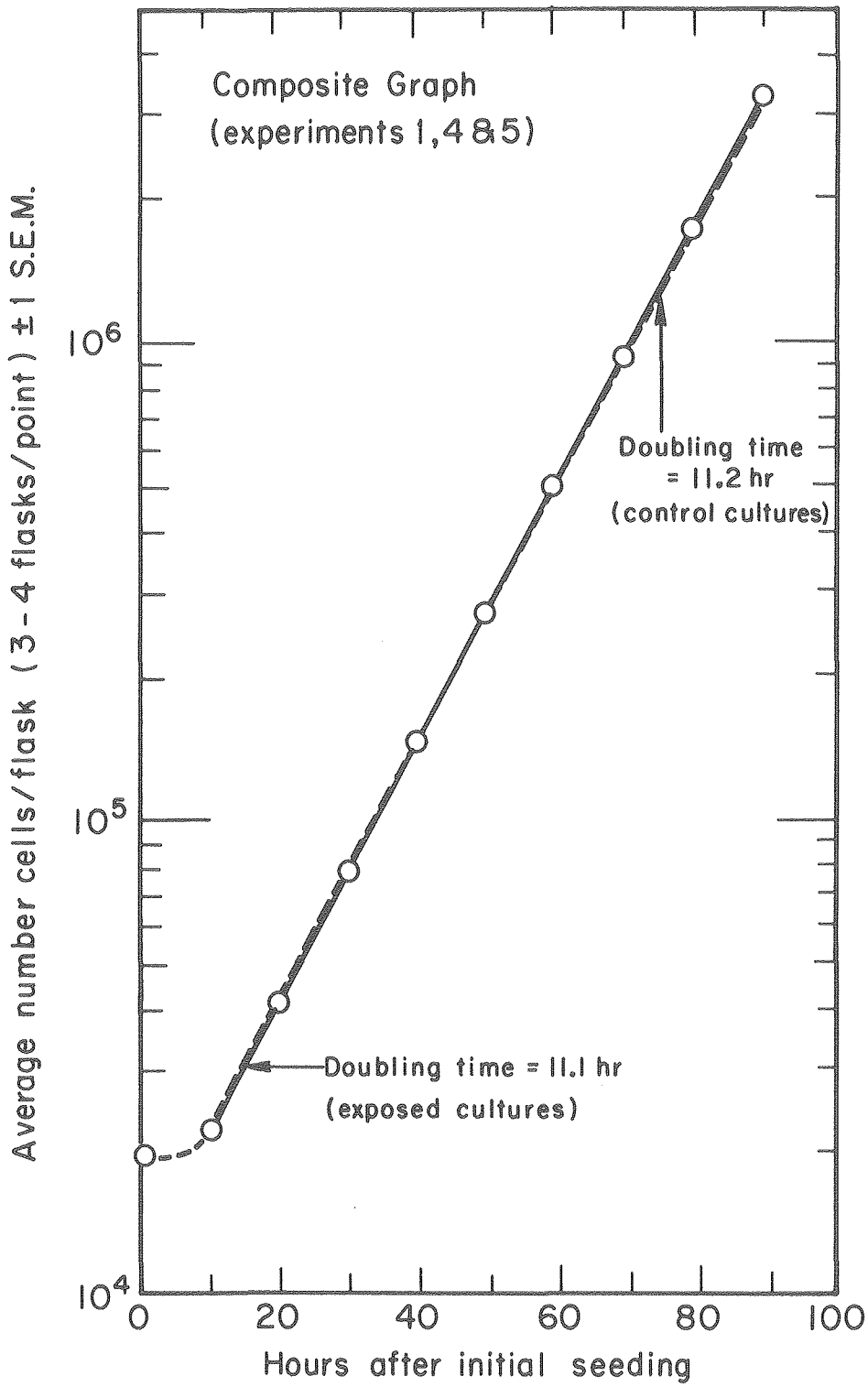

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FIGURE 10

U.S.GPO;1980-698-169/F-123