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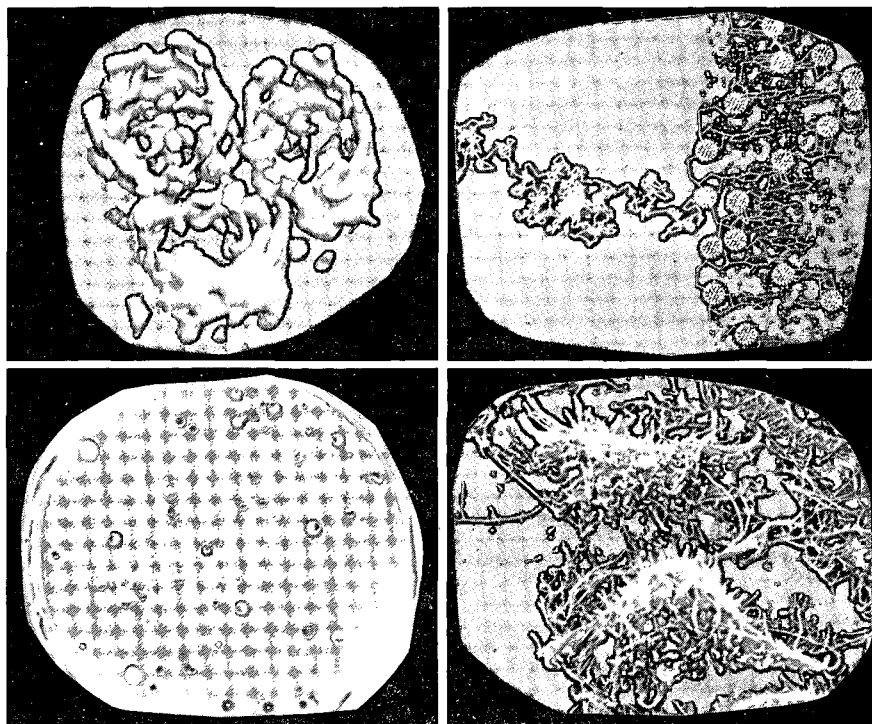
CELL & MOLECULAR BIOLOGY DIVISION

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ELF Magnetic Fields and Melatonin-Induced Growth Inhibition of ER+ Breast Cancer Cells

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ABSTRACT Exposure to ELF fields is reported to depress or time-shift melatonin secretions in animals [1-4], and may also be a risk factor in human breast cancer epidemiology [5-8]. In this study we whether an ELF magnetic field act at the cellular level to influence growth of the breast cancer cell MCF-7 in the presence of melatonin, a known oncostatic agent. Continuous exposure to a sinusoidal 9.5-15.0 mG 60 Hz magnetic field over a 7 days growth period resulted in blocking of melatonin's oncostatic effect on MCF-7 proliferation; this was not observed in a background field of 1.6-3.5 mG. These findings link ELF fields, melatonin, and in vitro growth of a breast cancer cell line. The mechanism(s) of interaction is unknown and may involve modulation of signal transduction events associated with melatonin and MCF-7 cell growth.

INTRODUCTION

Since ELF field exposure has been associated with mammary cancer risk and incidence, and ELF fields influence melatonin secretion in animals, we decided to examine whether ELF fields could directly influence melatonin-induced growth inhibition of a breast cancer cell line, MCF-7, in vitro. Melatonin, the pineal gland's major hormone, exhibits oncostatic activity on human estrogen-receptor-positive (ER⁺) breast cancer cells such as MCF-7 [9,10]. MCF-7 cells are derived from the pleural effusion of a mammary adenocarcinoma, and, when grown as a monolayer, these cells possess an epithelial-like morphology [ATTC HTB-22].

MATERIALS AND METHODS. Cells, Hormones and Drugs. MCF-7 cells at passage 315 were a gift of Dr. David Blask of the Mary Imogene Bassett Hospital Research Institute, in Cooperstown, NY. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM H-21), supplemented with 10% Fetal Bovine Serum, penicillin (100 units/ml), streptomycin (100 mg/ml), and L-Glutamine (2mM) (UCSF Cell Culture Facility, San Francisco, CA), at 37C in a humid atmosphere containing 5% CO₂. Melatonin was purchased from Sigma Chemical Co. (St. Louis, MO).

Exposure Systems. Two double-wound, Merritt four-coil exposure systems were employed in matched Queue incubators (37 ± 0.1°C); parallel(active) and antiparallel(control) current flow generated the 60 Hz ELF magnetic field environments of 9.5-15.0 mG (active) and 1.6-3.5 mG (background), respectively, in the central volume of the coil systems. DC field values were

equivalent (130 ± 12 mG). Plate position in the central volume was systematically randomized during exposures, with matched active vs. control exposures conducted simultaneously.

Culture Techniques. MCF-7 cells were grown out in 60 mm plates or T-75 flasks prior to the experiment. Cells were harvested in a 0.2% EDTA phosphate buffer from the seeding vessel, and diluted in DMEM. Cells were employed at a density of 0.1×10^5 cells/35 x 15 mm culture dish. After cells became firmly attached, four hours after seeding, the plating medium was replaced with melatonin-enriched medium (10^{-11} M, 10^{-9} M, 10^{-7} M, 10^{-5} M) or DMEM (zero melatonin). Triplicate plates were used for each melatonin concentration. On alternate days, cells were harvested from plates in each treatment group by incubation with trypsin at 37°C for two minutes, and counted with the hemocytometer. Fresh medium, with or without melatonin as required, was fed to the remaining plates twice weekly.

RESULTS. The growth of MCF-7 cells in the presence of 10^{-11} M or with no melatonin in a background 1.6-3.5 mG magnetic field, is shown in **Fig. 1A**. Typical exponential growth was observed over the 7-day period. When melatonin was present an approximate 40% inhibition of growth on days 5 and 7 was detected ($p = 0.002$); this is consistent with reports from Blask and colleagues [9,10,12]. **Fig. 1B** presents data for cells continuously exposed to a 9.5-15.0 mG magnetic field. In the absence of melatonin we see an exponential growth curve for the MCF-7 cells that is superimposable with cells maintained in the background field (compare with **Fig. 1A**). However, when melatonin is present the ELF field is observed to block the oncostatic growth inhibition of melatonin.

We also investigated whether this field effect is melatonin-dose dependent. **Fig. 2A** depicts data (% of control) corresponding to growth of MCF-7 cells on day 5 at different melatonin concentrations in the presence of the background 1.6-3.5 mG field (solid curve) or the exposure 9.5-15.0 mG field (dashed curve). In the absence of melatonin, cell growth for both fields was indistinguishable. As melatonin concentration was increased for the background field, maximal growth inhibition occurred at 10^{-11} M melatonin. In contrast, melatonin's growth inhibition was

significantly blocked by the 9.5 -15.0 mG field at all melatonin concentrations. **Fig. 2B** indicates a similar pattern was observed for cell growth on day 7. ANOVA across all data yields $p = 0.0014$.

In these studies no gross morphological differences were noticed between background and exposed MCF-7 cells, whether in the presence or absence of melatonin.

DISCUSSION Our findings indicate that ELF magnetic fields at 9.5-15.0 mG act to block melatonin's growth inhibition of a breast cancer cell line, MCF-7. Magnetic fields had no effect on MCF-7 cells in the absence of melatonin. Thus, although the mechanism of ELF field action is unknown, it must be mediated at the cellular level through melatonin and its action the MCF-7 cell.

Several models have been proposed to elucidate the link between ELF field exposure and breast cancer incidence [1]. For example, one important aspect is an ELF-induced decrease in melatonin secretion in vivo and a concomitant enhancement in the production of prolactin and estrogen, which in turn may increase the growth of susceptible breast epithelial cells [11]. Our in vitro study suggests a direct interaction between ELF fields, the MCF-7 cell, and melatonin at the cellular level. This interaction most likely involves receptor activation and signal transduction (ST) pathways.

End-stage ST events include cellular proliferation and protein secretion. In in vitro studies with transformed HL60 cells, translation and transcription are altered in ELF magnetic fields [13]. Experimental evidence for a 60Hz field effect on an early-stage ST marker, $[Ca^{2+}]_i$ in mitogen-activated lymphocytes was first reported by us [14]. Alterations in $[Ca^{2+}]_i$ were not observed during 60 Hz field exposure of resting cells, but approximately 100s after addition of mitogen, changes in $[Ca^{2+}]_i$ were detected. We postulate that alterations of an early ST marker such as $[Ca^{2+}]_i$ will propagate down the ST cascade to influence transcription and expression of such proto-oncogenes as c-MYC; we have recently provided evidence for this interaction [15]. In this regard, we hypothesize that a possible mechanism for ELF field action on MCF-7 growth may involve (a) an interference with melatonin's activity and subsequent ST sequelae, and/or (b) an activation of estrogen receptors to overcome melatonin's anti-mitogenic action. Future studies should address such issues.

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FIGURE LEGENDS

FIG. 1. Effect of Melatonin (10^{-11} M) on MCF-7 Cell Growth in the Presence of an ELF Magnetic Field. **1A:** Melatonin exhibits typical inhibition of MCF-7 cell growth in a background field of 1.6-3.5 mG ($p = 0.002$). **1B:** Melatonin fails to inhibit growth of MCF-7 cells in a 9.5-15.0 mG field. Mean \pm S.E.

FIG. 2. Effect of Melatonin Dose (10^{-5} - 10^{-11} M) on MCF-7 Cell Growth at Days 5 & 7 in the Presence of an ELF Magnetic Field. **1A:** Day 5; Melatonin growth inhibition at 10^{-7} - 10^{-11} M is blocked by the 9.5-15.0 mG ELF magnetic field. **1B:** Day 7; Melatonin growth inhibition at 10^{-7} - 10^{-11} M is blocked by the 9.5-15.0 mG ELF magnetic field. Mean \pm S.E. Multivariate ANOVA across all data yields $p = 0.0014$ for blocking effect of the 9.5 - 15.0 mG ELF magnetic field.

Fig. 1A

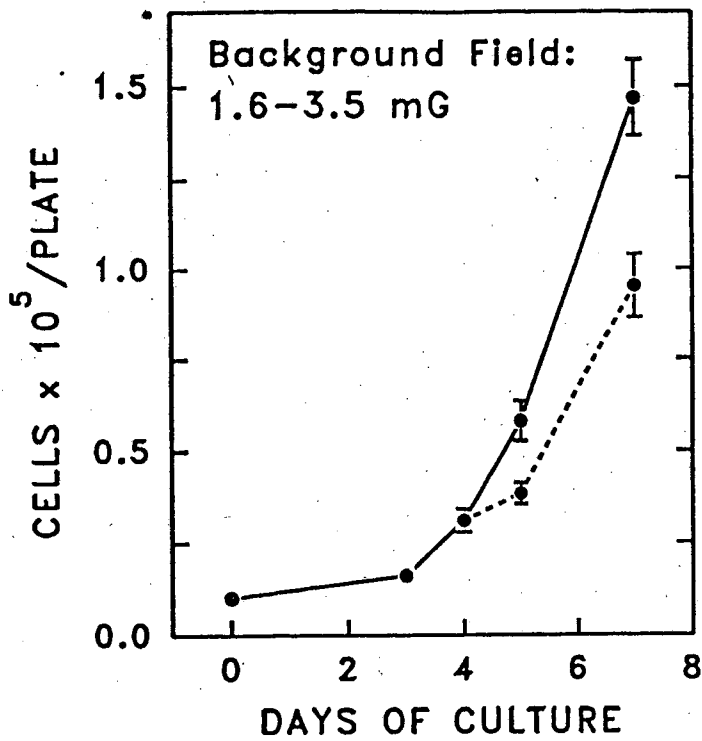
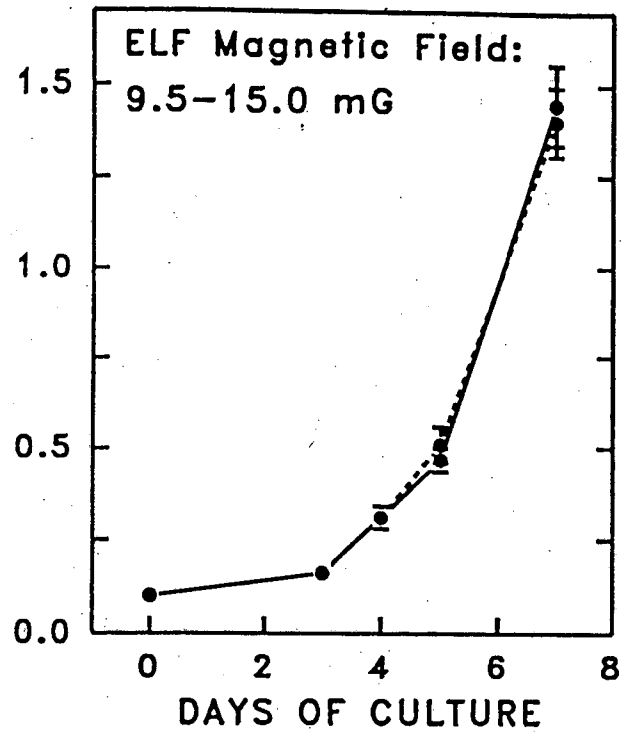


Fig. 1B



—●— no melatonin
 - - -●- - - 10⁻¹¹ M melatonin

Fig. 2A

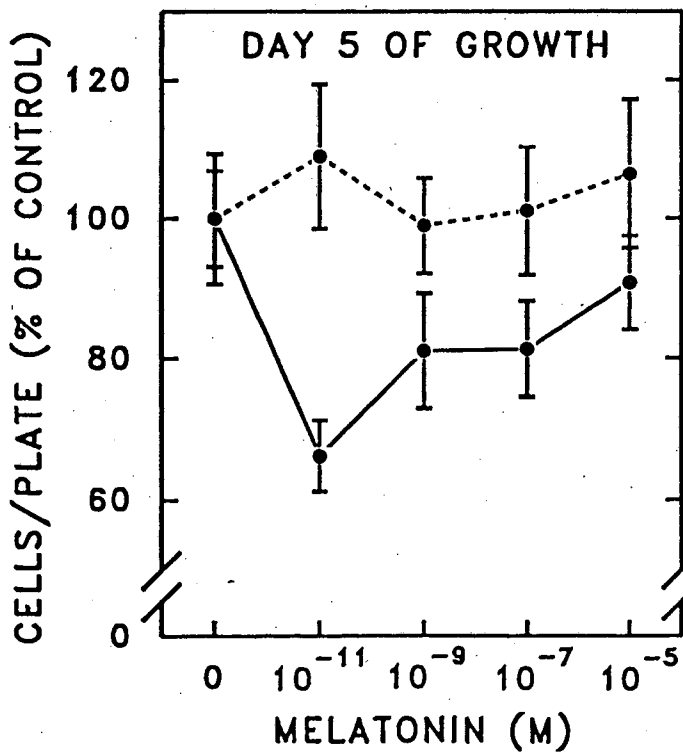
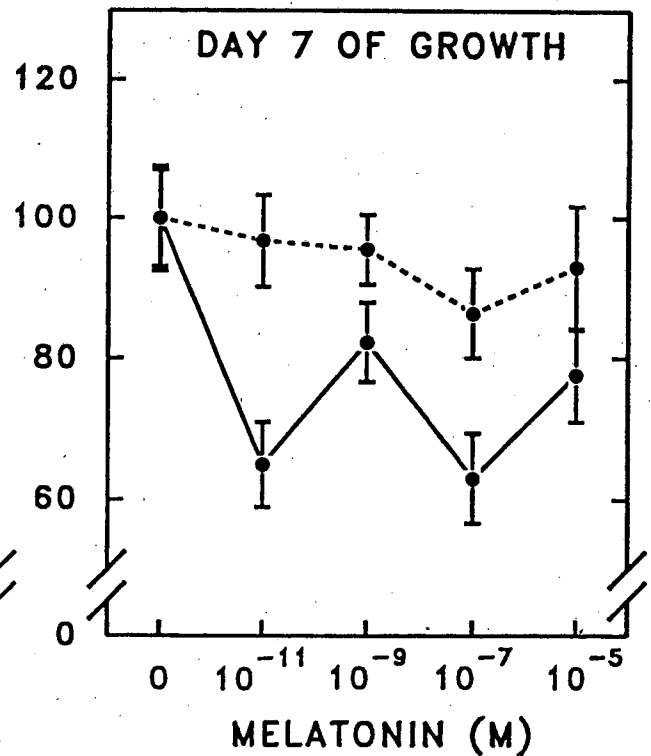


Fig. 2B



—●— Background Field: 1.5-3.5 mG
 - - -●- - - ELF Magnetic Field: 9.5-15.0 mG

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