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Evidence of Morphological and Physiological Transformation of Mammalian Cells by Strong Magnetic Fields

Abstract. *Cultures of L-929 and WI-38 cells, frozen to 4.2°K and exposed for 4 to 8 hours to 5000-oersted magnetic fields, were markedly inhibited in their growth as compared to controls. In cultures grown on cover slips, approximately 7 days after exposure, morphologically distinct cells emerged and were propagated from generation to generation; 3 weeks later, in flask cultures, contact inhibition was abolished. It is concluded that under certain experimental conditions, strong magnetic fields induce morphological and physiological transformations of target cells.*

There have been numerous attempts to demonstrate significant effects of magnetic fields on several biological systems (*1*). Magnetic fields seemingly alter a number of physiological indicators in intact animals (*2*), as well as certain aspects of cellular metabolism (*3*), but no unequivocal effects of magnetic fields on intact mammalian cells have yet been demonstrated.

Under certain experimental conditions, we have found that strong magnetic fields induce an apparently irreversible transformation of cultured eukaryotic cells. Heteroploid L-929 and diploid WI-38 cells were grown in plastic Falcon flasks under standard conditions [Eagle's minimum essential medium (MEM) supplemented with 10 percent calf serum, 2 mmole of glutamine per milliliter, and antibiotics]. The cells were harvested by trypsinization and frozen at a rate of 1°C per minute to –90°C in the presence of 10 percent dimethylsulfoxide (DMSO) in MEM. The frozen samples were then placed in liquid nitrogen and transported to the magnet. They were exposed to magnetic fields of 5000 oersted for 4 to 8 hours, using a standard iron core electron paramagnetic resonance magnet with 12-inch pole faces and a 4-inch pole gap. The samples were placed in the approximate center of the pole gap (field uniformity was 0.1 percent over a 15-cm^3 volume) in a double Dewar flask system, the outside flask containing liquid nitrogen, the inner holding helium and the cells in plastic tubes. After the samples came to thermal and mechanical equilibrium, the magnet was energized very slowly; it took approximately 1 minute to manually increase the current to its final value. After a predetermined elapsed time (4 to 8 hours), the magnet was slowly turned off over a period of 1 minute. The samples were then lifted from the inner Dewar flask, placed rapidly in liquid nitrogen, and transported back to the cell culture laboratory. The same procedure was repeated immediately, in the same Dewar system, with the control samples, with the exception that the magnetic field was never turned on for the controls.

Four sets of L-929 cells (with one control group and one magnetized group in each set) and three sets of WI-38 cells were investigated at approximately equal intervals over a period of 14 months. After a set of target and control cells was obtained, the cells were thawed and allowed to grow, as described below, for periods of time ranging up to 2 months. Some variation of the exposure time to the magnetic field was attempted. Three sets of L-929 target cells and one set of WI-38 target cells were exposed to the magnetic field at a temperature of 4.2°K for 8 hours. The remainder of the experiments involved exposure to 5000-gauss magnetic fields at 4.2°K for 4 hours. (No differences were observed in the results described below for the different exposure times, with the techniques used in this study.)

Experimental and control frozen cells were thawed by immersion in a 37°C water bath. Thawed cell suspensions were then immediately diluted tenfold with MEM and allowed to stand undisturbed until most cells had settled to the bottom of a conical tube. The DMSO-containing medium was then aspirated and the cells were resuspended in fresh MEM supplemented with 10 percent calf serum, 2 mmole of glutamine per milliliter, and antibiotics. At this time, viability of the cells was determined by vital dye (trypan blue) exclusion, and the cells were counted in a hemocytometer. There were no statistically significant differences in viability of control [81 ± 6 percent (standard error of the mean)] and experimental (79 ± 5 percent) cultures. They were then seeded in plastic Falcon flasks and Leighton tubes at a concentration of 300,000 or 40,000 viable cells per flask or Leighton tube, respectively. Randomly selected cultures were demonstrated to be free of contamination by pleuropneumonia-like organisms (*4*).

Cells exposed to the magnetic field attached readily, but their rate of growth was markedly inhibited compared to that of the controls. Thus, while the controls had to be split at approximately 10-day intervals, there was no need to split experimental cultures for as long as 30 to 40 days. Moreover, the experimental cultures failed to maintain a consistent monolayer, leaving extensive free surfaces not covered by cells. In contrast to the controls, approximately 4 weeks after exposure to the magnetic field, the cell cultures formed tightly packed aggregates which, in the case of L cells, coalesced to form ridges several layers deep. Piling up of cells was likewise observed in the experiments with WI-38 cell cultures. The changes described here and depicted in Fig. 1 led us to conclude that in the cells exposed to the magnetic field, contact inhibition was abolished or markedly altered.

Before the loss of contact inhibition and about 7 days after exposure to a magnetic field, distinct cell types emerged in all experimental cultures. In the case of L cells, they were characterized by en-

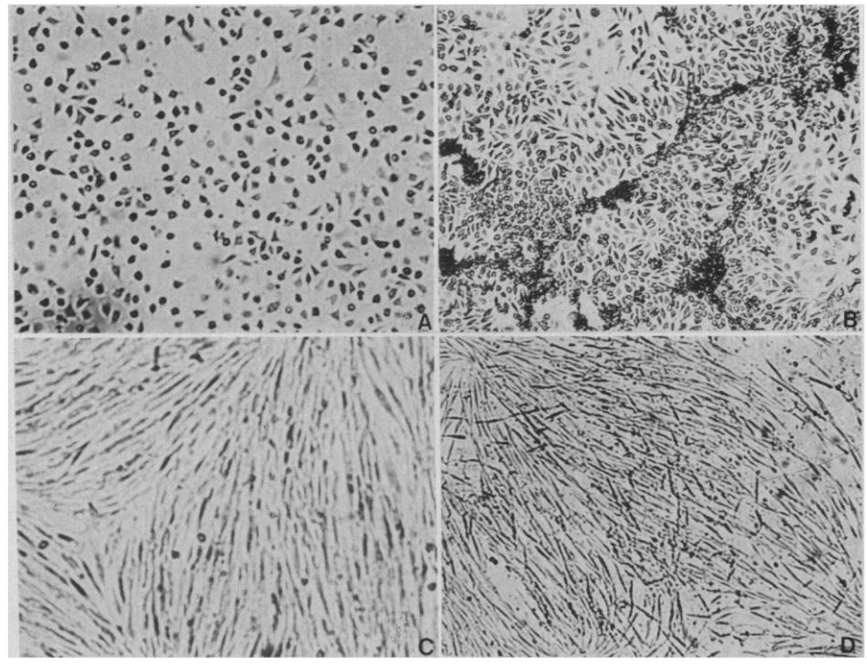

Fig. 1. (A) The control L-929 cell culture split three times in 27 days; this is a 3-day-old culture. (B) This experimental culture 28 days after exposure to the magnetic field has never been split. Note the agglomeration of densely packed cells forming ridges over the incomplete monolayer. (C) The control WI-38 culture split four times during 45 days. This confluent monolayer represents a 10-day-old culture. (D) A 45-day-old WI-38 cell culture that was exposed to the magnetic field. Note that confluence has not been reached and that the interdigitating cells are not contact-inhibited. All cultures were photographed by using the conventional transmission light microscope; × 250.

larged nucleoli and coarse chromatin, which jointly tended to form a symmetric intranuclear network. The cytoplasm of such cells was filled with tightly packed perinuclear granules, which persisted throughout the cell cycle.

In the case of WI-38 fibroblasts, morphologically distinct cells were likewise observed. Although the cytoplasm of these cells appeared to be free of granular material, it was enormously elongated. Strongly basophilic nuclei of the experimental WI-38 cells were characterized by coarse chromatin, which tended to display intranuclear axial symmetry. The nucleoli tended to lose their spherical shape and in their orientation tended to be associated with the axially symmetric chromatin network. Abundance of giant nuclei with the same characteristics was yet another striking feature. Since, with the passage of time, these changes were not abolished and the number of cells showing these characteristics tended to increase, it was concluded that these cells were, indeed, transformed (5). (These major changes are shown in Fig. 2.) At this time, it would be premature to postulate a mechanism for the results reported here, particularly as there are numerous plausible alternatives. However, it seems important to consider whether or not the observed effect was triggered solely by the magnetic field or by other possible contributing factors.

While the use of the control cultures allows one to eliminate most environmental differences, there are other possible differences that we attempted to eliminate. These are: (i) electromotive forces (emf's) due to electric fields induced by some variation of the magnetic flux in the Dewar system, and (ii) variations in the temperature of the bath due to induced eddy currents. The magnetic fields could change to produce emf's because of (i) changes of the current in the magnet itself, or (ii) motion of the sample in the field. Precautions were taken to change the current in the magnet sufficiently slowly to minimize the emf's induced in this manner. Also, the rectified alternating current used to drive the magnet was measured in attempts to estimate the size of induced emf's caused by this effect. Finally, the size of the emf's induced by the motion of the sample in the magnetic field was estimated. In all cases, we estimated that the upper limit of the emf's

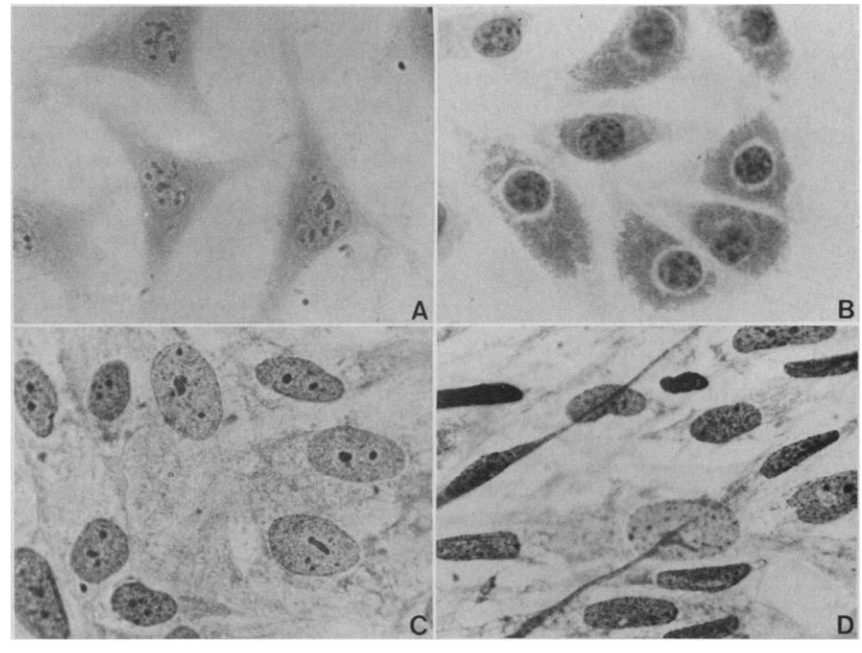

Fig. 2. (A) Control L-929 cells grown in Leighton tubes for 8 days and fixed in Carnoy's fluid. Note the agranular cytoplasm of these cells. (B) Experimental L-929 cell culture grown in Leighton tubes for 8 days after exposure to the magnetic field. Note the alteration in cellular morphology and granular cytoplasm. (A and B) Cultures were fixed in Carnoy's fluid. Feulgen reaction; light green counterstain. (C) Control WI-38 cell culture, 35 days old. (D) Experimental WI-38 cell culture, 35 days old. Note the increase in nuclear basophilia, changes in nuclear morphology, and enormously elongated cytoplasm of some cells. (C and D) Cultures were grown for 30 days in Falcon flasks. They were then harvested and cultured in Leighton tubes for 5 days. Carnoy fixation; stained with 0.1 percent toluidine blue at *p*H 4.5; × 1200.

produced by all these sources was 10^{-3} volt/cm. This is within the limits of the ambient emf's in an urban environment and, therefore, was the same for target cells and controls. Also, the temperature of the samples, estimated from the vapor pressure of the cooling liquid and the evaporation rate of the bath, was constant to $\pm$ 0.1°K for both target and control cells.

We have also estimated the effect the magnetic field should have on a system of independent magnetic moments of the size of the Bohr magneton. For the fields and temperatures used, no more than 20 percent of the spins would be preferentially aligned with the field.

We conclude that the observed transformations are due to magnetic field effects alone. Also, from the estimate given above of the alignment of noninteracting magnetic dipoles at the temperatures and magnetic fields used, it seems unlikely that these effects could be due to magnetic moment alignments in small portions of the cellular material. Permanent magnetically induced changes in the cellular material would seem to require much larger participation of cellular material because of energy considerations—that is, cooperative magnetic field interactions analogous to the cooperative dielectric phenomena postulated by Fröhlich (*6*). There is some evidence in the literature that such interactions are possible in cellular material such as DNA (*7–9*).

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Thermal Stability of Human DNA and Chimpanzee DNA Heteroduplexes

Abstract. *The base pairing fidelity of heteroduplexes formed from human DNA and chimpanzee DNA has been studied by the criterion of thermal stability to test the evolutionary conservation of repeated DNA base sequences.*

The eukaryotic genome is known to have a regular arrangement of repetitive and single copy sequences (*1*). In general, short repetitive DNA sequences, 300 nucleotides in length, are interspersed with short single copy sequences of a gene-sized length (*1*). This sequence arrangement is highly conserved and has been observed in organisms ranging from mollusks to mammals. The only documented exception to this sequence arrangement is that in *Drosophila* (*2*). In *Drosophila* the average lengths of the repeated and single copy DNA sequence classes are much longer than those in other organisms. It has, therefore, been concluded that the basic interspersion pattern of short repetitive and short single copy sequences is biologically important as suggested by its evolutionary conservation (*1*).

We have found that the DNA sequence organization in humans also follows this pattern (*3*). By an electron microscope method (*2*), we have determined the distribution of lengths of repeated DNA sequences in humans. There is good agreement between this distribution—both its width and mean—and the distribution of corresponding lengths found in *Xenopus* (*4*) by the same electron microscope technique. In contrast to the strong selection which maintains the lengths of the repeated sequences, the base sequence is not, in general, highly conserved. Duplex DNA prepared by renaturing repetitious DNA sequences is known, by the criterion of thermal stability, to have numerous sequence mismatches. The primary structures of the repetitive sequences have diverged since their original repetition, and repetitive sequences are similar but not identical in base sequence (*5*).

These observations suggest two extreme possibilities that we can test. The first is that the biological function of the repetitive DNA sequences is based exclusively on length and arrangement, and that detailed primary structure is not important to this function. One could imagine several structural functions for which this might be the case. The second possibility is that the primary structure is also important to the function of the repetitive sequences. According to this view, any useful mutations in repetitious sequences would be conserved.

These two possibilities can be distinguished by the methods which Kohne, Hoyer, and co-workers developed to compare the extent of divergence of the single copy sequences found in the primate genome (*5*). This method consists of cohybridizing the DNA of two different species to form heteroduplex DNA. In forming this heteroduplex, the DNA of one species, for example, chimpanzee, is present as a radioactive tracer at infinite dilution so that its concentration does not contribute to second order renaturation. The DNA of the other species, for example, human, is present at high concentration so that this component dominates the second order renaturation. Radioactive duplex DNA formed by this procedure is an interspecific DNA heteroduplex. Nonradioactive duplex DNA—monitored by ultraviolet absorbance—is the result of any intraspecific hybridization. The two types of duplexes are next bound to hydroxylapatite under conditions where single-strand DNA elutes. The thermal stability of the duplex DNA is then determined by the temperature at which the DNA elutes from hydroxylapatite as denatured single-strand DNA. The thermal stability of the hybrids depends on the fidelity of base pairing. A 1 percent mismatching of base pairs depresses the thermal stability of a duplex by approximately 1°C (*6*).

The evolution of repeated DNA sequences in rodents has been studied by the method we propose (*7*). Because of the extensive divergence of all sequence classes in rodents, the results of that study are not suitable for the comparison of divergences of sequences in humans and primates. Rice noted that repetitive DNA sequences in primates—rhesus and human—have undergone relatively less divergence than in rodent (*7*). We compared the relative divergence of repetitive and single copy DNA sequences in the primate line. We have chosen human and chimpanzee for this comparison of repetitive DNA sequences. The single copy DNA sequences of primates have been extensively compared (*5*). Furthermore, comparisons of the DNA's of chimpanzees and humans are of interest from an evolutionary viewpoint (*5, 8*).

The procedures for DNA renaturation and thermal stability studies are adapted