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## Cell transformation of frozen human fibroblasts by a strong magnetic field? – A reinvestigation

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**Summary.** A magnetic field of 5000 Gs did not induce morphological transformations of human skin and lung cells in culture as reported previously by Malinin et al.

Recently Malinin et al. reported 'evidence of morphological and physiological transformation of mammalian cells by strong magnetic fields'<sup>1</sup>. They exposed frozen cells at 4.2 °K to a magnetic field of 5000 Gs for 4–8 h. For human lung fibroblasts (WI-38 cells) changes were described as follows: The rate of growth was markedly inhibited; cultures failed to maintain a consistent monolayer; cells piled up; contact inhibition was abolished or markedly altered; morphologically distinct, enormously elongated cells were observed; strongly basophilic nuclei were characterized by coarse chromatin which tended to display intranuclear axial symmetry; nucleoli tended to lose their spherical shape and to be oriented in association with the axially symmetric chromatin network and there was an abundance of giant nuclei with the same characteristics.

We tried to reproduce these phenomena with human lung fibroblasts (WI-38 cells, obtained from Flow Laboratories, Irvine, Scotland), and with human skin fibroblasts established from healthy adults in our laboratory. Cells were cultivated in Falcon plastic flasks (75 cm<sup>2</sup>) under standard conditions (37 °C, 95% air, 5% CO<sub>2</sub>) in Eagle's minimal essential medium (MEM) to which were added fetal calf serum (GIBCO), glutamine, penicillin, streptomycin, amphotericin-B and sodium bicarbonate to give final concentrations of 10%, 2 mM, 100,000 units/l, 100 mg/l, 0.25 mg/l and 12 mM respectively. The WI-38 cells were in passages 29–31, the human skin fibroblasts in passages 15–16. All cultures were free of mycoplasma as tested by the fluorochrome procedure<sup>2</sup>. Cells from each flask were harvested by trypsinization, suspended in 1.8 ml culture medium supplemented with 0.2 ml of dimethylsulfoxide (DMSO), frozen slowly and stored in 2-ml vials in liquid nitrogen. Vials were inserted into a rack which fitted the cryostat inside the bore of a superconducting magnet and secured immobility of the samples. The vials were transported in

liquid nitrogen to the precooled (78 °K) cryostat, immediately mounted into the center of the superconducting solenoid and the compartment was further cooled with liquid helium. The temperature of the cells was never above 80 °K, and they were cooled to liquid helium temperature within approximately 15 min. The temperature was monitored by means of a resistance thermometer mounted on the rack. When the temperature of the rack had reached 4.2 °K the magnetic field was slowly activated to 5000 Gs within approximately 1 min. The cells remained exposed to this stationary magnetic field for 4 h. After removing the magnetic field with about the same sweep rate, the cells were transferred back to the liquid nitrogen container. Control cells were handled identically but the magnetic field was not activated. 8 vials of each cell line were exposed to the magnetic field and 8 vials of each line served as controls.

Treated cells were thawed after storage periods varying from 20 h to 1 week to 4 months and transferred at different concentrations to small petri dishes (Falcon plastics) for culture. Cells of each vial were also grown on glass cover slips in petri dishes. Cultured cells were examined for morphological appearance and growth pattern. Nuclei of cells grown on cover slips were inspected before and after fixation with methanol/glacial acetic acid (3/1; vol/vol) and staining with orcein.

None of the cell lines, neither the WI-38 lung cells nor the human skin fibroblasts, showed any of the changes of growth or form described previously<sup>1</sup>. Sample cells could not be distinguished from control cells nor from cells that had never been exposed to liquid helium. Rate of growth, cell shape and contact inhibition appeared normal. It seems clear that the effects observed by Malinin et al.<sup>1</sup> cannot be ascribed solely to the influence of the strong magnetic field applied<sup>3</sup>.

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- 3 We assume that the glutamine supplement to MEM was not '2 mmole per ml' as indicated in Malinin et al.<sup>1</sup> but in fact 2 µmole per ml.