Low-energy electromagnetic frequencies induce conspicuous, reproducible, and lasting effects in human and animal cells

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SUMMARY

Electromagnetic frequencies (EMF) are information: this hypothesis has been tested by experiments with cell cultures. Mammalian cells were treated with seven to eight EMF at low energy. THP-1, 3T3-L1, and B16-F1 cell lines as well as a primary culture of rat fibroblast cells were used for these experiments. Seven to eight EMF were applied electrically for three minutes per frequency, thirty times or more over a period of two months. This procedure led to lasting changes in the cells without damaging them. The induced properties were specific to the cell lines: After treatment, the THP cells grew better in a medium containing newborn bovine serum (NBS), and the 3T3 cells grew to two-fold higher cell density. The changes were stable and usually measurable over a period of four years. The new cell properties were adaptive to different growth conditions. On the basis of these results, a model for the mode of action of the EMF was developed: *The information of the frequencies provides a regulation system for the cooperative activity of the gene products. This regulation system is not dependent on the genetic system.* The model is discussed in some details in the final section.

INTRODUCTION

This work is based on the hypothesis that EMF are information. The existence of EMF information is demonstrated through frequency treatment of cells in culture, based on the appearance in the treated cells of new growth parameter and new structures. EMF as information builds on the concept that all living beings consist of energy with EMF, or in other words, all energy units or quanta are frequencies. If the frequencies of quanta can be influenced by EMF treatment, then no observable changes in the cells would be expected, unless many quanta of the treated cells had the same frequencies. Presumably only then and, in addition, according to unknown properties of the EMF in organisms would it be likely for changes to occur that could be analyzed cytologically. This in turn would mean that the EMF of quanta are information of a type that could be decoded by means of the responses of treated cells. Furthermore, it seemed preferably to use EMF that are not, to the best of our knowledge, used technically in order to minimize interfering electromagnetic effects on the investigated cells. The hypothesis that EMF are information is based on the discovery by Royal Rife in 1920 (Lit. 1).

Cell lines of human and animal origin were used for these investigations. The cells were cultivated according to standard cytological practice. Treatment of the cells with specific EMF was standardized. The frequency generators used were 2 precision instruments, F-SCAN 2, from TB-Electronics, CH-9443 Widnau, Switzerland. Thomas Böhme developed, fabricated and provided these oscillators. We thank Thomas for his generous support.

METHODS

The frequencies were transmitted using two F-SCAN 2 oscillators, one was a prototype and the other a series instrument. The frequency generator was connected to a potentiometer with 12 steps that reduced the voltage of the frequencies from 10V (maximum output voltage) to 0.8V (step 1). The potentiometer was connected to a spider box with 6 parallel pairs of wires with a length of 1m each. This enabled parallel treatment of six cell cultures. The EMF were transmitted to the medium with the cells using sterilized wires of 24 carat gold with a diameter of 0.4mm. The gold electrodes were attached to glass platelets of 10x26x1mm, which were made from microscope slides. Two flattened gold wires were wound around the ends of the glass platelets and glued with Araldite so that the length of the free end of the gold wires was 4cm long. The distance between the two wires on the glass platelet was 1.8cm. Each gold wire was connected at a distance of 2 to 3cm from the glass platelet to an insulated tin wire (inner diameter, ID 0.4mm) with a length of 30cm. The junctions were isolated from the surroundings by a 2cm long glass capillary each whose end was sealed with Araldite. This design should allow EMF transmission to the medium exclusively via the gold wires. The free ends of the insulated wires were pulled through two holes next to the membrane of the lid of the culture bottle. These free ends were then connected to one pair of cables from the spider box for the EMF treatment. The cells were treated in 25cm^2 Falcon cell culture bottles made of plastic in a non-sterile environment at room temperature. The control cultures without frequency treatment were also kept at room temperature for the same length of time, which was up to one hour. The controls did not have electrodes. After EMF treatment, the wires to the spider box were disconnected, and the culture bottles with the electrodes and tin wires were incubated again at 37°C. During the EMF treatment, care was taken to ensure that the glass platelets lay flat on the bottom and in the middle of the culture bottle. They were completely covered by the nutrient solution; the length of the gold wire above the medium was at least 0.5cm. During the two-month treatment period, the cells grew around and also on the electrodes.

The frequency treatments of the cell cultures were carried out in a medium with ¼ of the usual serum concentration to keep the cell growth rate low. As soon as the cultures were confluent, the cells were partially resuspended with EDTA solution. About 10% of the cells were transferred to a new culture bottle, which was then fitted with a new sterile electrode. The cell groups were not completely dissociated in this step. All work with the cells was carried out in a sterile bench. There were cases of contamination, but they were rare. After completion of the frequency treatments, the cells were cultivated for 10 passages under standard conditions at 37°C to re-accustom them to the standard medium. Microscopic examinations were already possible during these ten passages.

Each experiment comprised a total of 30 to 35 EMF treatments. The first 18 treatments were performed every second day and the followings every day. Before each frequency treatment, a sweep was carried out for 2 minutes over the entire frequency range of the subsequent EMF. The sweep excited every 1000th frequency in the target oscillation range from 1.0 to 6x10^6 Hertz three times for a fraction of a second. The excitation of the EM field of the cells is supposed to increase their sensitivity to the subsequently applied EMF. If the time between the sweep and the EMF treatment was more than 1 hour, the sweep was repeated.

Each EM treatment of the cells comprised 7 or 8 frequencies: frequency group A with or without final frequency C and frequency group B, without a final frequency. Frequency group B was originally intended as a control EMF treatment. After completion of the overall treatment with A, frequency C

was applied on its own on three consecutive days after the sweep and without EMF A. Each frequency was applied for 3 minutes. Standard group A comprised the following frequencies: 5,555,555.4Hz; 5,555,554.3Hz; 555,555.6Hz; 555,555.1Hz; 555.7Hz; 111.1Hz; 112.2Hz, and final frequency C with 122.2Hz. Frequency group B: 5,555,556.9Hz; 5,555,556.2Hz; 555,554.0Hz; 555,553.2Hz; 556.7Hz; 109.2Hz and 110.0Hz. The actual treatment time for the sweep plus EMF A was 23 minutes.

Three established cell lines and one primary cell line were used: 1) THP-1, human monocytes; this cell culture was obtained from a patient with acute monocytic leukemia, acquired from ATCC; #TIB-202. 2) 3T3-L1 mouse fibroblast cells; pre-adipocyte cells (ATCC #CRL-173). 3) B16-F1 mouse melanoma cells (ATCC #CRL-6323). 4) Rafi; primary cell culture of rat fibroblasts. These were established from a one day-old laboratory rat. In contrast to the Rafi cells, THP-1, 3T3-L1, and B16-F1 are mutated to cancerous cells. The rat fibroblasts were cultivated after EMF treatments at low oxygen concentrations to minimize the likelihood of genetic changes (Lit. 2). The cell lines were assigned new designations after completion of the EMF treatment. After treatment with A frequencies, the designations of the cell lines changed to THP-J, 3T3-J, B16-J, and Rafi-J. After treatment with B frequencies, the designations changed to 3T3-K and Rafi-K (see Tab.3, page 13).

The EMF treatments of the cells were carried out with several culture flasks (n = 2 to 4) in parallel, and the identically treated cultures were pooled after completion of the treatment. Exceptions were 3T3-K1 and 3T3-K2, the Rafi and most of the B16-J/K cells. For the EMF treatments, the number of cells used to start a culture in 25 cm^2 Falcon culture flask was 40 cells for B16-F1, 400 for THP-1, and 500 for 3T3-L1. The treatments were carried out in 2005 and 2006, with exception of the B16-J cells for the investigations on melanin synthesis which were carried out in 2007. Rafi cells were treated in 2007 with 400 cells per culture flask. For Rafi and the second experiment with B16, the batch was extended from 2–4 to 6 culture flasks with parallel treatment. They were not pooled at the end of the EMF treatment.

Culture conditions: The cell cultures were kept at 37°C in standard incubators for mammalian cells with 5% CO_2. Where stated, the cells were cultivated in nitrogen with 1 to 3% oxygen and 5% CO_2. THP-1 cells were cultivated in RPMI medium (Gibco 1640) with 25mM Hepes, 50 units/ml penicillin/streptomycin, 7.5% NBS (without thermal inactivation), and 1mM Glutamax; 3T3-L1 cells were cultivated in DMEM high glucose medium (Gibco) with 25mM Hepes, 50 units/ml penicillin/streptomycin, 10% NBS (without thermal inactivation), and 1mM Glutamax; B16-F1 cells were cultivated in MEM-Earle medium (Biochroma) with 25mM Hepes, 50 units/ml penicillin/streptomycin, 8% NBS (without thermal inactivation), 1mM Glutamax, and 1% vitamin solution (MEM non-essential amino acids) of a 100x stock; Rafi were cultivated in MEM high glucose medium (Gibco) with 25mM Hepes, 50 units/ml penicillin/streptomycin, 5% NBS (without thermal inactivation), and 1mM Glutamax. The EDTA solution consisted of 10mM EDTA in a phosphate-buffered isotonic solution containing 0.1% BSA. Serum-free cell cultivation was carried out in Ultraculture (12–725F) medium from Cambrex containing 1mM Glutamax and 50 units/ml penicillin/streptomycin. The PMA stock solution was dissolved in chloroform at a concentration of 0.1mg/ml and then diluted to 10ng per ml medium for treatment of the cells.

Various counting methods were used for cell growth analyses: The most sensitive method was cell count with the counter from Beckmann (Z2 Cell Coulter). At least 1000 cells in three independent samples were determined. Great care was taken with all cell lines to ensure that all cell groups were completely dissociated into individual cells using EDTA solution. Loosening of the adherent cells by EDTA solution from the flask and from attachment to each other required up to about one hour incubation at 37°C, before dissociating the cells by aspirating gently with a pipette. The cell number of the resuspended cells remained stable in the EDTA solution containing BSA at 4°C for 24 hours.

To simplify the growth assay, microtiter plates were used with 96 wells instead of 12 or 24 wells. Growth was determined indirectly by measuring the color intensity after staining the fixed cells with methylene blue. Although a growth assay in microtiter plates and measurement with a plate reader is much less labor-intensive, providing a greater throughput for triplicate determinations, this method was significantly less sensitive: a difference of 100% in the direct determination of the cell count decreased to 30% or less in the methylene blue assay. Nevertheless, the reproducibility of the methylene blue assay was good. Many growth tests with 3T3, B16, and all of the experiments with Rafi cells were carried out with the methylene blue assay in spite of the lower sensitivity.

Confidence intervals in the statistical evaluations were calculated with a P value of 95%.

Fluorescence staining of the DNA of giant cells was carried out with DAPI (4',6-diamidino-2-phenylindole dihydrochloride).

Determination of the survival rate: The cells were resuspended and stored in EDTA solution at 4°C at identical cell density. The fraction of viable cells was determined microscopally once a day by staining with methylene blue.

The seeding efficiency SE was determined as follows: cells of a culture with about 80% confluency were resuspended to individual cells and kept on ice. 50,000 cells were distributed into 4 wells each of a 6-well plate in MEM medium containing 4% NBS and 1% FBS. The wells were pretreated with 5ml PBS containing 0.1% BSA solution, kept overnight at 37°C, then rinsed once with MEM medium. 4ml medium was added to each well for the assay and the plate equilibrated to 37°C. After 30 minutes, 50,000 cells in 1ml MEM medium were added to each well and the cell cultures incubated at 37°C for 2 hours. Cells that had not adhered were aspirated along with the incubation medium. The adherent cells were washed once and then counted: 100,000 adherent cells gave an SE of 50% per experiment.

The growth assays of the Rafi cell lines were carried out as follows: 6000 cells with an SE of 35% were added to each well in the first row of a 96-well plate and diluted 1:2 in each of the following rows. If the SE value was less than 35%, correspondingly more cells were used, and for a higher SE value correspondingly fewer cells.

RESULTS

Which cellular effects could be expected by the frequency treatments? A representative parameter for the whole cellular activity was sought. Growth is a good candidate because it can be assumed that it is affected by most metabolic functions and vice versa. In addition, attention was paid to the emergence of new or modified structures or properties of the cells. And there were some: aggregation into larger clusters for THP-J cells, more adherent cells for THP-J, more giant cells for 3T3-K, and more dendrites for 3T3-J and 3T3-K.

THP cell lines

THP-1 monocytes grow in suspension as individual cells or in small groups. After EMF treatment with the frequencies A+C, some of the THP-J cells aggregated into clusters comprising 100 or more cells instead of the usual 2–15, maximal 40 cells (see Table 1 and Figure 1). Most groups formed in the middle of a culture flask. The clusters were present under all cultivation conditions. The extent of aggregation varied depending on the age of the cell culture and on the type and amount of serum.

Growth analyses showed that the THP-1 cells proliferate almost twice as fast as THP-J in FBS (Figure 2). Differential changes in the growth of these cell lines occurred depending on the type and amount of serum and on other variables, such as number of cells used to initiate a culture and frequency of medium exchange. A low cell count at the start of the experiments was decisive for a significant difference in growth (200% and more). THP-1 cultures starting with 50,000 or less cells per ml nutrient solution grew well in the presence of 10% FBS, but very little in the first 20 days in a medium containing 7.5% or 15% NBS. In contrast, THP-J cultures initiated with 50,000 or less cells per ml nutrient solution proliferated well in all 3 media right from the start. If the concentration of cells was increased to 100,000 cells per ml at initiation of growth, the differences between THP-1/J disappeared.

It was not possible to repeat these specific growth properties 4 years later with the identical cell lines: all THP-1 and THP-J cultures started at low cell concentration died after a few weeks.

An examination of the THP-1/J cells after staining with the vital stain CFSE for laser cell analysis of individual cells showed a differential growth pattern: a small fraction of the THP-J cells, about 20%, divided more rapidly than the other THP-J cells and 99.5% of the THP-1 cells. In contrast, 80% of the THP-J grew more slowly than the THP-1 cells. The adherent cells, a small fraction of THP-1 and THP-J, are not included in these numbers. The fraction of apoptotic cells in both cell lines was comparable. Both cell lines were started for the CSFE experiment with more than 100,000 cells pro ml medium.

THP-1 monocytes, stimulated by the phorbol ester PMA, differentiate into adherent macrophages. All THP-1 and THP-J transformed into macrophages with PMA. THP-J spontaneously formed more adherent cells (macrophages) than THP-1, even without the addition of PMA (see Table 1): THP-J spontaneously formed up to 20% adherent cells, THP-1 less than 10%. Specific staining of alpha-tubulin showed a different pattern for the two cell lines: after stimulation with PMA, the THP-J cells were stained much more intensively, as compared to THP-1 or non-stimulated THP-J.

The THP cell lines were adapted to grow in NBS instead of FBS. Subsequently, at long and irregular intervals, some apoptoses occurred in the THP-1 and THP-J cultures. In the case of THP, this adaptation from FBS to NBS was performed in the first 3 months after EMF treatment, while this was done before EMF treatment for all other cell lines (3T3-L1, B16-F1, and Rafi).

Storage of THP-J in liquid nitrogen at -180°C did not have any effect on the new phenotype.

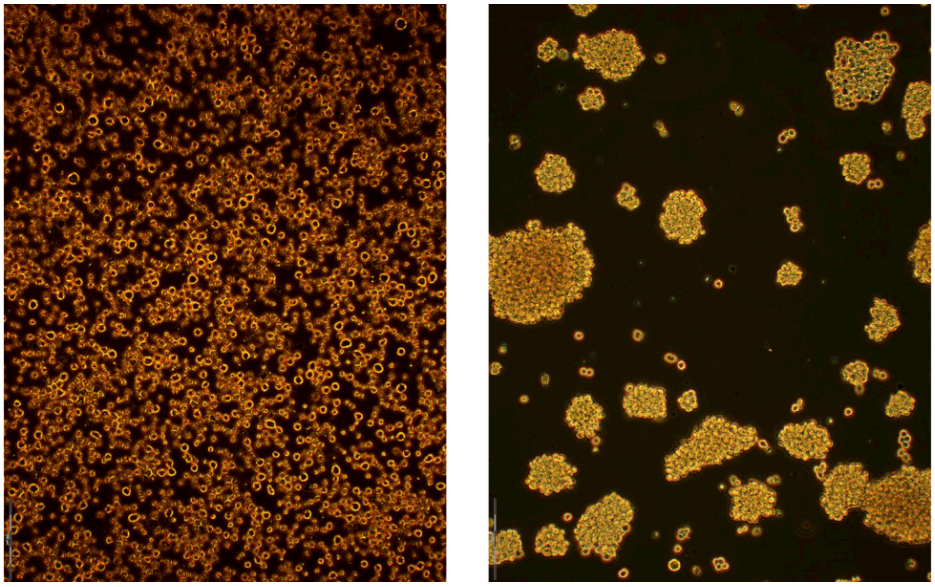

Figure 1
Typical appearance of densely grown THP-1 (left) and THP-J (right) cell cultures.

A parallel reduction in the viability of THP-1 and THP-J may have occurred; however, it only became apparent after cultivation these cell lines for more than 3 years.

Table 1

		THP-1	**THP-J**
a)	Medium containing 4% NBS		
	Number of individual cells, in %	40–80	40–50
	Number of cells per group	4–8	6–25
	Adherent cells in %	3	8–20
b)	Medium containing 10% NBS		
	Number of individual cells, in %	40–50	30–40
	Number of cells per group	40	100
	Adherent cells in %	3–4	8–9
c)	Medium containing 10% FBS		
	Number of individual cells, in %	5–6	6–7
	Number of cells per group	7–15	50–110
	Adherent cells in %	5–6	8–10

Table 1: Mean values for the growth pattern of THP-1 and THP-J cells ten days after starting the cultures.

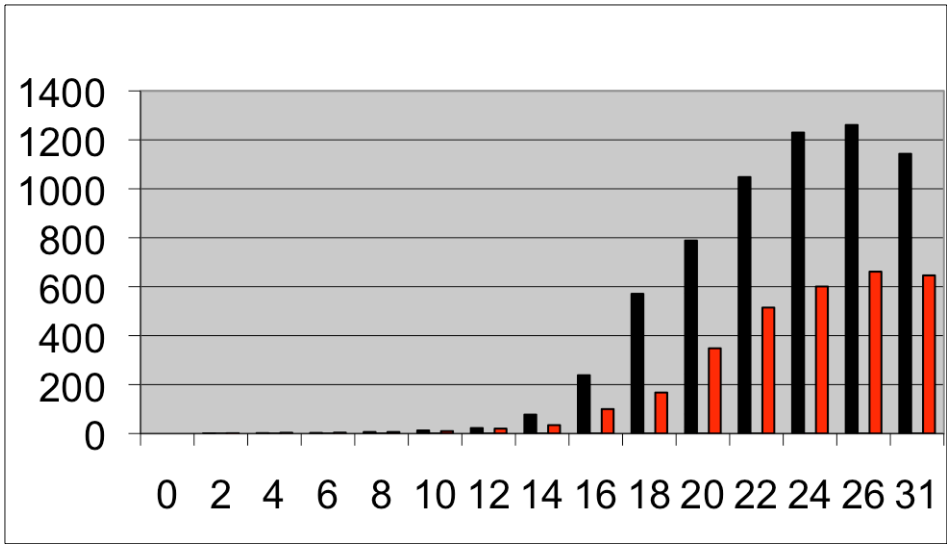

Figure 2
Characteristic growth curves of THP-1 (black) and THP-J2 (red) over a period of 31 days. The bars show the number of cells per 10-3 ml culture in the nutrient solution containing 10% FBS. Both cultures were started with 40,000 cells per ml.

3T3 Cell lines

In contrast to THP, the 3T3, B16, and Rafi cells grow as adherent cells. The notable feature of 3T3-J and 3T3-K was the higher cell count at confluency as compared to 3T3-L1 (Figure 3). A microscopic examination showed that the 3T3-J/K cells had formed multilayers. 3T3-L1 also showed growth of cells on top of each other, but to a much lesser extent. Stronger magnification revealed that the 3T3-J and 3T3-K cells formed two to three times as many dendritic cell processes as the 3T3-L1 cells. Owing to the many dendrites, the treated cell lines had a characteristic appearance under the microscope and could easily be distinguished from 3T3-L1.

The high cell density of 3T3-K2 at confluency decreased a few days after and dropped back to the level of the control cells or even below if the medium was not replaced. This was not the case with the 3T3-J1, 3T3-J2, and 3T3-K1 cultures: they retained their high density for several days even if the growth media were not renewed. 3T3-L1, 3T3-J, and 3T3-K cells did not differ regarding their initial growth rate.

Depending on the growth conditions, the cell count at confluency for 3T3-J1/2 and 3T3-K1/2 was 160 percent and more compared to that of 3T3-L1. These values remained reproducible over the analysis period of more than 4 years.

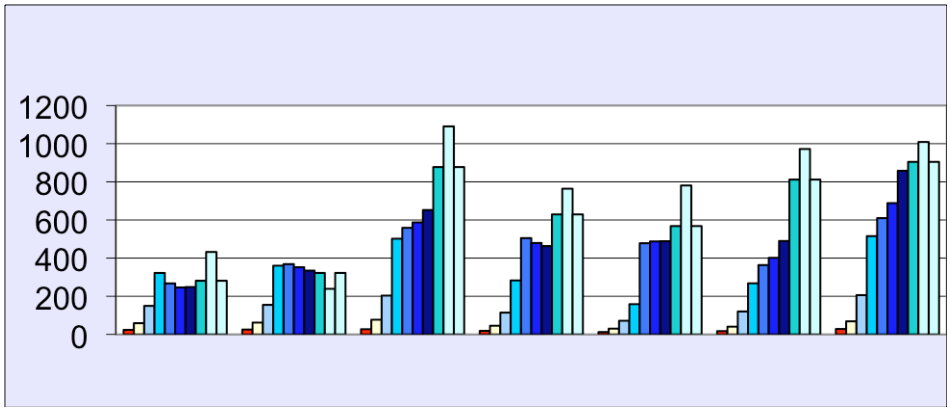

Figure 3
Growth curves of 3T3-L1 (2x), 3T3-J1, 3T3-J2 (2x), 3T3-K1, and 3T3-K2 cells (from left to right, respectively) in a nutrient solution containing 10% NBS. The height of the bar corresponds to the number of cells per well (1/1000) determined on day 4, 5, 6, 7, 9, 11, 13, 19, 23, and 27 after starting culture at 150 cells per well in 24-well plates (shown values are means from 3 wells).

3T3-K1/2 exhibited a large number of giant cells containing many nuclei. 3T3-L1/J also produced giant cells, but they were only slightly enlarged and rare with around 0.3% of all cells. In the case of 3T3-K, up to 2% of the cells were multinucleate, and cells with 8 to 12 nuclei were often observed. This did not occur with 3T3-L1/J, whose large cells generally contained only 2–3 nuclei. The number of nuclei in the giant cells corresponded to their cell size. Giant cells already formed a few days after starting a new culture, and were not a feature of an advanced or overaged culture. The giant cells, like all other 3T3 cells, were easily detached from the bottom of the culture flask. In EDTA solution, they formed large round cells that were conspicuous and stable.

3T3-K cells had a slightly changed appearance compared to 3T3-L1 or 3T3-J: they were somewhat flattened and similar to cells that are on the verge of converting to fat cells. However, induction of 3T3-L1, -J, or -K into fat cells was not successful, possibly due to the culturing condition with NBS.

There were also differences in the cell survival rates. In this test, the 3T3-J cells showed a significant statistical difference compared to 3T3-L1 and 3T3-K, and had a clearly superior survival rate by a factor of 2.

Growth of 3T3 cell lines under metabolic stress was extensively investigated in 2008 (for culturing conditions, see the section on Rafi cells). No differential growth changes were observed in assays performed in microtiter plates.

B16 cell lines

The growth properties of the B16-J cells were changed by EMF treatment with A frequencies without C. The cells in 5 of 7 experiments grew slightly faster than the control cells, as measured by the methylene staining assay. The frequency-related change in growth was maintained as measured over 2 years. EMF treatment of the B16-F1 with B frequencies was not carried out.

The survival rate of the B16 cells was inconsistent.

B16-F1 cells synthesize melanin if they are stimulated by alpha-MSH. It was investigated whether the sensitivity of B16-F1 for alpha-MSH is changed by EMF treatment. The melanin test, which was developed by the research group of Prof. A. Eberle and which was used in the laboratory while this work was carried out, is extremely sensitive, precise, and well standardized. Three parallel batches of cells are grown for a few days in a microtiter plate before adding different amounts of alpha-MSH to stimulate synthesis of melanin, which is then measured. This test is a measure for the sensitivity of the cells to synthesize melanin by different concentrations of alpha-MSH.

New EMF treatments were carried out with B16-F1 cells in order to investigate a larger number of independently generated cell lines by the melanin test. Six cultures were treated in parallel with EMF A in a metal enclosure that shielded them from external EM fields during EMF treatment. In addition, six cultures were treated with the same EMF without shielding, and a third group of six cultures were not treated and used as controls. After EMF treatment, all cultures were cultured separately. The shielding enclosures CFL4 were from Perancea (London). Melanin synthesis by all cell lines was investigated after 11 and 32 EMF treatments of the cells. The results of the melanin synthesis showed minor differences that did not correlate with EMF treatment nor with the presence of shielding enclosures nor with the number of treatments. Furthermore, no significant differences in growth were found. Thus, the weak initial results of B16-F1/J cells could not be reproduced.

Rafi cell lines

THP-1, 3T3-L1, and B16-F1 are cell lines originally established from cancerous tissues. The question was open whether changes due to EMF treatments can also be obtained for primary, non-mutated cells. Experiments with primary lens fibroblasts (porcine) were initially carried out using a cell line obtained from Kerstin Wunderlich. Since these cells stopped growing after a few passages and died by apoptosis, growth analyses after EMF treatment were not possible. Primary rat fibroblasts on the other hand can be cultivated for a long time without genetic changes (Lit. 5,22). A culture of primary rat fibroblasts (Rafi) was established in February 2007 from a one-day-old laboratory rat. Treatments with frequency groups A (without C) or B were carried out after passage 25 in June to August 2007, and growth analyses were started 6 passages afterwards.

The first analyses did not show any differential growth behavior of the cells. It was observed however, that the number of viable cells in new cultures greatly varied from day-to-day. Only after this fluctuation was taken into account in the establishment of growth assays was it possible to document differential growth (Table 2). The procedure was as follows: the number of viable cells in a culture was determined by measuring seeding efficiency (SE) prior to the growth test. Taking the number of viable cells into account gave reproducible values for the growth assays with a variation of +/- 2 % between growth cultures prepared in parallel.

Daily SE of Rafi cells showed extremely large daily variations with values ranging between 13 and 98 %. The daily fluctuations of SE were low in 3T3-L1 and B16-F1 cell lines. SE of 3T3 and B16 cell lines had mean values of 80 to more than 95 %, which was significantly higher than that of Rafi with 55–60 %. The mean SE values were similar for Rafi-J, Rafi-K or untreated Rafi cells.

All the sterile plastic materials and the various nutrient solutions used in the work were excluded as a possible cause of the daily fluctuations of SE. It was not possible to discover its likely cause. Test series in which SE was determined regularly over a period of several months indicate the presence of different rhythms in the cells (results not shown).

It was investigated whether suboptimal compositions of the nutrient solutions increased differential growth of the Rafi cell lines. Various nutrient solutions were tested: a) hypotonic nutrient solutions, b) serum-free or low-serum media, c) nutrient solutions containing mixtures of FBS and NBS, and d) a hypertonic nutrient solution containing EGTA and ammonium phosphate. The cells grew at various rates in the different nutrient solutions. 20% of the medium had to be replaced every five days.

The growth experiments in the presence or absence of metabolic stress were carried out for all Rafi cell lines, four to six times in 96-well plates, and growth determined by the methylene assay. The results are based on triplicate experiments (Table 2). In a rich culture medium containing 5% NBS and 1.5% FBS, the growth of all cell lines was comparable. In a nutrient solution containing 30% or 42.5% water, the control cells and Rafi-K grew faster than Rafi-J. In the artificial serum-free medium, the Rafi-K cells grew more slowly. And finally, the controls grew somewhat faster than the Rafi-J/K cells in media containing 5% NBS and significantly faster in media with 1.5% FBS. The significant differences amounted to maximally 20 to 30% growth difference. The growth experiments in hypertonic nutrient solutions could not be evaluated since, in spite of the added calcium and fixation with formaldehyde, the cells detached from the bottom of the culture plates and were washed away in the staining procedure.

Table 2

Culture conditions	**Results**	**Statistics**
Medium with 5% NBS + 1.5% FBS	Rafi=Rafi-J=Rafi-K	no significant variance
Serum-free medium	Rafi=Rafi-J>Rafi-K	significant
Standard medium + 30% water	Rafi=Rafi-K>Rafi-J	significant
Standard medium + 42.5% water	Rafi=Rafi-K>Rafi-J	significant
Medium + 1.5% FBS	Rafi>Rafi-J=Rafi-K	significant
Standard medium	Rafi>Rafi-J=Rafi-K	not significant
Medium + 2% NBS	Rafi-J>Rafi=Rafi-K	not significant

Table 2: Growth analyses of the Rafi cell lines in the presence or absence of metabolic stress

DISCUSSION AND FREQUENCY MODEL

Summary of the main results (Table 3): After EMF A/C treatments, both the THP-1 and the 3T3-L1 cells showed conspicuous and lasting changes. The most diverse changes were observed in the human cell line THP-1.

The first part of the discussion deals with the results of this work: How should the results be evaluated? The second part discusses a model for the possible mode of action of EMF.

Table 3

Cell type	Frequencies	Cell lines	Oscillator	Effects
THP-J	A/C	3	S	G 1; A 1
THP-J	A/C	2	P	G neg; A neg
3T3-J	A/C	2	S	G 1;SR 3
3T3-J	A/C	2	P	G neg
3T3-K	B	2	P	G 1; GC 1
B16-J	A	2	S	G3; MA possible SR 3
B16-J	A	2	P	G neg; MA na
B16-J	A	2x6	S	G neg; MA neg
Rafi-J	A	6	S	G 2
Rafi-K	B	6	S	G 2

Table 3: Summary of the EMF results

Abbreviations
Frequencies: A/C = Group A with final frequency C; B = Group B;
Cell lines: Number of cell lines generated by EMF
Oscillator: S = F-SCAN 2 series instrument; P = F-SCAN 2 prototype;
EMF Effects: 1 = Strong, 2 = medium, 3 = weak; neg = Negative; na = Not analyzed; G = Growth;
A = Aggregation; GC = Giant cells; MA = Melanin assay; SR = Survival rate

DISCUSSION, PART 1

None of the cell lines generated by the EM treatments with frequency group A had a reduced viability. On the contrary, the survival rate of 3T3-J was improved. The division rate of the treated cells was also not reduced: THP-J showed a higher proliferation rate, albeit only for a fraction of the THP-J. The EMF treatments did not induce changes for which the respective cell type is not adapted, as far as this can be evaluated.

Summary of the observed changes of the cells due to low energy EMF treatment:

1. Frequencies lead to *reproducible* changes in growth.
2. Frequencies lead to changes that are *specific* to each cell type.
3. Frequencies lead to *stable* cellular changes.

THP cell lines

All standardizations for the frequency treatments were carried out with THP-1 cells. THP-J showed the first positive results that helped to drive the project forward experimentally. Several results, such as differential growth in various nutrient solutions should have been investigated in depth. It is likely that the whole project could have been limited to the analysis of THP-1. Some obvious results were not investigated further:

- Which frequency/ies lead to which changes in the cells?
- What impact do treatments with EMF A plus/minus C respectively with B have?
- Do THP-1 and THP-J differ in their ability to differentiate into macrophages?

THP-1 did barely grew at low cell concentration in a medium containing NBS only in the first 2 to 3 weeks, whereas THP-J1 and J4 were able to proliferate well at all cell concentrations in NBS or FBS. These growth experiments were carried out in 2005 and 2006. An attempt was made in 2009 to verify differential growth of THP-1 and THP-J cells in NBS with a low initial cell count. However, this was not possible because all the low density cultures died in all attempts (N=11). The THP-J cells in 2009 were still changed by the frequency treatment carried out in 2005 since they had kept the property of growth in large groups. It is possible that ageing phenomena was the cause as this affected both THP-1 and THP-J kept in similar conditions during all this time.

THP-J cells grew more slowly than THP-1 in FBS. Analysis of CFSE-stained cells revealed that 1/5 of the THP-J grew faster than THP-1, whereas the remainder cells proliferated significantly slower. The growth behavior of the heterogeneous THP-J population is evidence for a regulative effect of the EMF treatment, showing the effect of adaptive growth by the different cell populations.

Part of the culture was always adherent and presumably no longer proliferated. It can be assumed that adherent THP had differentiated from monocytes to macrophages. The influence of EMF treatment on the differentiation capacity of THP-J was not investigated in depth.

The formation of large groups of THP-J cells was particularly conspicuous. It was thus possible to distinguish between treated and untreated cells without magnification. A description of a comparable cell line corresponding to the characteristics of THP-J was not found in the literature. With the growth

into large clusters, the THP-J cells showed an enhanced interactivity between each other. The tendency to grow in large groups was influenced by the growth environment. In overaged cultures, the groups disintegrated, and in nutrient-rich environments they were larger.

Which genes were affected by the EMF treatment? Since FBS and NCS differ in their concentration of growth factors and since it is known that cells in culture secrete such factors and influence the neighboring cells, it can be assumed that the EM information affects among other genes those responsible for regulation of the concentration of growth factors and/or their receptors (Lit. 9).

Giant cells were also observed with THP cell lines; however, they were rare. Their prevalence did not change after EMF treatment. It is possible that giant cells are commonly found.

3T3 cell lines

The 3T3 cell lines were robust and their growth characteristics did not change over the 4 years of cultivation after EMF treatment. The differences due to EMF treatment of 3T3 were not manifold, but they were stable. They consisted in an extensive growth effect and in the appearance of giant cells. Attempts were also made to stimulate the transformation of 3T3 cells into fat cells; however, this was not successful in nutrient solutions containing NBS. Overall, the potential of 3T3-L1 cells for investigations by frequency treatments with A/C or B are considered as good.

Rare giant cells have been reported in the literature (Lit. 3,4). Cell lines with up to 2 % giant cells were found after EMF treatment. The largest cells were detected in 3T3-K cultures only.

The growth characteristics of 3T3-L1, 3T3-J, and 3T3-K at confluency was repeatedly confirmed, remaining unchanged over the entire duration of the project. The different growth characteristics were also retained when the cells were stressed using suboptimally composed media as used for Rafi cells (results not shown).

3T3 cells form dendritic processes. Since 3T3-J and 3T3-K produced 2–3 times more dendrites than 3T3-L1, they were presumably better adapted to grow in multilayers. The composition of the dendrites was not investigated in details. The changes of 3T3 after EMF treatment demonstrate how information from a few frequencies are able to influence complex processes in a reproducible and stable way.

B16 cell lines

The effect of the applied EMF on the B16 cells was low and not always reproducible. It was hoped that the B16-J would grow more slowly than B16-F1. This, however, was not what happened: the B16-J cells grew somewhat faster than the already fast-growing B16-F1. However, the EMF treatment effect was minor and only detectable in 5 out of 7 experiments. Thus, B16-F1 are considered not suited for EMF experiments with the frequencies that were used.

Metabolic stress did not lead to differential growth differences of the B16-F1/J cells. This was only observed with Rafi, but not with the 3T3 or B16 cell lines. It is possible that the B16 are genetically modified cells with a limited response range for growth adaptations.

Extensive investigations were carried out on the inductive sensitivity of the B16-F1/J cells to synthesize melanin. The first observation was that the EMF-treated B16 showed a higher sensitivity to alpha-MSH. The enhanced alpha-MSH sensitivity effect could not be reproduced, however. Further analyses with a total of 6 controls and 12 EMF-treated cell lines showed that differential melanin synthesis can be attributed to selection occurring by the EMF treatment process. The lower the number of B16-F1 used to start the cultures for the frequency treatments, the more the cell lines lost their characteristic sensitivity to alpha-MSH. In other words, B16-J cell lines did not show any changed alpha-MSH sensitivity to melanin synthesis if the initial number of cells used in the EMF treatment was greater than 50 cells per culture flask.

In the same experiments, the B16 cells were also used to investigate the effect of EM shielding applied during the frequency treatments. Due to the absence of cellular changes in these experiments, no results were obtained on this issue.

The small or lacking differences in phenotype between B16-F1 and B16-J could have many causes:

- B16-J cells were treated with frequency group A only, without C.
- The applied growth assay with microtiter plates was not sensitive enough.
- Mouse melanoma cells are not sensitive to frequency group A.

The work with the B16 cells led to the conclusion that selection processes in the experiments can be a source of artifacts. Overall, the B16 cell lines were investigated much more intensively than the THP.

Rafi cell lines

The Rafi cells showed good cultivation performance up to at least passage 34. This was the decisive property for using these cells in the experiments. Nevertheless, apoptotic death of the cell lines at high passage numbers repeatedly made the replacement by frozen cell culture aliquots necessary. This occurred at irregular time points. Furthermore, it would have been interesting to repeat the growth experiments with A+C frequencies. In retrospect, a higher oxygen concentration as well as a medium containing FBS would also have been advisable for cultivation. All in all, the rat fibroblasts are considered not suited for EMF experiments with the selected frequencies.

The experiments with the Rafi were carried out with the aim of detecting the influence of frequency treatments on primary cells, established from non-cancerous tissue. This was successful to some extent. However, differential growth was only observed when the cells were under metabolic stress: the phenotypic alterations of the cells were minor. In addition, it was not possible to find a clear pattern for the growth differences of the Rafi, Rafi-J, and Rafi-K cells using various metabolic stress conditions.

Primary rat cells were used because they are known for their longevity in cultures without mutating, which is promoted by a low oxygen concentration in cell cultivation. The incubators for these cultures were set to 3 % oxygen, or even to 1 % in some cases. In retrospect, this appears to be too low for skin fibroblasts. It is assumed that an oxygen concentration of 10–15 % would have been better suited.

Since the oxygen concentrations in the cells greatly vary according to the tissue (lung 13–17 %, fatty tissue 1–2 %), the THP, 3T3, and B16 cells were also investigated at low oxygen concentrations. Differential growth of these cell cultures did not increase however.

The small and variable differences in growth of the Rafi cells with and without EMF treatment prompted a refined analysis of the growth conditions. This led to the discovery of the variations in seeding efficiency. Nevertheless, the growth differences with and without EMF treatment were slight compared to THP and 3T3. The question remains whether this is due to omitting the application of frequency C or to the possibility that growth changes induced by EMF treatments are generally of low extent in primary cells. In addition, it is although possible that the A or B frequencies are not ideal for experiments with Rafi cells.

Finally, the use of EM shielding enclosures for the Rafi cells did not yield results.

Reproducibility

Reproducibility of cell line-specific EMF effects was observed a number of times: THP-J1, THP-J4, and J12; 3T3-J1 and 3T3-J2; 3T3-K1 and K2; two test series each with 6 Rafi cell lines. Repeated treatments with the same frequencies always led to the same effects, specific for the cell type.

The cooperativity effect of EMF treated cells

A phenomenon was detected as a result of various EMF experiments that is interpreted as an enhanced interaction or as a cooperativity effect between the cells or between the cells and their environment:

- THP-J cells had a tendency to form large clusters. The preliminary results on alpha-troponin should have been further investigated: after treatment with PMA and staining with anti – alpha-troponin, the THP-J cells were found to contain much more alpha-troponin than the THP-1 cells. It is also conceivable that the enhanced cell interactions of THP cells are mediated by tight junctions (Lit. 10). However, this was not analysed.
- 3T3-J/K cells grew to denser cell layers and produced multilayers.

Prototype and series instruments of F-SCAN 2

Two THP-J and two 3T3-J cell lines were generated with the F-SCAN 2 *prototype instrument* using the A+C frequencies. However, these cells lines did not show any difference in their growth compared to the controls. All previously described positive results apply to cell lines obtained with the F-SCAN 2 *series instrument* (Tab. 3). It thus seems possible that the prototype instrument was not suitable.

However, the prototype instrument was used to generate the two 3T3-K lines that were obtained by applying B frequencies: the 3T3-K1/2 cells grew to a greater density and also had more giant cells.

Some possible explanations are:

1. The phenotype of the 3T3-K1/2 cells was the result of a selection process involving a preexisting mutation in 3T3-L1.
2. The prototype F-SCAN 2 transmitted only B frequencies, but no A frequencies.

The two 3T3 cell lines treated with A+C frequencies using the prototype instrument did not show any enrichment of giant cells: This speaks against Explanation 1. Explanation 2 seems not likely because A and B frequencies are very similar. It thus seems likely that the negative results were caused by an unknown error in the experiment.

Regarding the signal characteristics of the individual frequencies
The F-SCAN 2 oscillator can generate frequencies whose shape is rectangular or sinusoidal. Rectangular signals contain the corresponding EMF overtones of the basic signals, but sinusoidal signals do not. All EMF treatments of the cells were carried out in the rectangular mode. An analysis of the output signals from the F-SCAN 2 series instrument confirmed that the signals had the correct shape. However, the signals were no longer rectangular in the medium. The frequencies in the megahertz range were almost sinusoidal, and in the low Hertz range the originally rectangular signals were considerably lowered on one side without turning to a sawtooth form.

The voltages transmitted by the EMF treatment to the RMPI medium were checked in situ for the F-SCAN 2 series instrument. The voltage in the medium at various locations in the culture flask varied between a maximum of 800mV and a minimum of 233mV, depending on the frequency. The measurements were carried out at the furthest possible distance from the electrodes, which were located in the middle of the culture flask, as usual. Thus all cells in the culture flask were exposed to the EMF, but at different strengths. It must be assumed that the transmission pathway of the EMF signal from the oscillator via the potentiometer and spider box to the electrodes was the cause of the voltage loss and the loss of some overtones. Measurements in the RMPI medium gave the following in situ currents for the individual frequencies of frequency group A: 344 µA at 111.2Hz and 112.2Hz; 359 µA at 555.7Hz; 299 µA at 555,555.1Hz and 555,555.6Hz; 5.9 µA at 5,555,554.3Hz and 5,555,555.4Hz. The measured values were constant. It is assumed that these values are similar for the other nutrient solutions with comparable salt concentrations.

In summary, it can be stated that the strength of the EMF signals and the presence of overtones was reduced to a considerable extent by the transmittance from the oscillator to the point of action, the reduction depending on the exact frequency used.

DISCUSSION, PART 2

Are the described EMF effects due to mutations?
THP-J and 3T3-J/K were changed in a way that is presumably not due to a single mutated gene. In addition, the same phenotypes repeatedly occurred independently of each other. Did the starting cell lines of THP-1 and of 3T3-L1 already contain mutated cells that were selected by the experimental procedure? This is unlikely. The new phenotypes did not occur in several experiments and, in particular, in none of the controls. For the 7 cases in which changes were observed, they were frequency-specific and, with respect to the cell type, they were specific and reproducible.

It is not obvious how a single mutation could have caused all the changes of THP-J: formation of large clusters, differential growth of cell subpopulations, differential growth of THP cell lines depending on the serum and initial cell count as well as an increased probability of becoming adherent. If the new phenotype of 3T3-J and 3T3-K could be attributed to one mutation, the question arises as to why 3T3-J did not produce more giant cells. The fact that different phenotypes of 3T3-J and K were observed after different EMF treatments of 3T3-L1 is conclusive evidence. It is also unlikely that the same mutation would lead not only to giant cells, which are caused by endomitosis, but also to a higher cell density at confluency, a property that occurred alongside increased dendrite formation.

The following findings also contradict the hypothesis of mutation and/or selection as the cause for the different THP phenotypes present after EMF treatment:

1. In 2009, four years after THP-J had been induced, the growth capacity of THP-1 and J had obviously decreased, but not the ability of THP-J to form large clusters. The growth properties and the cluster formation of THP could thus change independently of each other.
2. The cells of 3T3-J and THP-J were cultivated separately in two to four flasks for EMF treatments and pooled afterwards. This procedure was used for the following reason: cells with phenotypes that were not generated by EMF treatment, which would have affected only a portion of the cell culture, should thus have been identified more easily before they could become predominant as a result of selection. The results of the EMF treatments, however, indicate that all cells had the new phenotype.
3. If the EMF treatments had led to mutations, the viability of the treated cells would probably have been reduced, since random mutations are statistically mostly negative. This was not the case.

Nevertheless, could the applied EMF have generated mutations in the DNA by some other means? Let us assume that this is indeed the case: Several genes would have had to be affected by mutations in order to explain the obtained phenotypes: the mutations would have to be suitable, cell-specific and reproducible. In other words, an explanation of the results on the basis of EMF-induced mutations and/or selection can be excluded.

The mechanism for the various cellular changes through EMF treatment must have other explanations.

All the effects described were induced with EMF below 6,000,000Hz and less than 0.4mA and less than 0.8V. It is likely that this energy is not sufficient to stimulate molecular receptors (Lit. 6). If the organism is defined as an EM unit that also responds accordingly: e.g. after an applied injury, tiny electric currents are detectable in the injured area (Lit. 7), then an influence exerted by EMF appears plausible. US Patent 3924609 demonstrates the fact that such weak EM properties can be used technically by an electrical ovulation test in humans for the determination of the precise fertilization time (Lit. 8).

The treatment of cells using EMF with very low energy corresponds to the EM influences of the natural environment: the EM field of the Earth is weak. Extremely low frequencies (ELF) of less then 50 or 60 Hertz with very low voltages are unlikely to have sufficient energy to reconfigure chemical bonds. But how can the Earth's 10Hz EM field control the 24-hour cycle of humans, animals, and plants (Lit. 13,14,15)? All organs, cells, and genes of organisms are subject to the 24-hour rhythm, as chronobiological research on humans and various animals (Lit. 16) and plants has shown. The many biological parameters that follow the 24-hour cycle of an organism, such as motor activity, blood pressure, or body temperature, as well as the rhythmic changes in the concentrations of glucose, sodium, calcium, melatonin, etc., demonstrate the all-encompassing influence of the internal clocks. The finding that not only the Schumann frequency, but also other EMF are able to affect organisms thus expands well-known facts.

To the best of our knowledge, the EMF used in this work do not occur in nature. It thus seems plausible that the new frequencies A, B, and C are able to induce new properties. But how could the EMF used in these experiments have been able to affect the metabolism of the cells? It is possible that there are information present in a cell, or in an entire organ that are common to a biological structure formed by many cells. On the basis of this assumption, it is possible to understand the responses of the cells obtained by the EMF treatments.

Extreme sensitivity to weak EM fields has been observed in organisms (Lit. 12). Voltage differences of only 0.5µV/m can be detected by the lateral line system of sharks and rays (Lit. 17). Although the mode of action of the lateral line system has been discussed, its extreme sensitivity remains a mysterious phenomenon (Lit. 19). Mammals also have a highly sensitive sensory organ: their eyes are said to be capable of perceiving single photons (Lit. 18). It has been proved that shielding of the Earth's magnetic field desynchronizes the internal clock in humans and animals (Lit. 13,14). How do living beings without eyes that also live in the dark keep their internal clock functioning without the usual daily adjustment by sunlight (Lit. 20)? Many people respond to changes in the weather conditions or moon phases by feeling unwell. What is the mechanism of this response? In this work, the lasting changes in cells are reproducibly induced by EMF treatments with very low energy. The following model could explain these findings:

MODEL FOR THE CELLULAR EFFECTS BY ELECTROMAGNETIC FREQUENCY TREATMENT

Parallel to the genetic system that is responsible for synthesis and metabolism of the structures of organisms, there exists an information field for the regulation of the genetic system. Information is encoded as electromagnetic frequencies of the quanta. The quanta are the units of matter of all structures. All quanta have specific and unique information for the respective structures of which they are part. The electromagnetic frequencies form an information field for the structures. The information field acts according to the resonance of all frequencies of a structure. The extent of the resonance determines the energy transmitted. The energy of a structure corresponds to the amplitude of its frequencies. All structures have quanta with specific frequencies and information that are needed for its establishment and regulation. As the frequencies of the quanta form the information field and the material structures, no energy transmission is necessary: The energy is generated at the place where it is used. The information field acts harmoniously because it acts by means of resonance of the involved quanta.

The proposed model in one sentence: *The electromagnetic frequencies are an interactive information field that forms structures from information and supplies energy in accordance with the resonance of its frequencies.*

The key results of this work are: Treatment of THP-1 and 3T3-L1 cells with EMF A+C and 3T3-L1 with frequency group B led to large and reproducible changes. The changes found are specific to the respective cell type. Most changes were stable for years. The following discussion is limited to the results obtained with THP and 3T3 cells.

EMF treatments caused changes to THP-1 and 3T3-L1: what sort of changes? Already existing properties of the cells are reinforced: THP-J forms larger cell groups and more frequently; 3T3-K forms larger giant cells and more frequently. 3T3-J and 3T3-K form more dendrites per cell and presumably also have in this way changed growth properties. *All these changes are only possible due to a cooperatively*

acting regulation mechanism for complex processes. These changes, comprising many cellular structures with many different molecules, involve interactive regulation of many different gene products. The growth at different rates of THP-J involving different cell populations is also regulated cooperatively. The fast-growing THP-J cells do not overgrow the slowly growing cells: the THP-J cell culture is in equilibrium.

The frequency-induced changes are specific for a given cell type: 3T3-J and B16-J, both of which are mouse cells, respond differently to the same EMF.

Changes were induced with 30 and more EMF treatments and remained stable for years. This means that the EMF of the information field, once it has been activated, probably remains in the new activity mode until it is influenced by new frequencies or new information. In other words, the activity of the information field is stable if there is no influence by any kind of energy. Repeated EMF treatments are thus expedient: presumably the amplitudes of the target information is reinforced. This in turn means that a large number of EM treatments of the cells tend to cause strong EMF effects.

The model raises many questions. Some of the following questions could probably be answered today using the A/C or B frequencies, with suitable cells; whereas other frequencies would have to be used for other cells. It is probably advantageous to carry out EMF experiments with at least two different cells lines if the difference in responses between primary and established cell lines has not been elucidated and if the EMF-specific sensitivity of a given cell type has not be investigated. Open questions that presumably could be tackled to some extend:

1. How often must THP-1 cells be treated with the A +/- C respectively the B frequencies to induce complete activation?
2. Can EMF effects also be obtained with cells using magnetic transmission of the frequencies?
3. If cells were to be treated with EMF to which they had been chronically exposed for an extended period, it is likely that no further behavioral changes would be induced: the target frequencies are already present in an active form. This phenomenon could occur due to electrosmog (Lit. 21).
4. Is it possible to treat cells successfully with EMF but without a sweep?
5. Is it possible to induce apoptosis in established cell lines using a suitable EMF?
6. Which EMF can reduce growth of cancerous cells?
7. Could cancerous cells be converted into benign cells using a particular EMF?

In summary: the model proposed here leads into an unexplored field. Although the model fulfills the necessary functions, it does so in a new way.

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ABBREVIATIONS

BSA	bovine serum albumin
EM	electromagnetic
EMF	electromagnetic frequencies
Frequencies	definition of EMF A, B, and C: see page 4
Hz	Hertz, frequency per second
FBS	fetal bovine serum
alpha-MSH	melanin-stimulating hormone
NBS	newborn bovine serum
PMA	phorbol ester
SE	seeding efficiency
3T3-L1	untreated mouse fibroblast cells, pre-adipocytes
3T3-J	3T3-L1 cells treated with EMF group A+C
3T3-K	3T3-L1 cells treated with EMF group B
B16-F1	untreated mouse melanoma cells
B16-J	B16-F1 cells treated with EMF group A
Rafi	untreated primary rat fibroblasts
Rafi-J	Rafi cells treated with EMF group A
Rafi-K	Rafi cells treated with EMF group B
THP-1	untreated human monocytes
THP-J	THP-1 cells treated with EMF group A+C

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