Scanning Microscopy

Volume 5 | Number 4 Article 24

9-9-1991

Effects of Modulated and Continuous Microwave Irradiation on the Morphology and Cell Surface Negative Charge of 3T3 Fibroblasts

Z. Somosy
"Fréderic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

G. Thuróczy
"Fréderic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

T. Kubasova
"Fréderic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

J. Kovács
Eötvös Loránd University

L. D. Szabó
"Fréderic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene

Scanning Microscopy, Vol. 5, No. 4, 1991 (Pages 1145-1155)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/91$3.00+.00

EFFECTS OF MODULATED AND CONTINUOUS MICROWAVE IRRADIATION ON THE MORPHOLOGY AND CELL SURFACE NEGATIVE CHARGE OF 3T3 FIBROBLASTS

Z. Somosy*, G. Thuróczy, T. Kubasova, J. Kovács[1] and L.D.Szabó

"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest and
[1]Department of General Zoology, Eötvös Loránd University, Budapest, Hungary

(received for publication March 4, 1991, and in revised form September 9, 1991)

Abstract

Mouse embryo 3T3 cells were irradiated with 2450 MHz continuous and low frequency (16 Hz) square modulated waves of absorbed energy ranging from 0.0024 to 2.4 mW/g. The low frequency modulated microwave irradiation yielded more morphological cell changes than did the continuous microwave fields of the same intensity. The amount of free negative charges (cationized ferritin binding) on cell surfaces decreased following irradiation by modulated waves but remained unchanged under the effect of a continuous field of the same dose. Modulated waves of 0.024 mW/g dose increased the ruffling activity of the cells, and caused ultrastructural alteration in the cytoplasm. Similar effects were experienced by continuous waves at higher (0.24 and 2.4 mW/g) doses.

Key Words: Microwave irradiation, modulation, ultrastructure, morphology, 3T3 cells, negative charges, electron microscopy.

*Address for correspondence:
Z. Somosy,
"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene,
P.O. Box 101,
H-1775 Budapest,
Hungary

Telephone No: 36-1-226-0026
FAX No: 36-1-226-6974

Introduction

The biological effects of microwave irradiation have been investigated extensively (1, 2, 4, 8, 20, 25, 36, 38, 39, 49, 70, 71, 80, 97, 104) and data are available about its effect on membrane organization (10) as well as membrane functions. Thus, microwave irradiation is known to alter the cation permeability and transport of plasma membrane (26, 27, 47, 49) trans-membrane protein channels (24), membrane bound enzyme activity (1, 2, 12-15, 97), ligand-receptor interactions (44, 52, 67, 73, 76), signal transduction (15, 52), cell communication (29), and immunity (11, 21, 33, 46, 50, 70, 76, 79, 87, 89, 103). Microwave irradiation caused membrane shedding (46, 67) and altered the phagocytotic activity of macrophages (56).

The morphological changes of the plasma membrane and the cell organelles upon exposure to microwave fields were investigated on cells of the central nervous system (6-8, 42, 65, 66, 94), lens (104), blood barriers (4), lymphocytes (34), and in cell culture (18, 28, 86, 102). Data were published about cell shape alteration following microwave irradiations (18, 28, 86, 102). The mechanisms leading to the membrane injury following microwave irradiation depend on the dose and mode of irradiations (1, 2, 6, 8, 19, 28, 36, 70, 71, 97). Thus, it is well known that high intensity microwaves (higher than 10-15 mW/cm) exert direct and indirect thermal effects (18, 45, 70, 71, 79, 86). However, the biological response also contains a non-thermal component, which came into prominence as low doses were applied (below 10 mw/cm) (11, 21, 28, 102). Recently it was proposed that the membrane effects of continuous and modulated electromagnetic (microwave) irradiations might not be identical. Chang (16) found that the oscillating electric field (from a few kHz to 1 MHz, the peak amplitude of oscillating field 0.5 to 5 kV/cm) caused cell poration and cell fusion. Some changes of membrane functions (i.e., cation transport and membrane permeability, ligand binding, signal transduction, cell communication (6, 8, 11-15, 19, 21, 24, 26, 27, 29, 49, 52, 60, 67, 73, 76, 96, 103) occur upon weak, low frequency

(below 100 Hz) modulated irradiation but continuous fields of the same intensity do not cause any effects on the cells. These changes in membrane function may be explained by a specific interaction between the weak low frequency modulated electromagnetic fields and the charged groups of membranes as well as the potential profile of the membrane (1, 2, 73). The electric potentials of the membrane play an essential role in the membrane organization and functions [i.e., protein conformation and enzyme activity, lipid composition, signal transmission, ion carriers and channels (22, 41, 59, 77, 78, 95)], some membrane effects of electromagnetic fields can be explained by that mechanism.

Since the difference in membrane effect between the low frequency modulated and continuous microwave fields are not clarified in all respects, we decided to investigate this problem by electron microscopy, we supposed that the charged groups of membranes may serve as primary targets of modulated microwave irradiation. Therefore, we studied the amount and distribution of fixed negative charges visualized by cationic ferritin (CF) binding (23) upon different kinds of microwave fields. Parallel changes exerted by modulated and continuous microwave fields on cell shape and ultrastructure in the low (0.0024 to 2.4 mW/g specific absorption rates (SAR) intensity range were investigated.

Materials and Methods

Cell culture

Mouse embryo 3T3 cells were cultured in Eagle MEM medium supplemented with glutamine (4 mM final concentration), and 10% fetal calf serum. They were maintained at 37 °C in a humidified 5% CO_2-95% air atmosphere. The cells were used in confluent monolayers.

Microwave irradiation and dosimetry

The cell cultures on the glass petri dishes were irradiated with 2450 MHz continuous (CW) and 16 Hz square modulated waves (modulation depth 75%) at field intensities 0.798, 7.98, 79.8 $\mu W/cm^2$, SAR were 0.0024, 0.0244, 0.24 and 2.4 mW/g; energy absorptions were 4.32, 43.2, 432 mJ/g and 4.32 J/g, the calculated electric field strength: 0.1168, 1.168, 11.68 and 116.8 mV/cm^2. The exposure facility is shown in Fig. 1. The pattern of the power density in TE_{10} mode has a $\sin^2$ standing wave across the long transverse axis of the wave guide (broadwall):

$$P_{av} = P_{max} \int_0^b \int_{-a/2}^{+a/2} \sin^2(\pi x/a)\, dx dy \qquad (1)$$

where P_{av} is the average power density given by the input power and the cross-section area of the waveguide, P_{max}, the maximum power density of the wave-guide area, and a and b are the size of the walls of wave guide. From the equation (1):

$$P_{max} = 2P_{av} \qquad (2)$$

The sample holder plates were placed on the central area of the broadwall of the wave guide and by measuring continuously the average input microwave power of microwaves the power density was calculated. The specific absorption rate (SAR) was measured in the culture plates by a thermocouple using the equation [2]:

$$SAR = 4.186\ c\ dT/dt \qquad (3)$$

where dT is the temperature rise during the short period of dt time interval, and c the specific heat of the irradiated sample. The dT was measured by a thermocouple microprobe (d=0.018 mm, Cole-Parmer J-8606-70 implant probe) connected to an on-line computer controlled measuring system [93]). In the dosimetry

Figure 1. Experimental setup for radiation and dosimetry. The microwave power generator was connected to a wave guide section by directional coupler (20 dB) and coax wave guide adapter. The cell culture was placed on a dielectric matching load at the center of the wave guide cross-section. The SAR was measured by a thermocouple using on-line computer evaluation (see text).

Figure 2. Electron micrograph of control 3T3 cells. Intact mitochondria (M), a few rough endoplasmic reticulum (RER), microfilaments (f) are seen. The cationic ferritin bound to plasma membrane in some clusters. The contact regions of membranes (→) labelled by ferritin particles heavily. Bars = 0.25 μm.

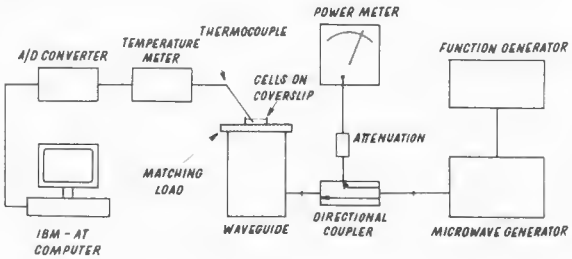
Figure. 3. Control 3T3 cells. G = Golgi complex, M = mitochondria, V = autophagic vacuole, L = lipid droplets, (→) =rough endoplasmic reticulum. Bar: A = 0.5 μm, B = 0.2 μm.

Figure. 4. Binding of cationized ferritin to continuous microwave field treated (2.4 mW/g) cell. The amount and distribution of bounded cationized ferritin did not change (*). Vacuolization of cytoplasm (V), increased number of Golgi vesicles (G), altered mitochondria (M) were evident. Bar = 0.25 μm.

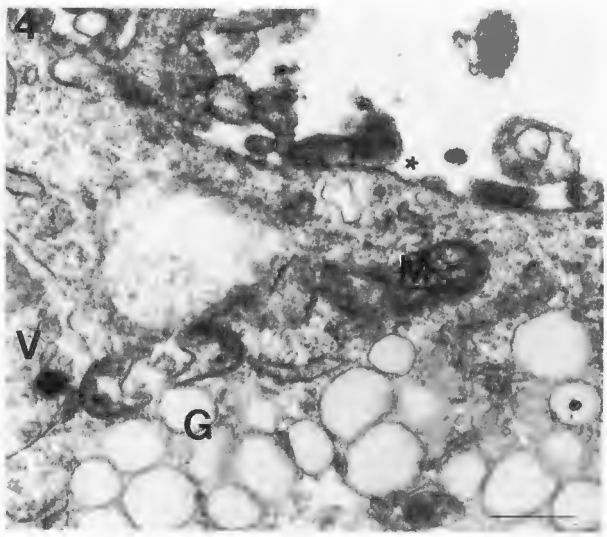
Figure 5. Binding of cationized ferritin to modulated microwave irradiated cell (0.024 mW/g). The amount of bound ferritin decreased markedly. N = nucleus, (→)= rough endoplasmic reticulum. Bar = 0.25 μm.

Figure 6. Scanning electron micrograph of control 3T3 cells. Bar = 1 μm.

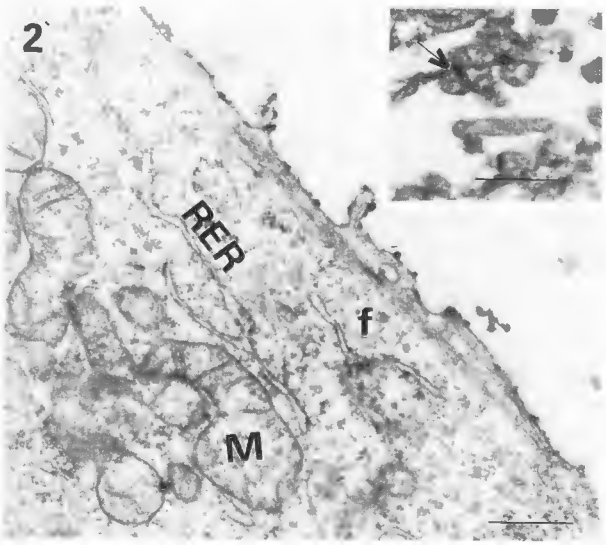
1


A/D CONVERTER
TEMPERATURE METER
THERMOCOUPLE
POWER METER
FUNCTION GENERATOR
CELLS ON COVERSLIP
ATTENUATION
MATCHING LOAD
IBM - AT COMPUTER
WAVEGUIDE
DIRECTIONAL COUPLER
MICROWAVE GENERATOR

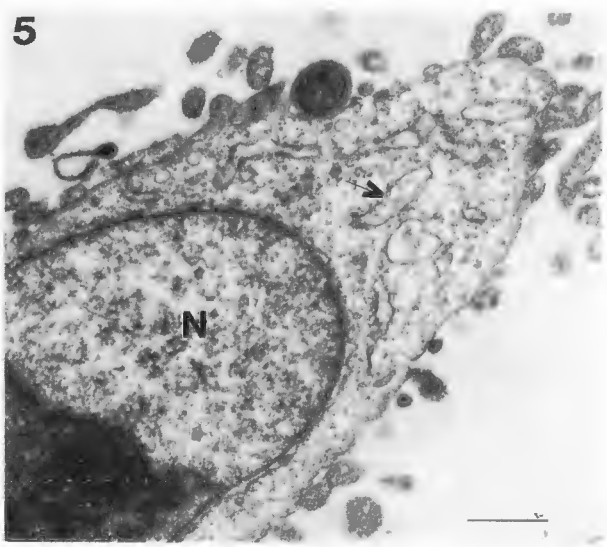
4


M
V
G

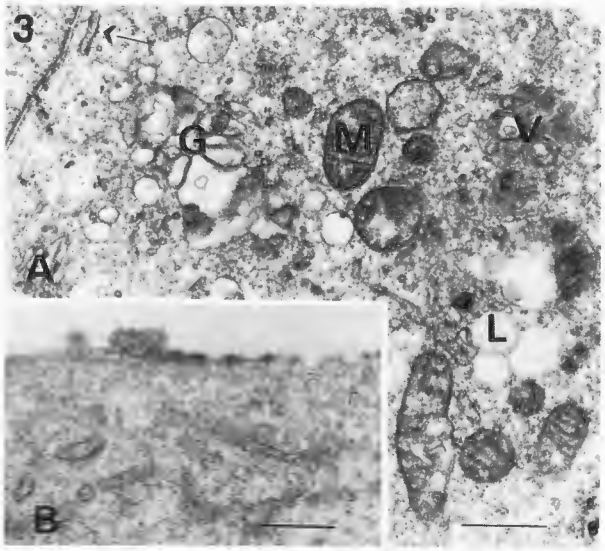
2


RER
f
M

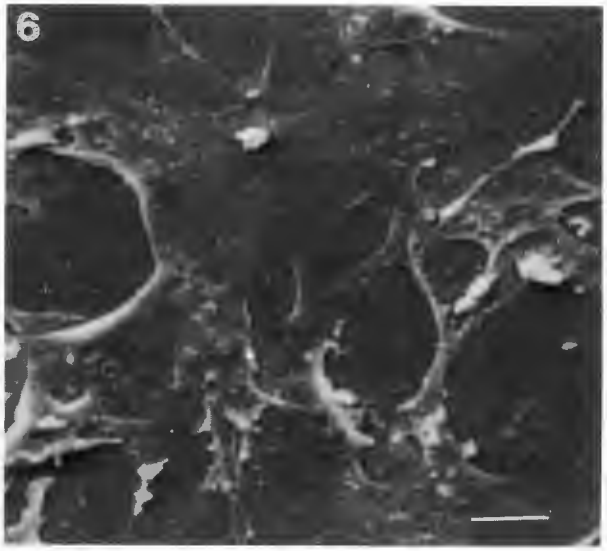
5


N

3


G
M
V
A
L
B

6



measurement the thermal level of irradiation was applied, using 10 W input power. However the irradiation during the experiment remained below the thermal power reached density and SAR level. The attenuation in the sample is exponential:

$$P_{in} = P_O\, e^{-2\alpha x} \quad (4)$$

where P_{in} is the power in the sample, P_0 the power on the surface (on the layer of cells), α the attenuation coefficient, x the depth in the sample. The measurement point of the SAR by thermocouple is 0.02 mm distance from the surface layer of the cell culture and P_{in} at this point:

$$P_{in}(0.02mm) = 0.9655\, P_O \quad (5)$$

and the measured SAR in the solution is as follows:

$$SAR_{cell} = 1.017\, SAR_m \quad (6)$$

where SAR_m is the measured absorbed power, the SAR_{cell} is the SAR at the cell layer surface and α = 0.876 Neper/m. From the equation (6), the error of the SAR is negligible because of the distance of the measurement point of the SAR from the thermocouple and the irradiated cell layer. The internal electric field strength was calculated by the equation:

$$SAR = \sigma/2pE_i^2 = \omega\, \epsilon_o\epsilon''/2pE_i^2 \quad (7)$$

where E_i is the internal electric field in the solution, σ the conductivity of the solution (σ = 2.79 mho/m [25]), ϵ'' the loss factor, $\omega = 2\pi f$, f is the frequency in hertz and p the mass density in kg/m^3.

Cytochemistry

Negatively charged sites were visualized by cationic ferritin binding (23). Prior to ferritin binding, the cells were fixed for 30 minutes in 0.1 M phosphate buffered 0.025% glutaraldehyde (pH 7.3).

Transmission and scanning electron microscopy

The cells were fixed *in situ* in a glass petri dish for 1 hour in 0.1 M phosphate buffered 2.5% glutaraldehyde (pH 7.3), at 4 °C, postfixed in 1% OsO_4, dehydrated with alcohol or acetone, and embedded in Durcupan AC (Fluka). Ultrathin sections were cut with glass or diamond knives on an LKB ultramicrotome. The sections were examined by Tesla BS-500 and JEOL 100CX transmission electron microscopes. For scanning electron microscopy, the samples were dehydrated and dried in a Sorvall critical point drying apparatus and coated with gold. Specimens were viewed and photographed using a JEOL 50A scanning electron microscope operating at an accelerating voltage of 20 kV, and a tilt angle of 45°.

Figure 7. Micromorphology of 3T3 cells after modulated microwave irradiation (0.024 mW/g). The cells have some cytoplasmic projections or exhibit spindle shaped forms. Bar = 1 μm.

Figure 8. Scanning electron micrograph of continuous microwave irradiated (0.024 mW/g) cells. The cell shape and micromorphology similar to the control. Bar = 0.5 μm.

Figure 9. 3T3 cells after higher intensity (2.4 mW/g) continuous microwave irradiation. Some attached cells however, exhibit a few ruffled cells with cytoplasmic projections. Bar = 0.5 μm.

Figure 10. Part of the cytoplasm of microwave treated cell (modulated, 0.024 mW/g). The nucleus (N) are invaginated. The cell has some autophagic vacuoles (→) and altered mitochondria. Bar = 0.3 μm.

Figure 11. Invaginated nucleus (N) after continuous microwave irradiation (2.4 mW/g). Cationized ferritin binding did not change upon microwave field (→). Bar = 0.3 μm.

Figure 12. Part of a cytoplasm of modulated microwave treated cell (0.024 mW/g). Altered mitochondria (M) and dilated endoplasmic reticulum (→). The endoplasmic reticulum cisternae contained amorphous material. Bar = 0.2 μm.

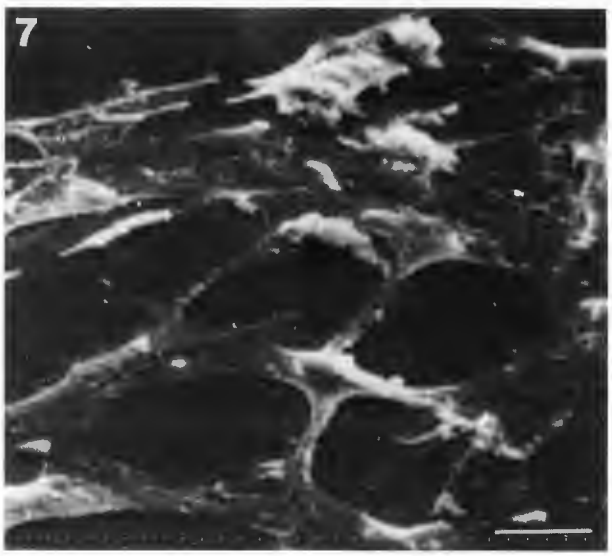
Results

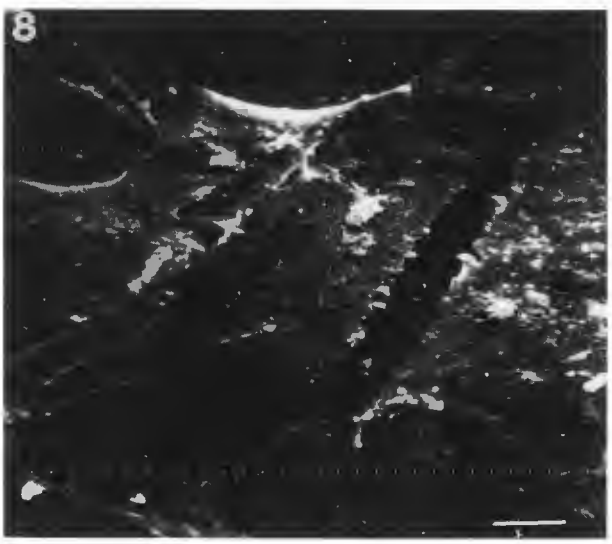
Binding of cationized ferritin

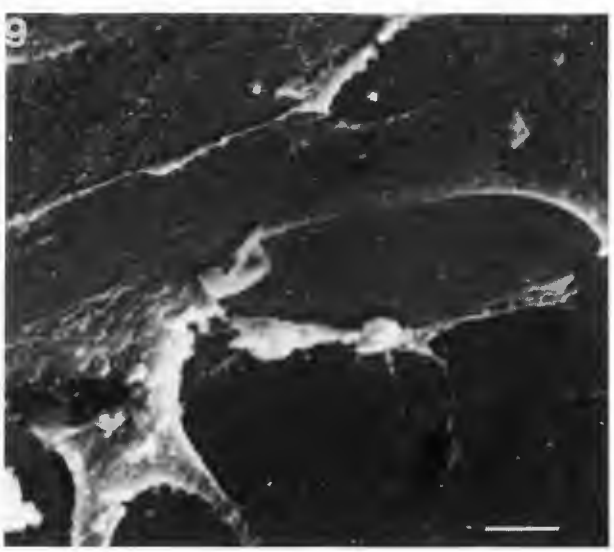
The cationized ferritin (CF) particles were mainly bound in the patches to the apical surface of the control cells (Figs. 2, 3). Binding of CF on the surface of villi and at sites of cell contact areas also was observed (Fig. 2 insert). The continuous microwave irradiation did not cause any changes in CF binding capacity of the plasma membrane at the used dose range (Fig. 4, also see Fig. 11). However, following low frequency modulated microwave irradiation (0.024 mW/g) the capacity of the cells to bind CF markedly decreased (Fig. 5)

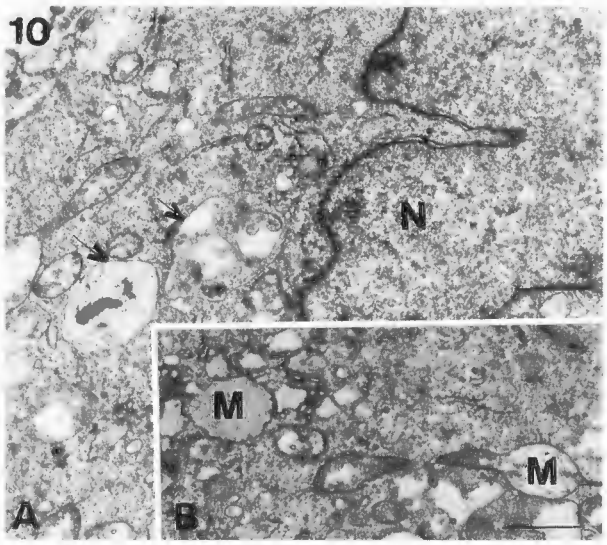
Micromorphological and fine structural alterations

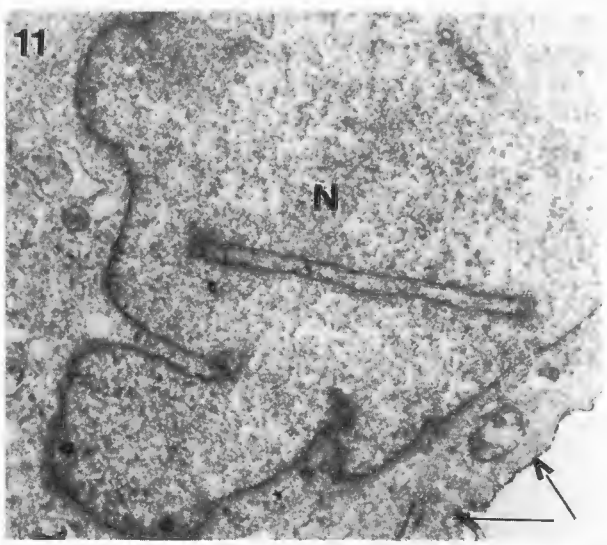
The control 3T3 cells exhibited a flat typical polygonal form (Fig. 6). Rounded or elongated cells represented only 3-5 per cent of the total cell population. The modulated microwave treatments from 0.024 mW/g increased the amount of altered cells to 50-60%. The cells showed increased ruffling activity, cell edges elevated from the substrate, and the cells exhibited long cytoplasmic extensions (Fig. 7). Continuous microwave fields did not cause any changes in cell shape and micro-

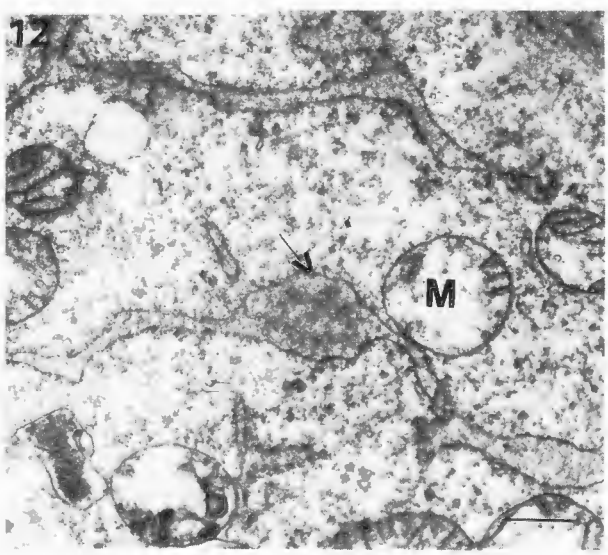

7

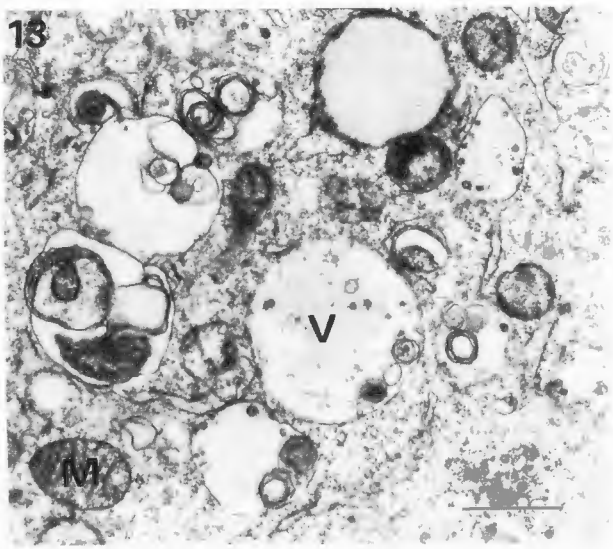

8


9


10
N
M
M
A
B


11
N


12
M



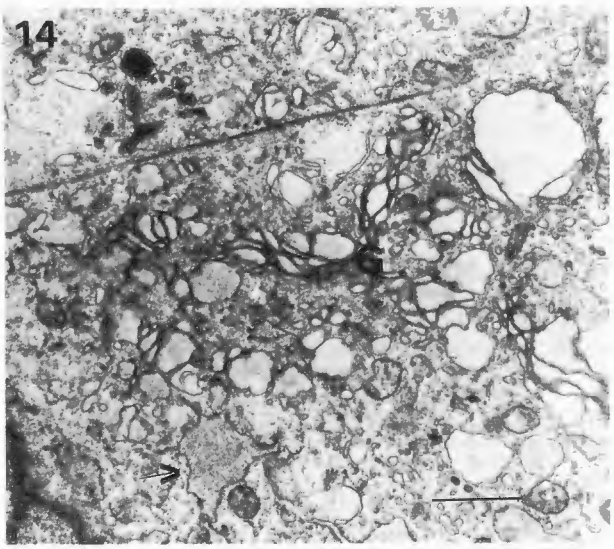


Figure 13. 3T3 cell after modulated microwave treatment (0.24 mW/g). There are some autophagic vacuoles (V) in the cytoplasm. M = mitochondria. Bar = 0.3 μm.

Figure 14. Dilated and vacuolized Golgi complex (G) after continuous microwave irradiation (2.4 mW/g) Dilated endoplasmic reticulum (→). Bar = 0.3 μm.

---

morphology when applied in 0.024 and 0.24 mW/g intensities (Fig. 8). However 2.4 mW/g intensity caused a elevated ruffling activity (Fig. 9).

In addition to cell shape alterations, the modulated microwave fields elicited changes in the ultrastructure of the cytoplasm which were already produced at 0.024 mW/g. On the other hand, marked structural changes of the cells after continuous wave exposure, were only found at 0.24 and 2.4 mW/g SAR. In spite of differences in the sensitivity of cells to the two modes of irradiation, the changes in structure of cell organelles elicited by the effective doses of either modulated or continuous wave were similar. We observed dilation of the cisternae of rough endoplasmic reticulum (Figs. 12, 14) and Golgi elements (Figs. 4, 14). The number of lysosome-like bodies and autophagic vacuoles increased (Figs. 4, 10, 12, 13). Disruption of mitochondrial internal membranes and appearance of electrolucent vacuoles containing myelinated figures and amorphous material of unidentifiable origin (Figs. 4, 10, 12) was observed. The nuclei of the cells showed deep indentations (Figs. 10, 11).

Discussion

The two main findings of our studies are that microwave irradiation may damage 3T3 cells and that these cells are more sensitive to modulated microwave fields than to continuous microwave irradiation. One of the effects of microwave irradiation was the disappearance of CF binding sites from the cell membrane. The negative surface potential plays an essential role on the membrane organization and functions (22, 33, 59, 78, 96). Physical agents including ionizing radiations (43, 82-84) heat treatment (75), ultrasonic insonications (3, 40) are all known to decrease the amount of negative charges on the cell surface. Redistribution of some membrane proteins (acetylcholine-, ricin-, Concanavaline-A- and *Phaseolus vulgaris* lectin receptors, intramembrane particles) was observed in various cells subjected to weak external electric fields (19, 64, 90, 98).

The electric pulses or modulated magnetic and electrical fields caused changes of transmembrane potential, induced effects to ion transport, reversible or irreversible poration of the membrane, electrofusion, and electrotransfection (17, 19, 30, 35, 37, 51, 53-55, 60, 61-63, 68, 73, 74, 91, 95, 105). For example, the electric pulses caused an increase of negative charges on the cell wall in yeast cells (95). Sinusoidal modulated electric and magnetic fields may exert different effects on the cell surface of *Physarum polycephalum*. The exposure to an electric field increases the negative charge on cell surface while the magnetic-field decreased the hydrophobic character of the surface, as shown by aqueous partition chromatography (54). Microwave electromagnetic radiation caused a higher electrophoretic mobility of erythrocytes 30 min after exposures at SARs greater than 10 W/kg (37). Low frequency modulated electromagnetic fields (below 100 Hz) may disturb the cell membranes with mechanisms of transductive coupling

(1, 2). Our experimental results may be explained by a structural rearrangement of the plasma membrane, which caused orientation changes of negatively charged molecules because these may be "sinking" into the membrane material. The changes in cell shape and micromorphology similar to these observed in this study, were observed in cells after ionizing radiation (81, 82, 84, 85), laser treatments (100), ultrasonic insonication (48), heat treatments (5, 101), and application of steady state or pulsed electric fields (9, 16, 31, 32, 57, 58, 64, 88, 99). It was shown recently that the long term treatments with electromagnetic fields (72) or with Helium-Neon lasers (69) can transform fibroblasts. Limited data are available on the morphological effect of microwave irradiation after high doses of continuous waves (18, 28, 34, 86, 87). The increased ruffling activity of the cells observed in our experiments may be related to the decrease of the amount of negatively charged surface sites. This assumption is based on our earlier observations (84) showing that blocking of the negatively charged surface sites results in a rapid increase of ruffling activity of human primary fibroblasts. The correlation between the low frequency microwave field induced change of membrane potential and the decrease of the cell's negative surface charge together with changes of cell shape under the influence of microwave irradiation remains to be elucidated.

As reported earlier, a short exposure of low intensity microwave at 10 mW/cm (SAR = 0.2418 W/kg) caused marked changes in fine structure of Chang liver cells (28). We found similar ultrastructural changes following irradiation by continuous waves in the same intensity range; however, the modulated microwave fields were more effective, as shown in the present paper. The mechanism by which the low intensity microwave field damages the cells is not known. According to Webber *et al.* (102), the observed fine structural alterations are not due to heat effects. Liburdy and Vanek (47) think that reactive oxygen species are involved in the mechanism of membrane damage during exposure of the cells to microwave irradiation. It is known that free hydroxy radicals can also cause membrane damage (92). Taken together our observations, and the above mentioned data of the literature strongly suggest that the cell membranes are targets of microwave irradiation.

Acknowledgements

The authors highly acknowledge the interest and support of Professors Dr. L.B. Sztanyik and Dr. G.J. Köteles.

References

1. Adey WR (1990) Nonlinear electrodynamics in cell membrane transductive coupling. In: Membrane Transport and Information Storage, Alan R. Liss. Inc. New York, pp. 1-27.

2. Adey WR (1990) Electromagnetic fields and the essence of living systems. In: Modern Radio Science 1990, Ed: Andersen JB, International Union of Radio Science and Oxford Univ. Press, Oxford, pp. 1-36.

3. Adler J, Spurná V, Hrazdira I (1988) The effect of low intensity ultrasound on mammalian cell cultures. II. Direct and indirect effects of ultrasound. Studia Biophys. **128**, 13-20.

4. Albert EN (1977) Light and electron microscopic observations on the blood-brain barrier after microwave irradiation. In: Symposium on Biological Effects and Measurement of Radio-frequency / Microwaves, Ed: Hazzard DG, US HEW (FDA) 77-8026, pp. 294-304.

5. Arancia G, Trovalusci PC, Mariutti G,Mondovi B (1989) Ultrastructural changes induced by hyperthermia in Chinese hamster V79 fibroblasts. Int. J. Hyperthermia **5**, 341-350.

6. Arber LA, Lin JC (1985) Microwave-induced changes in nerve cells: Effects of modulation and temperature. Bioelectromagnetics **6**, 257-270.

7. Baransky S (1972) Histological and histochemical effects of microwave irradiation on the central nervous system of rabbits and guinea pigs. Am. J. Phys. Med. **51**, 182-191.

8. Blackwell RP, Saunders RD (1986) The effects of low-level radio frequency and microwave radiation on brain tissue and animal behavior. Int. J. Radiat. Biol. **50**, 761-787.

9. Blangero C, Rols MP, Teissie J (1989) Cytoskeletal reorganization during electric field induced fusion of Chinese hamster ovary cells grown in monolayers. Biochem. Biophys. Acta **981**, 295-302.

10. Bagolyubov VM, Zubkova SM, Frenkel ID, Sokolova ZA, Laprum IB (1988) The functional state of thymus cells following microwave exposure of endocrine glands. Rad. Res. **115**, 44-53.

11. Budd RA, Czerski P (1985) Modulation of mammalian immunity by electromagnetic radiation. J. Microw. Power Electromagn. Energy **20**, 217-231.

12. Byus CV, Lundak RM, Adey WR (1984) Alteration in protein kinase activity following exposure of cultured lymphocytes to modulated microwave fields. Bioelectromagnetics **5**, 34-39.

13. Byus CV, Pieper SE, Adey WR (1987) The effects of low-energy 60-Hz environmental electromagnetic fields upon the growth-related enzyme ornithine decarboxylase. Carcinogenesis **8**, 1385-1396.

14. Byus CV, Kartun K, Pieper S, Adey WR (1988)

Increased ornitine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. Cancer Res. **48**, 422-433.

15. Cain CD, Adey WR Luben RA (1987) Evidence that pulsed electromagnetic fields inhibit coupling of adenylate cyclase by parathyroid hormone in bone cells. J. Bone Mineral Res. **2**, 437-442.

16. Chang DC, Reese TS (1990) Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. Biophys. J. **56**, 1-12.

17. Chang DC (1989) Cell poration and cell fusion using an oscillating electric field. Biophys. J. **56**, 641-652.

18. Chen K-C, Lin CJ (1978) A system for studying effects of microwaves on cells in culture. J. Microwave Power **3**, 251-256.

19. Chiabrera A, Grattarola M, Viviani R (1984) Interaction between electromagnetic fields and cells: Microelectrophoretic effects on ligands and surface receptors. Bioelectromagnetics **5**, 173-191.

20. Chou CK, Guy WA (1975) The effects of electromagnetic fields on the nervous system. Scientific Report No.6. Bioelectromagnetics Research Laboratory, Univ. Washington, Seattle, pp. 67-73.

21. Cleary SF, Liu L-M, Merchant RE (1990) *In vitro* lymphocyte proliferation induced by radio-frequency electromagnetic radiation under isothermal conditions. Bioelectromagnetics **11**, 47-56.

22. Clementz T, Christiansson A, Wieslander A (1988) Effects of electrical potential on membrane organization and function. In: "Physiological Regulation of Membrane Fluidity", Alan R. Liss Inc. (New York) pp. 41-71.

23. Danon D, Goldstein L, Marikovsky Y, Skutelsky E (1972) Use of cationic ferritin as label of negative charges on cell surface. J.Ultrastruct. Res. **38**, 500-510.

24. D'Inzeo P, Bernardi F, Eusebi F, Grass F, Tamburello C, Zani BM (1989) Microwave effects on acetylcholine-induced channels in cultured chick myotubes. Bioelectromagnetics **9**, 363-372.

25. Durney CH, Iskander MF, Massoudi H, Allen SJ, Mitchell JC (1980) Radio frequency Radiation Dosimetry Handbook, USAF School of Aerospace Medicine, San Antonio, Texas, Report SAM-TR-80-32, pp. 15-31.

26. Dutta SK, Subramoniam A, Ghosh B, Parshad R (1984) Microwave radiation-induced calcium efflux from brain tissue, *in vitro*. Bioelectromagnetics **5**, 71-78.

27. Dutta SK, Ghosh, Blacman (1989) Radio frequency radiation-induced calcium ion efflux enhancement from human and other neuroblastoma cells in culture. Bioelectromagnetics **10**, 197-202.

28. Dwivedi RS, Dwivedi V, Chiang B (1989) Low intensity microwave radiation effects on the ultrastructure of Chang liver cells. Exp. Cell Res. **180**, 253-265.

29. Fletcher WH, Shin WW, Ishida TA, Haviland DL, Ware CF (1987) Resistance to the cytolytic action of lymphotoxin and tumor necrosis factor coincides with the presence of gap junctions uniting target cells. J. Immunol. **139**, 956-963.

30. Fuhr G, Muller T, Hagedorn R (1989) Reversible and irreversible rotating field-induced membrane modifications. Biochim. Biophys. Acta **980**, 1-8.

31. Grasso RJ, Heller R, Cooley JC, Haller EM (1989) Electrofusion of individual animal cells directly to intact corneal epithelial tissue. Biochim. Biophys. Acta **980**, 9-14.

32. Hamada SH, Witkus R, Griffith R (1989) Cell surface changes during electromagnetic field exposure. Exp. Cell Biol. **57**, 1-10.

33. Honig BH, Hubbell WL, Flewelling RF (1986) Electrostatic interactions in membranes and proteins. Ann. Rev. Biophys. Biophys. Chem. **15**, 163-193.

34. Huang AT, Engle ME, Elder JA, Kinn JB, Ward TR (1977) The effect of microwave radiation (2450 MHz) on the morphology and chromosomes of lymphocytes. Radio Sci. **12**, 173-177.

35. Huang J-C, Nicolson C, Okada YC (1990) Distortion of magnetic evoked fields and surface potentials by conductivity differences at boundaries in brain tissue. Biophys. J. **57**, 1155-1166.

36. Illinger K (Ed.) (1981) Biological effects of non-ionizing radiation. Washington: American Chemical Society. pp. 37-56.

37. Ismailov ES (1978) Effects of ultrahigh frequency electromagnetic radiation on the electrophoretic mobility of erythrocytes. Biophysics **21**, 961-963.

38. Joines WT (1976) Reception of microwaves by the brain. Med. Res. Engineer **12**, 8-11.

39. Joines WT (1987) Biological effects of non-ionizing radiations: Cellular properties and interactions. In: "The L. S. Taylor Lecture Series, 10, by NCRP", National Council on Radiation Protection and Measurements, Bethesda, Maryland, pp. 9-42.

40. Joshi GP, Hill CR, Forrester JA (1983) Mode of action of ultrasound on the surface charge of mammalian cells *in vitro*. Ultrasound Med. Biol. **1**, 45-48.

41. Karshikov AD, Engh R, Bode W, Atanasov BP (1989) Electrostatic interactions in proteins: Calculations of the electrostatic term of free energy and the electrostatic potential field. Eur. Biophys. J. **17**, 287-297.

42. Kása P, Bánsághy K, Gulya K (1982) Ultrastructural changes and diffusion of acetylcholine in rat brain after microwave irradiation. J. Neurosci. Meth. **5**, 215-220.

43. Köteles GJ, Somosy Z, Kubasova T (1987) Radiation-induced changes on cell surface charges. Radiat. Phys. Chem. **30**, 389-399.

44. Lai H, Horita A, Guy AW (1988) Acute low-level microwave exposure and central cholinergic activity: Studies on irradiation parameters. Bioelectromagnetics **9**, 355-362.

45. Liburdy RP (1979) Radio frequency radiation alters the immune system: Modulation of T- and B-lymphocyte levels and cell-mediated immunocompetence by hyperthermic radiation. Rad. Res. **77**, 34-46.

46. Liburdy RP, Penn A (1984) Microwave bioeffects in the erythrocyte are temperature and pO dependent: Cation permeability and protein shedding occur at the membrane phase transition. Bioelectromagnetics **5**, 283-291.

47. Liburdy RP, Vanek PF (1985) Microwaves and the cell membrane. II. Temperature, plasma, and oxygen mediate microwave induced membrane permeability in the erythrocyte. Rad. Res. **102**, 190-205.

48. Liebeskind D, Padawer J, Wolley R, Bases R (1982) Diagnostic ultrasound: Time-lapse and transmission electron microscopic studies of cell insonated *in vitro*. Br. J. Cancer **45**, Suppl. V, 176-186.

49. Lin-Liu S, Adey WR (1982) Low frequency amplitude modulated microwave fields change calcium efflux rates from synaptosome. Bioelecromagnetics **3**, 309-322.

50. Lin-Liu S, Adey WR, Poo M-M (1984) Migration of cell surface concanavalin A receptors in pulsed electric fields. Biophys. J. **45**, 1211-1218.

51. Lojewska Z, Farkas DL, Ehrenburg B, Loew LM (1989) Analysis of the effect of medium and membrane conductance on the amplitude and kinetics of membrane potentials induced by externally applied electric fields. Biophs. J. **56**, 121-128.

52. Luben RA, Cain CD, Chen MC-Y, Rosen DM, Adey WR (1982) Effects of electromagnetic stimuli on bone and bone cells *in vitro*: Inhibition of response to parathyroid hormone by low-energy low-frequency fields. Proc. Natl. Acad. Sci. USA **79**, 4180-4184.

53. Markov MS, Todorov NG (1984) Electromagnetic field stimulation of some physiological processes. Studia Biophys. **99**, 151-156.

54. Marron M, Goodman E, Sharpe P, Greenebaum B (1988) Low frequency electric and magnetic fields have different effects on the surface. FEBS Lett. **230**, 13-16.

55. Maurel P, Gualandris-Parisot L, Teissie J, Duprat A-M (1989) Electric field-induced permeabilization and fusion of embryonic amphibian cells. Exp. Cell. Res. **184**, 207-218.

56. Mayers CP, Habeshaw JA (1973) Depression of phagocytosis: a nonthermal effect of microwave radiation as a potential hazard to health. Int. J. Radiat. Biol. **24**, 449-461.

57. McCaig CD (1990) Nerve branching is induced and oriented by a small applied electric field. J. Cell Sci. **95**, 605-615.

58. McCaig CD (1990) Nerve growth in a small applied electric field and the effect of pharmacological agents and rate and orientation. J. Cell Sci. **95**, 617-622.

59. McLaughliin S (1989) The electrostatic properties of membranes. Ann. Rev. Biophys. Biophys. Chem. **18**, 113-136.

60. McLeod BR, Liboff AR (1986) Dynamic characteristics of membrane: ions in multifield configurations of low-frequency electromagnetic radiation. Bioelectromagnetics **7**, 177-189.

61. McCloskey MA, Liu Z-Y, Poo M-M (1984) Lateral electromigration and diffusion of Fc receptors on rat basophilic leukemia cells: Effect of IgE binding. J. Cell Biol. **99**, 778-787.

62. Mishima K, Morimoto T (1989) Electric field-induced orientation of myelin figures of phosphatidylcholine. Biochim. Biophys. Acta **985**, 351-354.

63. Neumann E (1989) Electric field-induced structural rearrangements in biomembranes. Studia Biophys. **130**,139-143.

64. Nuccitelli R (1983) Transcellular ion currents: Signals and effectors of cell polarity. In: "Modern Cell Biology 2", Alan R. Liss, Inc. New York, pp. 451-481.

65. Oldendorf WH (1949) Focal neurological lesions produced by microwave irradiation. Proc. Soc. Exp. Biol. Med. **72**, 432-443.

66. Paulsson L-E, Hammerius Y, McLean WG (1977) The effects of microwave radiation on microtubules and axonal transport. Rad. Res. **70**, 212-223.

67. Philippova TM, Novoselov VI, Bystrova MF, Alekseev SI (1988) Microwave effects on Camphor binding to rat olfactory epithelium. Bioelectromagnetics **9**, 347-355.

68. Polk C (1990) Electric fields and surface charges induced by ELF magnetic fields. Bioelectromagnetics **11**, 189-201.

69. Pourreau-Schneider N, Ahmed A, Soudry M, Jacquemier J, Kopp F, Franquin JC, Martin PM (1990) Helium-Neon laser treatment transforms fibroblasts into myofibroblasts. Am. J. Pathol. **137**, 171-178.

70. Roberts NJ (1983) Radio frequency and microwave effects on immunological and hematopoietic systems. In: "Biological Effects and Dosimetry of Non-ionizing Radiation", Ed: Grandolfo M, Plenum Press New York, pp. 442-460.

71. Roberts NJ, Michaelson SM, Lu S-T (1986) The biological effects of radio frequency radiation: a critical review and recommendations. Int. J. Radiat. Biol. **50**, 379-420.

72. Rodemann HP, Bayreuther K, Pfleiderer G (1989) The differentiation of normal and transformed human fibroblasts *in vitro* is influenced by electromagnetic fields. Exp.Cell Res. **182**, 610-621.

73. Rotkovska D, Vacek A (1982) An increase in colony forming capacity of the bone marrow stem cells after microwave radiation *in vitro*. Folia Biol. **28**, 138-141.

74. Sakurai I, Kawamura Y (1989) Effect of a magnetic field on charge flow along the interface between phosphotidylcholine monolayer and water. Biochim. Biophys. Acta **985**, 347-350.

75. Sato C, Nakayama T, Kojima K, Nishimoto Y, Nakamura W (1981) Effects of hyperthermia on cell surface charge and cell survival in mastocytoma cells. Cancer Res. **41**, 4107-4110.

76. Schlagel CJ, Sulek K, Ho HS, Leach WM, Ahmed A, Woody JN (1980) Biological effects of microwave exposure II. Studies on the mechanisms controlling susceptibility to microwave-induced increases in complement receptor-positive spleen cells. Bioelectromagnetics **1**, 405-415.

77. Sharp KA, Honig B (1990) Electrostatic interactions in macromolecules: Theory and applications. Ann. Rev. Biophys. Biophys. Chem. **19**, 301-332.

78. Seelig J, Macdonald PM, Scherer Y (1987) Phospholipid head groups as sensor of electric charge in membranes. Biochemistry **26**, 7535-7541.

79. Smialowicz R (1984) Hematologic and immunologic effects. In: Biological Effects of Radio frequency Radiation", Eds: Elder JA, Cahill DF, Report EPA-600/8-83-026F Springfield, VA: National Technical Information Service, pp.513-528.

80. Smialowicz RF (1979) Hematologic and immunologic effects of nonionizing electromagnetic radiation. Bull. N. Y. Acad. Med. **55**, 1094-1118.

81. Somosy Z, Kubasova T, Köteles GJ (1983) Scanning electron microscopic appearance of X-irradiated human fibroblasts. J. Radiat. Res. **24**, 109-117.

82. Somosy Z, Csuka O, Kubasova T, Kovács J, Köteles GJ (1989) Surface heterogeneity of tumor cells and changes upon ionizing radiation. Scanning Microsc. **3**, 895-906.

83. Somosy Z, Kubasova T, Ecsedi GS, Köteles GJ (1986) Radiation-induced changes of negative charge on the cell surface of primary human fibroblasts. Int. J. Radiat. Biol. **49**, 968-978.

84. Somosy Z, Kubasova T, Köteles GJ (1987) The effects of low doses of ionizing radiation upon the micro-morphology and functional state of cell surface. Scanning Microsc. **1**, 1267-1278.

85. Somosy Z, Kubasova T, Kovács J, Köteles GJ (1991) Effects of low energy beta-irradiation from tritiated water on the morphology of 3T3 fibroblasts. Scanning Microsc. **5**, 127-134.

86. Stensas LJ, Partlow LM, Bush LG, Iverson PL, Hill DW, Hagmann MJ, Gandhi OP (1981) Effects of millimeter wave radiation on monolayer cell cultures II. Scanning and transmission electron microscopy. Bioelectromagnetics **3**, 141-150.

87. Stodolnick-Branska W (1967) Lymphoblastoid transformation of lymphocytes *in vitro* after microwave irradiation. Nature **214**, 102-103.

88. Stump RF, Robinson KR (1983) Xenopus neural crest cell migration in an applied electrical field. J. Cell Biol. **97**, 1226-1233.

89. Sulek K, Schlageel CJ, Ho HS, Leach WM, Ahmed A, Woody JN, Wiktor-Jedrzejczak W (1980) Biological effects of microwave exposure I. Therehold energy requirements for the induction of an increase in complement receptor positive (CR^+) mouse spleen cells following exposure to 2450 Hz microwaves. Rad. Res. **83**, 127-137.

90. Tank DW, Fredericks WJ, Barak LS, Webb WW (1985) Electric field-induced redistribution and postfixed relaxation of low density lipoprotein receptors on cultured human fibroblasts. J. Cell Biol. **101**, 148-157.

91. Tekle E, Astumian RD, Chock PB (1990) Electro-permeabilization of cell membranes: Effect of the resting membrane potential. Biochem. Biophys. Res. Comm. **172**, 282-287.

92. Thaw H, Hamberg H, Brunk VT (1983) Acute and irreversible injury following exposure of cultured cells to reactive oxygen metabolites. Eur.J.Cell Biol. **31**, 46-54.

93. Thuróczy G, Szabó LD, Ballay L, Almássy G, Kenderessy M, Szász E, Bakos S (1986) Biological effects of electromagnetic fields I. An irradiation and measuring system for the study of biological effects of microwaves. Proc. URSI Int. Symp. Electromagnetic Theory, Barcel T (ed.), Akad. Kindo, Budapest, Hungary, pp. 258-259.

94. Tolgskaya MS (1959) Morphological changes in animals exposed to lo cm microwaves. Vopr. Kurortol. Fizioter. Lech. Fiz. Kult. **1**, 21-33.

95. Tomov TC, Tsoneva IC (1989) Changes in the surface charge of cells induced by electrical pulses. Bioelectrochem Bioenerg. **22**, 127-133.

96. Tsong TY (1990) Electrical modulation of membrane proteins: Enforced conformational oscillations and biological energy and signal transductions. Annu. Rev. Biophys. Biophys. Chem. **19**, 127-133.

97. Tyazhelov VV, Alekseev SI (1983) On primary mechanisms of microwave biological effects. In: Problems of Experimental and Practical Electromagnetic-biology, pp.61-81 (copy available from Z. Somosy).

98. Yang W-P, Onuma EK, Hui S-W (1984) Response of C3H/10T1/2 fibroblasts to an external steady electric field stimulation. Reorientation, shape change, ConA receptor and intramembranous particle distribution and cytoskeleton reorganization. Exp.Cell Res. **155**, 92-104.

99. Yaoita M, Aizawa M, Ikariyama Y (1989) Electrically regulated cellular morphological and cytoskeletal changes an optically transparent electrode. Exp. Cell Biol. **57**, 43-51.

100. Yew DT, Zheng DR, Au C, Li WWY (1990) Responses of astrocytes in culture after low dose laser-irradiation. Scanning Microsc. **4**, 151-159.

101. Wachsberger PR, Coss RA (l990) Effects of hyperthermia on the cytoskeleton and cell survival in G and S phase Chinese hamster ovary cells. Int. J. Hyperthermia **6**, 67-85.

102. Webber MM, Barnes FS, Seltzer LA, Bouldin TR, Prasad KN (1980) Short microwave pulses cause ultrastructural membrane damage in neuroblastoma cells. J. Ultrastruct. Res. **71**, 321-330.

103. Wiktor-Jedrjejczak W, Ahmed A, Czerski P (1980) Effect of microwaves (2450 MHz) on the immune system in mice: Studies of nucleic acid and protein synthesis. Bioelectromagnetics **1**, 161-170.

104. Williams R, McKee A, Finch ED (1975) Ultrastructural changes in the rabbit lens induced by microwave radiation. Ann. N. Y. Acad. Sci. **247**, 166-174.

105. Zimmermann U (1986) Electrical breakdown, electroporation, and electrofusion. Rev. Physiol. Biochem. Pharm. **105**, 175-256.

Discussion with Reviewers

J.R. Trevithick: Have any acoustic measurements been performed to show whether thermoelastic transduction produces pressure waves in the sample in the modulated mode?

Authors: The acoustic measurement have not been performed. The 16 Hz square wave modulation with 50 % filling coefficient and low level intensity was not able to cause thermoelastic pressure wave propagation in the sample. This pressure wave acoustic propagation exists in the short microwaves pulses (i.e., radar pulse with the μs range) with high power of pulses [Lin JC, Su J-L, Wang Y (1988) Microwave-induced thermoelastic pressure wave propagation in the cat brain. Bioelectromagnetics **9**, 141-147].

J.R. Trevithick: Do the changes observed seem similar to those found when cells are exposed to ultrasound or sound waves at similar repetition rate?

Authors: Yes, preliminary experiments have been performed with similar changes of cell surfaces negative charges at low frequency pulsed ultrasound.

H.P. Rodemann: Is there any information available from the experiments done so far whether 3T3 fibroblast cultured on collagenized glass petri dishes react differently, e.g., in ultrastructural changes etc., in response to microwave irradiation than cells seeded on pure glass petri dishes?

Authors: It is known that the various extracellular matrix components (i.e., collagen, fibronectin, proteoglycans) can modulate some functions of fibroblasts and at the same time can cause altered responsiveness of cells for some environmental factors. We did not investigate these problems upon microwave irradiation.

H.P. Rodemann: Does the clonogenic activity of the cells irradiated with 16 Hz square modulated waves decrease as compared to controls?

Authors: We have not investigated the clonogenic activity of the cells irradiated with 16 Hz square modulated waves.