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EFFECTS OF MODULATED AND CONTINUOUS MICROWAVE IRRADIATION ON PYROANTIMONATE PRECIPITABLE CALCIUM CONTENT IN JUNCTIONAL COMPLEX OF MOUSE SMALL INTESTINE

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Abstract

The pyroantimonate precipitable calcium content of intestinal epithelial cells was investigated in mice following total body irradiation with 2450 MHz continuous and low frequency (16 Hz) square modulated waves. In the control animals the reaction products appeared in the intercellular space of adjacent cells including intermediate junctions and desmosomes and were absent in the area of tight junctions. Immediately after low frequency modulated microwave irradiation at 0.5 and 1mW/cm^2 power densities, a rapid distribution of pyroantimonate precipitable calcium content was observed. The pyroantimonate deposits were located on the cytoplasmic side of lateral membrane, in the area of junctional complex, including tight junction, and in other parts of lateral plasma membrane. These changes were reversible and 24 hours after the irradiation the distribution of pyroantimonate deposits was similar to the control. Continuous waves with same energy not altered the distribution of precipitable calcium. We conclude the low frequency modulated microwave irradiation can modify the calcium distribution without heat effects.

Key Words: Small intestine, modulated and continuous microwave fields, tight junction, pyroantimonate precipitable calcium, irradiation, electron microscopy.

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Introduction

The biological effects of electric and magnetic fields as potentially hazardous environmental factors have been investigated extensively (Williams et al., 1984; Roberts et al., 1986; Adey 1990 a, b; Blackman, 1990; Tenforde, 1991; Somosy et al., 1991). The mechanisms leading to subcellular effects of electromagnetic radiation depend on the dose and mode of irradiation (Adey, 1990 a, b; Tenforde, 1991). The high intensity waves (>1mW/cm^2) exert thermal effects (Roberts et al., 1986), however, weak, extremely-low-frequency (ELF) (below 100 Hz) electric and magnetic fields can interact with living material via non thermal interactions (Adey, 1990 a, b; Tenforde, 1991; McLeod et al., 1992).

The plasma membrane is a sensitive target of ELF radiation (Blackman et al., 1979; Williams et al., 1984; Roberts et al., 1986, Adey 1990 a, b; Somosy et al., 1991; Tenforde 1991). The ELF microwave and electromagnetic field irradiation modulate the amount of charged sites on the cell surfaces (Luben et al., 1988; Smith et al., 1991; Somosy et al., 1991), and the distribution of cell surface receptors and expression of cell surface markers (Wiktor-Jedrzejczak et al., 1977). They can modify the binding and/or response of biological active substances as mitogenic compounds (Roberts et al., 1987; Cossarizza et al., 1989, Walleczek, 1992), hormones (Luben et al., 1982; Adey, 1990a, b), alter the membrane hydrophobicity (Smith et al., 1991), and cause conformational rearrangement of membrane macromolecules (Bogolyubov et al., 1988). All these effects lead to the alteration of membrane-related cell functions, including the increase of the blood-brain barrier permeability (Williams et al., 1984), modify the potassium permeability (Liburdy and Penn, 1984; McLeod et al., 1992), change the transport of insulin, and other hormones (Roberts et al., 1986; Tenforde, 1991), and alteration some membrane bound enzyme activities (reviewed by Adey 1990a, b). Since calcium plays an essential role in a variety of cell activities, it is reasonable to suppose that the effects of ELF irradiation are somehow connected to disturbances in intracellular calcium homeostasis. Reports published on this subject support this notion. An increase of calcium efflux upon modulated microwave irradiation was observed in nervous tissues (Blackman et al., 1979; Lin-Liu and Adey 1982; Dutta et al., 1984; Blackwell and Saunders 1986; Roberts et al., 1986) and an elevated calcium uptake

Table I. The applied averaged SAR and SA of the experiments as calculated for 200 μW/cm^2, 500 μW/cm^2 and 1000 μW/cm^2 power density respectively.

Power density (μW/cm^2)	SAR* (mW/g)	SA** (J/g)
200	0.33 ± 0.05	1.78 ± 0.27
500	0.82 ± 0.12	4.43 ± 0.65
1000	1.64 ± 0.25	8.86 ± 1.35

(* Calculated from the SAR measurement at 11 mW/cm^2)
(** Calculated from SAR multiplied with exposure time)

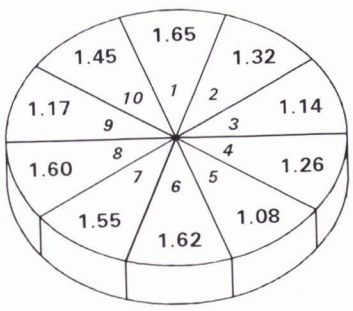


Figure 1. The SARs of the phantoms in different separated area of plastic cage as normalized to 1 mW/cm^2 power density.

induced by modulated electromagnetic fields was in the mucosa of the chick small intestine and lymphocytes (Walleczek and Liburdy, 1990, Klavinsh et al.,1991; Lyle et al., 1991, Liburdy, 1992). However, data on the redistribution of calcium inside the cells and in the areas of their contacts following either continuous or modulated microwave irradiation are not available in the literature. This prompted us to study the calcium content of low intensity microwave irradiated intestinal epithelial cells by electronmicroscopic histochemical methods.

Materials and Methods

Animals

Male CFLP mice were maintained under in standard laboratory conditions for our experiments. Experimental groups consisting of 3 animals each were irradiated as indicated below. The experiments were carried out in three parallels.

Microwave exposure and dosimetry

The mice were total body microwave [continuous (CW) and a amplitude modulated mode (AM)] irradiated for 3 hours with either 0.1, 0.5 and 1 mW/cm^2 energies, and were killed by cervical dislocation immediately, or 30 minutes, 1, 3 and 24 hours after irradiation. The irradiation were performed in anechoic room (2.55m x 1.8m x 2.9m) with a standard horn antenna (G=14 dB). The microwave generator (TKI model TR) was used in CW or external modulation mode. A function generator (OMKER model) was coupled to the microwave source. The carrier frequency was 2.45 GHz. The 16 Hz rectangular waveform (on/off ratio 50-50 %) amplitude modulated and CW microwave signal were amplified by a traveling-wave tube amplifier (TWT, Hyghes model). The incident power toward the horn antenna was measured by directional coupler (NARDA model) and microwave power meter continuously (Fig.1). The spatial pattern of power density and the field uniformity at the animal's location was determined with NARDA Model 8616 broadband isotropic radiation monitor with 8623D field probe. The field was mapped at 5 cm intervals across 45x45 cm plane. The power density of time averaged microwave exposure at the location in the center of plastic cage was 200, 500 and 1000 μW/cm^2 respectively. The alteration of power density pattern inside the exposure area (23 cm diameter) is below ± 5% (Thuróczy et al., 1986).

The irradiation was carried out in a circular plastic container (23 cm diameter, 5 cm high) which was divided into 10 separated cages. The control animals were kept for 3 hours in similar boxes without irradiation. The animals in each group were placed head to head in the opposite cages of the container in a way that the long axis of their bodies was parallel to the electric field vector of the incident planewave, in the same position number (Fig.1).

In order to quantify the Specific Absorption Rate (SAR) in the exposed objects a phantom model (e'= 48, e"= 17, c~ 1 cal/ Cg) was made according to Andreucetti et al. (1988) and Surowiec et al. (1992). In the mouse-shaped tissue equivalent polyacrilamide phantom (25.5 g +/- 4.5 g) the SAR was measured by triple-junction thermocouple (diameter 0.011 mm, constantine/manganin) (Hand and Johnson, 1984). The SAR measurements were made at 11 mW/cm^2 with 30 sec exposure duration. The multiprobe (3 junctions) thermocouple was inserted into the phantom tissue at 1.2 +/- 0.2 cm depth. The average SAR and specific absorption (SA) of phantom models in the plastic cage at the used power densities is shown in the Table I.

Electron microscopy, cytochemistry

From every single animal, two pieces of small intestine from the duodenal region (about 0.5 cm) were cut. One of them was fixed by 0.1 M phosphate buffered 5% glutaraldehyde (pH 7.3) at 4 C, and postfixed in 1% OsO_4. Another sample, was immersed in ice cold 2% glutaraldehyde (Merck) buffered with potassium acetate (Reanal) containing 0.05M potassium pyroantimonate (Merck) for 1-2 hours, rinsed in same buffer and postfixed in 1% osmium tetroxide containing 0.05 M potassium pyroantimonate for 1 hour (Eisenman et al. 1979). After fixations the pieces of tissues (five from one sample) were dehydrated through a graded

ethanol series to propylene oxide and embedded in Durcupan AC (Fluka). The samples were cut with diamond knives on an LKB ultratome and the sections were examined in a JEOL 100CX transmission electron microscope.

Results

In the control animals the reaction products of the potassium pyroantimonate treatment appeared as dense deposits on the microvilli and in the intercellular space between adjacent cells as well as in the area of desmosomes and intermediate junctions (Figs. 2, 3). They were, however absent in the tight junctions (Fig. 3). Limited number of dense deposits were also seen in the cytoplasm and on the mitochondria. The goblet cells were heavily labeled by pyroantimonate precipitates (Fig. 2 insert).

Continuous microwave fields in the investigated energy range (Fig. 4) and modulated microwave irradiation with 0.1 mW/cm^2 (not shown) did not alter the morphology of junctional complex or distribution of calcium containing pyroantimonate deposits on small intestine. However of modulated microwave irradiation at higher energies (0.5 and 1 mW/cm^2) caused marked changes in the distribution of pyroantimonate precipitates (Figs. 5, 6). Which were localized on the cytoplasmic leaflet of plasma membranes, both in the junctional area, including tight junctions, and lateral plasma membranes. One day after modulated microwave irradiation, at either 0.5 and 1 mW/cm^2 power densities the distribution of pyroantimonate deposits became similar to that of the control (Fig. 7).

Discussion

The pyroantimonate method provides information about state of calcium in tissues and cells in pH range 7.2-7.8 as shown some microanalytical data (Simson and Spicer, 1975; Mentré and Escaig; 1988, Mentré and Halperen, 1989; Eisenman et al., 1979; Bonhomme et al, 1993). The presence of calcium in the pyroantimonate deposits on small intestine was shown in our earlier electron spectroscopy and electron energy loss spectrometry results (Somosy et al., 1993).

Several reports have shown that calcium pyroantimonate precipitates are localized in the lateral membrane regions of a variety of epithelial cells, i.e. in hard tissue forming cells (Kogaya and Furushi, 1988), liver (Mentré and Halperen,1989), intestinal membranes (Satir and Gilula, 1970; Oschman and Wall, 1972). The calcium probably binds to structural proteins of intermediate junctions and desmosomes (Garrod 1986; Geiger and Ginsberg, 1991), and to other less well characterized sites on the cell surface e.g. to cell surface's polycationic molecules (Adey et al., 1969, Matsukubo et al., 1981). According to theoretical models the interaction between ELF field and electrical double layer of cell surface may cause currents in pericellular fluids and may modify intrinsic conformational equilibrium of membrane domains (enzymes) (Adey 1990 a, b; Tenforde, 1991). These

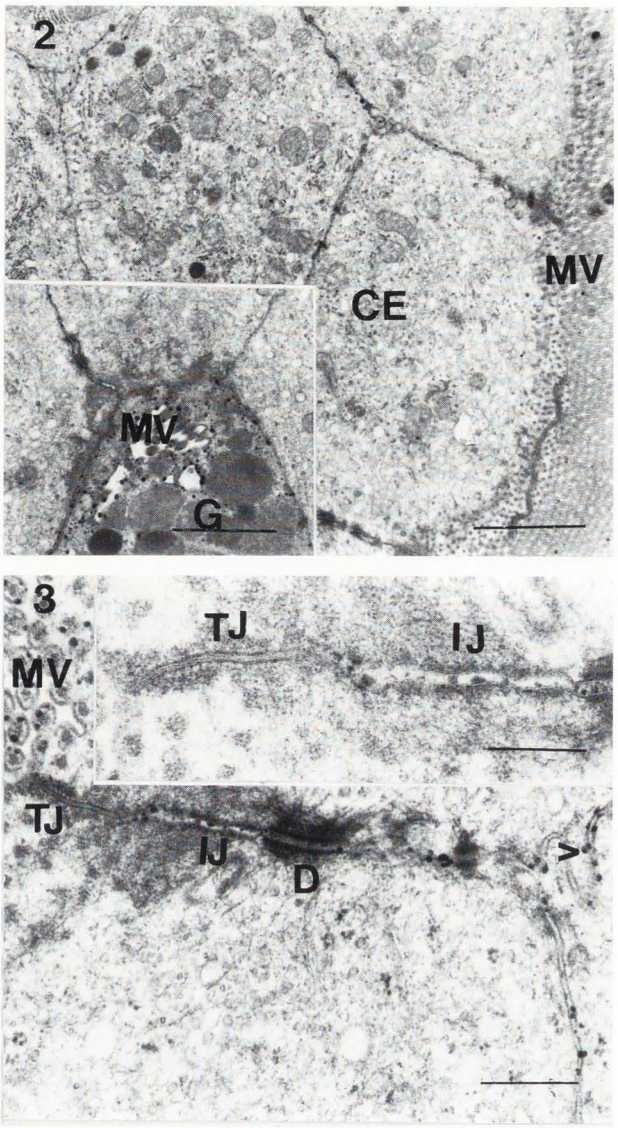
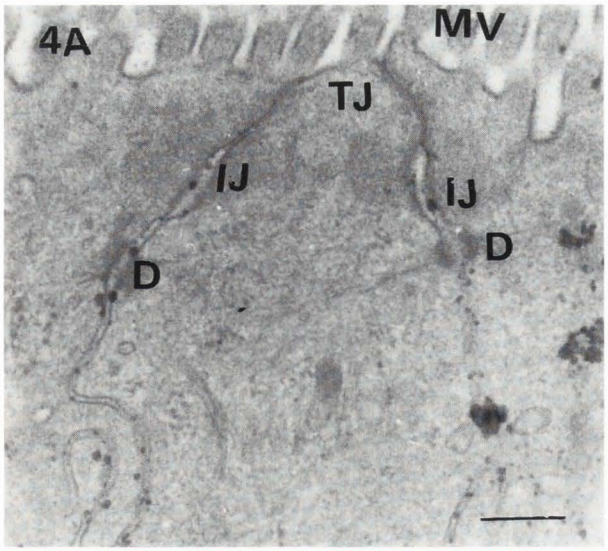


Figure 2. Low magnification pictures of unirradiated small intestine. The sections were cut parallel with the plane of epithelial layer. The pyroantimonate deposits were located in intercellular space of intermediate junctions as well as other intercellular regions. CE = columnar epithel cells, G = goblet cell, M = mitochondria, IJ = intermediate junction, D = desmosome. Bar: 1 μm, insert: 2 μm.

Figure 3. Junctional complex of unirradiated small intestine. TJ = tight junction, IJ = intermediate junction, D = desmosome. Reaction products were located within intercellular space of IJ and D, however the TJ was not labeled by pyroantimonate precipitates . Bar: 0.5 μm.

biophysical alterations may modify calcium content and/or distribution in the cell membrane and disturb cellular functions regulated by the calcium signal transduction pathway (Adey 1990 a, b). Our experimental data support these suggestions. The rapid redistribution of pyroantimonate precipitable


4A
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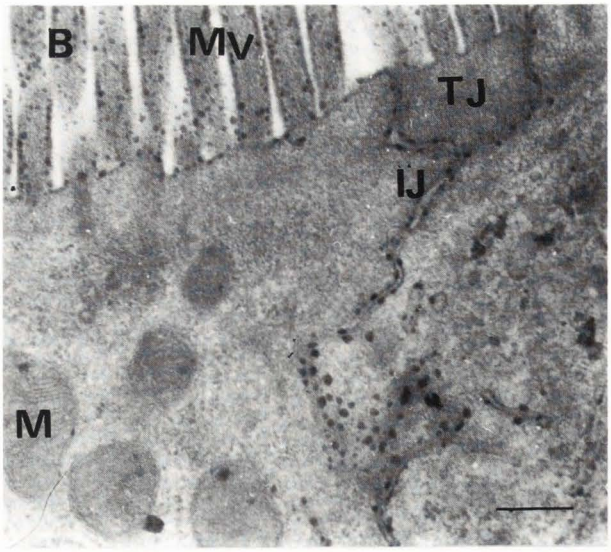
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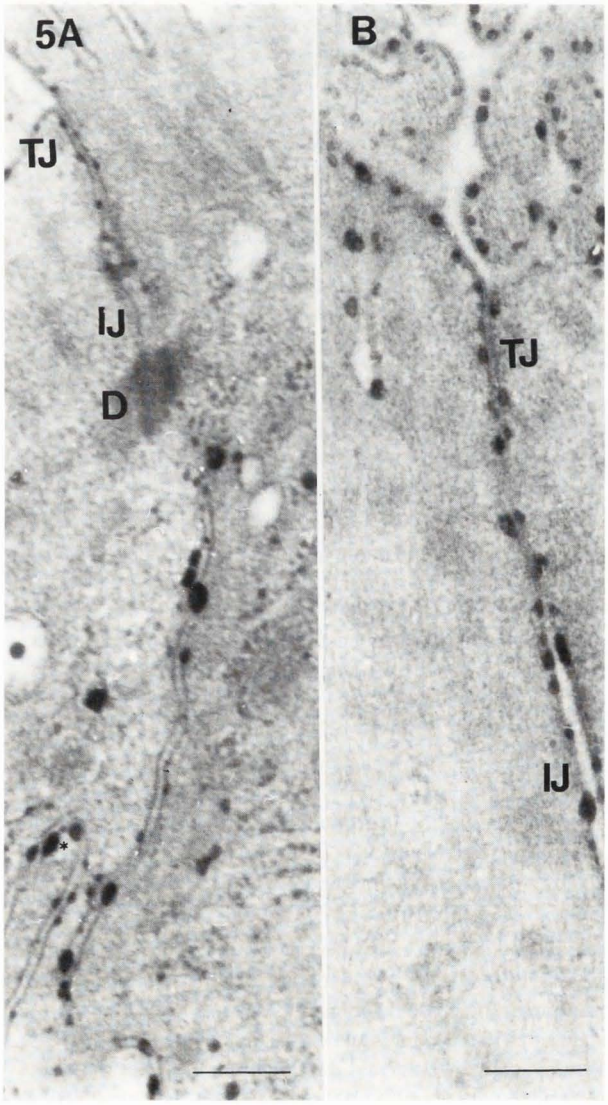
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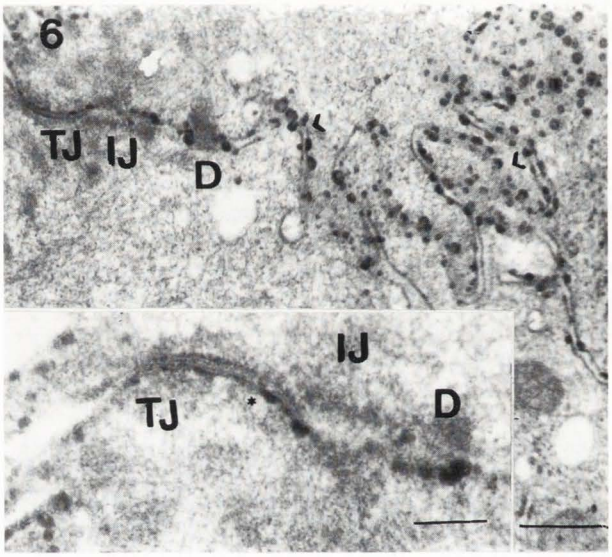
6
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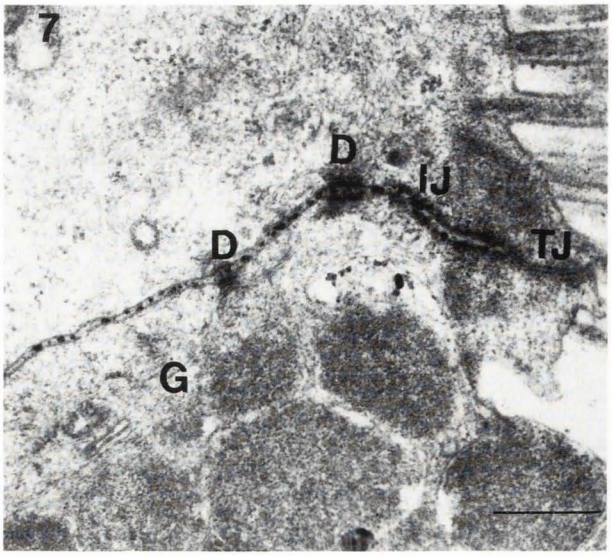
7
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Figure 4. Junctional complexes of continuous (A) and modulated (B) microwave irradiated small intestine. The power densities were identical (1 mV/cm^2) and the animals were killed immediately after irradiation. Continuous wave did not cause any changes of morphology and distribution of pyroantimonate precipitable calcium, however pulsed waves with the same energy altered the distribution of reaction products. MV = microvilli, TJ = tight junction, IJ = intermediate junction, D = desmosome, M = mitochondria. Bar: A = 0.5 μm, B = 0.4 μm.

Figure 5. Junctional complexes of modulated microwave irradiated small intestine. Power densities were 1 mW/cm^2, the animals were killed immediately after irradiation. The sections were cut approximately perpendicular (A) and parallel with the plane of the junctional complex (B). The pyroantimonate precipitates were localized at the inner side of tight junctions (TJ) and intermediate junctions (IJ) and at the cytoplasmic surface of lateral membranes (*), too. D = desmosome. Bar: A = 0.4 μm, B = 0.3 μm.

Figure 6. Junctional complex of modulated microwave irradiated small intestine. The power density was 0.5 mV/cm^2, the animals were killed 30 minutes after irradiation. The tight junctions (TJ) were also labeled by pyroantimonate precipitates (*) (insert), the amount of reaction products increased, and they also localized at the cytoplasmic surface of plasma membrane (→). IJ = intermediate junction, D = desmosome. Bar = 0.5 μm, insert = 0.2 μm.

Figure 7. Junctional complex of modulated microwave irradiated (1mV/cm^2) small intestine after 24 hours. The distribution of reaction products was similar to the control. TJ = tight junction, IJ = intermediate junction, D = desmosome. Bar: 0.5 μm.

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calcium upon exposure to low frequency-modulated, nonthermal microwave field observed in this study, points to an enhanced calcium uptake from the lateral extracellular space. Similar observations were recently published by Walleczek and Liburdy (1990); Klavinsh et al. (1991); Lyle et al. 1991; Liburdy (1992). The mechanism of rapid dislocation of calcium from intercellular space to the cytoplasmic side of plasma membrane remains to be elucidated as an effect of irradiation (similar to vinblastine effects) on Ca-pump enzymes, which normally maintain the low level of intracellular calcium (Eisenman et al., 1992). Since the local concentration of calcium modulates the structural stability and functions of the elements of junctional complex (Garrod 1986; Geiger and Ginsberg, 1991, Nilsson, 1991), the observed decrease of calcium content in the junctional area may explain the loss of tight junction mediated barrier functions (Bawin et al., 1975; Hecht et al., 1988; Nilsson, 1991). A similar explanation was suggested by Williams et al. (1984) for the increased permeability of the blood-brain barrier following microwave irradiation. Our preliminary data showing an increased ruthenium red permeability of an epithelial layer upon 1 mW/cm^2 irradiation with pulsed microwave are also in agreement with this suggestion.

Acknowledgment

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Discussion with Reviewers

D.R. Eisenmann: What was the basis for choosing the level of radiation utilized in these experiments? How does this compare with environmental levels?

Authors: The basis for choosing the level of radiation was as follows: The radiation protection standards in the microwave frequency range are between 0.05 - 5 mW/cm^2 (as a permissible levels for public or occupational workers) depending on the country where it is applied. There are very large differences in the value of permissible exposure levels of standards between the US and European countries and inside the European region as well. The ANSI and NCRP guidelines recommend 5 mW/cm^2 for 8 hours, the standards in the European countries are about 1 mW/cm^2, the permissible level in the Hungarian standard is 0.1 mW/cm^2, and the guidelines of ICNIRP (former IRPA/INIRC) is 1 mW/cm^2. Only the NCRP pointed out that the permissible level of extremely low frequency (below 300 Hz) amplitude modulated exposure has to be reduced in comparison to the continuous wave exposure. The applied exposure level in our experiment is comparable to the permissible levels of guidelines and standards in the European countries and US. The above standards and guidelines are under very heavy debate concerning the permissible levels especially on the question of health risk of mobile phone systems (GSM). However, work place the environment (the area of radars, microwave industrial machines) and even the front of microwave ovens may create an exposure level comparable to that used in our experiments.

D.R. Eisenmann: Is it possible that the calcium dislocation is occurring because of an effect on Ca-pump enzymes which normally maintain the low level of intracellular calcium as compared to surrounding tissue fluids?

Authors: Yes. We plan to investigate Ca-ATP-ase enzyme activity upon irradiation.

W.R. Adey : There has been no consideration of possible diffusion of calcium away from the original *in vivo* cellular and subcellular compartments before fixation occurred.

Authors: The diffusion and /or distribution changes of any ions or molecules after fixation are a fundamental problem with some histochemical reactions. According to literature data (Simson and Spicer, 1975, Mentré and Escaig, 1988, Mentré and Halperen,1989, Eisenman et al. 1979, Bonhomme et al, 1993) in this case the precipitation of calcium is rapid and localization of calcium does not change after fixation procedure.

W.R. Adey: What quantification have the authors made of the reported disposition of increased calcium in the vicinity of tight junctions?

Authors: It is possible to quantify histochemical reactions by morphometric methods or direct measurement of calcium content by microanalytical (i.e., electron spectroscopy, or electron energy loss spectrometry) investigations. Taking in to consideration the fact that the tight junction area did not contain calcium in the control samples, our observations of calcium in this region upon irradiation, may be of significance without quantification.