EFFECTS OF RF LOW LEVELS ELECTROMAGNETIC FIELDS ON PARAMECIUM PRIMAURELIA*

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INTRODUCTION

In the last years many studies have been performed to examine biological effects of prolonged exposure at electric field low levels (1-2).
This great interest is linked to a specific interaction possibility, also related to the exposure length, between electromagnetic fields and biological systems without remarkable enhancement of organism's temperature.
Hence the need to investigate in vitro the possible cellular regulation mechanisms involved in these interactions, varying physical exposure parameters.

For these esperiments we choose ciliata protozoa Paramecium primaurelia as biological system.This is an interesting experimental model because:
a) share certain membrane properties with nerve and muscle cells such as electrical excitability, presence of a Ca++ transport system, exocytotic ability and mobility.
b) its division rate can be easily directly determined.
c) there are several mutant types involving alterations in ion permeability and Ca++ transport.

In a first moment the influence of some physical parameters at constant frequency of 27.12 MHz (at various power density)has been studied on growth rate;the growth rate is able to give a comprehensive description of the cell system situation.
At present we are trying to correlate the found effects with cytoskeleton structural modifications using immunofluorescence techniques.

MATERIALS AND METHODS

Cells of the wild type strains of Paramecium primaurelia were grown at 25°C according to the usual procedure (3) in Cerophyl inoculated the day before with Klebsiella aerogenes.
For the experiments 24 depression plates with a single cell in each depression have been used. Plates had been exposed at 0 (sham-exposed), 1, 10, 100 W/m2 CW and square wave modulated at 72 Hz in a TEM cell. Modulation at 72 Hz has been choosen by many Authors (4)

* This work was supported by Regione Piemonte - Grant n° 144 for Health Research and by the National Institute of Nuclear Physics - Section of Torino.

since it is similar to that employed in clinical applications to enhance bone healing.

The TEM cell was connected to a signal generator and an amplifier through the 24 hours. The instrumental chain composed by a signal generator, an amplitude modulator , an amplifier, a bidirectional coupler, power sensors and a power meter allows the generation inside TEM cell of an electromagnetic field at a frequency of 27.12 MHz with an accuracy of $\pm$ 0.7 dB (5).
The whole system is connected to a computer that assures constant conditions of electric field strength.

By daily isolation before the exposure we had obtained isogenic lines used for every couple of plates: one for control group and one for the exposed one.

The TEM cell had been maintained at 25°C $\pm$ 0.2°C by a hot air flow set by a feed-back system and placed in a climatized room.
Temperatures of exposed cultures and of control groups were measured by an optical fiber probe connected to a fluoroptic thermometer interfaced with a computer.
The 0.4°C range has not influence on growth division rate (4).
The cell cycle of Paramecium primaurelia takes nearly 8 hours.

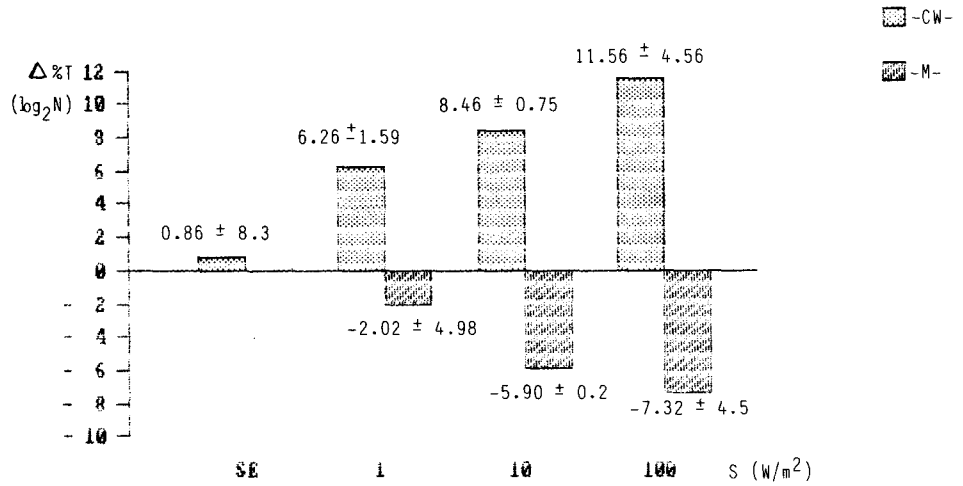
Cell number was determined by a microscopic examination. The daily division rates was calculated by taking the $\log_2$ of the number of cells produced in each depression by a single cell after 24 hours. Those depressions where we had the death of the cell, or we have no cell division (may be cell in autogamy) were not taken into account.
We choose the 24 hours period because after this time the average number of paramecia in every depression is optimal for counting and statystical analysis.
The experimental unit is the growth rate averaged on the set of 24 depressions in a plate.
Statistical analysis of the results was by Mann-Whitney's U test to compare means of growth rates between plates exposed to the same power density to the correlated isogenic control plates.

RESULTS

The advancement of percent variation of growth rate versus power density for CW and modulated exposures is shown in Fig.1.
Tab. 1 reports the numerical consistency of experimental groups and the statistical significance of the different tests.

We can observe that the percent variation of growth rate raises with increase power density as regards CW fields and decreases as regards modulated fields.

Fig. 1 - Growth rate percent variation (Δ % T) of Paramecium primaurelia versus power density in air, under 27.12 MHz continuous wave (CW) and square wave modulated (M) conditions. As regards square wave modulated fields, power density value regards to the "on" phase.



Tab. 1

	SE	1 CW	1 M	10 CW	10 M	100 CW	100 M	W/m²
N. plates	6	10	10	6	6	10	10	
N. isolated Paramecia	144	240	240	144	144	240	240	
N. counted Paramecia	1152	1972	1872	1195	1036	2280	1680	
Mann Whitney	N.S.	N.S.-5%	N.S.-5%	1-5%	5%	1-5%	1-5%	(N.S.= Not Significant)

DISCUSSION AND CONCLUSIONS

The first data emerging from analysis of above results, is the opposite pattern of growth rate variation depending on CW or modulated exposure.

The pattern suggests that the wave shape of the electromagnetic field is more important with regard to induction of effects on the cell than the amount of the energy carried by the wave. Concerning the electromagnetic field strength to which Paramecia are exposed , it must be underlined that the true value of power density in culture medium is about 6400 times lower than in air.

Previous studies show the influence of electromagnetic fields on Ca++ ions influx inside various type of cells (6), and in particular the Paramecium (7).

There's evidence for the involvement of Ca++ ion in regulation of a lot of different cellular mechanisms. In particular it influences polymerization of tubulin, polymer playing an important part in cytoskeletal organization of the cell (8).

A first hypothesis of work concerning biophysical interpretation of obtained results, is that variations of growth rates is connected

to an influence of electromagnetic fields on mitotic spindle micro tubules organization, mediated by Ca++ ion concentration variation.

To verify this hypothesis we are setting up a series of immuno fluorescence experiments with the use of monoclonal antibodies anti -α and anti-ß tubulin conjugated with FITC, in order to detect the presence of some morphological alterations of Paramecia at mitotic stage depending on incident electromagnetic field features (9). In this way we think to have more useful data for the understanding of the opposite effects of growth rate of CW fields and of modulated fields.

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