A SEARCH FOR FREQUENCY-SPECIFIC BIOEFFECTS CAUSED BY MICROWAVE IRRADIATION

ABSTRACT

A method recently developed in this laboratory was used in order to directly expose cells grown in tissue culture to high levels of microwave irradiation without significant microwave-induced heating (<0. 1° C). Monolayer cultures of BHK-21/C13 cells were grown on microwave-transparent polystyrene coverslips, placed directly on the open end of a waveguide which terminated in an incubator held at 36°C, and irradiated at frequencies in the E-band (average power density = 292 mW/cm^2) or in the U-band (average power density = 177 mW/cm^2). Each culture was exposed for 15 minutes and four cultures were irradiated at each frequency. Frequencies corresponding to 0.1 GHz increments in the ranges from 37 to 48 GHz and from 65 to 75 GHz will be studied.

Incorporation of ^3H-methionine into protein during the period of microwave irradiation was assessed by autoradiography. Incorporation by the cell monolayer was quantified by measurement of the optical density of the autoradiograms in a set of contiguous 0.1 mm wide regions which spanned the long axis of the waveguide. Since microwave power density along the major axis of the waveguide varies from its maximum at the center to zero at either edge, these data provide information on the extent of protein synthesis in BHK cells subjected to 16 or 25 different power densities in the E- or U-bands, respectively. These data sets were examined both by visual inspection and computer analysis for the presence of narrow "power windows" in which microwave bioeffects might be expressed.

Data have already been compiled on 100 frequencies ranging from 43 to 48 GHz and from 65 to 70 GHz. In agreement with earlier studies in this laboratory at the fixed frequencies of 41.80 and 73.95 GHz, amino acid incorporation by irradiated cultures did not differ significantly from that of nonirradiated "sham controls" at any frequency and no evidence of a "power window" was found. (Data on other frequencies will be presented at the meeting.)

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SUMMARY

A method recently developed in this laboratory was used in order to directly expose cells grown in tissue culture to high levels of microwave irradiation in the E- and U-bands without significant microwave-induced heating ($< 0.1^{\circ}C$). BHK-21/C13 cells obtained from The American Type Culture Collection were grown at low passage levels in Eagle's MEM supplemented with 10% heat-inactivated calf serum and 0.1% tryptose. These cells were incubated at $36^{\circ}C$ in a CO_2 incubator until they had formed nearly confluent monolayers on microwave-transparent polystyrene coverslips (Lux). The cultures were then irradiated by placing the culture dishes directly on the open end of either an E- or U-band waveguide which terminated within the incubator (Fig. 1). Thus, the first absorbing material encountered by the microwaves was the monolayer of cells on the upper surface of the polystyrene. Alignment markers on the lower surface of the polystyrene coverslips outside of the irradiated area were used both to position the dishes on the waveguide and to determine what portion of the cell monolayer was directly over the waveguide for autoradiographic analysis. Equipment for microwave irradiation is shown diagramatically in Fig. 1.

Monolayer cultures of BHK cells were irradiated at an average power density of 292 mW/cm^2 in the E-band or at an average power density of 177 mW/cm^2 in the U-band. (These specific power densities were chosen because they were the highest levels which could be maintained over the entire frequency range of the relevant klystron.) Each culture was exposed for 15 minutes and four cultures were irradiated at each frequency. This exposure period was chosen in order to minimize the time required to examine the effects of microwave irradiation in 0.1 GHz increments in the frequency ranges from 37 to 48 GHz and from 65 to 75 GHz. This frequency increment (0.1 GHz) was selected as being the smallest practical value because the accuracy of the best available frequency meter was $\pm$ 0.05 GHz.

Incorporation of labelled amino acids into proteins was selected as a potentially sensitive indicator of microwave bioeffects for the following reasons. First, this process involves active transport of labelled amino acids across the plasma membrane. Second, protein synthesis itself requires energy and is subject to control by numerous regulatory mechanisms. Thus, microwave-induced changes

in (a) the state of the plasma membrane, (b) the bioenergetic status of the cells, or (c) any of the multiple regulatory pathways associated with protein synthesis might result in an alteration in the level of incorporation of the labelled amino acid.

Incorporation of ^{3}H-methionine into protein during the period of microwave irradiation was assessed by autoradiography. Incorporation by the cell monolayer was quantified by measurement of the optical density of the autoradiograms in a set of contiguous 0.1 mm wide regions which spanned the long axis of the waveguide. Optical density measurements were made using a Zeiss microscope connected by a light pipe to the photomultiplier assembly in a Gilford model 240 spectrophotometer. The autoradiograms were advanced in 0.1 mm increments along the long axis of the waveguide under computer control and data were collected in real time using an H/P 9825A computer. Control experiments verified that optical density of autoradiograms made with special tritium-sensitive film (CEA, Verken, Sweden) was directly proportional to the amount of radioactivity within the range of interest.

Since microwave power density along the major axis of the waveguide varies from its maximum at the center to zero at either edge, these data sets provide information on the extent of protein synthesis in BHK cells subjected to 16 or 25 different power densities in the E- or U-bands, respectively. These data sets were examined both by visual inspection and computer analysis for the presence of narrow "power windows" in which microwave bioeffects might be expressed. Such a "power window" would be detected by observation of a pair of affected bands of cells lying in the same relative position between the center and edges of the waveguide.

Data have already been compiled on 100 frequencies ranging from 43 to 48 GHz and from 65 to 70 GHz. For example, a three-dimensional graph is presented in Fig. 2 for data gathered at frequencies from 65.0 to 65.9 GHz. The x-axis represents position along the long axis of the waveguide, the y-axis represents frequency in 0.1 GHz increments, and the z-axis represents percent of control optical density. Solid portions of each curve represent values >100% while dotted portions represent values <100%. The data demonstrate that microwave irradiation does not significantly alter protein synthesis at any point along the major axis of the waveguide (i.e., at any power level between zero and maximum.) Similar data sets were obtained at other frequencies. In summary, amino acid incorporation into proteins by irradiated cultures did not differ significantly from that of nonirradiated "sham controls" at any frequency and no evidence for a "power window" was found.
(Supported by NIH grant CA20419.)

FIG. 1

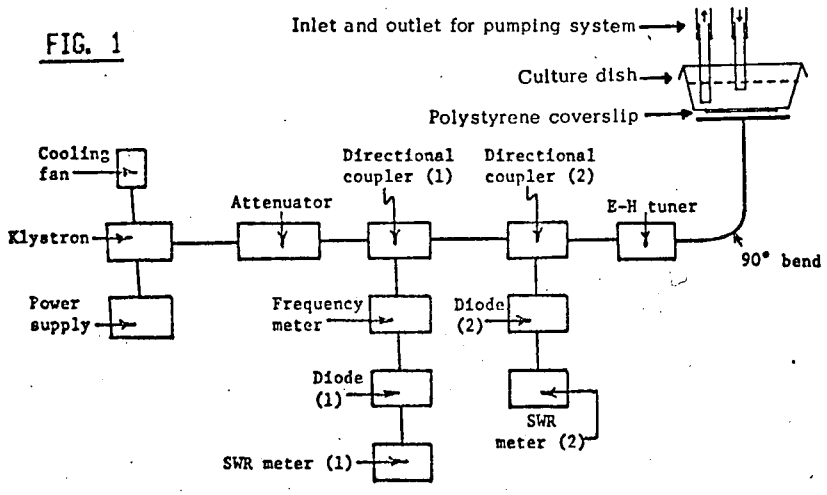
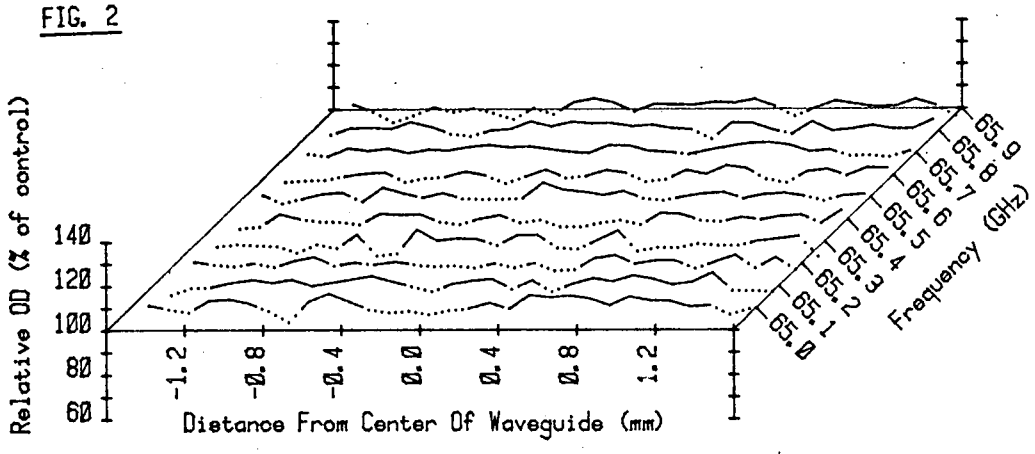

Inlet and outlet for pumping system
Culture dish
Polystyrene coverslip
Cooling fan
Directional coupler (1)
Directional coupler (2)
Attenuator
E-H tuner
Klystron
90° bend
Power supply
Frequency meter
Diode (2)
Diode (1)
SWR meter (2)
SWR meter (1)

FIG. 2


Relative OD (% of control)
140
120
100
80
60
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Distance From Center Of Waveguide (mm)
65.0
65.1
65.2
65.3
65.4
65.5
65.6
65.7
65.8
65.9
Frequency (GHz)