A NOVEL IN VITRO METHOD FOR STUDY OF THE BIOLOGICAL EFFECTS OF MICROWAVE IRRADIATION

ABSTRACT

A method has been devised whereby cells grown in tissue culture can be directly exposed to exceedingly high levels of microwave irradiation without significant heating ($< 0.1^{o}C$). BHK-21/C13 cells were grown on microwave-transparent polystyrene coverslips and irradiated by placing the culture directly on the open end of a waveguide which terminated in an incubator held at $36^{o}C$. Thus, the first absorbing material encountered by the microwaves was the monolayer of cells on the upper surface of the polystyrene.

Microwave-transparent sheets of plastic coated with temperature-sensitive liquid crystals were used in order to determine the extent of heating in the cell monolayer caused by microwave irradiation. Such studies revealed that local heating to temperatures as high as $42^{o}C$ occurred at the center of the waveguide. As a result, a pumping system was devised which recirculated the culture medium over the cell monolayer rapidly enough to completely dissipate any microwave-induced heating. This allowed the use of high average power densities (320 mW/cm^2 at 41.80 GHz and 450 mW/cm^2 at 73.95 GHz).

Biological effects of microwave irradiation were sought by two different procedures. First, electron microscopy was used to evaluate the ultrastructural characteristics of cells at chosen points within the exposed field after one hour of irradiation. Second, autoradiography was used to assess incorporation of either ^{3}H-uridine into RNA or ^{3}H-methionine into protein both during and after one hour of irradiation. Since power density varies along the major axis of the waveguide, incorporation was quantified by measurement of optical densities of the autoradiograms in contiguous 0.17 mm wide regions along that axis. No evidence was found for microwave "power windows". The results of all experiments demonstrate that, under carefully controlled conditions explicitly designed to eliminate localized heating, even very massive doses of microwave energy at the two specified frequencies were without effect on either cell ultrastructure or the synthesis of two types of macromolecules.

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A NOVEL IN VITRO METHOD FOR STUDY OF THE BIOLOGICAL EFFECTS OF MICROWAVE IRRADIATION

SUMMARY

A method has been devised whereby cells grown in tissue culture can be directly exposed to exceedingly high levels of microwave irradiation without significant heating (< 0.1°C). BHK-21/C13 cells were obtained from The American Type Culture Collection and 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°C in a CO_2 incubator until they had formed a nearly confluent monolayer 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. 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.

The extent of local heating in the cell monolayer caused by microwave irradiation was determined by use of microwave-transparent sheets of plastic coated with temperature-sensitive liquid crystals in place of the plastic coverslips. When such tissue culture dishes were filled with culture medium and irradiated at maximum power, a complex pattern consisting of a set of concentric ellipses of different colors was formed (Fig. 2). Color calibration indicated that the temperature at the center of the waveguide rose as high as 42°C when cultures were irradiated in the E-band and as high as 39°C when cultures were irradiated in the U-band. Furthermore, irradiation with less than 10 mW/cm^2 caused discernable increases in the temperature in the cell monolayer. As a result of these observations, a pumping system was devised which recirculated the culture medium over the cell monolayer rapidly enough to completely dissipate any microwave-induced heating. A Sage peristaltic pump set at 650 ml/hr was used to recirculate the medium through inlet and outlet tubes mounted in the lid of this culture dish in order not to interfere with microwave irradiation (Fig. 1). This system allowed use of very high average

power densities (320 mW/cm^2 at 41.80 GHz and 450 mW/cm^2 at 73.95 GHz). In this configuration, sheets coated with liquid crystals showed no discernable heating at any point within the microwave field.

Monolayer cultures were irradiated at either frequency for one hour and 3-5 cultures were exposed at each frequency. "Sham controls" were also run which were treated identically to experimental cultures except that no microwave power was delivered. Biological effects of microwave irradiation were sought by two different procedures. First, electron microscopy was used to evaluate the ultrastructural characteristics of cells at chosen points within the exposed field after one hour of irradiation. One or more levels from each of 70 specimens were examined. Electron microscopy revealed a homogenous monolayer of well-preserved BHK cells which were virtually confluent. BHK cells in both sham control and microwave-irradiated cultures (either at 41.80 or 73.95 GHz) were characterized by an oval nucleus with heterochromatin and a central nucleolus, a moderately well developed Golgi apparatus, numerous mitochondria, and "stringy" rough endoplasmic reticulum containing an electron-dense material. Ribosomes were uniformly distributed throughout the perikaryal cytoplasm and were situated in a light cytoplasmic matrix. Microfilaments and microtubules formed electron-dense aggregates in the cellular processes at sites of attachment to the substrate. Direct comparison of the morphology and frequency of these cellular organelles failed to demonstrate any changes directly attributable to exposure to microwave irradiation. Second, autoradiography was used to assess incorporation of either ^{3}H-uridine into RNA or ^{3}H-methionine into protein. Specific inhibitors of RNA synthesis (actinomycin-D) or of protein synthesis (cycloheximide) were utilized to demonstrate that radioactivity detected by autoradiography represented label incorporated into RNA or protein, respectively. Incorporation of these precursors over the period of one hour was studied both during irradiation in order to study transient effects and after exposure in order to study cummulative effects. Incorporation was quantified by measurement of optical densities of the autoradiograms in contiguous 0.17 mm wide regions along the major axis of the waveguide using a Quantimet Image Analyzing 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 power density varies along the major axis of the waveguide (i.e., power density at either edge is zero and that at the center is maximum), determination of the amount of incorporation in each region along that axis might make it possible to detect "power windows" in which a microwave bioeffect could be manifested. 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. No evidence was found for such microwave "power windows" by either visual inspection or computer analysis. On the contrary, microwave irradiation never resulted in any significant deviation from the levels of incorporation observed in cells outside the field of exposure. In summary, the results of all experiments demonstrate that, under carefully controlled conditions explicitly designed to eliminate localized heating, even very massive doses of microwave energy at the two specified frequencies were without effect on either cell ultrastructure or the synthesis of two types of macromolecules. (Supported by NIH grant CA20419.)

FIG. 1

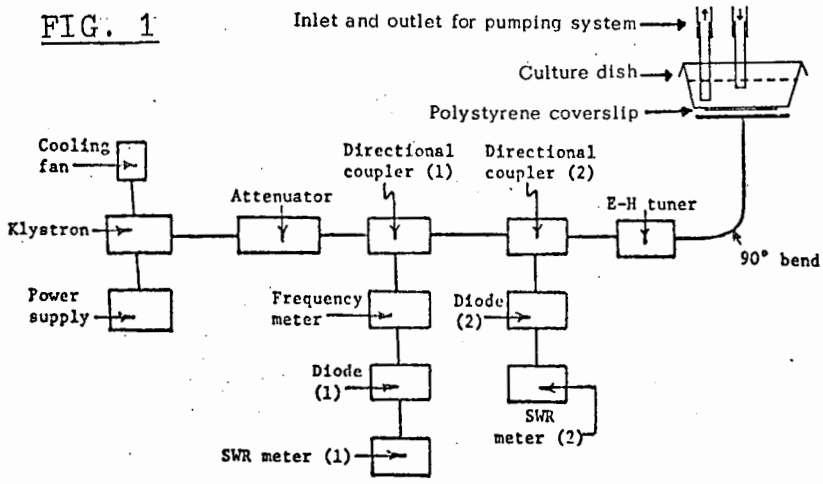

FIG. 2 HEATING PATTERN FOR E-BAND