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Mechanism of Lethal Action of 2,450-MHz Radiation on Microorganisms

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Various bacteria, actinomycetes, fungi, and bacteriophages were exposed to microwaves of 2,450 ± 20 MHz in the presence and in the absence of water. It was found that microorganisms were inactivated only when in the presence of water and that dry or lyophilized organisms were not affected even by extended exposures. The data presented here prove that microorganisms are killed by "thermal effect" only and that, most likely, there is no "nonthermal effect"; cell constituents other than water do not absorb sufficient energy to kill microbial cells.

The effects of electric currents on microorganisms have been of interest to microbiologists for more than 75 years (2, 4, 7, 12). Whereas the first experiments dealt with instantaneous discharges in air, the invention of generators capable of producing continuous fields of electromagnetic radiation which could be propagated through space made possible the study of the effects of these radiations on microbial cells in isolated systems. The principal studies focused on those wave bands which held promise for use in instruments and appliances for general utility or popular applicability. Today, those microwave bands called UHF (300 to 3,000 MHz) are used in many commercial devices, including television, microwave communications, microwave ovens, medical diathermy, radio navigation, long-range radar, and an abundance of special equipment designed for specific uses.

One of the major areas of specialized application has been that concerned with food preparation, sanitation, and storage (3, 5, 14, 15). Many reports show that microwaves can be successfully used for blanching, sanitizing, and even sterilizing many foods. Some of the older reports address themselves to the mechanism of lethal action of microwaves on microorganisms, but these reveal a certain degree of ambiguity. Unfortunately, the more recent work has served to accentuate ambiguity and inconsistencies. The major area of confusion concerns studies on the distinction between "thermal" and "nonthermal" effects. Many reports show that the bactericidal action of microwaves resides in the thermal effect, i.e., energy transfer from microwaves to the specimen material with a consequent rise in temperature. Other reports show bactericidal action in the absence of temperature increase. Lystsov et al. (9) state "... we found no specific non-thermal lethal or mutagenic effects of UHF fields," whereas Olsen (14) reports "... the excellent results with microwaves are probably not due to conventional thermal kill." The most recent report on this topic (3) states plainly that the killing of bacteria by microwaves is not entirely due to the heat produced by microwave energy. It also states that the nature of the lethal effects of microwave radiation on living things is still not known.

In a previous paper (17), it was shown that bacteria, actinomycetes, and fungi became more susceptible to microwaves when irradiated in moist soil and, also, that the degree of susceptibility varied as a function of the physiological condition of the irradiated cells. This is the report of studies designed to show, unambiguously, the nature of the lethal effect of microwaves on microbial cells.

MATERIALS AND METHODS

Irradiation. All irradiations were carried out in a specially constructed stainless steel cavity (66 by 41 by 46 cm) coupled to a magnatron tube capable of emitting 1.5 kw of forward power at 2,450 ± 20 MHz. In normal operation, power output was adjusted so that the cavity and material therein absorbed a measured amount of energy. A Teflon platform rotating at 60 rpm was adjusted for uniformity of exposure and used as a sample holder. Energy output was calculated and verified by using temperature increase of distilled water. It was shown that energy output was a flux of 1 kw (1,000 J/s) at the sample locations. Air movement, air temperature, cavity loading, and other pertinent factors were kept constant during the course of this investigation, and repeated experiments gave identical results.

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Preparation of microorganisms. Samples of dry soils were brought to the laboratory and prepared for irradiation by a previously described method (16). They were then placed in desiccators with sufficient water to provide predetermined quantities of soil moisture. Other samples of the same soils were placed in desiccators with NaOH to remove moisture, whereas still others were moistened directly to produce soil-water slurries. Approximately 250 g of each prepared soil was placed in a beaker for irradiation, whereas other portions were used to determine the exact moisture content. All samples from a given lot were irradiated simultaneously, and soil temperature was determined by plunging a rapid-indicating mercury thermometer (20 to 100°C range) into the sample immediately after irradiation.

Commercial yeast (Fleischmann's Active Dry; Standard Brands, Inc., New York) was obtained in 7-g packets and used immediately after opening. The contents from several packets were mixed, moisture content was determined, and the yeast cells were placed on a Teflon platform in formed Styrofoam dishes supported by Styrofoam blocks. It had been previously established that these Styrofoam holders do not become hot even after 30 min in the microwave cavity.

Cultures of different bacteria were grown at their optimal temperatures in tryptic soy broth. The cells were harvested by centrifugation at late log phase, washed three times in distilled water, and suspended in an aqueous medium containing 10% (vol/vol) skim milk and 5% (wt/vol) lactose. They were then lyophilized and stored until needed. *Azotobacter* cultures were grown in Burk medium at 28°C and lyophilized in the same manner.

A bacteriophage for *Escherichia coli* K-12 and another for *E. coli* were isolated from sewage. They were grown on these hosts, separated by differential centrifugation and filtration through 0.45-μm membranes, collected by centrifugation, and lyophilized after resuspension in the milk–lactose medium.

Moisture content. Moisture content was determined by weighing a representative portion of the specimen to be irradiated. The weighed sample was placed in the 105°C oven overnight and weighed after cooling. In some experiments, only the distinction "wet" and "dry" was deemed necessary. In this case, wet means that sufficient water was added to completely soak the specimen.

Lethal effects. Lethal effects of microwaves were determined by comparing counts of viable cells in irradiated samples and in nonirradiated controls. Total bacterial populations were determined by triplicate spread plate counts, using tryptic soy agar and incubating the plates aerobically at 30°C for 2 days. Actinomycetes were counted on a medium prepared as follows: glycerol, 10 ml; $CaCO_3$, 3 g; K_2HPO_4, 1 g; DL-aspartic acid, 1 g; agar, 20 g; and water, 1 liter. Spread plates were incubated at room temperature for 10 days. Fungi were plated on fungi count agar (11) and incubated at room temperature for 5 to 10 days at 30°C. Estimates of soil *Azotobacter* populations were obtained by plate counts on a medium previously described (18). *Bdellovibrio* no. 1 and *Bdellovibrio* no. 2 were counted on lawns of *Pseudomonas aeruginosa* and *E. coli*, respectively. Bacteriophage K-12 was counted as plaque-forming units on lawns of *E. coli* K-12, and another bacteriophage was counted on an *E. coli* culture isolated for this study.

RESULTS

To examine the thermal component of the bactericidal properties of microwaves, a series of experiments was performed in which the rate of inactivation of viable microorganisms was compared with the temperature of the suspending medium. The system of choice was considered one in which microorganisms could exist in either the presence or the absence of water. In a previous work (18), it was shown that natural populations of *Azotobacter* in dry soils could be counted by plating on a special medium. Figure 1 shows that inactivation of soil *Azotobacter* depends on the presence or the absence of water and/or on the temperature of the specimen after irradiation. These experiments were repeated eight times with different soils, and the results were essentially identical to those shown here. In 14 other experiments in which the total soil population (heterotrophic, aerobic, mesophilic) was counted, the results (not shown) were essentially the same as those shown in Fig. 1 for *Azotobacter*. We interpreted these results as showing clearly that, in the presence of water, the temperature of the suspending medium and bacterial inactivation are dependent functions of the total amount of radiation delivered into the cavity by the magnatron tube and, also, that temperature increase in the sample depends on the presence of water.

Since the loss factor (dielectric constant × loss tangent) of water at 2,450 MHz is much greater than that of soil or that of the glass beakers, it seemed reasonable to assume that the major part (more than 98%) of microwave energy was absorbed by moisture in the soil.

The results of experiments in which diverse

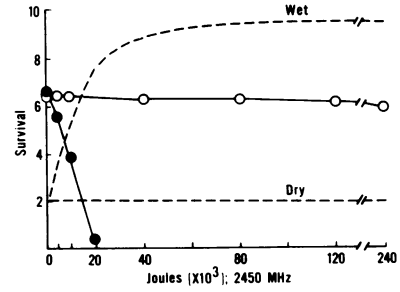

FIG. 1. *Colony counts of Azotobacter that survived irradiation in dry soil* (○) *and in wet soil* (●). *Survival is indicated as the log number of viable cells per gram of soil. The dashed lines indicate the temperature of the irradiated sample; ordinate* × *10 =* °*C.*

microbial populations were monitored showed that bacteria, actinomycetes, and fungi were inactivated as a function of the moisture content of the soil (Fig. 2). These results proved that the bactericidal effect of microwaves was due to the total amount of water in the soil sample. In more than 70 repetitions of this experiment, the results showed that there was a distinct loss of microbicidal activity as the moisture content of the sample approached zero.

Since it is obvious that cooling devices such as those used in previous experiments (8, 9) or any suspending medium (10) must act as a barrier for the microwave energy or in some other way interfere with the interaction between microwave energy and biological target, the only valid experiments which could be employed were those in which the biological material could be irradiated directly. To circumvent the problem of energy absorption by water and soil, lyophilized organisms were prepared and irradiated in the dry condition and, for comparison, after moistening. The data obtained from these experiments also show that cell killing is a function of moisture content; lyophilized yeast cells were inactivated when irradiated in the presence of water but survived large doses of radiation in the dry state (Fig. 3).

A variety of bacteria and two bacteriophages were also irradiated in the presence and in the absence of water. The results of these experiments clearly show that bacteria and viruses were not killed by microwave radiation at 2,450 MHz (Table 1 and Fig. 4). All of the organisms tested failed to absorb sufficient energy in the dry state to bring about significant reduction in cell populations although they were exposed for prolonged periods of time.

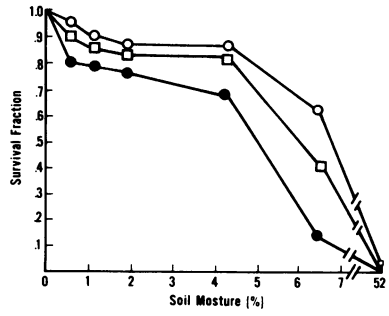

FIG. 2. *Effect of moisture on survival of natural soil populations of bacteria* (○), *actinomycetes* (●), *and fungi* (□) *in the microwave cavity after absorbing 240 kw of energy at 2,450 MHz. Ordinate indicates survival fraction, i.e., irradiated sample ÷ unirradiated control.*

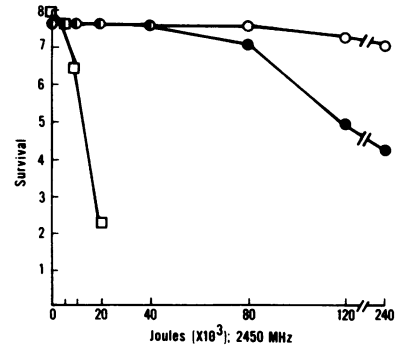

FIG. 3. *Effect of moisture on yeast cells exposed to various amounts of radiation. Freshly opened packages of commercially dried yeast contain approximately 0.1% moisture* (○); *about 3 h later the cells contain approximately 7.5% moisture* (●). *Cells from freshly opened packages were suspended in distilled water to make a slurry and were also irradiated* (□). *Survival is indicated as the log number of viable cells per milliliter of slurry or per gram of dry yeast cell preparation.*

TABLE 1. *Effect of microwave radiation (2,450 MHz) on microorganisms in the lyophilized state (Dry) and after moistening (Wet)*

Organism	$LD_{99.9\%}$ ($J \times 10^3$)[a]	
	Dry	Wet
Escherichia coli	>240	16
Pseudomonas aeruginosa	>240	8
Salmonella typhimurium	>240	11
Serratia marcescens	>240	10
Staphylococcus aureus	>240	11
Bacillus cereus	>240	12
Azotobacter vinelandii	>240	12
Azotobacter chroococcum	>240	10
Bdellovibrio spp.	>240	22
Bdellovibrio spp.	>240	20
Bacteriophage (*E. coli* K-12)	>240	18
Bacteriophage (*E. coli*)	>240	18

[a] The 99.9% lethal dose, $LD_{99.9\%}$, was obtained from graphs depicting the surviving fraction, i.e., irradiated/nonirradiated control.

DISCUSSION

A report by McRee (10) suggests that microwave energy absorption by biological materials can be measured by temperature increase of the specimen material. McRee proposed the following equation, which shows the suggested correlation between change in temperature, ΔT, and the energy-time exposure parameter: $\Delta T = 0.185P\,(1 - e^{-0.10t})$, where ΔT is the temperature increase (degrees Celsius) of the specimen during time t; P is the incident microwave energy in units of milliwatts per centimeter squared; and t is the time of exposure in minutes. In this equation, the parameter 0.185, in units of degrees

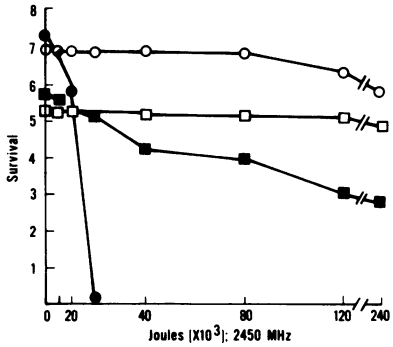

FIG. 4. *Effect of microwave radiation on vegetative cells of Bacillus cereus irradiated in the lyophilized state* (○) *and after wetting* (●). *Spores were also irradiated in the dry state* (□) *and suspended in distilled water* (■). *Survival is indicated as the log number of viable cells or viable spores per milliliter of suspension or per milligram of dry weight.*

Celsius per minute per milliwatt per centimeter squared, is defined as "a measure of the absorbing characteristics of the specimen." The data presented in this report (Fig. 1) can be used to show that the equation is not a general equation applicable to all biological materials, but rather a special one applicable only to biological materials that contain certain quantities of water. According to McRee's equation, ΔT for the experiment described in Fig. 1 (soil in water) should be as follows: $T = 0.185 \times (10^7/10^3)\ (1 - e^{-0.1 \times 0.1667}) = 30.5°C$. The observed values ranged from 29 to 36°C and averaged 32.5°C for 14 measurements. On the other hand, if the specimen is dry soil (also Fig. 1), the observed value was $\Delta T = 0$ for exactly the same time-energy exposure. This clearly shows that, in the absence of water, microwave energy is not absorbed, at least by biological material in the form of bacterial cells. Curiously, bacterial cells (suspended in water) were the biological material used in McRee's report. The remainder of the data presented here support this observation and the concomitant fact that, in the absence of water, biological specimens, i.e., microbial cells, are not damaged. These data give license for speculation: McRee's time-temperature profiles are due to energy absorption by water in the biological specimen and not by the specimen. It follows, therefore, that only the amount of water in the specimen is important in this regard and not the nature of the biological material.

In summary, it is obvious from these studies that microbial cells are not inactivated by large amounts of microwave radiation. A search of the pertinent literature shows that these are the first experiments in which microorganisms were directly exposed to microwaves. The data presented here clearly show that microwaves kill microorganisms only by thermal effect and that, if there is a nonthermal effect, it is not a bactericidal one, although the possibility exists that water may be necessary to potentiate the nonthermal effect.

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