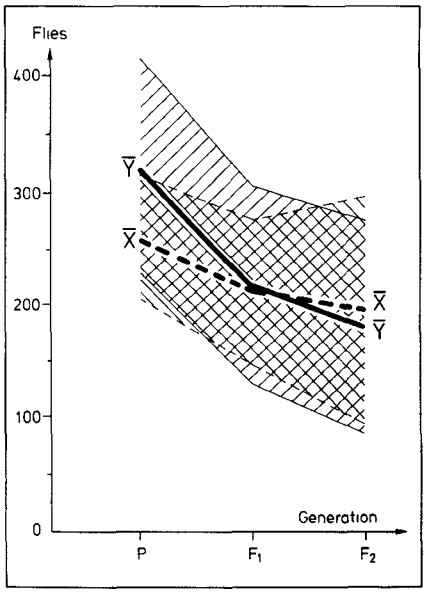


Fig. 2. The average offspring of all the six experiments. The solid lines give the value of irradiated, the broken lines those of untreated insects. The prime standard deviation of both populations is displayed. They are overlapping in a broad area.

is a nonspecific decrease of vitality as a consequence of the experimental plan who insisted on in-breeding.

6) The decrease of vitality is covered by the irregular distribution of offspring (see 4)). The statistically expected values of experimental parameters are changing with generations. As a consequence we have infectious distributions (Neyman). The result of an experiment is dependent on the outcome of the previous one.

7) Autonomous internal processes in test populations may suggest relations between microwave exposure and changes in fertility. However, as has been shown, nonthermal microwave-induced effects could not be detected in this extensive study of the fertility of Drosophila melanogaster. This result is contrary to recent reports on microwave-induced changes in fertility [1], [2].

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# Changes in Liposomes Permeability Induced by Gramicidin D After Microwave Irradiation

D. YOVA, S. LOUKAS, AND G. BOUDOURIS

***Abstract*—The response of model membranes (liposomes) to microwave irradiation (9.2 GHz, energy absorption 20 mW/g, continuous 1.5-h exposure in an orthogonal waveguide) was determined spectrophotometrically by recording after irradiation the gramicidin D-induced cation permeability. The irradiation modified the gramicidin D-induced permeability to the cations $K^+$, $Na^+$, and $Rb^+$ through the liposomes and seemed to facilitate the movement of $Na^+$ and $Rb^+$. These results are discussed in relation with the hypothesis that microwave radiation may induce changes to the structure of liposomes.**

## I. INTRODUCTION

Many experiments have demonstrated microwave irradiation influences on membrane transport processes in the blood–brain barrier [1], rabbit erythrocytes [2], [3], and human blood platelets [4]. Several theories have been proposed which suggest new modes of interaction between microwaves and biological systems [5]. However, there are few studies concerning microwave irradiation effects on model membranes transport processes.

The experiments described in this paper investigated the effects of 9.2-GHz microwave irradiation on liposomes by recording spectrophotometrically the gramicidin D-induced permeability to the cations $K^+$, $Na^+$, and $Rb^+$ after irradiation.

The irradiation changed the gramicidin D-induced transport of the cations across the liposomes and seemed to facilitate the transport of $Na^+$ and $Rb^+$.

## II. MATERIALS AND METHODS

### A. Materials

Phosphatidylcholine (egg lecithin), phosphatidic acid, and gramicidin D were purchased from Sigma Chemicals. The phosphatidylcholine was purified by silica gel column chromatography. The purity of phosphatidylcholine and phosphatidic acid appeared to be better than 99 percent by thin-layer chromatography.

### B. Preparation of Liposomes

Solutions in chloroform of phosphatidylcholine and phosphatidic acid (36:4 w/w) were reduced to dryness on a rotary evaporator. Liposomes (15 $\mu$M of total lipid/ml) were then allowed to form by shaking (2 h, 48°C) in an aqueous solution (40 mM KCl/5 mM tris-phosphate $p$H 7.2). External KCl was removed by centrifugation (1 h, 105.000xg, 20°C) and pellet washed (twice) with the same buffer. Liposomes containing potassium were finally resuspended in isoosmolar buffer (40 mM choline chloride/5 mM tris-phosphate, $p$H 7.2) [6].

### C. Instrumentation

The microwave source was a 8-12.4-GHz $x$-Band YIG-Gunn oscillator and driver (Systron Donner Mod. SDYX-3000-130

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HP). The microwave radiation at 9.2 GHz was delivered through an isolator (Systron Donner, DBG-480-A) to a directional coupler and then to a rectangular waveguide section (Systron Donner, DBG-210) with a variable matched load (Systron Donner, DBG-969-1) at the end. One-hundredth of the incident microwave power was delivered through the directional coupler to a power meter (Hewlett-Packard 432-B). The directional coupler was connected in various configurations to measure the incident, reflected, and transmitted power levels.

A test tube containing the liposomes was inserted through the wall (0.5-cm-diameter hole) of the waveguide. The tube was so positioned that the long axis was aligned with the center of the waveguide as described by Livingston *et al.* [7]. The sample of liposomes was irradiated for 1.5 h. Energy absorption was determined calorimetrically according to the method of Allis *et al.* [8]. A chromel-constantan thermocouple with a 0°C reference junction was used to monitor the temperature rise of the sample during irradiation. The thermocouple output was connected to a digital thermometer (Omega 412A). The response time (2–3 s) was small compared to the time scale of the events studied.

A 1.5-h exposure time at an incident power of 60 mW at 9.2 GHz increased the temperature of the sample approximately 3°C above the ambient temperature, and the energy absorbed by the liposomes was calculated to be 20 mw/g.

A second test tube containing the same sample was heated for 1.5 h to the same temperature above ambient (3°C) by a heat-regulated (±0.1°C) water bath. A third sample was maintained at room temperature (ambient ±0.1°C) and used as a control.

### *D. Measurements of Cation Permeability*

The induced cation permeability by gramicidin D was studied for liposomes maintained at room temperature, conventionally heated, and microwave irradiated by measuring the optical extinction at 450 nm ($E_{450}$) according to the method of Bangham *et al.* [9]. This method has correlated volume changes in liposomes with changes in absorbance reading at 450 nm. The volume of liposomes is proportional to the reciprocal of the extinction coefficient.

The absorbance of liquid dispersions was continuously recorded on a Perkin–Elmer model 356 dual-beam spectrophotometer using quartz cuvettes of 1-cm path length at 450 nm.

## III. Results

A 40-$\mu$l aliquot of the suspension of liposomes containing $K^+$ (remained at room temperature, irradiated, and waterbathed) was added to 2.45 ml of 40-mM NaCl or RbCl buffered at *p*H 7.2 with 5-mM tris-phosphate. The suspension was equilibrated for 15 min, after which time 1 ml of it was added in a cuvette, and an absorbance reading was performed at 450 nm. The $E_{450}$ of the three samples was the same and remained unchanged over a period of 30 min, so any passive $K^+$-cation exchange was excluded.

The same experiments were again performed, but after equilibration, 0.5 $\mu$g of gramicidin D was added in the cuvette containing liposomes, and spectrophotometric changes in $E_{450}$ were recorded. The rate and extent of the shrinking depends on the ease of $K^+$-cation exchange mediated by gramicidin D.

In Fig. 1, it is seen the gramicidin D caused volume changes for nonirradiated liposomes containing potassium. The initial $E_{450}$ was 0.52. At point *G* in Fig. 1, 0.5 $\mu$g of gramicidin D was added. The extent of the volume changes indicated that gramicidin D facilitated movement of cations in the order $K^+ > Rb^+$, $Na^+$.

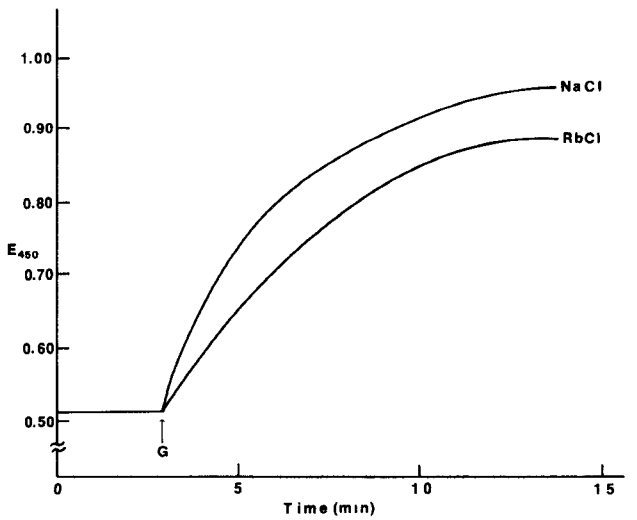


Fig. 1. Gramicidin D-induced volume changes of liposomes remained at room temperature, containing $K^+$, and resuspended in NaCl or RbCl (preparation as described in the text).

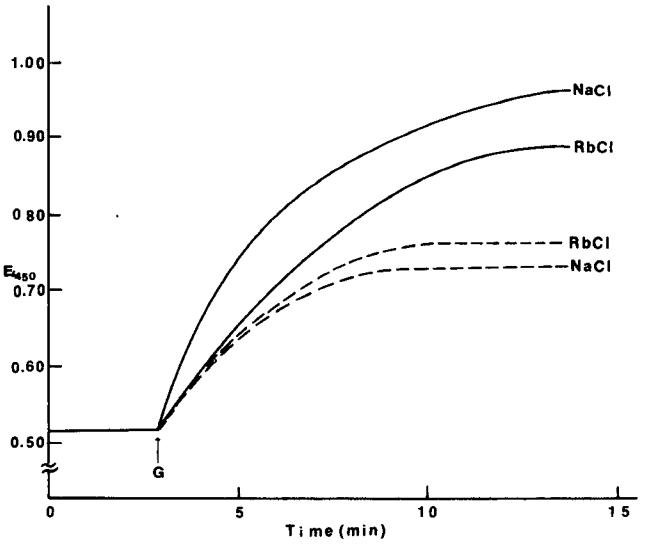


Fig. 2. Gramicidin D-induced volume changes of irradiated liposomes containing $K^+$, and resuspended in NaCl or RbCl (preparation as described in the text), in comparison with liposomes remained at room temperature. Curves for irradiated liposomes are represented with dotted lines (-----).

In Fig. 2, the gramicidin D caused volume changes for irradiated in comparison with nonirradiated liposomes. The extent of the volume changes indicates that microwave irradiation causes a modification at the action of gramicidin D. Although the order of cation selectivities remains the same, i.e., $K^+ > Rb^+$, $Na^+$, the radiation seems to facilitate the transport of $Na^+$ and $Rb^+$. The exact extent of the volume changes varies from one lipid dispersion to the other, and all comparisons were made with the same preparation.

Samples that were heated in a water bath for 1.5 h to a temperature 3°C above ambient did not show any change at the action of gramicidin D in comparison with liposomes maintained at room temperature.

The $E_{450}$ at 10 min was compared for irradiated, heated, and nonirradiated liposomes after addition of gramicidin D (Table I),

TABLE I
OPTICAL EXTINCTION AT 450 nm OF LIPOSOMES TREATED WITH GRAMICIDIN D

| Liposomes containing $K^+$ | $E^1_{450}$ at 10 min | |
|---|---|---|
| | $Na^+$ | $Rb^+$ |
| Irradiated | 0.72±0.03[2] | 0.76±0.03 |
| Heated | 0.94±0.02 | 0.87±0.02 |
| Room-temperature | 0.95±0.02 | 0.88±0.02 |

1. Absorption range 0.3 full scale
2. Data are means of three experiments ±S.D.
The initial $E_{450}$ was 0.50-0.53.

where values represent the mean of three experiments ±S.D. The student t-test analysis was used and the values were found to be significantly different for irradiated liposomes compared to those maintained at room temperature. No significant difference was observed for liposomes heated by the water bath compared to those maintained at room temperature ($p \leqslant 0.01$).

## IV. CONCLUSIONS

The results presented in this work are for an exposure of liposomes containing $K^+$ and 9.2 GHz continuous irradiation for 1.5 h with an energy absorption of 20 mW/g. We found that irradiation modifies the gramicidin D-induced permeability to the cations $K^+$, $Na^+$, and $Rb^+$, through the liposomes and seems to facilitate the movement of $Na^+$ and $Rb^+$ across the membrane. Liposomes heated to the same temperature by a water bath did not react any differently to the action of gramicidin D than did those maintained at room temperature.

Our results provide evidence that microwave radiation induces changes to the structure of liposomes and, as a consequence, the conducting state of gramicidin D channels is modified.

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# A Multifrequency Water-Filled Waveguide Applicator: Thermal Dosimetry *In Vivo*

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***Abstract*—A new horn-shaped waveguide hyperthermia applicator, operating in the range 300–1000 MHz, has been designed. The applicator is filled with deionized water acting as both a dielectric and a cooling fluid. Preliminary tests indicate that proper heating can be achieved at frequencies of about 340, 440, 560, and 690 MHz. A very low level of environmental pollution was observed. Thermodosimetry has been carried out on two young female sheep. Measurements *in vivo* have been carried out using up to 5 temperature sensors in different positions. The results indicate the occurrence of different temperature trends if the water is maintained at 15, 23, 30, and 35°C.**

## I. INTRODUCTION

Reliable measurements in the field of radio frequency hyperthermia can be performed only on the condition that one is able to heat to a desired constant temperature level a satisfactorily thick stratum of living tissue.

Roughly, if we temporarily neglect tissue inhomogeneity, power deposition—and therefore temperature elevation—owing to medium attenuation, decreases exponentially from skin-applicator contact surface to inner layers. Therefore, it is necessary, as a first step, to reduce the temperature peak that will develop in the skin, by efficient cooling means characterized by an exponential decrease steeper than radiofrequency heating decrease. So it is possible to get a much smoother maximum—hence, a wider working range—at a depth depending on the external parameters: frequency, RF power, and temperature imposed on skin surface by cooling system (Fig. 1).

## II. THE APPLICATOR

Efficient microwave applicators have to provide the following:

- deep heating and variable surface cooling,
- matching when in contact with the body and low environmental RF pollution,
- best fitting to irregular skin surfaces,
- large bandwidth for frequency diversity applications.

As an outcome of these requirements, the implementation of a ridged horn (Fig. 2) filled with high-permittivity liquid dielectric and working in the bandwidth from 300 to 1000 MHz has been chosen.

The reason leading to this choice is straightforward. To heat deeply and yet to get a reasonably sized applicator, it is necessary to use relatively low frequencies and a high-permittivity dielectric. This latter characteristic is very useful also to make easier the matching to living tissues (and/or relevant phantoms), while radiation in air is hindered by the heavy discontinuity between water and air permittivity and by the very small dimensions of the applicator with respect to air wavelength. Consequently, very

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