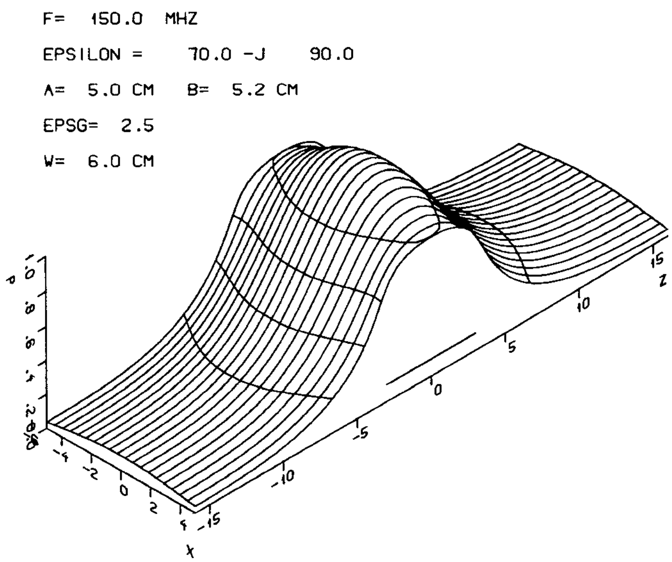


Fig. 2. Plot of relative power distribution in muscle cylinder excited by quasistatic field distribution in gap in metallic cylinder. A layer of lossless dielectric separates the tissue from the metal. The contour lines correspond to $P = 0.8, 0.6, 0.4$, and $0.2$.

constructive interference between the waves creates a local maximum.

Near the metallic edges the fields are singular, ($E \sim (1-(2z/w)^2)^{-1/2}$) so if $b = a$ ($d = 0$), excessive heating takes place near the edges. As $d$ is increased, this effect disappears, and the power distribution becomes insensitive to the precise aperture fields. If $d$ becomes very large, the usual coax-line mode becomes dominant with low attenuation in the axial direction, and the axial confinement of the hot spot is lost. Thus, there is an optimum value for $d$, which in the 100-MHz range is of the order a few millimeters. The edge fields are avoided, and the shielding of the tissue outside the volume of interest is effective.

The width of the gap is important in the sense that too narrow a gap leads to excessive amounts of nearfields destroying the focus obtained with the other parameters. Numerical simulations show that $w$ should be larger than approximately a quarter of a wavelength in the lossy medium.

Fig. 2 shows the theoretical result for a muscle cylinder of 10-cm-diameter and a complex permittivity of $\epsilon = 70 - j90$. The lossless dielectric has a thickness of only 2 mm, which seems to be sufficient for suppression of the singular edge fields. The aperture has a width of 6 cm, and there is a clearly developed focus which is in marked contrast to the standard coil excitation of a cylinder with a zero on the axis.

## III. Experimental Results

Experiments were performed in phantom material simulating muscle tissue. The diameter was 10 cm and the length about 40 cm. The lossy material was surrounded by a thin shell (2 mm) of low-loss dielectric, $\epsilon \sim 2.5$, which had a close fit to the metallic shield. The gap may be excited in many ways, but for this experiment a balanced RF voltage was applied between the two sleeves four points around the circumference. Lagendijk [4] has used an additional outer conductor for axially symmetric excitation of the gap. The power distribution was determined by measuring the temperature gradients in time after the application of power. A probe of eight thermistors was placed in a radial direction at three different axial positions (see Fig. 1). For a discussion of the temperature measurement problems, the reader is referred to [5]. Results of the measurements are shown in Fig. 3, together with theoretical results. Although there are some discrepancies, a well-defined focus is clearly seen. The results obtained cannot be directly scaled to other dimensions and other frequencies since the material constants of tissue are frequency dependent, but the general philosophy of a gap-excited cylinder is valid.

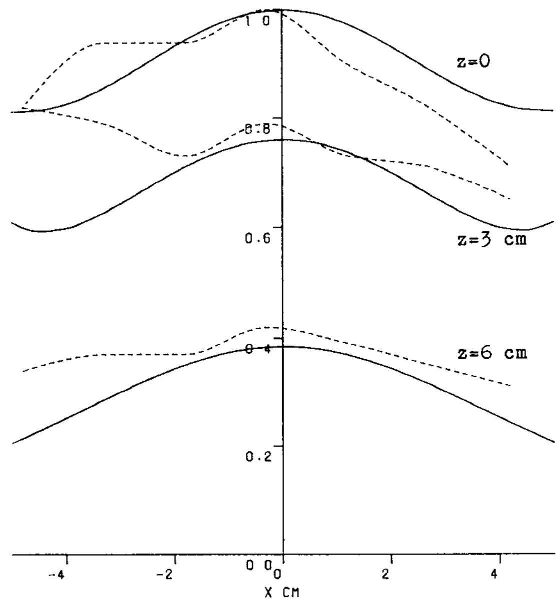


Fig. 3. Relative power distribution as in Fig. 2 for different $z$-values. $z = 3$ cm corresponds to the position of the edge ——— theoretical results (as in Fig 2), ----- experimental results.

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# On The Nonthermal Microwave Response of Drosophila Melanogaster

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***Abstract*—The fertility of microwave-irradiated fruit flies was investigated in an experiment conducted at 40 GHz and at a low power level to**

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TABLE I
TIME OF EXPERIMENTS AND NUMBER OF OFFSPRING OF CONTROL "$x$" AND OF IRRADIATED INSECTS "$y$" OF SIX EXPERIMENTS I TO VI

| | I | | II | | III | | IV | | V | | VI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ | $\Sigma\bar{x}$ | $\Sigma\bar{y}$ |
| Time of experiment | Oct.-Dec. 1981 | | Jan.-Apr. 1982 | | July-Oct. 1982 | | July-Nov. 1982 | | Jan.-Mar. 1983 | | Jan.-Apr. 1983 | |
| P | 2059 | 4343 | 2591 | 4148 | 3504 | 3296 | 2121 | 2354 | 2474 | 2016 | 2746 | 3167 |
| $\Sigma\bar{y}/\Sigma\bar{x}$ | 2.11 | | 1.6 | | 0.94 | | 1.11 | | 0.82 | | 1.15 | |
| $F_1$ | 2524 | 1931 | 2905 | 3767 | 1078 | 1995 | 1652 | 1058 | 2179 | 2352 | 2372 | 1905 |
| $\Sigma\bar{y}/\Sigma\bar{x}$ | 0.77 | | 1.3 | | 1.85 | | 0.64 | | 1.08 | | 0.80 | |
| $F_2$ | 2718 | 1624 | 3689 | 3662 | 1513 | 1272 | 1110 | 1308 | 1156 | 1062 | 1492 | 1867 |
| $\Sigma\bar{y}/\Sigma\bar{x}$ | 0.60 | | 0.69 | | 0.84 | | 1.18 | | 0.92 | | 1.25 | |

$P$, $F_1$, and $F_2$ are the offspring of the paternal, first, and second filial generations, respectively.

**avoid thermal effects. In a series of six experiments with 82 910 flies, the fertility of irradiated flies and of their first and second filial generation was determined. No radiation effect was found.**

**This result is contrary to previously published data.**

## I. INTRODUCTION

Recently, numerous and speculative reports have been published on nonthermal microwave-induced biological effects. Some reports have been devoted to effects observed with irradiated fruit flies (Drosophila melanogaster) [1]–[4]. These studies concern properties such as fertility, tumor incidence, viability, and mutagenic action. The proof of such hypothetical microwave-induced nonthermal effects in biological systems is of vital importance for at least two reasons: 1) ecological problems and 2) basic research.

We report on an interdisciplinary investigation of nonthermal microwave effects in Drosophila melanogaster (strain Berlin). The investigative team consisted of physicists, biologists, and a physiologist. The study deals with the fertility of irradiated insects over three generations after microwave exposure. A total of 82 910 insects were observed in this study lasting two years.

## II. EXPERIMENTAL PROCEDURE AND RESULTS

Pupae were exposed to microwave intensities of about 10 $\mu W/cm^2$ at a frequency of 40 GHz for 120 h. Calorimetric measurements proved that a potential temperature rise could be ruled out since it could not exceed 0.05°C. Control insects were bred in the same environment except without microwave radiation. The experiments proceeded as follows: Two groups of pupae were taken from the same egg deposition. One group was irradiated for 120 h in the pupae state. The other group was kept as controls. A few hours after becoming an adult fly, one female and two male insects from both groups, respectively, were crossed to start a family. From each group, ten such families were founded and their offsprings were counted. The number of offsprings, i.e., the children of the irradiated generation and of the corresponding control insects, represented the fertility of the $P$ generation. In order to measure the fertility of the two successive generations $F_1$ and $F_2$, the previous procedure was repeated by always crossing one female and two males from the offsprings of the irradiated and untreated insects, respectively. If in the filial generations $F_1$ and $F_2$ no significant changes in fertility occurred, belated effects due to radiation exposure could be excluded. The complete experiment was repeated in six series I, $\cdots$, VI during two years.

In Table I, the results of the six experiments are presented. The number of offsprings of control insects "$x$" and of irradiated insects "$y$" are given in this table. The data are displayed as the relative deviation of the fertility of irradiated from that of control insects in Fig. 1. Recently, such a presentation of data was often used in order to give evidence of potential nonthermal microwave effects in biological systems, see for instance [1], [2], [4]–[6]. The data on fertility shown in Fig. 1 inclines one to recognize an increase in fertility in the generations $P$ and $F_1$ and a decrease in generation $F_2$ due to microwave irradiation. However, as seen by inspecting Table II, the number of offsprings are characterized by a high standard deviation.

The magnitude of variance indicated a special primary unknown influence. To test the constancy of the experimental conditions, the primary information base was expanded to include a series of six experiments. The complete material will be analyzed in the following.

## III. DISCUSSION AND CONCLUSION

1) There was no correlation between irradiated and untreated families. The correlation coefficient for all combinations $\bar{r}$ was not significant ($\alpha = 0.05$). The same held for the correlation coefficients of single groups (see Table II). Therefore, it can be assumed that no general influences (as a variation of air pressure, season, cosmic rays, etc.) were effective for both irradiated and

TABLE II
RANDOM SAMPLES OF FERTILITY IN THE GENERATIONS $P$, $F_1$, AND $F_2$

| Families | P (IV) x | P (IV) y | $F_1$ (III) y | $F_1$ (III) y | $F_2$ (VI) x | $F_2$ (VI) y |
|---|---|---|---|---|---|---|
| 1 | 171 | 92 | 100 | 72 | 198 | 172 |
| 2 | 285 | 286 | 75 | 128 | 248 | 81 |
| 3 | 14 | 400 | 30 | 443 | 141 | 83 |
| 4 | 273 | 317 | 83 | 126 | 379 | 266 |
| 5 | 0 | 95 | 165 | 194 | 63 | 42 |
| 6 | 168 | 430 | 385 | 134 | 0 | 168 |
| 7 | 99 | 0 | 30 | 88 | 71 | 267 |
| 8 | 429 | 254 | 62 | 83 | 167 | 455 |
| 9 | 212 | 218 | 65 | 134 | 128 | 79 |
| 10 | 470 | 262 | 83 | 593 | 97 | 154 |
| n | 10 | | 10 | | 10 | |
| $\bar{x};\bar{y}$ | 212 | 235 | 108 | 200 | 149 | 187 |
| ±s | 158 | 128 | 105 | 175 | 108 | 116 |
| $V_k$ | 74 | 59 | 97 | 88 | 72 | 62 |
| r | 0.21 n.sig. | | - 0.15 n.sig. | | 0.15 n.sig. | |
| D | 23.3 | | 91.7 | | 37.5 | |

$x$ = control and $y$ = irradiated insects; $n$ = numbers of families; $V_K$ coefficient of variance = $(s/\bar{x}\cdot 100)$; $r$ = correlation coefficient $(x, y)$; $D = |\bar{x} - \bar{y}|$. For roman numerals see Table I.

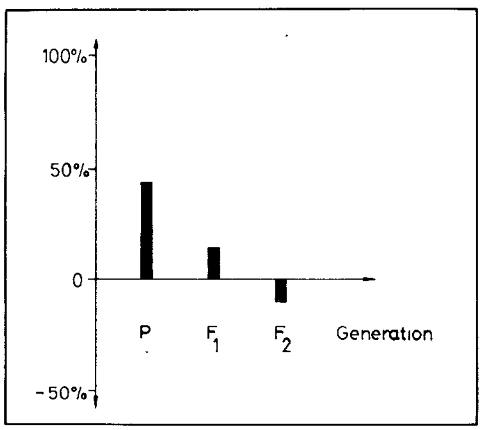


Fig. 1. Deviation $[100((\Sigma y/\Sigma x)-1)$ percent] of the fertility of irradiated insects $P$, of their nonirradiated children $F_1$, and of their nonirradiated grandchildren $F_2$ relative to untreated insects. The data are representing the experiments I to IV.

untreated groups simultaneously. It is possible to combine pairs of offspring of irradiated and untreated ancestors stochastically.

2) All six experiments show the same variability during a time of experimentation of two years. This was proven by constructing a temporal series of the coefficients of variance (see legend Table II) and testing it with two independent nonparametric methods (Runs and Spearman's Range Test). Therefore, there was no indication of a systematic change of the experimental procedure dependent on time.

3) In spite of strong differences in both directions between the absolute numbers of offspring of irradiated and untreated insects (e.g., $P_{I,\bar{y}}/P_{I,\bar{x}} = 434/206$ or $F_{2,I,\bar{y}}/F_{2,I,\bar{x}} = 162/272$), there was not one single case where the Null hypothesis was to be rejected. There was no significant change of fertility due to microwave irradiation (see Fig. 2).

4) The number of offspring per family was extremely variable under all experimental conditions. The observed span was 0 to 669 for one family. Both extrema, however, were not outliers in statistical terms. 13.3 percent of 360 families had no offspring, and 18 percent of these families had between 0 and 50 children. In the range between 551 and 669 children, there were still 4.4 percent of the families. The distribution was nonGaussian and irregular to the extent that the prime standard deviations and arithmetic means were close to equality. Thus, the applied tests all had to be nonparametric.

5) There is a significant influence on the number of offspring by the succession of generations of both the irradiated and untreated insects. This was revealed by analysis of variance (see Table I). The relative reduction of offspring of all groups is in $F_1 = 74$ percent and in $F_2 = 64$ percent ($P = 100$ percent). There

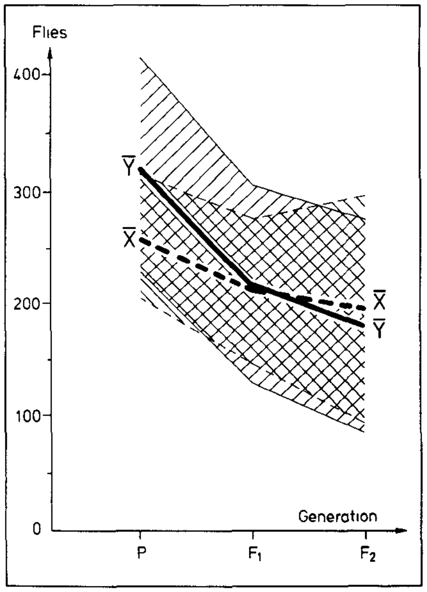


Fig. 2. The average offspring of all the six experiments. The solid lines give the value of irradiated, the broken lines those of untreated insects. The prime standard deviation of both populations is displayed. They are overlapping in a broad area.

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].


## Acknowledgment

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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 KC1/5 mM tris-phosphate $p$H 7.2). External KC1 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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