the design of these experiments, and the Amperex Electronic Corporation, Hicksville, N. Y., for the two magnetrons that were used in this work.

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Evidence for Nonthermal Effects of Microwave Radiation: Abnormal Development of Irradiated Insect Pupae

RUSSELL L. CARPENTER AND ELLIOT M. LIVSTONE

Abstract—Several investigators have reported experiments in which microwave radiation caused biological damage at tissue temperatures which were not harmful when brought about by means other than microwaves. To study the effects of 10-GHz CW radiation on a poikilothermic invertebrate animal, we irradiated early pupae of the mealworm beetle, *Tenebrio molitor*. Each pupa was inserted in a waveguide and irradiated therein at waveguide powers of 80 mW for either 20 or 30 min or at 20 mW for 120 min, after which their subsequent development was observed. In control groups similarly treated, except that no microwave power was applied, 90 percent metamorphosed to become normal adult beetles. In the irradiated groups only 24 percent developed normally; 25 percent died and 51 percent developed abnormally. In half of the abnormal animals, the front half had undergone metamorphosis to form a normal beetle head and thorax but the hind part remained in the pupal state. Temperature increases within pupae were recorded during irradiation. When these thermal conditions were duplicated by means of radiant heating, subsequent development of pupae was normal in 80 percent of the experiments. We therefore concluded that the abnormalities induced by microwave radiation were not a thermal effect.

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R. L. Carpenter is with the Northeastern Radiological Health Laboratory, Bureau of Radiological Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Winchester, Mass.
E. M. Livstone is with the Presbyterian University Hospital, Pittsburgh, Pa.

Introduction

In a recent evaluation of the biological effects of exposure to microwave radiation, Cleary [1] stated, "In general, the results of experiments carried out in this country suggested that thermal damage was the primary effect of excessive exposure to microwave radiation." He included in his review, however, a number of studies which tended "to suggest that nonthermal microwave fields at various frequencies interact with intracellular moieties to alter metabolic and/or genetic processes." Pointing out that "interpretation of the significance of these findings is difficult, since mechanisms are not specified," he concluded, "In view of the many uncertainties, additional research should be performed in the area of nonthermal effects of UHF and SHF radiations."

As a result of experiments dealing with the induction of cataracts in the eyes of rabbits exposed to 2450-MHz radiation [2], we suggested in 1958 that "the cataractogenic effect of microwave radiation at this frequency is not primarily a thermal effect." Further evidence supporting this view came from a study of cumulative effects of repeated exposure of the eye to microwave doses which were below the cataractogenic threshold [3].

Experiments on the effect of microwave radiation on insect development, which involved irradiation of pupae of the mealworm beetle, *Tenebrio molitor*, have yielded results which also suggest a nonthermal effect. These experiments have been referred to previously [4], but have been reported only in part. Before presenting them more completely here, and in view of the discussions which periodically erupt concerning thermal versus nonthermal effects, it may be appropriate first to cite some additional evidence from a variety of animal experiments reported in the literature in which microwave radiation has caused biological damage at tissue temperatures which are not harmful when brought about by other means.

Abnormal Development of Chick Embryos

Van Ummersen [5] subjected chick embryos at the 48-h stage of development to 2450-MHz CW radiation through the intact shell from a dipole antenna with reflector. After $4\frac{1}{2}$ min of irradiation at 400 mW/cm², 7 to $8\frac{1}{2}$ min at 280 mW/cm², or 13 to 14 min at 200 mW/cm², the eggs were returned to the incubator at 39°C for another 48 h. The embryos were then studied at the 96-h stage of development. Of 183 embryos so treated, 16 had continued to develop normally, 48 had died, and 119 had developed abnormally. In general, the abnormalities appeared to be the result of inhibition of growth and/or differentiation, so that many embryos were only as large as 72-h rather than 96-h embryos. In many cases, further differentiation of the brain, eye, wing buds, and heart had been inhibited. Development of hind limbs, tail, and allantois was suppressed. The temperature of the yolk at a position immediately beneath the embryo was recorded at all three power densities which were employed. There was an increase in temperature and the higher the power, the more rapid was the increase. In all cases, a yolk temperature of approximately 59°C proved to be lethal.

Van Ummersen [6] obtained similar results when the embryos were irradiated within a terminal section of waveguide maintained under incubator conditions of temperature and humidity, and with provision for tuning out any mismatch resulting from the presence of the egg in the waveguide. At the waveguide powers employed, abnormalities of development resulted from 5- to 12-min irradiation or, at a lower power, from 14- to 16-min irradiation. Yolk temperature reached 56° to 57°C during the period of exposure.

This system made it possible to irradiate embryos at very low powers for long periods of time while they were still maintained under incubator conditions. With waveguide power reduced by a factor of 10 and exposure period increased to 280 or 300 min, irradiation still caused the embryos to develop abnormally. During this long exposure period, the temperature of the yolk was increased to 42.5°C, a rise of 3.5° above normal incubator temperature.

To test whether this 3.5° temperature rise could have caused the abnormal development, Van Ummersen incubated 62 fertile eggs at 39°C for 48 h and then transferred 41 of them to a second incubator having a temperature of 42.5°C for 5 h. Continuous recording from one of the eggs showed a rise in yolk temperature corresponding closely with that which had been recorded during the 5-h waveguide irradiation of embryos.

After 5 h in the warmer incubator, the eggs were returned to the 39°C normal incubating temperature. When they were examined at the end of the total incubation period of 96 h, no abnormalities were found to have occurred; they differed in no respect from the 21 control embryos which had not been subjected to the elevated temperature. Van Ummersen therefore concluded that since irradiation at a low power level for a long period induced abnormal development of chick embryos, while the amount of the accompanying temperature increase was demonstrably not harmful, then some factor other than a thermal one must have operated to bring about the observed biological effect.

Electrocardiogram Changes in Embryonic Hearts

Paff *et al.* [7] tested the effect of 24-GHz radiation on isolated hearts from 72-h chick embryos. At a level of 75 mW/cm², the thermal effect was so slight that the cardiac rate did not deviate from normal limits. Nevertheless, changes were observed in the electrocardiogram in all cases and these persisted after the irradiation had been terminated. There was a consistent elevation of the *ST* segment and an inversion of the *T* wave. They concluded that the metabolic activity of the heart had been affected and that at the level of microwave power employed in their experiments, the operative factor was other than a thermal one.

Impairment of Male Endocrine Function in Rats

In a study of the effect of 24-GHz radiation on the male endocrine system of the rat, Gunn *et al.* [8] made use of the finding that the capacity of the dorsolateral lobe of the prostate gland to concentrate zinc is controlled by the male sex hormone, testosterone, the output of which depends, in turn, upon gonadotrophin secretion by the pituitary gland. By administering Zn-65, the uptake of this isotope by the prostate gland can be measured and used as an index of testosterone secretion by the interstitial cells of the testes.

When microwave radiation was applied to the testes of the rat, various degrees of testicular damage occurred, depending upon exposure time, and the capacity of the dorsolateral prostate to concentrate Zn-65 was diminished by 45 to 70 percent. This was an indication of reduced testosterone secretion, even though the interstitial cells responsible for such secretion appeared normal histologically. Administration of testosterone restored Zn-65 uptake to control levels, thus demonstrating that microwave radiation had indeed impaired testosterone secretion.

The question of whether this impairment was simply a thermal effect was next investigated, even though the bulk of reported experimental evidence indicates that it is the sperm-producing germinal epithelium, rather than the interstitial cells, which is peculiarly susceptible to thermal damage. In one group of rats the testes were exposed to microwave radiation for 5 min, at the end of which time the intratesticular temperature was found to be 41°C. A second group of rats was exposed to infrared radiation with the distance of the infrared source from the scrotal area adjusted so that the 5-min exposure also produced an intratesticular temperature of 41°C.

Two weeks later the animals of both groups were injected with tracer doses of Zn-65 and its uptake by the dorsolateral prostate gland was determined. In the microwave-exposed rats, Zn-65 uptake was depressed by 45 percent, indicating impairment of the male endocrine system. In the infrared-exposed animals, Zn-65 uptake was no different from control levels, indicating that there had been no damaging thermal effect from the increased intratesticular temperature. They felt that these experiments pointed toward an athermal effect of microwaves.

Reduction of Lens Ascorbic Acid Content

Merola and Kinoshita [9] reported certain biochemical changes in the crystalline lens of rabbits whose eyes had been exposed to microwave radiation of 2450-MHz frequency. The earliest observable change was a substantial drop in the ascorbic acid content of the lens which occurred within 18 h following irradiation. In order to determine whether this could have been caused by an increase in intraocular temperature during irradiation, lenses were examined ½ h and 6 h after exposure to microwave. In neither instance was there a change in ascorbic acid, indicating that it was during the period of 6 to 18 h after irradiation that the process bringing about the depression of ascorbic acid took place. These findings, they concluded, ruled out the possibility that the temperature increase during exposure could be the immediate cause of the disappearance of ascorbic acid.

Kinoshita *et al.* [10] provided further evidence that the destruction of ascorbic acid was not simply a thermal effect. They kept isolated rabbit lenses at 45°C for 8 min; this was the temperature attained in the eyes exposed to microwave for that length of time. There was no effect upon the normal ascorbic acid level of these lenses.

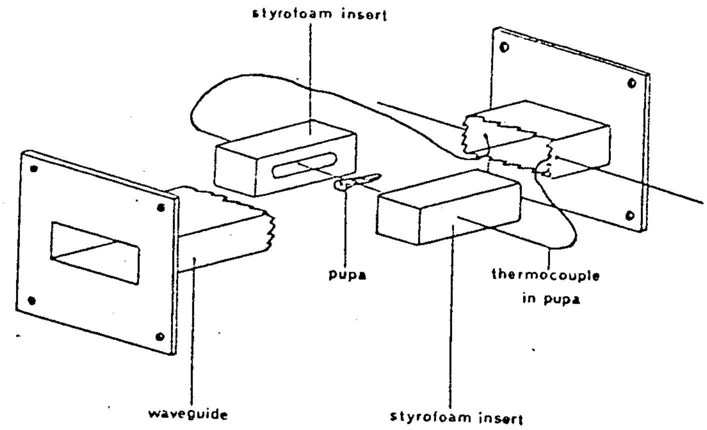

Fig. 1. Arrangement for irradiating *Tenebrio molitor* pupae in 10-GHz waveguide. Positioning of thermocouple is also shown.

Abnormal Development of Irradiated Insect Pupae

Van Ummersen [5] observed that microwave radiation appeared to inhibit cellular differentiation in the developing chick embryo. Structures which had already become differentiated continued to grow by cell proliferation but underwent little or no further differentiation during a 48-h period following irradiation. Structures which had not begun to differentiate prior to irradiation failed to do so subsequently, so that their development was effectively suppressed.

To discover whether microwaves might exert a similar effect on the development of a poikilothermic form such as an invertebrate, we performed a series of experiments on pupae of the "mealworm" beetle, *Tenebrio molitor*.[1] In the normal development of this insect, the pupation period lasts 9 or 10 days, at which time the adult stage, or beetle, emerges.

For the purpose of irradiation, individual pupae in the first or second day of pupation were placed in a depression centered in a two-piece styrofoam block which was then inserted in a section of waveguide (Fig. 1) so that the pupa was ventral side up with anterior end toward the radiation source, a klystron tube operating at 10 155 MHz. With the appropriate components in the waveguide train, it was possible to minimize the VSWR by tuning out the mismatch caused by the pupa and/or the waveguide termination, and to adjust the power to which the organism was subjected in the waveguide. Following irradiation, each pupa was returned to its individual glass container, in which emergence as an adult beetle occurred several days later.

In addition to the irradiated pupae, two other groups were followed through the pupation period. One group

[1] These experiments were carried out in the Microwave Radiobiology Laboratory, Department of Biology, Tufts University, Medford, Mass., and were supported by Public Health Service Research Grant GM 09495-03 from the National Institute of General Medical Sciences.

consisted of control animals which were raised in individual glass vials and received no treatment or manipulation during the entire pupation period. In a second group termed waveguide controls, all the pupae were treated in a manner similar to that accorded the irradiated ones, i.e., placed in a styrofoam block inside the waveguide for a similar period, but with the difference that no power was applied. All three groups were kept at room temperature.

The length of the pupal period, the condition in which the adult emerged, and the length of adult life were recorded for all animals. On the basis of their observed state at emergence, they were assigned to one of the following five categories (Fig. 2).

1) Pupal death during pupation period.
2) Abnormal adults in three grades of abnormal development:
   a) grade 1 anomaly: normal head and thorax; pupal abdomen with pupal case sometimes attached; wings and/or elytra (wing covers) absent, reduced, or shredded;
   b) grade 2 anomaly: normal adult head, thorax, and abdomen; wings and/or elytra rumpled or shredded;
   c) grade 3 anomaly: normal adult except for discrete holes in elytra.
3) Morphologically normal adult beetles.

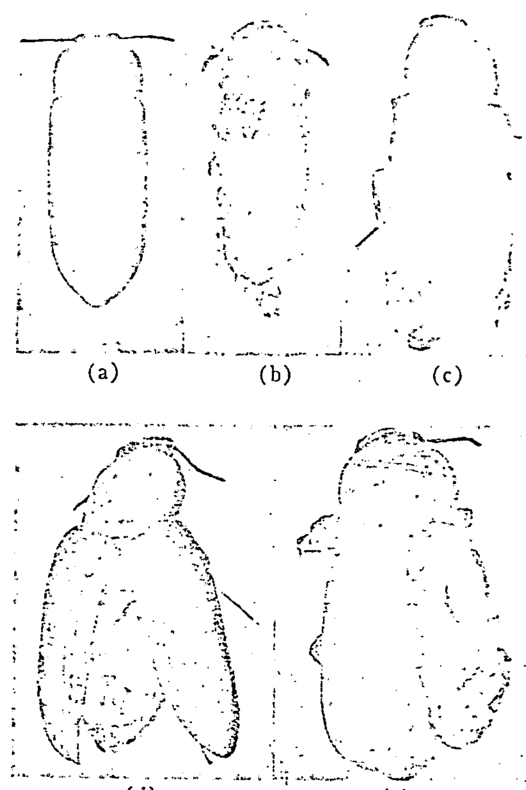

Fig. 2. Irradiation induced abnormalities in *Tenebrio molitor*. (a) Normal adult beetle. (b) Grade 1 anomaly. (c), (d), and (e) Grade 2 anomalies.

The results of 140 experiments are presented in Table I, together with data on the control series. From the 7 deaths during the pupal period and the 8 abnormally developed beetles which occurred in the 137 untreated controls, it is apparent that even under undisturbed conditions the process of metamorphosis was successful only 90 percent of the time. This proportion was also seen in the 44 waveguide control pupae which were placed in the waveguide but not irradiated.

In contrast, of the 90 pupae irradiated for 20 min at a waveguide power of 80 mW, 25 percent died before completing metamorphosis, 51 percent emerged and were abnormally developed, and only 24 percent accomplished normal development. In more than half of the affected animals, the anterior portion had undergone metamorphosis and formed the head and thorax of a normal adult beetle but the abdominal part of the body remained in the pupal state. In some cases, the wings and wing covers (elytra) had failed to develop; in other cases, they were small or in a shredded condition.

When waveguide power was reduced to 20 mW and the period of irradiation increased to 2 h, only 5 of 25 pupae developed normally, 1 died, and 19 underwent incomplete or imperfect development.

Another effect of irradiation was the prolongation of the pupation period by 3 days; the irradiated animals generally took 12 days to emerge as adults compared to 9 days for those of the two control series. The length of adult life was also affected. Under laboratory conditions, most of the normal adults lived for 13 to 20 days. The abnormal beetles had a shorter adult life, with those classed as grade 1 anomalies living only 3 to 9 days. The less severe grade 2 anomalies lived 9 to 14 days, and grade 3 anomalies lived 9 to 18 days.

A comparison of the thermal response of pupae was made for the 20-min exposure at 80 mW and the 120-min exposure at 20 mW. For this purpose, a fine copper-constantan thermocouple junction was placed in the abdomen and the pupa positioned in its styrofoam block in the waveguide. The thermocouple leads passed through small holes in the sides of the waveguide to a microvolt amplifier which fed into a strip-chart recorder (Fig. 1). Preliminary experiments in which the thermocouple alone in the waveguide continued to record room temperature when power was on indicated that the thermocouple itself was not affected by the microwaves.

At 80 mW, a temperature rise amounting to 11.5 to 12.5°C was observed in the pupae; the maximum was reached in 6 min and was maintained for the remainder of the 20-min irradiation period. At 20 mW, there was a rise of only 2.5 to 3°C in pupal temperature. This occurred during the first 7 min of irradiation and then was maintained through the rest of the 2-h period. It was found that varying the placement of the thermocouple junction in the abdomen made no difference, indicating that there were no local "hot spots" during irradiation.

Inasmuch as this amount of radiation (20 mW for 120 min) applied during the first or second day of pupation had been demonstrated to cause subsequent abnormal development, we sought to discover whether the tem-

TABLE I
EFFECT OF 10 000-MHz RADIATION ON *TENEBRIO MOLITOR* PUPAE

	Period of Time in Wave-guide	Micro-wave Power in Wave-guide	Pupal Deaths	Grade 1 Anomaly	Grade 2 Anomaly	Grade 3 Anomaly	Normal Adults	Total
Untreated controls	None	None	7 (5.1 percent)	6 (4.4 percent)	2 (1.5 percent)	0 (0 percent)	122 (89 percent)	137 (100 percent)
Waveguide controls	20 min	None	0 (0 percent)	0 (0 percent)	1 (3.4 percent)	0 (0 percent)	14 (93 percent)	15 (100 percent)
	30 min	None	1 (3.4 percent)	1 (3.4 percent)	1 (3.4 percent)	0 (0 percent)	26 (89.6 percent)	29 (100 percent)
Irradiated pupae	20 min	80 mW	20 (25 percent)	22 (27.5 percent)	10 (12.5 percent)	9 (11.9 percent)	19 (23.7 percent)	80 (100 percent)
	30 min	80 mW	8 (22.9 percent)	7 (20 percent)	1 (2.8 percent)	7 (20 percent)	12 (34.3 percent)	35 (100 percent)
	120 min	20 mW	1 (4 percent)	4 (16 percent)	10 (40 percent)	5 (20 percent)	5 (20 percent)	25 (100 percent)

perature rise of 3°C might by itself be sufficient to explain the experimental results, even though such a temperature excursion was obviously less than that which pupae might experience under natural conditions. To test the possibility, we subjected day-old pupae to an identical thermal experience achieved by means other than microwaves.

As a controlled-temperature chamber, we employed a cylindrical ceramic-coated high-power resistor, with voltage supplied through a variable transformer, so that the temperature within the cylinder could be precisely regulated. After determining that there was no appreciable temperature gradient within the resistor, we placed day-old pupae in styrofoam blocks and inserted them, five at a time, into the hollow core of the cylindrical resistor. A thermocouple junction was in the abdomen of one of the pupae and the voltage across the resistor was regulated according to the temperature recorded continually from this animal. In this manner, it was possible to duplicate the temperature conditions which were prevalent during the entire 2-h period of irradiation at 20 mW; there was the same 3° temperature rise during the first 7 min, and the elevated temperature was maintained for the remainder of the 120 min but there was no microwave radiation. Except for those used for monitoring pupal body temperature, the pupae were then returned to their individual containers for the remainder of the period of pupation.

Twenty such experiments were performed; of these, 17 metamorphosed to become normal adults and three had abnormalities. The normal development occurring in 85 percent of these experiments is in marked contrast to the 20 percent normal and 80 percent abnormal development in the case of pupae subjected to the same temperature conditions for the same length of time but under microwave radiation.

Another 20 pupae were similarly treated except that they were subjected to a temperature rise of 12°C and were kept in the resistor cylinder for 20 min, thus duplicating the temperature conditions which prevailed during irradiation of pupae at 80 mW for 20 min. In the 20 animals, there were no pupal deaths and at the end of the pupation period, 75 percent emerged as normal adults and 25 percent exhibited abnormalities. This contrasts with 25 percent pupal deaths, 25 percent normal adults, and 51 percent abnormally developed in the pupae which experienced the same temperature conditions and microwave radiation as well.

It seems logical to conclude from these results that the abnormal development induced in irradiated insect pupae cannot be explained as a thermal effect, a conclusion which is in agreement with that of Van Ummersen respecting the effect of microwaves on development of the chick embryo.

To speculate upon how microwave radiation exerts its effect upon insect development or to hypothecate as to its site of action in the pupa would be a fruitless endeavor, considering both how little we know with regard to the way microwaves react with living tissues and how much has been learned about the complex series of interrelated processes involved in postembryonic growth and metamorphosis in insects. Successful development and metamorphosis depend upon a system of delicate chemical balances involving so many enzymes and hormones and their interactions that there must indeed be almost countless targets for microwave radiation to strike. But exactly because so much is known about this complex process, we suggest that it may be a fertile and possibly rewarding field in which to explore the question of the biological action of microwave radiation.

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Quantifying Hazardous Electromagnetic Fields: Scientific Basis and Practical Considerations

PAUL F. WACKER AND RONALD R. BOWMAN

Abstract—As commonly recognized, the problem of quantifying hazardous electromagnetic (EM) fields is difficult and has not yet been satisfactorily solved. Essentially, this is because people are often exposed to emanations from powerful sources of EM fields at points close to the sources and at points where arbitrary polarization and multipath interference exist. However, the accepted concepts, standards, and most measuring instrumentation are based on simple plane-wave field propagation and so are inadequate for complicated fields.

The complications and problems of quantifying hazardous EM fields involving source-subject coupling, reactive near-field components, multipath components, and arbitrary polarization are examined in some detail. General discussion of dosimetric measurements and hazard survey measurements is given, and also some basic considerations for the design of field probes for these measurements. Recommendations are given for suitable parameters for quantifying complicated EM fields, and essential and desirable characteristics for hazard survey meters are stated. Several recently designed hazard survey probes are capable of measuring these recommended parameters in many complicated fields of interest, and improved instruments are anticipated.

Glossary

Hermitian magnitudes
: $\sqrt{s\bar{s}}$ for a scalar s and $\sqrt{V_x\bar{V}_x+V_y\bar{V}_y+V_z\bar{V}_z}$ for a vector V, where the subscripts indicate the rectangular components and the overbar indicates the complex conjugate.

Hermitian and anti-Hermitian parts of a matrix
: Hermitian: that part which is its own Hermitian conjugate (complex transpose); anti-Hermitian: that part which is the negative of its Hermitian conjugate.

Homogeneous plane wave
: Monochromatic infinite plane wave for which the planes of constant phase are planes of constant amplitude [30, p. 360].

Linearity
: (For a linear medium, see footnote 3; for a linear probe, see Section VII-B.)

Monochromatic
: Characterized by a single frequency.

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The authors are with the National Bureau of Standards, Boulder, Colo. 80302.

I. Introduction

Accepted concepts and standards for quantifying hazards from nonionizing electromagnetic fields (EM) are quite satisfactory under certain idealized conditions. However, these idealized conditions do not obtain for most hazardous situations or even for many exposure experiments; in these cases, the usual formulations must be regarded as simplistic. The authors believe that inapplicable concepts and inadequate instrumentation are responsible for much of the controversy and confusion in the study of nonionizing EM field hazards, as well as for the use of conjecture