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Effect of pulsed microwaves at X-band on skin metabolism

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Apparatus used to expose skin to a frequency of 9.6GHz with a pulse duration of 0.25μs and a repetition frequency of 4kHz is described. This gave a ratio of 1000:1 of peak to mean power. With this apparatus it was found that an exposure of 6,000mJ/cm² reduced respiratory activity of skin by 50%. Other experiments were made to determine the effect of pulsed microwave energy on certain specific aspects of skin biochemistry, especially biosynthesis of intercellular materials and specific cell components. The histology of skin after exposure to microwaves was also investigated. The findings of these experiments are compared with those obtained previously using a continuous source of radiation.

AS PART OF A PROGRAMME on the possible effects of non-ionizing radiation on biological tissues we recently reported experiments in which skin was exposed to a continuous source of microwaves at X-Band to discover what effect this radiation might have on various aspects of skin metabolism (Lawrence, 1968; Carney, Lawrence & Ricketts, 1968). Respiration of skin cells, the incorporation of phosphate into various cell components and the biosynthesis of intercellular materials were all altered after exposure to microwaves and a relationship was found between the energy density of microwaves and the change in metabolic activity. These alterations in skin metabolism were thought to be caused by heat produced within the tissue from absorbed microwave energy.

Much microwave equipment in common use emits pulses of energy rather than a continuous output, consequently materials irradiated by such a source may receive peak power levels considerably in excess of mean power. The appropriate form of averaging for biological purposes was in doubt and it was possible, in this circumstance, that effects other than those caused by heat could be produced. We therefore repeated the previous experiments with a pulsed microwave generator operating at X-Band instead of a continuous source.

Materials and methods

Thin slices (0.1 to 0.2mm) of skin cut free-hand from guinea-pig ears were used for all the experiments (Lawrence, 1961). Red-eyed white guinea-pigs of the Portland strain were used, aged 6 to 9 months. The skin slices were incubated on a standard medium which consisted of

homologous serum	5 parts
Krebs-Ringer phosphate	3 parts
glucose, 5% w/v	1 part
dihydrostreptomycin sulphate, 50γ/ml	1 part

Measurements of skin respiration were made with a differential micro-respirometer (Cruickshank, 1954). The technique was similar to that used previously to study the effect of laser energy (Lawrence, 1967) and continuous microwaves at X-Band (Lawrence, 1968) on skin. Respiration of the skin slices was measured for two hours then the tissue was removed from the respirometer and exposed to pulsed microwaves at the selected energy density. The skin

was then replaced in the respirometer and measurements continued for a further 24h. Any alteration in metabolic activity was assessed by expressing the mean respiratory rate of the 22–24h period as a percentage of the initial (0–2h) control period.

A schematic diagram of the apparatus used for exposure of the skin to microwave energy is shown in **Fig. 1**. The generator was a magnetron operating at a frequency of 9.6GHz with a pulse duration of 0.25μs and a repetition frequency of 4kHz. This gave a ratio of 1000:1 of peak to mean power. The mean power output of the magnetron was 100W. giving a peak power of 100kW. The output was obtained via a waveguide, size 16 measuring 2.286 X 1.016cm. (internal dimensions). This waveguide was smaller than that used for the rest of the system (size 15, internal dimensions 2.850 X 1.262cm), consequently it was necessary to connect the generator to the remainder of the apparatus by means of a tapered adaptor. The upper frequency limit of size 15 waveguide is 9.99GHz, so it was necessary to recalibrate all the couplers and attenuators at 9.6GHz (the original calibration being for a lower frequency).

The power divider enabled the required power to be transmitted down the main channel, remaining power being dissipated in the high power load on the power divider. Two 10dB couplers were attached to the main channel and specimens to be irradiated were introduced into the waveguide at the point X – X. The first coupler enabled any power reflected by the specimen to be measured (by attenuator *A*, crystal detector and meter M1). The second

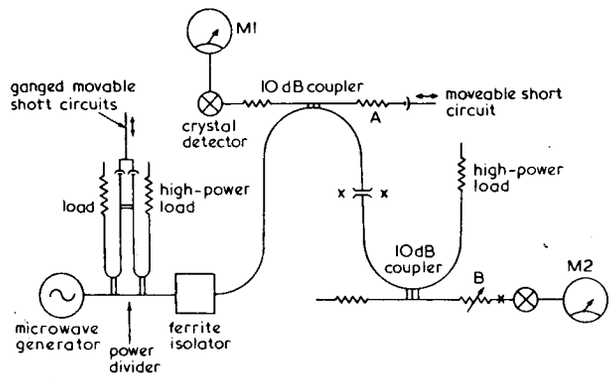

Fig. 1 Schematic diagram of microwave apparatus

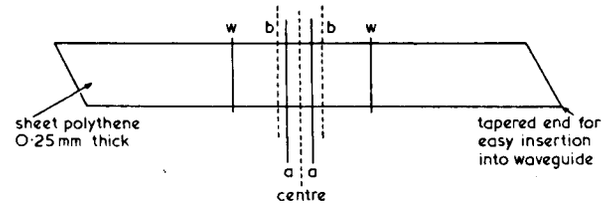

Fig. 2 Plan of polythene specimen carrier. See text for explanation of symbols

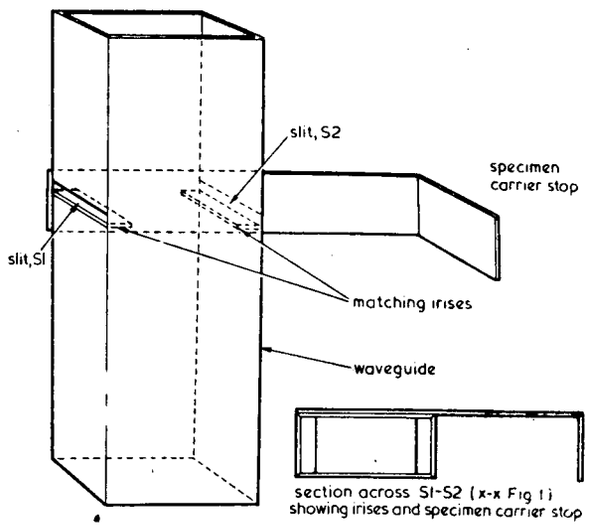

Fig. 3 Modification of waveguide to receive specimen carrier shown in Fig. 2

10dB coupler (in conjunction with attenuator *B*, crystal detector and meter M2) provided measurement of power passing through the specimen. Remaining power was dissipated in the load at the end of the waveguide.

Skin samples were introduced into the waveguide on a specimen carrier made from polythene sheet 0.25mm thick (**Fig. 2**). The waveguide at the position X – X (**Fig. 1**) was modified to receive the specimen carrier; this is shown in **Fig. 3**. Two slits (S1 and S2, **Fig. 3**) were cut across the short sides of the waveguide; S1 was 1.25mm high and S2 0.5mm. A metal strip with one end bent at a right angle was fastened to a long side of the waveguide; this provided a stop for the specimen carrier. The dimensions were such that when the polythene specimen carrier was pushed as far as possible through slit S1 the area W – W (**Fig. 2**) corresponded exactly with the internal area of the waveguide. It is important that the specimen is located in the central region of the waveguide because power distribution within the guide is only uniform across the shorter side; it is not uniform in the other direction but its distribution can be calculated. Most of the power is concentrated in the centre zone and its level can be calculated from the formula

$$\text{power (centre)} = 2P_T\frac{\Delta a}{A}$$

where P_T is total power passing down the waveguide, Δa the area of the central zone, and A the internal area of the waveguide. The formula is not quite correct in practice so that over the area a – a (**Fig. 2**), which is $0.1A$, the power distribution is 97% of the theoretical value and for area b – b ($0.2A$) 95%. Most of the skin samples fitted within the area bounded by a – a, a few specimens were a little wider; consequently the calculated power density for exposure was at least 95% accurate.

The two irises were inserted into the waveguide to neutralise the small amount of energy reflected by the polythene specimen carrier. These irises were small tongues

of metal 3mm deep which also reflected a little energy but of opposite phase to that of the carrier; accordingly one reflection neutralised the other and reflection of power from a specimen introduced into the waveguide could be measured directly.

In all experiments a strict aseptic technique was observed. The polythene specimen carrier was sterilised by immersion in 70% ethanol for 10 minutes, washed in sterile water and dried with sterile filter paper. All tissue cultures were checked for sterility after each experiment by spotting drops of the medium on 4% blood-agar plates and incubating these overnight. The incidence of contamination was low (<1%); the occasional contaminated culture was discarded.

Results

The power levels chosen for transmission down the guide were based on those used previously for continuous irradiation of skin by X-Band (Lawrence, 1968). Five basic levels were used, these were 1.7, 3.4, 6.8, 13.6 and 27.2 watts which gave the following respective power densities over the central area: 0.97, 1.94, 3.89, 7.78 and 15.5W/cm^2. Exposure times of 1, 2 and 4sec were made at each of these power levels and the total radiation dose expressed in J/cm^2. This can only be done for short exposures; if times longer than 6sec are used cooling of the specimen occurs and any effect measured is no longer simply related to intensity and time (Carney, Lawrence & Ricketts, 1968).

A typical experiment is shown in **Fig. 4**. Control skin (i.e., not exposed to microwaves) respired steadily throughout the course of the experiment (line **A, Fig. 4**) but samples irradiated with microwaves show a decrease in respiratory activity (lines B, C & D, **Fig. 4**). As might be expected, the effect is greatest at the highest exposure level. It was convenient to measure this damage to skin respiration as a percentage of the initial rate and this was done by relating the mean rate of the 22 to 24h period to the mean respiratory rate of the initial 0 to 2h control period. When the percentage respiratory damage was plotted against the microwave dose a sigmoid dose-response curve was obtained (**Fig. 5**). This curve was linearised by transferring the percentage data to a probability scale and plotting these against the logarithm of the microwave dose (**Fig. 6**).

From this curve the median effective radiation dose (ED_{50}) can be estimated with some accuracy, the ED_{50} obtained from **Fig. 6** suggests that 6.0J/cm^2 would reduce skin respiration by 50%. The ED_{30} (i.e., 30% reduction in respiratory activity after 24h) is also of interest since previous experience suggests this is a maximum 'safe' level for explants cultured *in vivo* compatible with normal histology (Lawrence, 1961, 1968, 1969a). Power density required to produce this effect was 3.98J/cm^2.

An experiment was made to measure the amount of microwave radiation absorbed by skin. This was done by using the apparatus shown in **Fig. 1** and covering the area W – W on the polythene specimen carrier (**Fig. 2**) completely with skin. The carrier was then inserted into the modified waveguide (**Fig. 3**) and exposed to pulsed microwaves at a total power of about 0.2W – a low power was chosen to avoid undue heating of the specimen whilst measurements of transmitted and reflected power were

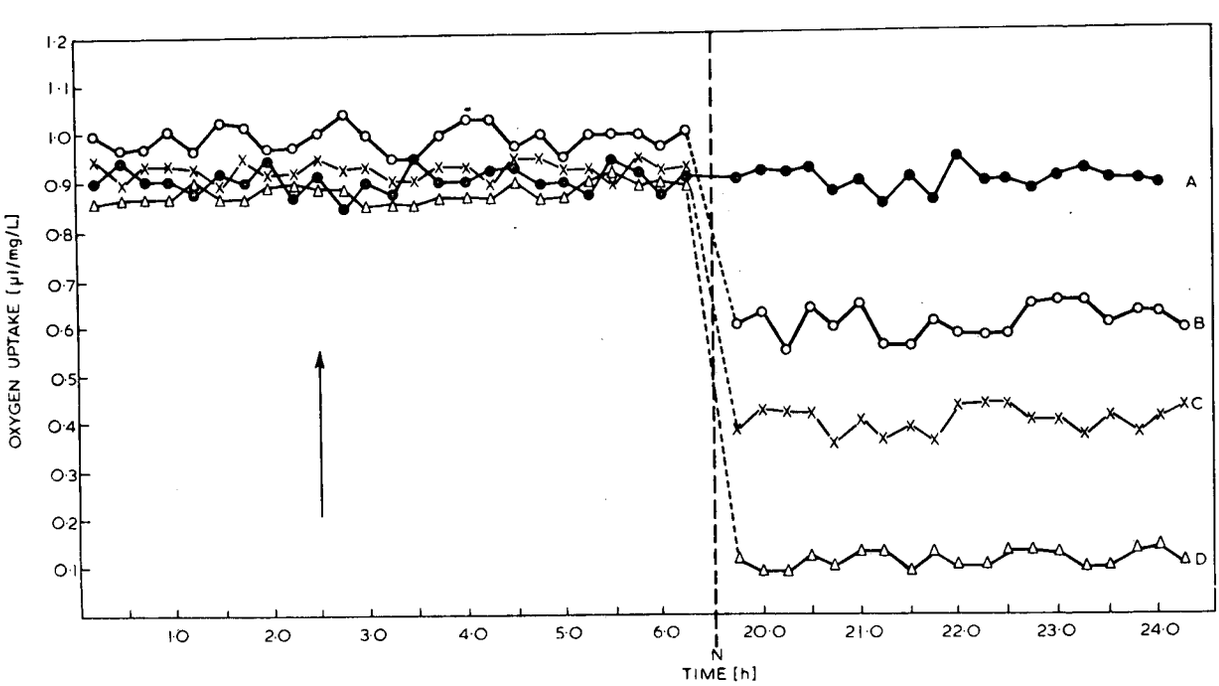

Fig. 4 Effect of pulsed microwaves at X-Band on the respiration of guinea-pig skin. Specimens were irradiated at time indicated by the arrow at the following energy levels: A, 0J/cm^2 (control); B, 3.9J/cm^2; C, 7.8J/cm^2; and D, 15.6J/cm^2. N denotes the overnight period when observations were discontinued

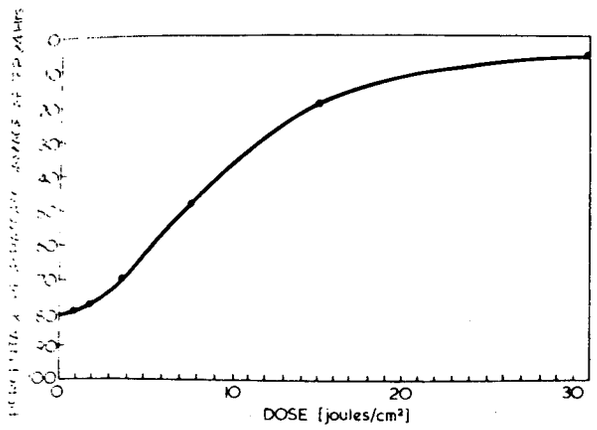

Fig. 5 Dose-response relationship for effect of pulsed microwaves on guinea-pig skin respiration

made. Standard slices of skin (about 0.15mm thick) absorbed 35% of the incident power, 30% was reflected and the remainder transmitted through the specimen.

Discussion

Sigmoid response curves relating mortality to the probability integral are commonly encountered in toxicity experiments on animals; this phenomenon can be considered as a normal distribution of susceptibility in the population and several methods for evaluating such data are available (e.g. Finney, 1947; Gaddum, 1948). A skin slice can be considered as a population of individual cells, which, due to variation in age and other characteristics, will differ in susceptibility to trauma of either physical or chemical type. The action of toxic materials, heat, laser energy and continuous microwave radiation on skin cultured *in vitro* has been evaluated by assuming such a relationship (Lawrence, 1961, 1967a, 1968, 1969); the same method was used in this investigation.

The dose-response curve for the effect of pulsed X-Band microwaves on skin respiration (**Fig. 6**) suggests that the median effective microwave (ED_{50}) dose is 6.0J/cm². Included in **Fig. 6** for comparison are the results of a previous study using a continuous source of microwave power (Lawrence, 1968); the ED_{50} of this curve was 4.75J/cm². This suggests, surprisingly, that pulsed microwaves at X-Band may be less damaging than continuous exposure. However, the difference between the two sets of observations is not statistically significant; also the ambient temperature in the current study was 2–3 deg C lower than that of the earlier experiments using continuous irradiation.

This difference in temperature can be quite important because the rise in temperature of the skin sample will be about 30 deg C above ambient temperature for an incident radiation dose of 5.0J/cm² (assuming about 1/3 of the energy to be absorbed and the specific heat of skin to be about 1.0). Thus in a typical experiment carried out in the laboratory at 22°C the final temperature of the skin will be 50–55°C. A further few degrees may well be critical: for instance skin immersed in saline at 50°C for 1sec was damaged less than specimens immersed for 2sec (Lawrence, 1969b); similarly Cruickshank & Hershey (1960) found that the respiratory activity of guinea-pig skin heated to 60°C for 1min decreased by 86% whereas reducing the heating time to ½min caused only a 20% decrease.

It seems, therefore, that expressing pulsed microwave dosage in terms of mean power is satisfactory for biological purposes; the choice of a very high peak to mean power ratio (1000:1) for this study could reasonably be expected to show some difference in effect on skin metabolism as compared with the damaging effect of continuous radiation. There is always the possibility that particular enzymes may be selectively destroyed by microwave radiation; for example, Bach (1965) reported that alpha amylase was inactivated to some extent after exposure to 12MHz. The respiration tests reported here reflect the overall effect of pulsed microwaves on skin metabolism, other experiments have been carried out on specific aspects of skin biochemistry; these included the biosynthesis of skin collagen, sulphated mucopolysaccharide (intercellular components), phosphoprotein, phospholipid and nucleic acids (intracellular components). These observations showed no specific effect of pulsed microwaves on any of these processes and will be reported in full (Lawrence, Carney & Ricketts, 1969).

All experiments to date have been made with guinea-pig skin, experience with the toxicity of therapeutic agents has shown that human skin exhibits a similar response to that of other mammals (Lawrence, 1959, 1961 & 1967(b)) and

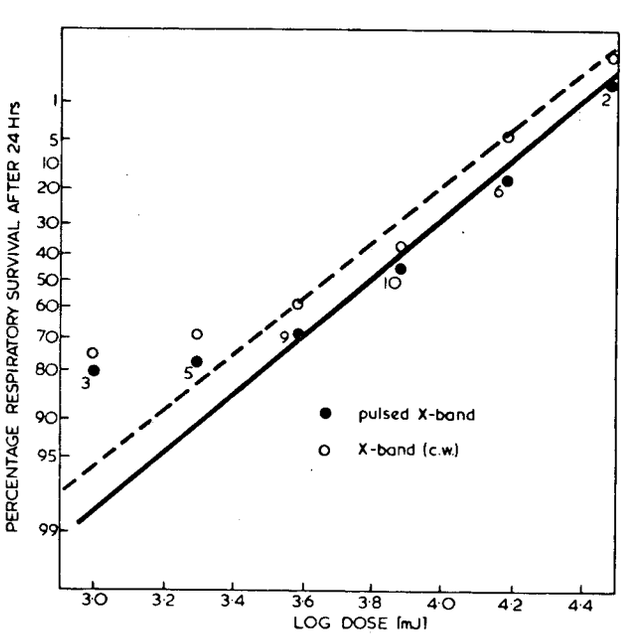

Fig. 6 Dose-response curve showing the effect of pulsed microwaves at X-Band on skin respiration. Ordinate is probability. Number of observations at each point is indicated. Broken line shows effect of continuous microwaves at X-Band on skin and is quoted from Lawrence (1968)

there is no reason to suppose that damage caused by microwaves would differ in this respect. We propose, however, to carry out some confirmatory tests with human skin in the future.

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Some aspects of laser safety

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Considerable study has been made over the past few years of the conditions under which damage can be sustained by living tissue due to laser light. This has led to limits of power density which should not be exceeded without caution. However, many applications have to be carried on where it is not possible to exclude all possibility of hazard. In such cases it is necessary to assess the probability of the hazard and to decide whether the event is sufficiently improbable to be acceptable or not. This paper deals with some of the factors useful in making this type of assessment.

LASERS SHARE with many other inventions some potential hazard to safety. One of the best features about the development of lasers has been the early appreciation of safety aspects and this has led to concerted action by users to reduce the possibility of accidents to a minimum. Several papers have dealt very fully with the experimental results of detectable damage caused by light energy on living tissue such as skin and the retina of the eye – notable among these being the work of Ham,[1] which has led to the recommendation of levels of laser radiation which should not be exceeded without caution[2 3] (**Fig. 1**).

The power levels specified in these recommendations are not very different from those for microwaves – for continuous wave radiation 100mW/cm^2 instead of 10mW/cm^2 for