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Effect of Microwaves at X-Band on Guinea-pig Skin in Tissue Culture

1. Microwave Apparatus for Exposing Tissue and the Effect of the Radiation on Skin Respiration

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An apparatus was designed which enabled small pieces of skin to be exposed to a uniform field of microwaves at X-band (8,730 MHz). This was used to investigate the effect of these microwaves at selected energy levels on the metabolism of skin.

It was shown that skin cultured *in vitro* exhibited a graded response to microwave energy, and a dose-response curve was constructed from this data. The ED_{50} of this curve was 4,740 mW./sq. cm. applied for 1 second.

Microscopical examination of three-day cultures of skin showed that histological abnormalities occurred if the specimens were exposed to intensities of microwaves causing more than 30% respiratory damage. The energy level at the ED_{30} was 2,880 mW./sq. cm. applied for 1 second.

Results were consistent with the hypothesis that tissue damage caused by irradiation with microwaves was due to the energy absorbed by the specimen being converted to heat.

Modern technology has produced a variety of devices emitting high-power beams of non-ionizing electromagnetic radiation at various wavelengths. For example, lasers emitting visible light or near infra-red radiation are becoming increasingly available for various purposes; also short radio waves (1 cm. to several metres) are frequently encountered in telecommunications and radar systems. Many generators of non-ionizing radiation have an output of several hundreds of watts and this power is often concentrated in a narrow beam. Consequently, there is a renewed interest in the possible hazard of this radiation to biological material.

Skin seems a suitable choice of tissue for experiment since it is the part of the body most immediately exposed. The effect of energy from a ruby laser on skin maintained *in vitro* has recently been investigated (Lawrence, 1967, 1968; Carney, Lawrence, and Ricketts, 1967), by means of techniques developed for studying changes in various aspects of skin metabolism caused by different forms of injury (Lawrence, 1961; Lawrence, 1959; Carney, Lawrence, and Ricketts, 1965; Lawrence and Ricketts, 1957). These tests seemed appropriate for investigating the effect of microwaves on skin. Accordingly a series of experiments was made in which skin slices were exposed to microwaves at X-band.

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Methods

The circuit diagram of the microwave apparatus used is shown in Figure 1. The microwave source was a Klystron oscillating at 8,730 MHz (wavelength 3·43 cm.) capable of delivering 10 watts continuously. The output was obtained via a waveguide measuring 2·8 × 1·25 cm. (internal dimensions); this was fed into an adjustable power divider which enabled the required power to be transmitted through the isolator down the main channel, the remaining power being dissipated in the higher power load of the power divider. The ferrite isolator ensures that the Klystron output encounters a matched load at all times. Two 10 dB couplers were attached to the main channel, and specimens for irradiation were introduced into the waveguide between the couplers at the point x——x.

The first 10 dB coupler enabled power reflected by the specimen to be measured by attenuator A, crystal

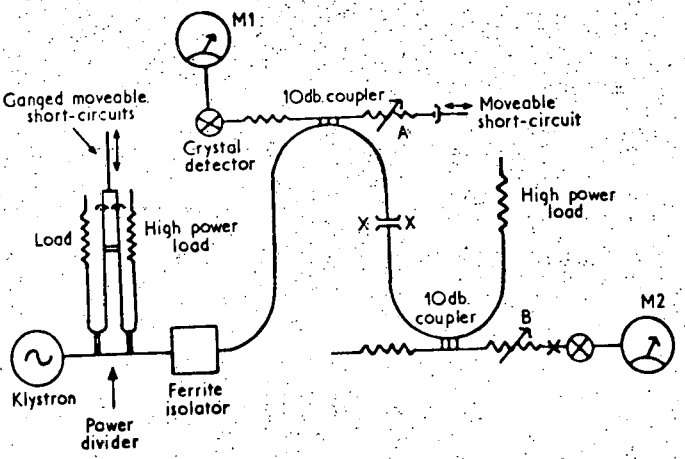

FIG. 1. Schematic diagram of the microwave apparatus used.

detector, and meter M1. The second 10 dB coupler in conjunction with attenuator B, crystal detector, and meter M2 measured the power passing through the specimen. The power was dissipated in the high-power load at the end of the waveguide.

Skin samples were inserted into the waveguide on a specimen carrier; this was made from polythene sheet, 0·25 mm. thick (Fig. 2). This material was chosen since its electrical properties enable it to be introduced

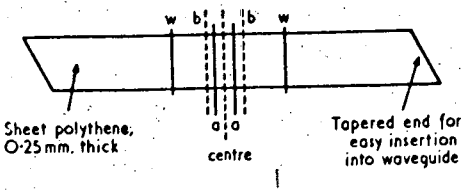

FIG. 2. Plan view of polythene specimen carrier. See text for explanation of the symbols.

into the microwave field with minimum reflection of the wave and virtually no absorption of energy. The waveguide at point x———x (Fig. 1) was modified to receive the specimen carrier; this is shown in Figure 3. Two slits, S1 and S2, were cut across the shorter sides of the waveguide. S1 was 1·25 mm. high and S2 0·5 mm. high. A strip of metal with one end bent at a right angle was attached to one long side of the waveguide; this strip acts as a stop for the specimen carrier. Thus when the specimen carrier is pushed as far as possible through slit S1 the area marked w———w (Fig. 2) corresponds exactly with the area of the waveguide, and the centre of

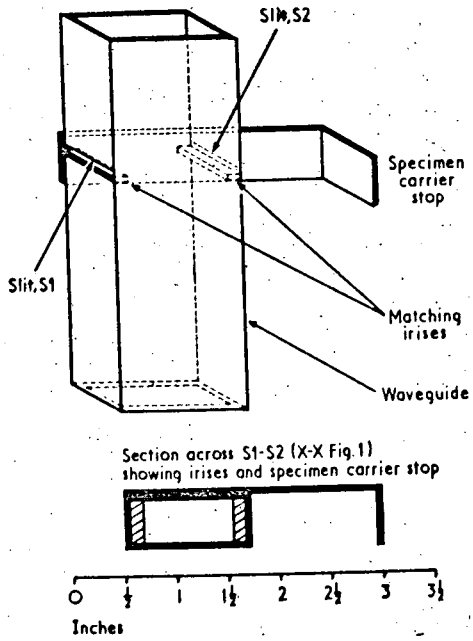

FIG. 3. Modification of waveguide to receive the specimen carrier.

the specimen carrier is in the centre of the waveguide. This is important because power distribution within the waveguide is uniform only across the shorter side; its distribution in the other direction is not uniform though it can be calculated. Most of the power is concentrated in the central zone and can be expressed by the formula

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

where P_T = total power passing down the waveguide; $\triangle a$ = area of the central zone, and A = area of the waveguide. In practice, this formula is not quite correct so that over the area a———a (Fig. 2), which is 0·1 A, the power distribution is 97% of the theoretical and for b———b (0·2 A) 95%.

The irises were inserted in the modified waveguide to neutralize the small amount of energy reflected by the polythene specimen carrier. These irises were small tongues of metal (Fig. 3) protruding into the guide by a depth of 3 mm.; they also reflect a little energy but of opposite phase to that of the specimen carrier. Accordingly, if the size of the irises is correct, one reflection neutralizes the other. Thus any reflection of power from a specimen introduced into the waveguide could be measured directly.

Slices of guinea-pig ear skin were used for all the experiments, these were cut free-hand from the ear with a very sharp scalpel (Cruickshank, 1954). Albino animals of the Portland strain, 6 to 9 months old, were used.

The skin was incubated on a standard culture medium of homologous serum, Krebs-Ringer phosphate, 5% w/v glucose, and dihydrostreptomycin sulphate 50 γ/ml. mixed 5:3:1:1 by volume (Cruickshank, 1951).

Measurements of skin respiration were made with the differential microrespirometer designed by Cruickshank (1954), and the technique to investigate the effect of microwaves on skin respiration was similar to that used by Lawrence (1967, 1968) to study the effect of laser energy on skin. Respiration was measured for two hours, then the skin slices were removed from the respirometer and exposed to microwaves at the selected power density. The skin was replaced in the respirometer and measurements were continued for a further 24 hours. Changes in respiratory activity were assessed by expressing the mean respiratory rate of the 22-24 hour period as a percentage of the initial control (0-2 hour) period.

To investigate the effects of microwaves on the migration of skin epithelium experiments involving three-day cultures of skin were made using the method described by Cruickshank and Lowbury (1952). Specimens of skin were also retained immediately after exposure for histological examination.

In all experiments a strict aseptic technique was observed. The polythene specimen carrier was sterilized by immersion in 70% ethanol for 10 min., washed in sterile water, and dried with sterile filter paper. All skin cultures were checked for sterility after incubation by spotting out drops of the medium on 4% blood agar plates and incubating these overnight. Very little bacterial contamination was detected (1·6%); no fungal contamination was observed. The occasional contaminated culture was discarded.

Location of the skin sample in the central region of the specimen carrier was achieved by placing the previously sterilized polythene over a marked plan (Fig. 2). The lines can be readily seen through the polythene, and the specimen was placed along the central line and within the area bounded by a———a. Very few samples were wider than this; consequently the calculated power density for exposure was at least 95% accurate.

Results

A series of experiments was made in which slices of skin were exposed to microwaves to see if this radiation affected skin respiration. Three basic power levels were chosen for transmission down the waveguide. These were 1,700, 3,400, and 6,800 mW. and gave the following power densities over the central area: 970, 1,940, and 3,890 mW./sq. cm. respectively. Exposure times of 1, 2, and 4 seconds were chosen at each of these power levels. Doses were expressed in millijoules per square centimetre; in doing so, it is assumed that the effect of, *e.g.*, 1,940 mW. for 1 second was equivalent to the effect of 970 mW. for 2 seconds. Some justification of this assumption is provided in Part 2 of this paper (Carney, Lawrence, and Ricketts, 1968).

Control skin (*i.e.*, skin not exposed to the microwave field) respired steadily for 24 hours; slices of skin irradiated with microwaves showed a decrease in respiratory activity after exposure, the effect being greater for higher energy densities. Changes in respiratory activity were assessed by calculating the mean respiratory rate of the 22-24 hour period and expressing this as a percentage of the initial 0-2 hour control period. If the microwave dose was plotted against percentage respiratory survival, a sigmoid curve was obtained. This was made linear by taking logarithms of the dose and transferring the percentage data to a probability scale. This dose-response curve is illustrated in Figure 4. The results used to construct this curve were obtained from five separate experiments, six respirometers being used in each. A control respirometer was included in each experiment.

The median effective power density (ED_{50}) estimated from Fig. 4 suggests that 4,750 mW./sq. cm. applied for 1 second would reduce the respiration of skin by 50%. The ED_{30} (30% respiratory damage after 24 hours) is also of interest since previous experience suggests that this is a maximum 'safe' level for explants of skin cultured *in vitro* compatible with normal histology (Lawrence, 1961). The power density required to produce this effect was found to be 2,880 mW./sq. cm. applied for 1 second.

Two separate experiments were carried out in

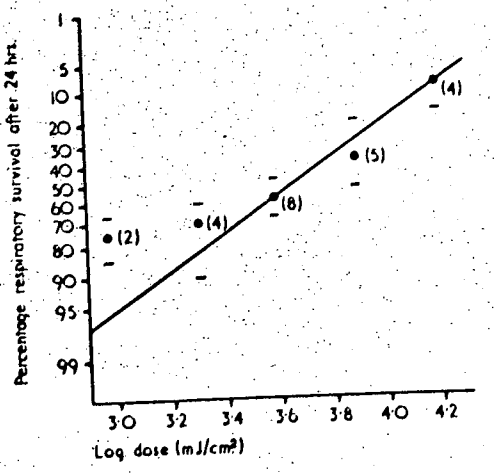

FIG. 4. Dose-response curve showing the effect of microwaves on skin respiration. The number of observations made at each point is indicated in brackets. The limits of the highest and lowest observation for each value are indicated. Exposure times for the doses were 1 to 4 seconds.

which groups of four skin explants were exposed to microwaves at energies of 1,940 and 3,890 mW./sq. cm. for periods of 1, 2, and 4 seconds. Groups of unexposed explants were included in each experiment. These served as controls. After incubation for three days at 37° C. sections of these cultures were prepared for microscopical examination. The appearance of these cultures was then classified as 'normal' (showing migration of epithelium over the cut surface of the dermis), 'inhibited' (showing no epithelial migration) or 'necrotic' (showing cellular abnormalities) as described by Cruickshank and Lowbury (1952). The results are summarized in the Table.

Skin sectioned immediately after exposure to microwaves appeared histologically normal at nearly all energy densities. Exposure to a total energy of 15,560 mJ./sq. cm. caused abnormalities in the staining properties of the epidermis. There was no evidence of damage to any specific portion of the skin.

TABLE

HISTOLOGICAL APPEARANCE OF 3-DAY CULTURES OF GUINEA-PIG SKIN AFTER EXPOSURE TO VARIOUS INTENSITIES OF MICROWAVES AT X-BAND

Microwave Exposure mJ./sq. cm.	*Total No. of Explants*	*No. of Cultures showing*			*Corresponding Respiratory Damage (%) (from Fig. 4)*
		Normal Appearance	*Inhibition*	*Necrosis*	
0	8	7	1	0	0
1,940	4	2	2	0	18
3,880	8	0	3	5	42
7,760	8	0	2	6	62
15,560	4	0	0	4	90

Discussion

It is well known that many materials become heated if exposed to a microwave field; this is the principle of many industrial applications of microwaves and of 'short wave diathermy' (widely used for medical purposes). It is also recognized that excessive exposure of the body to microwaves may be harmful and appropriate safety measures are officially recommended (Post Office, 1960). It is suggested that the energy density should not exceed 10 mW./sq. cm. for continuous exposure to frequencies between 30 and 30,000 MHz. Little attention has, however, been paid to the effect of microwaves on specific tissues other than the lens of the eye; it is, therefore, of interest to demonstrate that exposing skin to a microwave source alters its metabolic activity. The present investigation has been limited to an X-band frequency (8,730 MHz). This band has the advantage of giving a reasonable energy density in a waveguide of convenient size from a moderately powered microwave generator. Lower frequencies require larger waveguides and correspondingly greater power from the generator to produce a reasonable energy density: conversely, equipment employing shorter wavelengths use waveguides too small to enable skin specimens to be irradiated uniformly. As it happens, 8,730 MHz is in the central region of the frequency range commonly considered hazardous, and this particular frequency penetrates tissue to a moderate depth, and an appreciable proportion of the energy is absorbed.

Experiments on animals are commonly found to give a sigmoid response curve relating mortality to dose, equivalent to the probability integral. This is conveniently viewed as representing a normal distribution of susceptibility in the population. The sigmoid curve of such data is rendered linear if the ordinate is scaled in 'probability' units. Such probability paper has been successfully used previously to render linear the dose-response curves obtained for the action of toxic materials, heat, and laser energy on skin cultured *in vitro* (Lawrence and

Ricketts, 1957; Lawrence, 1959; Lawrence, 1961; Lawrence, 1967). It should be noted that the weighting of the points varies according to their position on the curve, this weighting being a minimum at 50%. These sigmoid curves may be more accurately fitted by Probit Analysis (Finney, 1947). However this method involves rather heavy calculation which may not be considered justified.

It was found that a power density of 2,880 mJ./sq. cm. reduced the respiration of skin by 30% after 24 hours. This was the limiting energy density that skin could tolerate consistent with a normal histological appearance of three-day cultures; higher energy levels invariably caused necrosis of the cells. This correspondence of respiratory damage with histological appearance is in agreement with previous experience concerning the toxicity of antibiotics to skin (Lawrence, 1959) and damage to skin caused by irradiation with a ruby laser (Lawrence, 1967; Lawrence, 1968). The ED_{30} appears to be the maximum 'safe' level for estimation of damage to skin *in vitro* and accordingly affords a convenient method for quantitatively comparing respiratory damage caused by various means (Lawrence, 1961).

The ED_{50} is possibly a more reliable level to select for comparison of data because this value can be accurately calculated from dose-response data (Finney, 1947). Previous experiments concerning the effect of heat on skin suggested that exposure to a temperature of 43·5°C. for 30 min. would reduce respiration 50% (Carney *et al.*, 1965). The ED_{50} for X-band microwaves on skin was found to be 4,750 mJ./sq. cm. Assuming that about 50% of the incident energy is absorbed by skin (muscle absorbs 45% at this frequency (Püschner, 1966)) and an ambient temperature of 20°C., a calculation from fundamental principles suggests that the temperature of the skin would rise to about 63°C. It must be remembered that this is the maximum temperature the specimen will reach during its brief heating period; it also assumes that no other heat losses occur during this time. Losses caused by conduction and convection are probably negligible due to the brief exposure time but some re-radiation may occur. This will be discussed in detail elsewhere (Carney *et al.*, 1968). Quite possibly, exposure of skin to a higher temperature for a short time will have the same effect as a lower temperature for a longer time. For example, Cruickshank and Hershey (1960) found that the respiration of guinea-pig skin fell to 14% by heating to 60°C. for 1 minute, but decreasing the time to half a minute resulted in 20% respiratory survival. Very brief exposures of skin to laser energy (about 1/500 sec.) may raise the temperature transiently by several hundred degrees but not cause excessive respiratory damage (Lawrence, 1967; 1968).

On the basis of these observations it is reasonable to assume that the changes in the metabolic activity of skin caused by microwaves are due to heat. It is possible that other damaging mechanisms occur. For example, resonance of specific molecular species leading to their disruption might occur at particular frequencies; for instance, Bach (1965) reported deactivation of the enzyme alpha amylase by frequencies around 12 MHz. Any effect attributable to molecular disruption in the microwave field might be expected to occur at a particular energy threshold rather than produce a graded response; alternatively, damage might be caused by energy densities too low to produce much heat within the specimen. The dose-response curve (Fig. 4) apparently shows more damage at low energy densities than might be expected on the basis of heat production, but it is thought that this can be attributed to poor accuracy in timing the 1-second exposures combined with the extreme sensitivity of the percentage scale in this region of the graph. The remainder of the graph is based on a higher proportion of longer exposures. These could be timed more accurately; also the percentage scale is less sensitive in the centre. This variation in the sensitivity of the percentage values in dose-response data is the basis of the recommendation by Gaddum (1948) that only points falling between 16% and 84% should be considered when constructing a graph. More accuracy might have been obtained if an automatic time switch had been used in the waveguide or to operate the specimen carrier.

The present experiments suggest that skin can tolerate 2,880 mW./sq. cm. for 1 second *in vitro* without any obvious harmful effect. This is considerably higher than the recommended maximum of 10 mW./sq. cm. for body exposure. This latter dose, however, is calculated from the amount of heat the body can dispose of in normal conditions (Püschner, 1966) and probably allows a considerable safety margin. The eye is thought to be particularly sensitive to microwaves since it lacks the advantage of a circulatory system which provides efficient cooling of other tissues. It is tempting to speculate that skin *in vitro* might behave similarly since it has been removed from a blood supply. Unfortunately, no information is to hand concerning the production of cataract with X-band microwaves. However Seth and Michaelson (1965) found that exposure of the eye to a frequency of 2,800 MHz for 1 hour consistently produced permanent cataract if energies above 240 mW./sq. cm. were used. Tissues absorb approximately equal amounts of energy at either 2,800 MHz or 8,730 MHz (Püschner, 1966) so,

allowing for the different exposure times used in this investigation for skin and those employed by Seth and Michaelson (1965), both tissues may well have similar thresholds of damage by microwave energy.

These preliminary experiments clearly demonstrate that exposure of skin to microwaves alters its metabolic activity, and it seems probable that the effect is caused by absorbed energy raising the temperature of the tissue, although it is possible that non-thermal effects might also be involved. Other aspects of skin biochemistry have been investigated and are the subject of a further communication (Carney *et al.*, 1968).

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Effect of Microwaves at X-Band on Guinea-pig Skin in Tissue Culture

2. Effect of the Radiation on Skin Biochemistry

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Small pieces of guinea-pig skin were exposed to a uniform field of microwaves at X-band (8,730 MHz). Measurements showed that 26% of the incident energy was reflected, 34% was absorbed, and the remaining 40% was transmitted. Absorbed energy was converted to heat, causing a rise in the temperature of the skin. After exposure to microwaves the skin was maintained *in vitro* on a nutrient medium. Uptake of radioactive substances from the medium into skin constituents was measured. A graded reduction in the uptake of sulphate ions into chondroitin sulphate, proline into collagen, and of phosphate into phospholipid, nucleic acid, and phosphoprotein fractions was found. The incident energy density causing 50% reduction of all these biochemical activities was approximately 4,750 mJ./sq. cm. under the thermal conditions of the experiment. The cooling rate of the tissue is important in determining the effect of microwaves.

In Part I an apparatus was described (Lawrence, 1968) for exposing small pieces of skin to microwaves of known power density at an X-band frequency of 8,730 MHz. The skin was heated by the radiation, and over an appropriate range of power density there was a graded reduction in respiration of the skin cells. This showed that metabolism was to some extent continuing in the affected cells. In previous work on the effects of heat (Lawrence and Ricketts, 1957; Carney, Lawrence, and Ricketts, 1962; Carney, Lawrence, and Ricketts, 1965) methods were developed for measuring various aspects of skin metabolism through the addition of radioactive tracer substances to the tissue culture medium. This paper reports on the uptake of ^{32}P-phosphate, ^{35}S-sulphate and ^{14}C-proline by the cells of skin exposed to microwaves and subsequently maintained *in vitro* on a nutrient medium.

Methods

Slices of guinea-pig ear skin were weighed and exposed to microwaves under sterile conditions as described (Lawrence, 1968). The skin slices were then incubated for 24 hours at 37°C. on a culture medium containing one of the following radioactive substances: 20μC ^{32}P disodium hydrogen phosphate (Carney *et al.*, 1962), 150μC ^{35}S sodium sulphate (Lawrence and Ricketts, 1957), or 5μC ^{14}C-L-proline per 10 ml. of medium (Carney *et al.*, 1965). All measurements of radioactivity (see below) were related to the fresh weight of each skin slice.

Phosphate Skin slices were fractionated by the trichloracetic acid method of Schneider (1945) into fractions containing phospholipids, nucleic acids, and phosphoproteins. The radioactivity of each fraction was measured using a Geiger counter for aqueous solutions.

Sulphate The cells in skin slices were killed by freezing to −79°C. ^{35}S-sulphate ions were removed by dialysis of the skin slice against sodium sulphate solution, and the remaining ^{35}S-sulphate was liberated by hydrolysis and precipitated as barium sulphate, as described by Lawrence and Ricketts (1957). Radioactivity was measured by a Geiger counter with a mica end-window.

L-Proline Skin samples were homogenized in ethanol to extract free proline and autoclaved to convert collagen to gelatine as described by Carney *et al.* (1965). The radioactivity of the gelatine extract was measured using a liquid scintillation counter and NE 220 scintillator.[1]

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[1]Nuclear Enterprises, Edinburgh.