Effect of microwaves at X-band on guinea-pig skin in tissue culture

3. Effect of pulsed microwaves on skin respiration and biochemistry

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Carney, S. A., Lawrence, J. C., and Ricketts, C. R. (1970). *Brit. J. industr. Med.*, 27, 72-76. **Effect of microwaves at X-band on guinea-pig skin in tissue culture. 3. Effect of pulsed microwaves on skin respiration and biochemistry.** Small pieces of guinea-pig skin were exposed to known power densities of pulsed microwaves at X-band (9.6 GHz). The pulse duration was 0.25 microsecond and the pulse repetition frequency 4 KHz. The peak power was thus 1,000 times the mean power. Otherwise conditions were closely comparable with those of previous experiments using continuous microwaves. After exposure the skin was maintained on a nutrient medium *in vitro*. The respiration of the skin and the uptake of ³⁵S-sulphate, ³²P-phosphate, and ¹⁴C-L-proline into skin constituents were reduced by exposure. The reduction was very similar to that observed after exposure to the same mean power density of continuous microwaves. The effects are believed to be attributable to heating of the skin.

Parts 1 and 2 of this series of papers (Lawrence, 1968; Carney, Lawrence, and Ricketts, 1968) have described the effect of continuous microwaves at an X-band frequency on the respiration and biochemistry of guinea-pig skin using tissue culture techniques. The kind and degree of cellular damage found were entirely consistent with the known effect of heat on the skin. There remains, however, the question of whether there is any other effect, such as might be due to an intense electrical field.

Much radar equipment uses pulsed microwaves. During the pulses very high powers and electrical field strengths are attained; these are followed by relatively long periods without radiation. It was therefore of very great interest to compare the effects of the same average power delivered in continuous or in pulsed form.

Through the courtesy of the Royal Radar Establishment we have had access to equipment delivering pulsed microwaves at X-band. This has enabled us to make a direct comparison with our previous work using continuous microwaves. The equipment chosen operated at a frequency of 9.6 GHz which was satisfactorily close to the frequency of 8.73 GHz of the continuous wave equipment previously used. The percentage absorption of energy by muscular tissue and, presumably, by skin is almost the same (about 45%) at these two frequencies (Puschner, 1966). The ratio of peak power to mean power was 1,000:1. The mean power and total energy input were kept the same in the two series of experiments so that the thermal experience of the skin would be similar. Hence if any effect were attributable to the very high peak powers it should become evident by comparison with the experiments previously reported.

Materials and methods

The circuit diagram of the microwave apparatus used was the same as in the continuous wave experiments but in this case the generator was a magnetron operating at a frequency of 9.6 GHz with a pulse duration of 0.25 microsecond and a pulse repetition frequency of 4 KHz. The mean power was 100 W and the peak power was therefore 100 kW. The power was obtained through a waveguide, size 16, measuring internally 2.286×1.016 cm. This waveguide was smaller than that used in the rest of the circuit which was size 15, internal dimensions 2.850×1.262 cm. Consequently, it was necessary to connect the generator to the remainder of the apparatus with a tapered adaptor. The upper frequency limit of a size 15 waveguide is 9.99 GHz so that it was necessary to recalibrate all the couplers and attenuators at 9.6 GHz, as the original calibration was for a lower frequency. A general description of the operation of the circuit and the considerations affecting the introduction of skin samples into the waveguide has already been given (Lawrence, 1968).

The power levels chosen for transmission along the waveguide were based on those used previously in the continuous wave experiments. Five levels were used: 1.7, 3.4, 6.8, 13.6, and 27.2 W, which gave the following power densities respectively over the central area of the waveguide within which the skin was placed: 0.97, 1.94, 3.89, 7.78, and 15.5 W/cm². Exposures lasting 1, 2, and 4 seconds were made at each of these power levels. The total radiation incident on each skin explant was expressed in millijoules per square centimetre. It should be noted that this is only valid for short exposures; if times longer than 6 seconds are used cooling of the specimen is appreciable, as was pointed out in Part 2 (Carney *et al.*, 1968).

As before, a strict aseptic technique was carefully maintained. Measurements of skin respiration for two-hour periods before and 20 hours after exposure to the pulsed microwaves were made using the differential respirometer devised by Cruickshank (1954). After exposure some of the skin explants were maintained *in vitro* on a nutrient medium containing one of the following radioactive substrates (bought from the Radiochemical Centre, Amersham, Bucks.): 2 μ Ci/ml. ³²P-phosphate (Carney, Lawrence, and Ricketts, 1962), 15 μ Ci/ml. ³⁵S-sulphate (Lawrence and Ricketts, 1957) or 0.5 μ Ci/ml. ¹⁴C-L-proline (Carney, Lawrence, and Ricketts, 1965). Uptake of these isotopes by skin cells and their incorporation into skin constituents was measured as described in those papers.

Results

Respiration rates were expressed as microlitres of oxygen per milligram fresh weight of skin per hour. Control skin, not exposed to microwaves at all, respired steadily throughout the 24 hours of the experiment. Exposed skin showed a decreased respiratory activity. As might be expected, the effect was greatest at the higher power levels. The rate after exposure was expressed as a percentage of the rate for the same piece of skin before exposure. When this percentage was plotted against the

FIG. 1. Percentage respiratory survival of skin cells exposed to pulsed microwaves plotted on a probability scale against logarithm of incident energy in millijoules. Respiration was measured 24 hours after exposure to microwaves. The number of observations at each point is indicated. The broken line shows the effect of continuous microwaves and is quoted from Lawrence (1968). ● Pulsed X-Band; ○ X-Band (CW).

logarithm of total energy incident upon the skin a sigmoid curve was obtained. By transferring the percentages to a probability scale and using points between 16% and 84% a straight line was fitted to the data, as shown in Figure 1. From this line the median effective radiation dose (ED_{50}) was obtained: 6 J/cm². reduced skin respiration by 50%. The ED_{30} , corresponding to a 30% reduction in respiration, is also of interest as previous experience suggests that this is the maximum level of damage at which explants cultured *in vitro* show a normal migration of epithelial cells round the edges of the explant as shown in a histological section (Lawrence, 1961). The power density required to produce this effect was 3.98 J/cm².

Two separate experiments were carried out on the incorporation of ³²P from orthophosphate into phospholipid, nucleic acid, and phosphoprotein fractions from guinea-pig skin exposed to pulsed microwaves. In each experiment four skin explants from a single guinea-pig were exposed at each power level and a further four explants, which were not exposed, formed the control group. The mean radioactivity of each fraction and the standard error of the mean are shown in Table 1. A reduced incorporation of ³²P into some fractions was found, and this was statistically significant at the higher power

TABLE 1
PHOSPHATE UPTAKE BY VARIOUS FRACTIONS FROM SKIN EXPOSED TO PULSED MICROWAVES IN THE X-BAND

Energy density (J/cm. ²)	Experiment 1				Experiment 2			
	Control	1.94	3.88	7.76	Control	3.88	7.76	15.56
Phospholipids								
Mean cts/sec./mg. fresh wt. . .	22.1	23.4	13.6	9.8	5.20	4.52	3.30	0.79
± S.E.M.	0.46	1.67	2.20	2.40	0.55	0.80	1.10	0.47
% uptake relative to control . .	100	105.8	61.5	44.3	100	86.9	63.4	15.2
	—	N.S.	S	S	—	N.S.	N.S.	S
Statistical comparison with control		0.5 <P <0.6	0.02 <P <0.05	0.01 <P <0.02		0.5 <P <0.6	0.2 <P <0.3	P <0.001
Nucleic acids								
Mean cts/sec./mg. fresh wt. . .	3.46	2.04	3.11	1.74	1.24	1.50	0.84	0.12
± S.E.M.	0.39	0.47	0.91	0.72	0.01	0.20	0.30	0.004
% uptake relative to control . .	100	58.9	89.8	50.2	100	112.2	67.8	9.7
	—	N.S.	N.S.	S	—	N.S.	N.S.	S
Statistical comparison with control		0.05 <P <0.1	0.6 <P <0.07	0.01 <P <0.02		0.2 <P <0.3	0.2 <P <0.3	P <0.001
Phosphoproteins								
Mean cts/sec./mg. fresh wt. . .	6.00	6.48	5.14	4.71	2.21	2.37	1.48	0.42
± S.E.M.	0.42	0.41	1.57	0.24	0.42	0.38	0.56	0.82
% uptake relative to control . .	100	108.0	85.6	78.5	100	107.2	33.1	19.0
	—	N.S.	N.S.	N.S.	—	N.S.	N.S.	S
Statistical comparison with control		0.4 <P <0.5	0.6 <P <0.7	0.05 <P <0.1		0.7 <P <0.8	0.3 <P <0.4	0.001 <P <0.01

levels, as shown in Table 1. The mean value for the control group was taken as 100% and the incorporation of ³²P and other values were expressed as a percentage of this.

Skin in tissue culture takes up ³⁵S-sulphate from the medium and incorporates it into chondroitin sulphate and other polysaccharide sulphates. Fixation of sulphate in this non-dialysable form by skin exposed to pulsed microwaves was measured in four separate experiments. In each experiment a group of four skin explants was exposed at three different power levels and a further group served as a control. This form of experiment was repeated so as to span the required range of energy density. The mean values and standard errors for sulphate uptake by all skin explants exposed to each energy density are shown in Table 2. Except at the two lowest power levels there was a graded reduction in sulphate uptake with increasing energy density. Differences from the control value were highly significant at the higher energy densities. The mean value for all controls was taken as 100% and the other values were expressed as percentages of this.

¹⁴C-L-proline is taken up from the medium by skin cells in tissue culture and incorporated into newly formed collagen. The radioactivity of a gelatin extract of the skin provides a measure of the radioactivity of the collagen. Two experiments were done on the incorporation of proline into collagen by skin exposed to pulsed microwaves. In each experiment

four skin explants from the same guinea-pig were exposed at three different power levels, and four explants remained unexposed to provide the control group. Table 3 shows the radioactivity of gelatin extracts from skin exposed to pulsed microwaves. Mean values and their standard errors for all explants irradiated at each energy density are shown. Taking the mean value of the control group as 100% and expressing other values as a percentage of this, the gradation was not as marked as in previous experiments of this type. Only at the highest power level was the reduction in proline uptake statistically significant.

One of the effects of heat and continuous microwaves on skin is the release of soluble substances with strong absorption of ultra violet light at 260 nm. Slices of guinea-pig skin, about 19 mg. in all, were exposed to pulsed microwaves for 4 seconds at 3.4 W, i.e., an energy density of 7.76 J/cm². The skin was then transferred to 1 ml. saline for 5 minutes to allow diffusion of soluble substances. The control sample of skin was treated in the same way except that it was not irradiated. The ultra violet absorption spectrum of the irradiated and control skin extracts is shown in Fig. 2 and corresponds to the effect obtained with continuous radiation.

The energy absorbed or reflected by skin slices of the kind and thickness (c. 0.15 mm.) used in these experiments was measured using the instrumentation described in Part 1 (Lawrence, 1968). For pulsed

TABLE 2
SULPHATE UPTAKE BY SKIN EXPLANTS EXPOSED TO PULSED MICROWAVES AT X-BAND

	Energy density (J/cm. ²)						
	Control	0.97	1.94	3.88	7.78	15.56	31.12
Mean cts/min./mg.	41.3	37.2	35.3	28.2	17.5	8.0	5.3
± S.E.M.	1.4	5.1	3.2	1.8	0.82	2.0	0.9
No. of explants	15	4	8	12	12	7	4
% uptake relative to control	100	90	85	68	42	19	12
Statistical comparison with control	—	N.S.	N.S.	S	S	S	S
		0.2 <P	0.05 <P	P <0.001	P <0.001	P <0.001	P <0.001
		0.3	<0.1				

TABLE 3
PROTEIN UPTAKE INTO COLLAGEN OF SKIN EXPOSED TO PULSED MICROWAVES AT X-BAND

	Energy density (J/cm. ²)					
	Control	0.79	1.94	3.88	7.76	15.56
Mean cts/sec./mg. fresh wt.	42.68	44.17	24.50	28.52	26.91	3.55
± S.E.M.	10.68	19.24	8.84	7.40	10.61	1.43
No. of explants	8	4	4	8	4	4
% uptake relative to control	100	103.5	57.4	66.8	63.0	8.3
Statistical comparison with control	—	N.S.	N.S.	N.S.	N.S.	S
		0.9 <P	0.3 <P	0.2 <P	0.3 <P	0.02 <P
			0.4	<0.3	<0.4	<0.05

FIG. 2. Ultra violet light absorption spectrum of soluble substances, possibly nucleotides, released from skin cells through the action of pulsed microwaves. Incident energy 7.78 J/cm.²

microwaves about 35% of the incident power was found to be absorbed, 30% was reflected, and the remaining 35% was transmitted through the skin. Corresponding figures for continuous waves were

34% absorbed, 26% reflected, and 40% transmitted so that there appears to be no great difference in this respect.

Discussion

It was interesting to find that the percentage of power absorbed by the skin explant in the waveguide was so very nearly the same, 34-35%, from continuous and from pulsed microwaves with such a very high ratio of peak power to mean power as 1,000:1. Release of soluble ultra violet absorbing substances from the skin cells was similar to that occurring with continuous microwaves and with heating. The dose response curve for the effect of pulsed microwaves on skin respiration (Fig. 1) suggests that the median effective microwave dose (ED_{50}) is 6 J/cm.² The lower power levels show a small constant degree of damage. In previous experiments with continuous microwaves this was attributed to imperfect timing of short intervals but, although this was remedied in the experiments described, the effect has persisted and remains unexplained. As suggested by Gaddum (1948), only points between 16 and 84% have been used in calculating the dose response curve. The broken line in Fig. 1 represents the dose response curve obtained in previous work using a continuous

source of microwave power; the ED_{50} of this curve was 4.75 J/cm^2 . This suggests somewhat surprisingly that pulsed microwaves at X-band may be less damaging than continuous waves. However, the difference between the two sets of observations is probably not meaningful. The ambient temperature in the current work was $2-3^\circ\text{C}$. lower than that of the earlier experiments using continuous irradiation. This difference in temperature may be quite important as the rise in temperature of the skin sample will be about 30°C . above ambient temperature for incident radiation of 5 J/cm^2 (assuming about one-third of the energy to be absorbed and the specific heat of skin to be about 1). Thus, in a typical experiment carried out in the laboratory at 22°C ., the final temperature of the skin will be $50-55^\circ\text{C}$.; a further few degrees may well be critical, for instance it has been found that skin immersed in saline at 50°C . for 1 second was damaged less than specimens immersed for 2 seconds (Lawrence, 1969, in preparation); similarly, Cruickshank and Hershey (1960) found that the respiratory activity of guinea-pig skin heated to 60°C . for 1 minute decreased by 86% whereas reducing the heating time to one half minute caused only a 20% decrease.

It seems therefore that expressing the dosage of pulsed microwaves in terms of mean power is satisfactory for biological purposes. The choice of the very high peak power to mean power ratio of 1,000:1 for this study could reasonably be expected to reveal any difference in effect on skin metabolism as compared with the effect of continuous radiation. There is always the possibility that a particular enzyme may be selectively destroyed perhaps by heat-denaturation or even as the result of a momentarily intense electrical field. The respiration measurements reported here reflect the overall effect of pulsed microwaves on skin metabolism. Other experiments have been done to examine specific aspects of skin biochemistry.

The incorporation of sulphate in skin in non-dialysable form, mainly into skin mucopolysaccharides, is known to be a biochemical process with a relatively low turnover rate; that is to say, the sulphate becomes fixed in the mucopolysaccharide which is broken down only slowly into sulphate ions again. Measurements of mean sulphate uptake by a number of skin explants are highly reproducible.

The incorporation of phosphate into cell constituents has a much faster turnover and the variations shown by skin explants have proved to be greater. This has been found particularly with the application of pulsed power either as 0.25 microsecond pulses of microwaves or as previously reported with the even more intense 2 millisecond pulses from a ruby laser (Carney, Lawrence, and Ricketts, 1967). Heating, e.g., 43°C . for 30 minutes and continuous microwaves applied for 1, 2, and 4 seconds, gave much narrower standard deviations about the mean value for the incorporation of phosphate into cell constituents (Carney *et al.*, 1962).

Although these experiments have been done with guinea-pig skin, previous work has shown that the thermal response of human skin is similar (Lawrence and Ricketts, 1957). In the intact animal or in man, other factors assist the dissipation of heat from the skin so there is reason to believe that more energy would be needed to do equivalent damage.

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References

- Carney, S. A., Lawrence, J. C., and Ricketts, C. R. (1962). The incorporation of phosphate by mammalian skin in tissue culture. *Exp. Cell Res.*, **27**, 587-591.
- , —, and — (1965). The incorporation of [^{14}C] proline by mammalian skin in tissue culture. *Biochim. biophys. Acta (Amst.)*, **111**, 154-158.
- , —, and — (1967). The effect of light from a ruby laser on the metabolism of skin in tissue culture. *Ibid.*, **148**, 525-530.
- , —, and — (1968). Effect of microwaves at X-band on guinea-pig skin in tissue culture. 2. Effect of the radiation on skin biochemistry. *Brit. J. industr. Med.*, **25**, 229-234.
- Cruickshank, C. N. D. (1954). Continuous observation of the respiration of skin *in vitro*. *Exp. Cell Res.*, **7**, 374-380.
- , and Hershey, F. B. (1960). The effect of heat on the metabolism of guinea pig's ear skin. *Ann. Surg.*, **151**, 419-430.
- Gaddum, J. H. (1948). *Pharmacology*. 3rd ed., Oxford University Press, London.
- Lawrence, J. C. (1961). Factors influencing skin metabolism as shown by tissue culture. In *Wound Healing*. Ed. Slome, D. Pp. 32-45. Pergamon Press, Oxford.
- (1968). 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. *Brit. J. industr. Med.*, **25**, 223-228.
- , and Ricketts, C. R. (1957). The metabolic uptake of sulphate ions by skin. *Exp. Cell Res.*, **12**, 633-638.
- Puschner, H. (1966). Body tissue in microwave range. *Heating with Microwaves*. Translated by Grubba, E. Pp. 226-238. Philips Technical Library, Eindhoven, Netherlands.

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