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THERMAL AND ATHERMAL EFFECTS OF MICROWAVE RADIATION ON THE ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN HUMAN BLOOD

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Abstract—Human blood and yeast glucose-6-phosphate dehydrogenase were treated with 2.8 GHz microwave radiation (CW, 1 KHz square wave modulation) at an incident power density of between 500 mW/cm^2 and 1000 mW/cm^2. The power absorbed was observed to be 230 $\pm$ 70 mW/cm^3. This corresponds to absorbed energies of 35 and 62 J/cm^3 for the two treatment times used. Treatments were carried out at fixed temperatures of 37°, 46.7° and 49.7°C in such a way as to distinguish between thermal and athermal effects on the activity of glucose-6-phosphate dehydrogenase. Thermally induced reductions in relative activity of up to 80%, which were dependent on treatment period, were observed. The observed average athermal effect on the relative activity was (0.4 $\pm$ 1.8)%. This is not statistically significant. Thus no athermal effect of microwave radiation on the activity of glucose-6-phosphate dehydrogenase was observed.

INTRODUCTION

THE number of devices using microwave radiation has been increasing rapidly over the last few years. At the same time the concern of military, industrial and government organizations with the possible health hazards associated with exposure to microwave radiation has also grown. Review articles by CLEARY[1] and MILROY and MICHAELSON[2] have made obvious the extensive lack of any consensus of opinion on the degree of hazard or the mechanism by which microwaves have their effect on biological systems. Various studies have shown that the biological effects of microwave radiation depend on frequency, power density, duration of exposure, conductivity and dielectric constant of the tissue exposed and its geometrical configuration.

Studies by MICHAELSON *et al.*[3] on dogs, rabbits and rats exposed to pulsed 2.8 GHz microwave radiation under controlled conditions have displayed characteristic physiological responses some of which were related to heating of superficial tissues. It has been shown by several workers[4–7] that microwave radiation at frequencies in the range of 2–3 GHz is particularly effective in inducing cataracts in animals.

In view of the possible development of biological defects induced by microwave radiation, attempts have been made by some workers to investigate biochemical changes in tissues that might occur as a result of the exposure. DAILY *et al.*[8] have observed that microwave exposures which caused severe damage to the rabbit eye greatly inactivated ATPase and pyrophosphatase enzymes of the lens. SHARP and PAPERIELLO[9] found decreased thymidine uptake in ovarian and intestinal tissues of rats at a power level of 32 mW/cm^2 but an increase in thymidine uptake in only the ovarian tissue at a power level of 16 mW/cm^2. KINOSHITA *et al.*[10] have observed a drop in the level of ascorbic acid in rabbit lens after 6–18 hr of microwave radiation at a frequency of 2.45 GHz. These authors stated that the loss of ascorbic acid does not seem to be a direct thermal effect.

This laboratory has undertaken to assess any possible hazards to the users of microwave devices such as microwave ovens, diathermy units and blood warmers. As yet there is not much information available regarding the possible implications of microwave exposures on biological tissues with special reference to any changes in biochemical processes. This communication presents data on the effect of 2.8 GHz microwave radiation *in vitro* on human

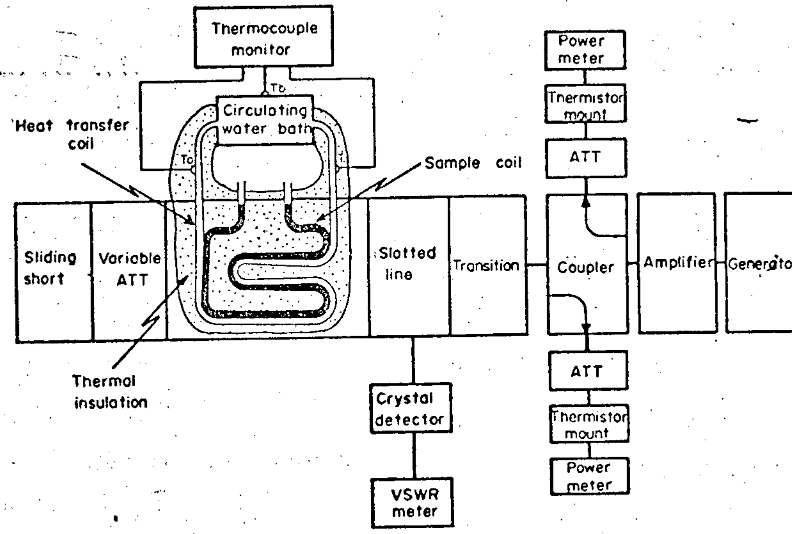

Fig. 1. Schematic diagram of the apparatus.

erythrocyte and crystalline yeast glucose-6-phosphate dehydrogenase (G-6-PDH).

APPARATUS

The exposures were carried out in a heat exchanging sample holder designed to fit inside S-band waveguide which has a nominal operating frequency range of 2.54–3.95 GHz. All the exposures were carried out at a frequency of 2.8 GHz, square wave modulated at 1 KHz with an average power of 10 W in the waveguide. A schematic diagram of the apparatus is shown in Fig. 1.

Assuming a uniform distribution of microwave power across the waveguide, the samples were exposed to a field having an average power density of 407 mW/cm². However, it must be recognized that the field within a waveguide is not uniform over the cross-section of the waveguide. Solution of Maxwell's equations in a waveguide where the coordinate axes and dimensions a and b are as indicated in Fig. 2, shows that for the fundamental mode (TE_{10}) the electric and magnetic fields have the form

$$\begin{aligned} E_z &= E_y = H_x = 0 \\ E_x &= E_o \sin\frac{\pi y}{a}\, e^{j(\omega t - z + \phi)} \\ H_y &= \frac{E_o}{\eta_o}\frac{\lambda_g}{\lambda} \sin\frac{\pi y}{a}\, e^{j(\omega t - z + \phi)} \\ H_z &= j\frac{E_o}{\eta_o}\frac{\lambda}{2a} \cos\frac{\pi y}{a}\, e^{j(\omega t - z + \phi)} \end{aligned} \tag{1}$$

where E_o is the amplitude of the electric field, η_o is the impedance of free space, λ is the wavelength of the radiation in free space and λ_g is the wavelength of the radiation in the waveguide. Since all biological materials only interact significantly with the electric field component of the electromagnetic field, the expression for E_x is of interest in the present experiment. It is evident that for the mode in question the transverse electric field E_x varies from zero at each side wall of the waveguide to a maximum E_0 at the centre. For the fundamental mode it can be shown using equations (1) that the total power transmitted down the waveguide is given by

$$P = \frac{1}{4}\frac{E_o^2}{\eta_o}\frac{\lambda_g}{\lambda}\, ab. \tag{2}$$

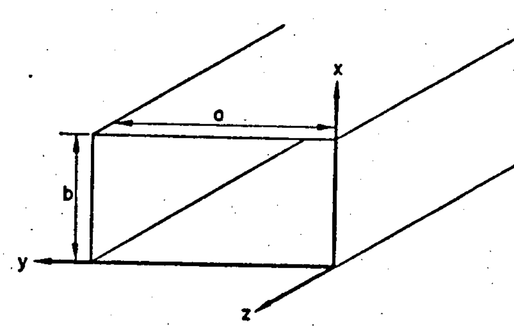

Fig. 2. Diagram of coordinate axes of waveguide.

now

$$\frac{\lambda_g}{\lambda} = \frac{1}{\sqrt{1-(\lambda/\lambda_c)^2}} = \frac{1}{\sqrt{1-(f_c/f)^2}}, \quad (3)$$

where λ_c and f_c are the cut-off wavelength and frequency respectively of the waveguide. Using this and equation (2) we can determine the electric field amplitude at the centre of the waveguide corresponding to a given power, P, from the expression

$$E_o^2 = \frac{4\eta_o P}{ab}\sqrt{1-(f_c/f)^2}. \quad (4)$$

For the present experiment $a = 7.21$ cm, $b = 3.42$ cm, $\eta_o = 377\ \Omega$, $f_c = 2.08$ GHz, $f = 2.80$ GHz and $P = 10$ W. Thus the electric field at the centre of the waveguide is 20.2 V/cm. This field strength in the waveguide corresponds to a power density in free space of 541 mW/cm^2. Because the samples contain substantial amounts of water which has a very high dielectric constant at microwave frequencies the situation is considerably more complicated than has been indicated above. The high dielectric constant of the samples being exposed has the effect of concentrating the fields inside the sample giving an enhancement of the effective exposure field. Because the geometry of the exposure chamber is quite complex in the present experiment an accurate calculation of the actual effective exposure field is not possible. However, the approximate geometrical cross-section of the exposure chamber is 10 cm^2. Assuming all the incident power passes through this area, the maximum exposure field will be about 1000 mW/cm^2. This figure represents an upper limit to the effective exposure field (spatially averaged) and the original spatial average value of 407 mW/cm^2 represents a lower limit. It should be noted that geometrical effects can give rise to localized exposure fields outside these limits even though the spatially averaged exposure field will remain within the limits noted above.

It was required to expose the samples in such a way that the difference between the temperature at any point within the sample and a predetermined temperature was as small as possible. From earlier measurements it was expected that the samples would absorb microwave energy at rates of up to 500 mW/cm^3. Some mechanism was therefore needed to remove this thermal energy from the samples. To achieve this requirement a thermostatically controlled circulating water bath was used in conjunction with a heat exchanging sample holder (see Fig. 1). Water at a preset temperature between 25° and 60°C was pumped continuously from the external water bath through a heat transfer coil within the waveguide and back to the bath. The heat transfer coil was in good thermal contact with the sample coil, while the entire apparatus was thermally insulated from the surrounding environment. The samples were thus held at a known, fixed temperature during exposure.

If a cylindrical sample of material with radius r cm and thermal conductivity k W/cm°C absorbs energy uniformly throughout its volume at a rate of σ W/cm^3 and is maintained at a fixed temperature at its periphery, the temperature on its axis will be higher than the peripheral temperature by an amount

$$\Delta T = 0.25\,\sigma\,(r^2/k)°\mathrm{C}. \quad (5)$$

Thus the conditions and requirements of this experiment ($\Delta T < 0.3°$ C, $\sigma \leq 500$ mW/cm^3, and $k = 5.0 \times 10^{-3}$ W/cm°C for whole blood)[11] set a maximum diameter for the sample of 0.22 cm. This constraint plus the requirement that the heat exchanging sample holder be microwave transparent suggested its construction from two parallel Teflon tubes each 350 cm long, with internal diameters of 0.15 cm. The parallel pair of tubes was wound on a Perspex spool to form a hollow coil of height 2.5 cm, i.d., 1.0 cm and o.d. 5.0 cm. One of the tubes formed the sample coil, with an internal volume of 6.2 cm^3. The other tube carried the circulating heat exchange water.

Accurate continuous measurements were required of the water bath temperature T_b, the temperature T_i at the inflow to the heat exchanger coil, and the temperature T_o at the outflow from the heat exchanger coil (see Fig. 1). Copper–constantan thermocouples were immersed in the flowing water at these points, enabling temperature measurements to be made with an accuracy of ± 0.1°C. In addition, a mercury thermometer was used to measure the water bath temperature.

Since no direct measurement was made of the sample temperature it was necessary to establish accurately the heat transfer coefficient κ from the sample to the circulating water, and the total microwave power absorption $\dot{q}$ in the sample.

The heat transfer coefficient κ was established by direct measurement of the circulating water inflow–outflow temperature difference $\Delta T_{io} = (T_i - T_o)$ following injection of a cooled water sample into the sample coil. After a time lag of a few seconds, the exponential return of ΔT_{io} to its steady state value occurs with a time constant equal to the thermal time constant for heat exchange between the sample and the circulating water. The equivalent heat transfer coefficient κ is then given by

$$\kappa = 2m_s c_p \{2\tau - (m_s/\dot{m}_c)\}^{-1}, \qquad (6)$$

where m_s is the mass of the sample, c_p and $\dot{m}_c$ are the specific heat and flow rate of the circulating water, and τ is the observed time constant. The sample and flow rate parameters used in this experiment were $m_s = 3.0 \pm 0.1$ g, $c_p = 4.2$ J/g°C, $\dot{m}_c = 0.61 \pm 0.03$ g/sec and $\tau = 25 \pm 2$ sec. These indicated a heat transfer coefficient of

$$\kappa = 0.56 \pm 0.05 \text{ W/°C}. \qquad (7)$$

The total power absorbed by the sample from the microwave field was obtained by observation of the change in the steady state value of ΔT_{io} with and without a sample in the sample coil. Defining two inflow–outflow temperature differences; $(\Delta T_{io})_1$ with no sample in the sample coil and $(\Delta T_{io})_2$ with a 3.0 g sample in the sample coil, the total power absorbed by the sample is

$$\dot{q} = \dot{m}_c c_p \{(\Delta T_{io})_1 - (\Delta T_{io})_2\}. \qquad (8)$$

For a total microwave power input at the waveguide of 10 W, measurements indicated a total power absorption by the sample of

$$\dot{q} = 0.7 \pm 0.2 \text{ W}. \qquad (9)$$

The heat transfer coefficient κ and the absorbed power $\dot{q}$ give the temperature difference ΔT_{sc} between the sample and the circulating heat exchange water. Therefore for the conditions used in the experiment

$$\Delta T_{sc} = \dot{q}/\kappa = 1.2 \pm 0.3\text{°C}. \qquad (10)$$

The spatial mean temperature of the circulating water within the heat transfer coil is

$$\bar{T} = \tfrac{1}{2}(T_i + T_o). \qquad (11)$$

The sample temperature T was therefore equal to $\bar{T}$ for the control samples (microwave field off) and was

$$T = \bar{T} + 1.2 \pm 0.3\text{°C} \qquad (12)$$

for the microwave exposed samples. In the course of the experiment T_b (and hence $\bar{T}$) was selected so as to treat the control samples and the exposed samples at identical temperatures.

METHODS

About 30 ml of blood was collected just prior to each experiment from normal healthy subjects and transferred into a conical flask containing acid–citrate–dextrose mixture (one part ACD mixed with five parts blood) which served as an anticoagulant. For irradiation purposes 3 ml blood (at 22°C) were taken in a syringe with a 15 gauge hypodermic needle. The sample was injected into the heat exchanger within the waveguide. Care was taken to see that the sample was entirely within the microwave field. An initial 90 sec warm-up period in the heat exchanger allowed the sample temperature to rise to the temperature of the circulating water. This was followed by a treatment period either with or without microwave radiation. The sample was then withdrawn and kept in an ice bath until ready for enzyme analysis. Control samples were maintained at 0°C during each experiment. The above procedure was also adopted when treating solutions of crystalline yeast G-6-PDH.

All the biochemicals required for the assay of G-6-PDH were purchased either from British Drug Houses Ltd., England, or from Calbiochem, Los Angeles. Crystalline yeast G-6-PDH (E.C. No. 1.1.1.49, Lot 121C-8580) was obtained from Sigma Chemical Company.

The assay of G-6-PDH was carried out as follows. One to two millilitres of blood was washed twice with 0.15 M NaCl at 4°C. The washed erythrocytes were hemolysed and diluted with water up to 10 ml. G-6-PDH activity was assayed by measuring the rate of increase of absorbance of NADPH at 340 nm.[12] The levels of enzyme activity were expressed in units per millilitre of blood. A unit is defined as that

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Table 1. Activity of glucose-6-phosphate dehydrogenase (G-6-PDH) *from human erythrocytes and from yeast with and without exposure to microwave radiation*

Enzyme source	Treatment period (sec)	Temperature (°C)	G-6-PDH activity (units/ml sample) With microwave radiation	Without microwave radiation	Control
Human erythrocyte	150	37	80.0	82.2	80.0
	270	37	83.3	81.6	84.4
	150	46.7	82.7	81.9	82.4
	270	46.7	65.7	70.4	75.0
	150	49.7	53.7	55.2	69.4
	270	49.7	42.6	41.9	72.1
Yeast	150	37	4.21	4.03	4.21
	270	37	3.84	3.86	3.79
	150	46.7	1.87	1.99	3.58
	270	46.7	1.50	1.46	4.30
	150	49.7	1.76	1.75	4.35
	270	49.7	1.04	1.01	4.01

quantity of enzyme which reduces 0.01 μmole NADP/min at 20°C. Crystalline yeast G-6-PDH was diluted with 0.005 M citrate buffer, pH 7.5, and used in experiments such that the rate of reduction of NADP did not exceed 0.015 μmole/min. In this case the levels of activity were expressed in units per millilitre of enzyme solution.

RESULTS

Representative results of the effect of microwave radiation on the level of G-6-PDH activity in human erythrocytes and yeast enzyme solution are given in Table 1. Exposure temperatures were chosen between normal human body temperature, 37° and 50°C beyond which temperature there is a great diminution in G-6-PDH activity. Each line of Table 1 gives the absolute activity for two of the 12 measurements made with identical treatment period and exposure temperature, and the relevant control value. Figures 3–5 show relative activity and include all the data. Figure 3 illustrates the relative activity following treatment of G-6-PDH from human erythrocytes. Figure 4 illustrates the relative activity following treatment of G-6-PDH from yeast. The relative activity is defined as the ratio of the activity remaining after treatment to the activity of the control. Each point on the graphs is the mean of six measurements.

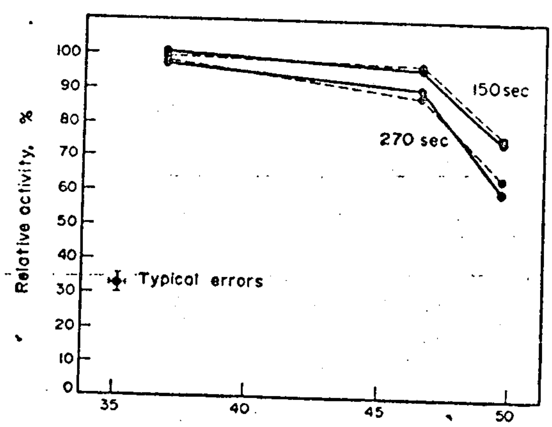

Fig. 3. Relative activity of human erythrocyte glucose-6-phosphate dehydrogenase as a function of temperature: – – – – – –, treatment with microwave radiation; ————, treatment without microwave radiation. Upper lines, 150 sec treatment; lower lines, 270 sec treatment.

At 37°C the G-6-PDH activity was not significantly different from control values. At 50°C, however, the G-6-PDH activity was substantially reduced and was a function of the treatment period. The activity of the yeast G-6-PDH decreased more rapidly with increasing temperature than did the activity of the G-6-PDH from erythrocytes.

Figure 5 shows the differences in mean relative activity of sample groups treated with

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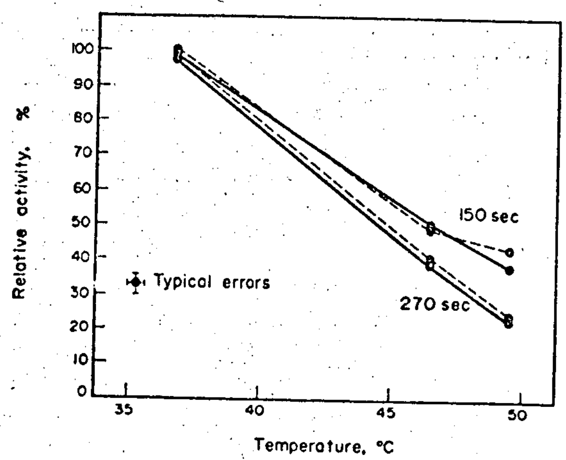

FIG. 4. Relative activity of yeast glucose-6-phosphate dehydrogenase as a function of temperature: ------, treatment with microwave radiation; ———, treatment without microwave radiation. Upper lines, 150 sec treatment; lower lines, 270 sec treatment.

and without microwave radiation. Each point was obtained by subtracting the mean relative activity of a sample group treated without microwave radiation from the mean relative activity of an otherwise identical sample group treated with microwave radiation. The error bars include the standard deviations of the means of the sample groups treated with and without microwave radiation and of the relative controls. The average relative activity difference of the sample groups and its standard deviation is $(0.4 \pm 1.8)\%$.

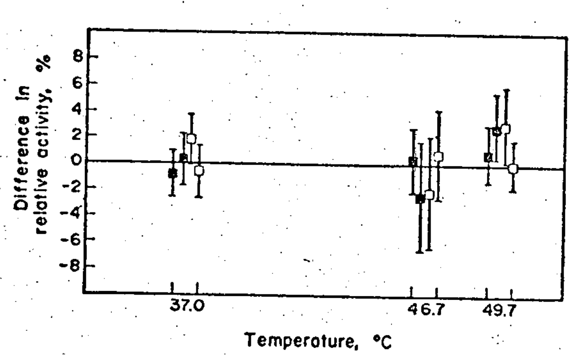

FIG. 5. Difference in mean relative activity of sample groups treated with and without microwave radiation: ▩, erythrocyte G-6-PDH; □, yeast G-6-PDH.

DISCUSSION

Human erythrocyte G-6-PDH was chosen to study as a possible biological indicator of microwave exposure. G-6-PDH is an enzyme which catalyzes the oxidation of sugars via the pentose–phosphate pathway. Solutions of crystalline yeast G-6-PDH were also studied.

Microwave radiation effects may be thermal or athermal, where it is understood that an athermal effect is one which is observed independently of the heating produced by the microwave radiation. This experiment allowed samples to be exposed to microwave radiation at a fixed temperature. Under these experimental conditions, any observed microwave effect must be athermal. Figures 3 and 4 show that the relative activity of G-6-PDH at 49.7°C is reduced by up to 80%. Figure 5, however, shows that the difference in relative activity between irradiated and non-irradiated sample groups averages 0.4%. This difference is not statistically significant. Thus under the conditions of the present experiment, no athermal effect of microwave radiation on the activity of G-6-PDH was observed. It should be noted that North American Agencies generally accept 100 mW/cm² as the level above which no human exposure is allowed and 10 mW/cm² as the level below which continuous human exposure is allowed. The microwave exposures in this experiment were 5–10 times the recommended maximum level and 50–100 times the recommended continuous level.

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