FACTORS AFFECTING THE INDUCTION OF LAMBDA PROPHAGES BY MILLIMETER MICROWAVES

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The induction of lambda prophages by millimeter microwaves has been found to be strongly dependent on frequency and dose and on the oxygen tension in the environment of the cells. An increase of a factor up to 10^5 over non-irradiated cells has been observed.

Introduction. Given high frequencies of microwaves, between 40 and 140 GHz, have been found able to increase or retard the division rate of both bacterial [1,2] and yeast cells [3], alter the oncogenic properties of some tumour cells [4] and affect the *in vivo* rates of synthesis of macromolecules [5]. The ability of the radiation to produce all of these effects, however, has been shown to depend strongly on the frequency of the radiation, and the age of the cells and their nutrition both before and after exposure to microwave fields [5]. In 1975 the induction of a lambda prophage, in cells of *Escherichia coli*, was reported to have occurred on the exposure of lysogenic cells to mm microwave fields [6]; a further study of this phenomenon, therefore, has been made.

Methods. Cell treatment: Lysogenic cells of *Escherichia coli K12*λ^+ were grown for 24 h at 35°C in either (a) DAVIS minimal glucose medium (MM); (b) Nutrient Broth (NB); or (c) Brain Heart infusion broth (BHIB). Suspensions of these cells were made, in fresh media, to a concentration of 10^6 cells/mℓ, and incubated at 35°C on a rotary shaker. At various times afterwards, the cells in a 10mℓ aliquot of a given culture were deposited on a Millipore filter and washed twice with 20 mℓ of the same medium; care was taken not to dry the cells on the filter. The filters then were placed on absorbent pads, soaked with the medium, and these placed in the bottom of a cavity, which could be held at temperatures from 25°C to 40°C by circulating medium. A Varian Klystron, manually swept from 69.1 to 72 GHz generated the microwaves which were fed through a tunable isolator, and a waveguide system of components, containing a loop for frequency stabilization, to a horn inside the cavity. In the cavity the waves impinged onto the filters and two horns, held at the same height, from the bottom of the cavity, as the filters. The horns monitored, by two separate crystals detectors, the field at the level of the cells. After given periods of exposure to this field each pad and filter was placed in 50 mℓ of fresh medium, stirred for 10 min to wash the cells from the filter and then all incubated at 35°C for 70 min with or without shaking. Following this the culture was filtered and the deposited bacterial cells washed twice, on the filter, with 20 mℓ each of medium and 0.85% NaCl (saline) to displace most bacteriophages. The filtrate was serially diluted in NB and 1.0 mℓ aliquots of each dilution plated onto four Tryptone-Saline agar plates seeded with 10^7 cells/plate of the indicator strain *Escherichia coli K12*; plaque counts were made after 24 h incubation at 35°C. Viable cell counts in all suspensions and cultures were made on Nutrient Agar.

Results. The above procedure using the incubation of the pad and filter (i.e. without removing them after the 10 min stirring) was adopted because after stirring

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a random number of the cells remained attached to the filter. Viable counts in suspensions free of pad and filter were made and direct plating of them onto seeded plates, before and after the 70 min incubation period, was performed. In this way the ratio of the number of plaques produced after incubation to that before incubation established the burst size (no. of phages produced/induced cell) which ranged from 14–26. A mean of 20 therefore, was used to calculate, from later plaque counts, the number of infective centres (i.e. the number of cells in which prophage induction had occurred), a procedure which produced a much smaller experimental error.

A second point established was that the Millipore filters must be washed with 10 to 20 mℓ of the medium in use immediately prior to their use to filter out the cells from the phages and they must not be allowed to dry. Such washing, before and after the depostion of the cells onto the filter, was found to reduce, to an insignificant amount, the number of phages which became or remained adsorped to either the cells or the filter.

With cells in MM no effect of the microwaves on the prophage was observed under any of the conditions tried. Lag phase and log phase cells, incubated without aeration (shaking), in NB also failed to show a response and in only a very small number, amounting to about 200 cells, of log phase, aerated cells did the prophage appear to be induced. All of these results, therefore, were considered negative. In BHIB, however, the prophage in 10^3 cells/10^7 cells non-aerated and in 3×10^5 cells/10^7 aerated cells was induced (fig. 1).

The use of BHIB medium, log phase cells, post irradiation aeration and a 30 min exposure at 0.25 mW/cm^2 revealed induction by microwaves to be sharply dependent on the frequency used. Between 69.1 and 71 GHz, with the field strength at each frequency maintained at 0.25 mW/cm^2, significant numbers of inductions were observed only between 70.3 and 70.5 GHz, i.e. within a narrow bandwidth of 200 MHz (fig. 2). This finding is in accord with earlier works in which the growth rate of yeasts was found to be disturbed by frequencies of mm waves within bandwidths of 10 to 20 MHz [3].

The sigmoidal shape of the curve for induction in BHIB cells (fig 1) indicated that either (1) a threshold level of absorbed energy was required to initiate

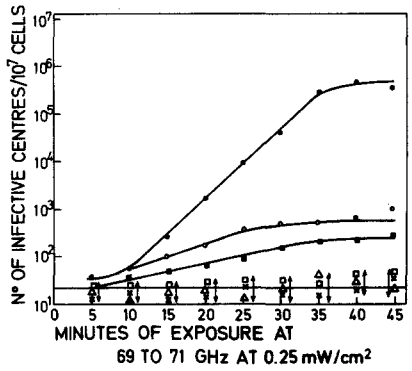

Fig. 1. The influence of nutrition on the induction of lambda prophage by microwaves. X, control no irradiation; △, all phases in minimal medium; □, nutrient broth (NB), no aeration log phase; ■, NB with aeration log phase; ○, heart infusion (BHIB), no aeration log phase; ●, BHIB aeration log phase. Sweep time 1.0/s.

induction and more inductions occurred as the propability of this increased with time of exposure (e.g. dose applied to the population) or (2) each cell had to reach a specific metabolic state, during its lifetime, before absorption of the microwaves and induction could occur. The latter was indicated because the maximum number of inductions was reached at 35 min and 30 min was the mean generation time of the cells under the conditions used.

To examine the above possibilities, 24 h cells in BHIB, were washed in saline, synchronized by alternate heat and cold shock at 40°C and 4°C, and exposed to 10 min of 70.4 GHz radiation at various periods following their resuspension in fresh medium and incubation at 35°C. As can be seen from fig. 3,

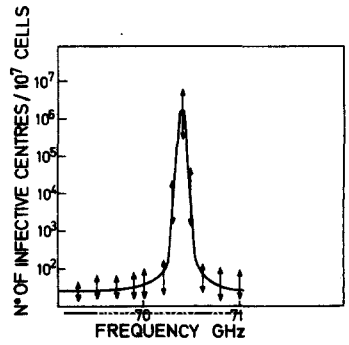

Fig. 2. The influence of frequency on the induction of lambda prophages by microwaves. Log phase cells in BHIB with aeration. Exposure 30 min at 0.25 mW/cm^2.

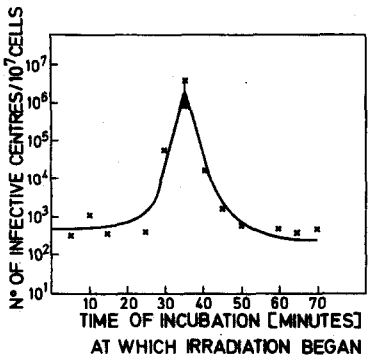

Fig. 3. The effect of incubation time on the induction of lambda prophages by microwaves in synchronized pupulations of cells. Medium BHIB; exposure 10 min at 0.25 mW/cm^2.

a dramatic increase in the number of inductions occurred between 30 and 40 min with the maximum number of affected cells, $5 \times 10^6/10^7$ cells or 50% of the population, occurring at 35 min.

The final stage of the investigation was to take 35 min, synchronized BHIB cells and expose them for 10 min to different field densities. The results (fig. 4) revealed that the maximum effect first was reached at a density of 0.2 mW/cm^2 and this remained constant through to 0.5 mW/cm^2; at higher densities a decline in the number of affected prophages occurred.

Discussion. The above results demonstrate that lambda prophages may be induced by mm microwaves but in order to observe this phenomenon specific nutritional and physical requirements are needed. First, the cells must be given a rich nutrition and supplied with adequate oxygen; this suggests that the processes involved in phage synthesis, after induction, are aerobic ones. Second, the cells must be in a specific metabolic state for the event, leading to induction by microwaves, to occur. Third, the field density to which the cells are exposed should not exceed 0.5 mW/cm^2. Fourth, during the experimental procedures the cells must not dry or be exposed to nutritional changes, especially step downs to weaker nutrition. Saline washes may be used only to remove adsorped phages from filters after their synthesis and release.

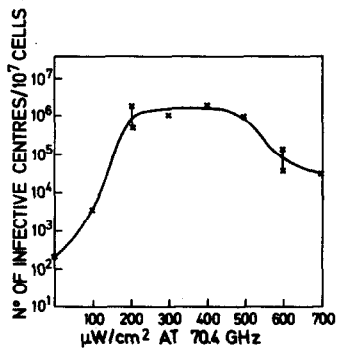

Fig. 4. The effect of field density on the induction of lambda prophages in synchronized populations. Exposure time 10 min; medium BHIB; cell age 35 min.

The first and second requirements taken together suggest that a specific metabolic energy state is set up *in vivo* by a given nutrition at a specific time in the life of the cell which directly or indirectly involves the prophage molecule and which reacts with a narrow 200 MHz waveband of mm waves between 70.3 and 70.5 GHz. Presumably this *in vivo* state is not produced under other nutritional conditions although similar ones, able to react with different microwave frequencies, may occur. The narrowness of the band able to induce the prophage suggests that the effect produced is a non-thermal one. No difference exists in attenuation by free water over the band studied and thus temperature increases, if they existed, would have been uniform over each of the frequencies employed, however, no significant temperature rise was observed throughout the experiments. It appears, therefore, that a particular resonance at 7.04×10^{10} Hz occurs, under these time and nutritional conditions, which involves the large prophage DNA molecule. The possible existence of such oscillations, at these frequencies, induced by *in vivo* metabolic events has been postulated by Fröhlich [7,8] on many occasions. Why such a state does not occur in other media is not understood although it is believed to be due to nutritionally induced differences in gross *in vivo* macromolecular motions which determine whether or not given metabolites and macromolecules may meet and interact [9].

Why high field densities should induce fewer prophages is not understood but it could be due to thermal effects overriding non-thermal ones [10]. Holding cells in weak media or salt solutions after induction by physical and chemical agents has been known for some 15 years to reverse the induction process, although the mechanism of this reversal, usually explained in terms

of DNA repair, still is unclear. This "holding" repair of induction appears to occur also after exposure to microwaves if weak media or salt solutions are used to resuspend the treated cells.

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