# Effects of Microwave Irradiation on Some Membrane-Related Processes in Bacteria

E. N. KAZBEKOV and L. G. VYACHESLAVOV

*Leningrad Institute of Nuclear Physics, Academy of Sciences of the USSR, 188350 Gatchina, Leningrad District, USSR*

**Abstract**. In a series of experiments performed on intact cells or spheroplasts of *E. coli* and *Bac. subtilis* a possibility of non-thermal effects induced by continuous microwave irradiation of a low power density (at wave length range from 6.0 to 7.8 mm) was studied. Thymidine and thymine uptake, leakage of potassium and hydrogen ions as well as the uptake of the transforming DNA by the component cells of *Bac. subtilis* were shown to be affected in a way typical of that due to heating of a sample. No specific dependence of the effects observed on wavelength was found.

**Key words**: Microwave irradiation — Bioeffects — Bacteria

## Introduction

The biological effects of microwave irradiation have been subject of great interest for many years (see e. g. reviews by Chipley 1980; Schneider et al. 1981). Non-thermal effects of microwave irradiation could not definitely be established, although numerous studies have been undertaken using various biological species (e. g. Webb and Booth 1968; Grundler and Keilmann 1978; Bannikov and Rozhkov 1980; Koschmitzke et al. 1983). There also are some reports showing that no similar effects occur in biological systems (e. g. Lystsov et al. 1965; Goldblith and Wang (1967; Wayland et al. 1977; Tuengler et al. 1979). In addition a satisfactory and comprehensive theoretical approach is lacking to predict the actual occurrence of non-thermal effects resulting from microwave irradiation of a biological species. Further investigations carried out with as high accuracy as possible are required, which should consider all the possible artifacts, to accumulate highly valid data.

In the present study we tried to detect non-thermal effects of microwave irradiation on some biological processes depending on the cell membrane activity in bacteria. Two bacterial species were used: *Escherichia coli* and *Bacillus subtilis*. The cellular wall structures of these two species are rather different

(*E. coli* is Gram-negative while *Bac. subtilis* is Gram-positive), and it was of interest to compare the responses of identical biological processes in these bacteria to microwave irradiation.

## Materials and Methods

*Microwave equipment.* A backward oscillator was used as the source of microwave irradiation. The oscillator was connected to a waveguide track (2.6 × 5.2 mm in section) through a ferrite valve and two variable attenuators. A part of the microwave energy was directed into a branch-guide coupler connected to a resonance wave meter which served to indicate approximate frequency values. A measuring line was used to determine the standing wave ratio (dBSWR). A termistor and a bridge test units were used to measure the incident power absorbed by a sample during the experiment. An impendance matching transformer served to match the sample to the waveguide. The frequency during irradiation was measured exactly with an electronic frequency meter coupled with a heterodyne conversion transducer unit.

*Irradiation procedure.* Samples were irradiated continuously with incident power of 5 mW/cm$^2$. The irradiation was applied for 1 or several minutes untill the effect obtained reached its maximal value.

*Cuvettes for samples.* Two identical cuvettes were used for the control and experimental (i.e. microwave irradiated) samples for thymidine and thymine uptake measurements. The cuvettes were made of stainless steel and were crafted like a flange with the window 2.6 × 5.2 mm. The bottoms and lids of the cuvettes were made of 0.02 mm thick mica. Potassium ($K^+$ leakage masurements were performed in polystyrene cuvettes identical to those described above. Two platinum electrodes were inserted through the side walls to monitor changes in the concentration of $K^+$ in the medium. Cuvettes for pH measurements had an agar bridge inserted through the bottom binding the cuvette with a pH recording unit. A calomel electrode was immersed directly into the sample cell suspension. Cells of *Bac. subtilis* were exposed to irradiation in standard commercial tips for automatic pipettes.

*Bacterial strains and cell cultures.* Two well known *E. coli* strains were used: the prototrophic strain B and the thymine-defficient strain $B_3$ thy$^-$. The strains were provided by Dr. F. Jacob. The strains of *Bac. subtilis* used included a prototrophic 168 strain, a thymine defficient 168 thy$^-$ strain and a tryptophan-requiring $T_3$ trp$^-$ strain. The strains were obtained from Dr. F. Rothman. Bacteria were grown on solid (1.5 % agar in Petri dishes) mineral medium supplemented with 0.5 % glucose (Spizizen 1958). Thymine of tryptophan were also added if necessary at the concentration of 20 $\mu$g/ml. Cells in an overnight culture grown at 32° are all in the early log phase. Cells taken from agar plates were suspended in an appropriate medium. The concentration of cells in a medium was adjusted to 5 × $10^8$ 1/ml as determined by turbidity assays.

*Metabolite uptake assays.* Fifteen $\mu$l aliquots of cell suspension were added into control and experimental cuvettes each. The medium contained radioactive thymidine ($^3$H, 120 mCi/mmol) or thymine ($^{14}$C, 76 mCi/mmol) in final concentration of 20 and 10 $\mu$g/ml respectively. Immediatelly after the addition of the label serial samples were taken to determine non-specific background incorporation into the TCA — (trichloroaceticacid) insoluble cell fraction. This was performed by mixing each sample with a standard volume of ice bath-chilled 7 % water solution of TCA. After 30—60 min of incubation in ice bath the samples were filtered through nitrocellulose filters (0.45 $\mu$m pore diameter). The label incorporated (1.5 – 2.5 × $10^3$ counts/min/sample) was counted in a liquid scintillation Intertechnique SL 30 counter.

*Preparation of spheroplasts.* Cells taken from solid medium were suspended with EDTA ($K^+$-EDTA solution, pH 8.0, $10^{-2}$ mol/l). After 30 min, the suspension was centrifuged and the pellet obtained was resuspended in the same medium (omitting EDTA) and lysozyme (1 mg/ml) was added. The samples were incubated with lysozyme for 1 hour at room temperature under slight shacking. The spheroplasts formed were then used for experiments.

*Potassium ions leakage assays.* An overnight culture of bacteria was suspended in a solution of $10^{-2}$ mol/l KCl and 0.5 % glucose, centrifuged and resuspended in $10^{-2}$ mol/l KCl. The suspension was filled in a conductometric cuvette and time-dependent changes in the concentration of $K^+$ in the medium were monitored. A highly sensitive balanced bridge conductometric unit was developed to detect relatively small changes in $K^+$ in the medium ($\pm 10^{-5}$ mol/l at a background concentration of $K^+$ of approximatelly $10^{-2}$ mol/l).

*pH measurements.* Acidification of the culture medium during bacterial growth (release of $H^+$) glucose. The sensitivity of the pH meter used was about $10^{-4}$ pH units for the whole scale (with a long-time baseline drift of about $10^{-4}$ pH units per hour).

*Transformation.* Cultures of competent *Bac. subtilis* cells were prepared as described elsewhere (Prozorov 1980). Transforming DNA was added in a saturating concentration of 5 $\mu$g/ml and 15 $\mu$l/aliquots were immediatelly sucked into pipette tips and the tips were placed directly into the outlet of a waveguide. The samples were heated to 25° to promote effective DNA uptake. After irradiation for 15 min the samples were transfered to agar plates and mixed with 0.2 ml of DNAse solution (1 mg/ml) to prevent further DNA uptake. The plates were stored in a thermostat at 37° untill colonies of cells transformed to prototrophy occurred. The colonies were counted and compared to control values. Control cells were processed parallelly omitting irradiation.

## Results and Discussion

*The effect of microwave irradiation on thymidine and thymine uptake.* Results of experiments performed at room temperature using a wavelength range 6.0—7.8 mm (in 0.1—0.2 mm steps) are shown in Fig. 1*a*. Microwave irradiation had no detectable effect on thymidine uptake and its incorporation into DNA of *E. coli* $B_3$ $thy^-$ cells at any of the wavelength used. Similar experiments were performed with thymine instead of thymidine. To be taken by bacteria, thymine has to be converted to thymidine (D'Donovan and Neuhard 1970; Beacham et al. 1971) by periplasmic thymidine phosphorylase (E.C. 2.4.2.4.) in the cell wall. If irradiation altered this step, this would result in changes in the thymine uptake kinetics. Data shown in Fig. 1*b* suggest that there were no definite differences in the level of thymine uptake between control and irradiated samples of *E. coli* $B_3$ $thy^-$ cells. Similar results were also obtained with the *Bac. subtilis* 168 $thy^-$ strain.

*The effect of microwave irradiation on the kinetics of $K^+$ leakage from E. coli and Bac. subtilis cells.* The results of these experiments are presented in Fig. 2. The

conductivity of the medium containing bacteria increased with the time, obviously due (at least in its substantial part) to leakage of $K^+$ ions from the cells in the absence of glucose as the energy source, this assumption was confirmed in an experiment in which valinomycin (at a final concentration of 1 $\mu$g/ml), a well known specific potassium ions carrier was added to the sample. In the

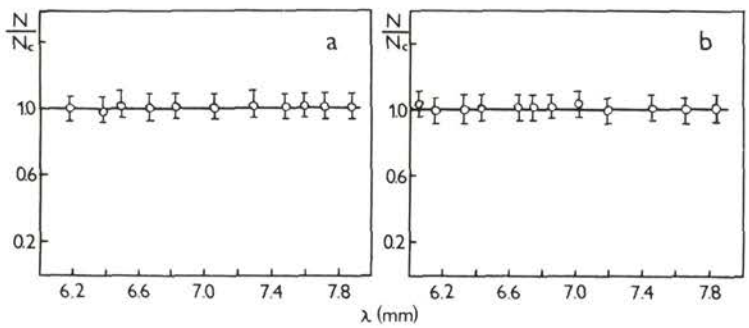


**Fig 1.** Effects of microwave irradiation on the uptake and incorporation of $^3$H-thymidine and $^{14}$C-thymine into DNA of cells of *E. coli* strain $B_3$ thy$^-$. ⓐ and ⓑ: incorporation of $^3$H-thymidine and $^{14}$C-thymidine respectively; $N/N_c$ — count ratio for irradiated and control samples respectively.

presence of valinomycin the kinetics of $K^+$ leakage was greatly enhanced (Fig. 2). Similar response was observed with valinomycin or in the absence of glucose with spheroplasts used instead of intact cells. Fig. 2 shows results of a typical experiment with irradiation of a cell suspension (*E. coli* strain B) at wavelength of 6.23 mm. It can be seen that the irradiation induced an immediate and distinct (by 20—30 %) increase in the conductivity of the sample. However, this effect was reversible: after the irradiation was interrupted the conductivity index returned to initial values. Experiments with spheroplast preparations of both *E. coli* and *Bac. subtilis* gave identical results. It should be emphasized that the effect of irradiation and the reversibility of the effect were standard at any wavelength chosen in all the experiments performed over a carefully checked band range (6.0—7.8 mm with 0.01—0.02 mm steps). A very similar response was obtained when the sample was heated to 1—2° higher than its actual temperature. It could be concluded that the irradiation effects observed were due to the heating of the sample.

*The effect of microwave irradiation on proton release during bacterial growth.* It is well known that bacteria produce large amounts of hydrogen ions ($H^+$, protons) during active growth, resulting acidification of the nutrient medium (e.g. Gunsalus and Shuster 1961). It was suggested that non-thermal effects of

microwave energy could change the general metabolism and thus interfere with the kinetics of $H^+$ accumulation in the cultivation medium. This suggestion was checked in a series of experiments carried out with both intact cells and spheroplasts of *E. coli* and *Bac. subtilis*. The wavelength range investigated was between 6.0 and 7.8 mm with 0.01—0.02 mm steps. The irradiation ($P = 5\,mW/cm^2$) was followed by a 60—80 % increase in the $H^+$ accumulation kinetics (Fig. 3). When irradiation was interrupted, the kinetic curve returned gradually to its pre-irradiation shape. No specific wavelength-associated responses could be found despite a detailed analysis within a band range of 6.0—7.8 mm. Similar as $K^+$ leakage release dynamics of $H^+$ could also be easily simulated in all details by heating the sample to 1—2° above the actual temperature. Obviously, the sharp increase in $H^+$ accumulation reflected an overall intensification of cell metabolism due to heat absorption.

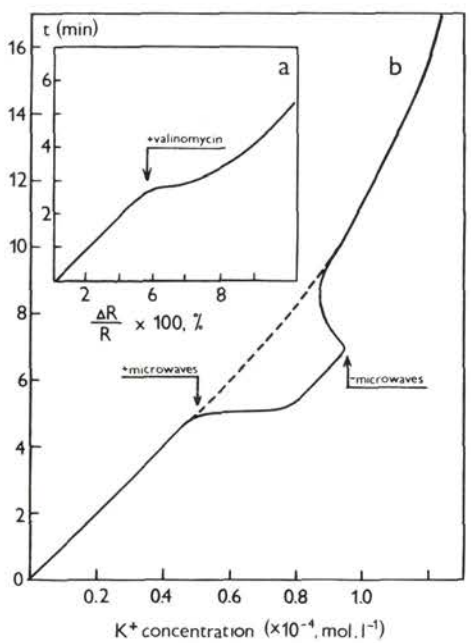


**Fig. 2.** The effect of microwave irradiation on $K^+$ leakage in *E. coli* strain B. ⓐ — control experiment with the cell suspension treated by valinomycin; ⓑ — effect of microwave irradiation on the kinetics of $K^+$ leakage.

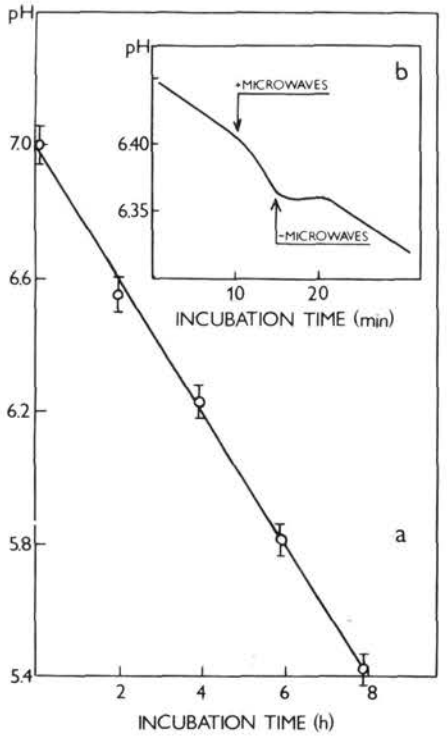


**Fig. 3.** The effect of microwave irradiation on the acidification of cultivation medium by *E. coli* strain B. ⓐ — kinetics of accumulation of $H^+$ ions in cell suspension at room temperature; ⓑ — the effect of microwave irradiation.

Wayland J. R., Brannen J. R., Morris M. E. (1977]: On the interdependence of thermal and electromagnetic effects in the response of *Bacillus subtilis* spores to microwave exposure. Radiat. Res. **71**, 251—258

Webb S. J., Booth A. D. (1968): Absorption of microwaves by microorganisms. Nature **222**, 1119—1201

Final version accepted March 6, 1986