LOW POWER 915 MHz CW MICROWAVE RADIATION ALTERS PROTEIN ARRANGEMENT IN *NEUROSPORA CRASSA*: COMPARISON OF EFFECTS ON HEAT DAMAGED AND UNDAMAGED CELLS

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ABSTRACT

915 MHz, CW at specific absorption rate 0.1 W/kg induces increased transcription of total RNA (as measured by the incorporation of ^{3}H-uridine), RNA polymerase I activity and total protein synthesis (as measured by the incorporation of ^{35}S-methionine) in heat damaged (up to 90% lethality) *Neurospora crassa* mycelial cells. Exposed cells not receiving any heat treatment show significantly enhanced synthesis of proteins and more RNA polymerase I activity, compared to heat damaged cells. Heat treatment or microwaves separately do not produce any heat shocked polypeptides or do not change the pattern of the polypeptides. However, if these heat-treated cells are exposed to microwaves the arrangement of polypeptides is changed. The translation of two polypeptides (13,000 and 51,000 dalton) was repressed almost completely, whereas synthesis of most polypeptides was enhanced. These studies suggest that heat damaged *N. crassa* cells are more sensitive to microwave exposure in inducing alteration in polypeptides arrangement. Some of these observations confirm our previous studies done with a neuroblastoma cell line NG108, indicating widespread phenomenon of low level microwave induced transcriptional and translational effects on both plant and animal cells.

INTRODUCTION

IT was reported earlier that 915 MHz, CW at 0.05 W/kg exposure to neuroblastoma cell line NG108 induced RNA and protein synthesis in heat damaged (up to 90% lethality) and undamaged cells[1]. When heat-treated NG108 cells were exposed to these radiations alteration in the pattern of the polypeptides was observed. Furthermore two heat shocked polypeptides were also synthesized. Neither heat nor radiations alone could alter the pattern of the polypeptides. Since these observations were made in cancerous cells (neuroblastoma) we were interested to know whether normal cells of other organism (in this report *Neurospora crassa*, a filamentous fungus) also behave in the same way. Our studies suggest that although the effect of a thermal micro-wave radiations and heat is the same in *N. crassa* cells but no heat shocked polypeptides were observed.

MATERIALS AND METHODS

Culture and maintenance of N. crassa

The wild type *N. crassa* 74A strain was obtained from the Fungal Genetics Stock Center (FGSC 587), Kansas City, USA. Details of culturing and maintenance of this culture are described by Dutta[2]. For all purposes, exponentially grown mycelia of *N. crassa* were grown under aeration overnight in 500 ml of Vogel's minimal medium[3] yielding approximately 20 g of squeezed mycelia/l growth medium.

Instrumentation and dosimetry

All the exposures were performed in a Crawford cell (Instruments for Industry, Model BC-100) energized with a Hewlett Packard Power Source at 915 MHz, CW and the details of equipment and conditions of exposure are described elsewhere[4]. The specific absorption rate (SAR) values of the samples were measured by detecting forward, reflected, and transmitted power via a 20 dB bidirectional coupler (Narda Microwave Model 3020A) as described, earlier[4].

Monitoring temperature rises

In these studies all the work were done only with relation to exposure of extremely low intensity (mostly below SAR 1.0 W/kg at which there is no detectable rise in temperature. In addition, the

results obtained by us so far, the biological effects (Ca^{2+}-ion efflux) occurred in three independent regions of SAR values at 0.005, 0.05 and 1.0 W/kg[4]. These results effectively rule out temperature rise as the causative agent.

In addition, we have conducted numerous experiments[4] to see if there is any difference in temperatures between inside the exposure chamber (called sham) of the Crawford cell and that exists within the incubator, i.e. outside the Crawford cell (called control). Similar experiments were done to see whether there is any difference in DNA synthesis between sham (with no exposure) and control experiments at 30°C. No difference in DNA synthesis was found.

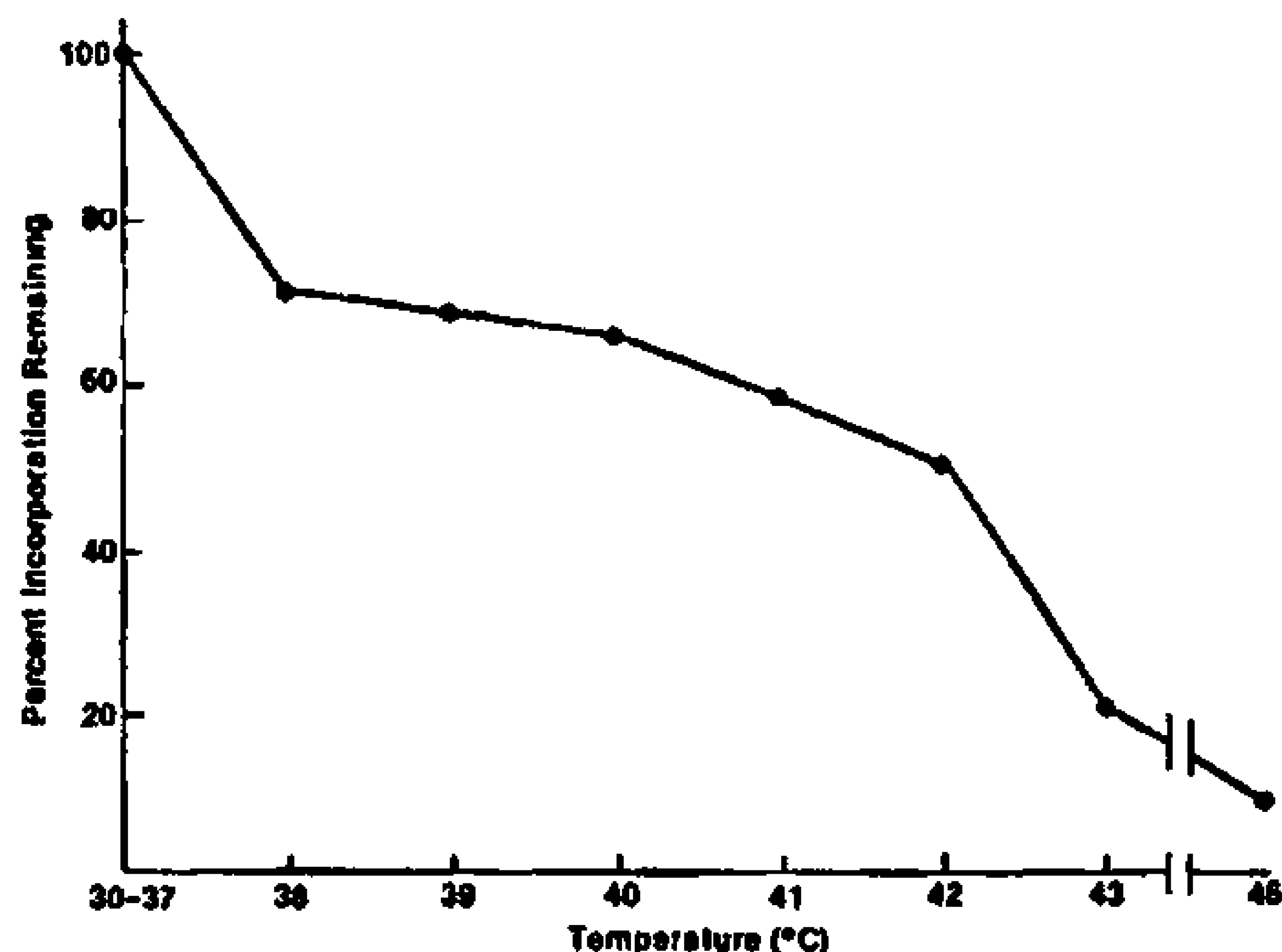

Figure 2. Per cent ^{3}H-uridine remaining after exposure for 120 minutes.

^{3}H-uridine incorporation of RNAs and conditions for heat damage of N. crassa cells

The optimum incorporation of ^{3}H-uridine was between 8 and 16 h (figure 1). To establish conditions to cause heat damage of ^{3}H-labelled *N. crassa* cells, exponentially growing mycelial cells were suspended in 5 ml Vogel's medium[3] in tissue culture flasks (Falcon, 25 cm^2 growth area). These cells were exposed at various temperatures for 120 min and the lethality of cells was measured each time. It was found that 120 min at 46°C (figure 2) caused 90% lethality which was chosen for all the subsequent experiments. Heat damage of macromolecules was confirmed by performing RNA gel electrophoresis[5] and protein gel electrophoresis[6,7]. The molecular weight markers were obtained from Bethesda Research Laboratories, USA.

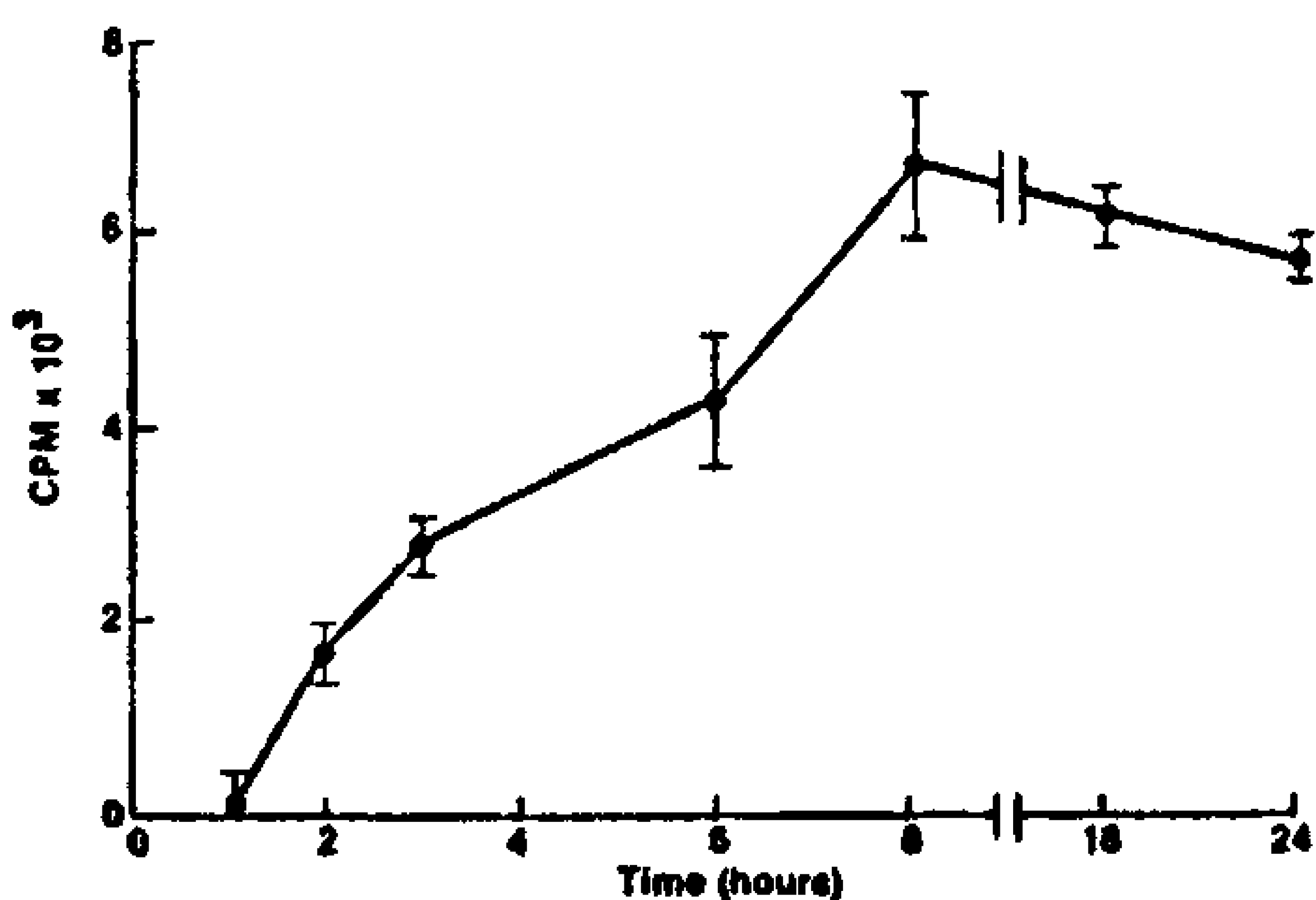

Figure 1. Incorporation of ^{3}H-uridine in *N. crassa* cells.

Incorporation of ^{35}S-methionine and ^{3}H-uridine during exposure of mycelial cells of N. crassa to microwave and PAGE of polypeptides synthesized

N. crassa was grown in Vogel's minimal medium (100 ml) by inoculating with a known number of conidia at room temperature with vigorous shaking for 18 h. The cells were heat damaged as described earlier. After the heat damage, 2.5 μCi of ^{35}S-methionine (990 Ci/mmol E & I Dupont Company) and 2.5 μCi ^{3}H-uridine was added to 100 ml of cell suspension. Five ml aliquots were removed and transferred to several T-flasks. Four such flasks were incubated at 4 C, four at 30 C, and four exposed in a Crawford cell (915 MHz, 0.1 W/kg SAR) at 30°C for 16 h. Thus cells at 4 C, 30 C without exposure and at 30 C with exposure were in contact of ^{35}S-methionine for 16 h. Afterwards cells from all batches, i.e. at 4 C, at 30 C with exposure, and at 30 C without exposure were collected by centrifugation (15,000 *g* for 30 min at 4 C) and washed thrice with 10 mM Tris-HCl, pH 7.5 containing 1 mM EDTA. From these samples proteins were extracted and analysed on 7.5% sodium dodecyl sulphate polyacrylamide gels[8]. Briefly this procedure involves slicing of gel (2 mm thickness) with automatic gel slicer (Fisher Scientific Co., Spring, N. J.) and counting each slice in liquid scintillation counter using toluene base solvent as described by Virula and Kapoor[7].

The procedure described by Grummt *et al*[9] was followed for RNA polymerase I assay.

RESULTS

Incorporation of ³H-uridine to N. crassa cells and percentage of ³H-uridine remaining after exposure to varying temperature

With increased time of incubation, more incorporation of ³H-uridine in *N. crassa* mycelial cells was observed (figure 1). The peak value is seen after 8 h of incubation in the presence of ³H-uridine. When ³H-uridine labelled cells were subjected to heat treatment (figure 2) the per cent incorporation remaining after the treatment started declining. Based on these results we selected that 120 min at 46 C caused 90% lethality.

Comparison of ³H-uridine incorporation in N. crassa mycelial cells between sham (Crawford cell) and incubator control

The results given in table 1 suggest that there is no difference in the sham and control samples. Based on these observations, all of the control treatments were done in the same incubator that also contained the Crawford cell.

Microwave induced RNA synthesis in normal and heat damaged N. crassa cells based on incorporation of ³H-uridine

Figure 3 indicates that microwaves induce RNA synthesis in both undamaged and heat damaged *N. crassa* cells although the incorporation of ³H-uridine

Table 1 *Comparison of ³H-uridine incorporation in N. crassa between sham (Crawford) and incubator controls*

Time of incorporation (h)	Average cpm* incorporated after heat damage at 46°C followed by 30 C recovery ± S E.	
	Incubator**	Sham***
0	87±10	90±8
1	1360±65	1547±70
2	2008±72	1954±75
4	3107±80	2849±78
8	4711±80	4905±95
16	3969±96	4025±98

Per cent uptake is calculated on the basis of total cpm added Each flask contained 500,000 cpm in 5 ml of cell suspension. Each of these tests was repeated five times. * Counts per min; ** Incubator is defined as the sample kept outside the Crawford cell without any exposure to microwave radiation and maintained at 30 C during the experiment; *** Sham is defined as the sample kept in the Crawford cell without exposure to MWR during experiment at 30 C.

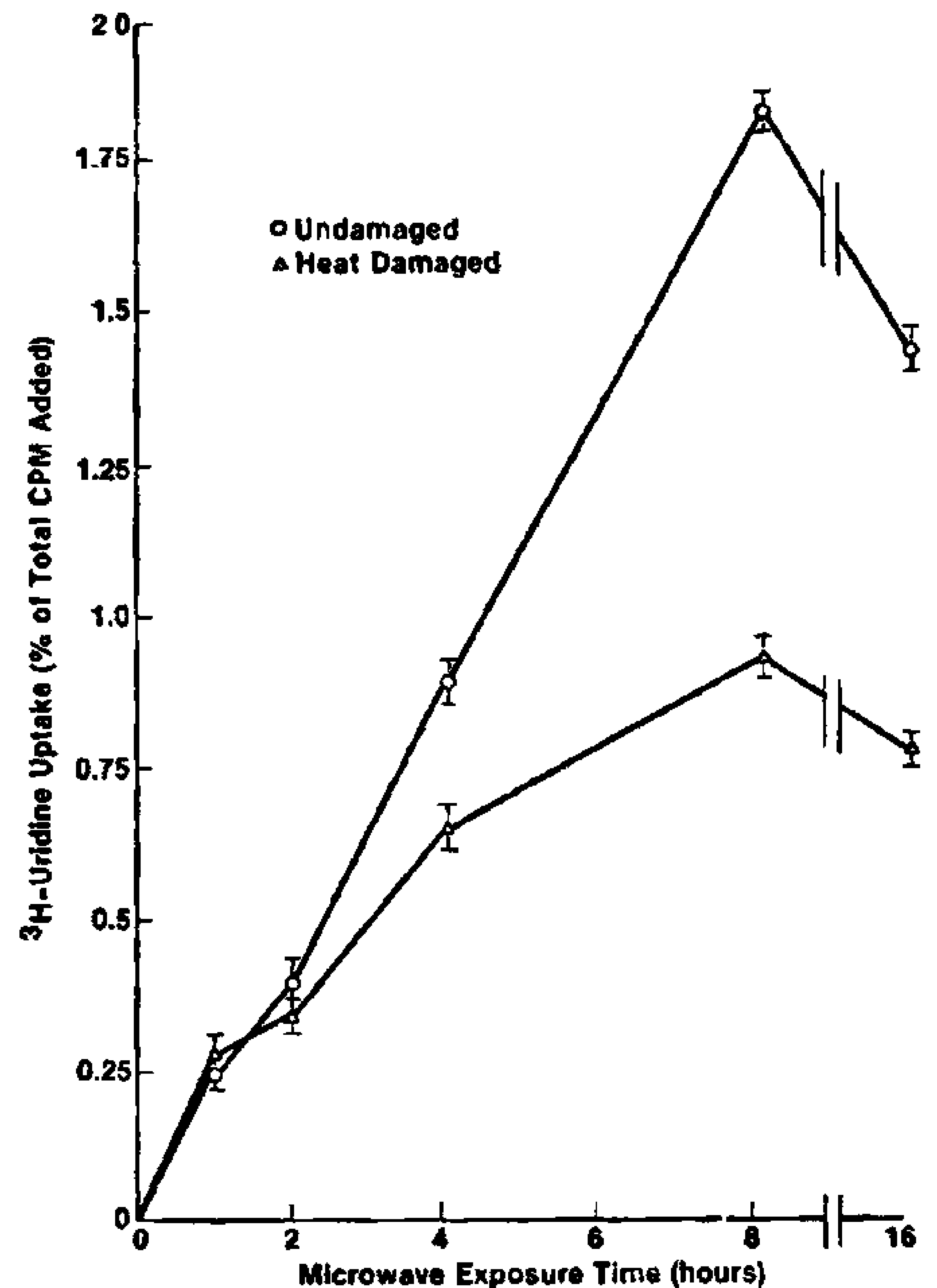

Figure 3. Incorporation of ³H-uridine in heat damaged and undamaged cells of *N. crassa*. *N. crassa* was grown in Vogels minimal medium (as described under materials and methods) and after 18 h of growth cells were divided into two batches containing constant number of cells in each batch. Cells of one batch were heat damaged (46 C for 2 h) and cells of other batch were maintained at 30°C for 2 h. Then ³H-uridine (2.5 μCi for 100 ml cells suspension) was added to each flask and aliquots (containing constant number of cells) were removed at different time intervals (as indicated in the figure). Trichloroacetic acid precipitable counts were determined in each aliquot. Symbols: (–O–), undamaged cells: (–△–), heat damaged cells. Error bars indicate the S.E.

is more in case of undamaged cells than in heat damaged cells.

Effect of microwave exposure on the activity of RNA polymerase I in normal and heat damaged N. crassa cells

Figure 4 shows a similarity between the results of ³H-uridine incorporation and as expected, the

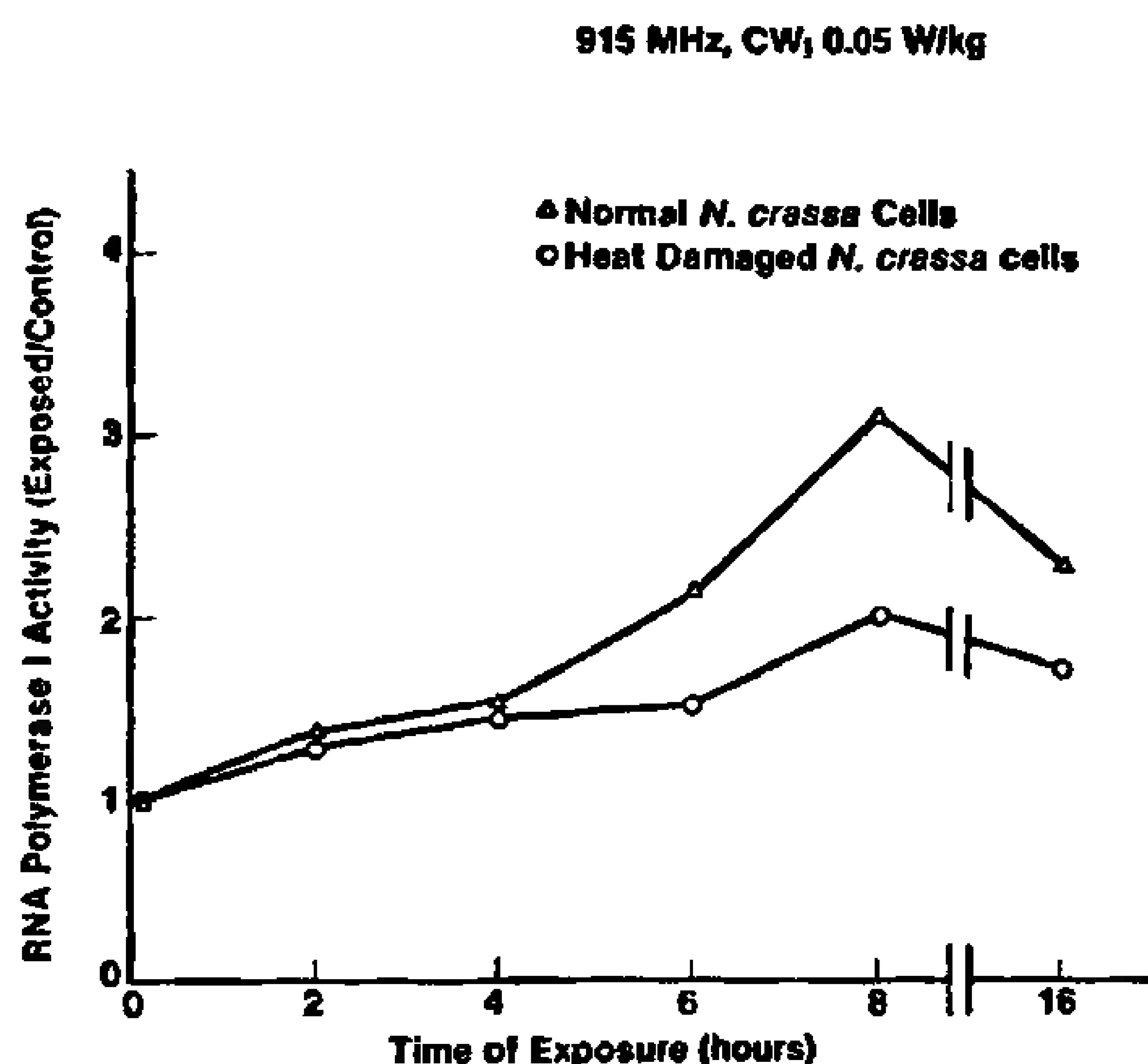

Figure 4. Activity of RNA polymerase I in *N. crassa* after exposure to 915 MHz, 0.05 W/kg. The detailed procedure is described under materials and methods. The activity of RNA polymerase I was determined in undamaged and heat damaged cells for different exposure times as indicated in the figure.

activity of RNA polymerase I in normal cells was higher than in heat damaged cells. Previously we conducted similar experiments in neuroblastoma cell line NG108 and obtained the same results[1].

Incorporation of ^{35}S-methionine in undamaged and heat damaged N. crassa cells

In our previous studies related with the dose response on the incorporation of ^{35}S-methionine in *N. crassa* cells (at 915 MHz, CW) we observed that the peak value comes at 0.1 W/kg. Similarly when the effect of the time of incubation of ^{35}S-methionine during exposure conditions (at 915 MHz, CW at 0.1 W/kg) was determined and the peak value was observed at 8 h. Similar experiments were performed with heat damaged (up to 90% lethality) *N. crassa* cells and it was concluded that 7-8 h time interval was the best for the incorporation of ^{35}S-methionine under exposure conditions (i.e. 915 MHz, CW, table 2). In all the cases the incorporation of radioactive amino acid was more in exposed sample compared to control (unexposed) sample. Enhanced protein synthesis due to exposure is shown in figure 5 where polypeptides have been separated on 7.5% acrylamide gel containing sodium dodecyl sulphate. These results suggest that exposure to low power level microwaves induces enhanced protein synthesis (as measured by the incorporation of ^{35}S-methionine).

SDS-PAGE of polypeptides synthesized under exposure conditions in undamaged and heat damaged N. crassa cells

The results of these experiments are shown in figures 6 and 7. As shown in figure 3, exposure of

Figure 5. SDS-polyacrylamide gel electrophoresis profile of polypeptides of *N. crassa* synthesized with and without exposure.

Table 2 *Microwave enhanced protein resynthesis based on incorporation of ^{35}S-methionine in N. crassa cells*

Time of exposure (h)	Average counts/min + S E.(N)			
	4 C	Control (30°C)	Exposed (30°C)	Exposed/control
0	214 ± 10 (6)	207 ± 015 (6)	208 ± 012 (6)	1.0 ± 0 01
1	231 ± 12 (6)	1304 ± 050 (6)	2230 ± 065 (6)	1.7 ± 0.30
2	299 ± 15 (6)	1876 ± 068 (6)	3308 ± 078 (6)	1.8 ± 0.18
4	343 ± 20 (8)	2532 ± 080 (8)	5505 ± 095 (8)	2.2 ± 0.15
8	431 ± 30 (10)	8308 ± 102 (10)	19997 ± 162 (10)	2.4 ± 0.32
16	242 ± 15 (6)	1798 ± 078 (6)	4878 ± 092 (6)	2.7 ± 0.25

915 MHz, CW at 0.1 W/kg SAR; N, number of times experiments were repeated; S.E., standard errors.

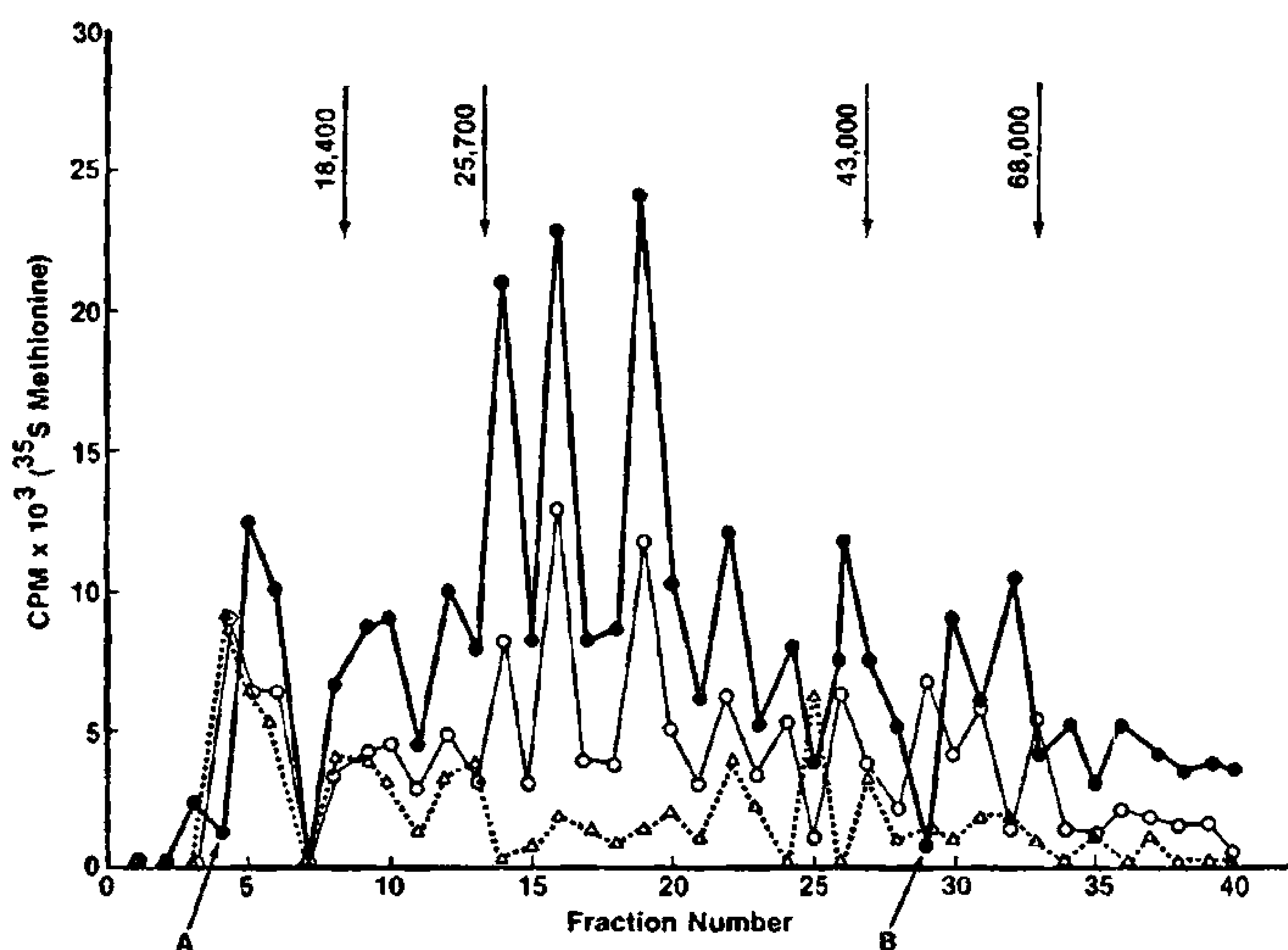

Figure 6. SDS-polyacrylamide gel electrophoresis profile of polypeptides of *N. crassa* synthesized during exposure to microwaves. Symbols: (–●–), heat damaged cells after exposure; (–O–), heat damaged cells without exposure and kept at 30°C; (–Δ–), heat damaged cells without exposure and maintained at 4°C. The numbers at the top represent the molecular weights of the marker proteins. Sign A (13.000 dalton) and B (51,000 dalton) are for two polypeptides which are repressed during exposure to microwaves.

N. crassa cells (undamaged) to microwaves induced enhanced synthesis of polypeptides. On the other hand, when heat damaged *N. crassa* cells were exposed to similar radiations for the same exposure time, the altered pattern of polypeptides was observed (figure 6). The synthesis of at least two polypeptides was repressed (molecular weight 13,5000 and 51,000 dalton). In order to find out whether these two polypeptides are heat shocked polypeptides (HSP) also, the samples were tested for the presence of HSP (as described under methods) and results are shown in figure 7. These results suggest that no heat shock polypeptide was synthesized under the conditions of heat treatment. A comparison of NG108[1] and *N. crassa* results suggests that under the same exposure conditions, four polypeptides are repressed in NG108 and two of them happened to be heat shock polypeptides.

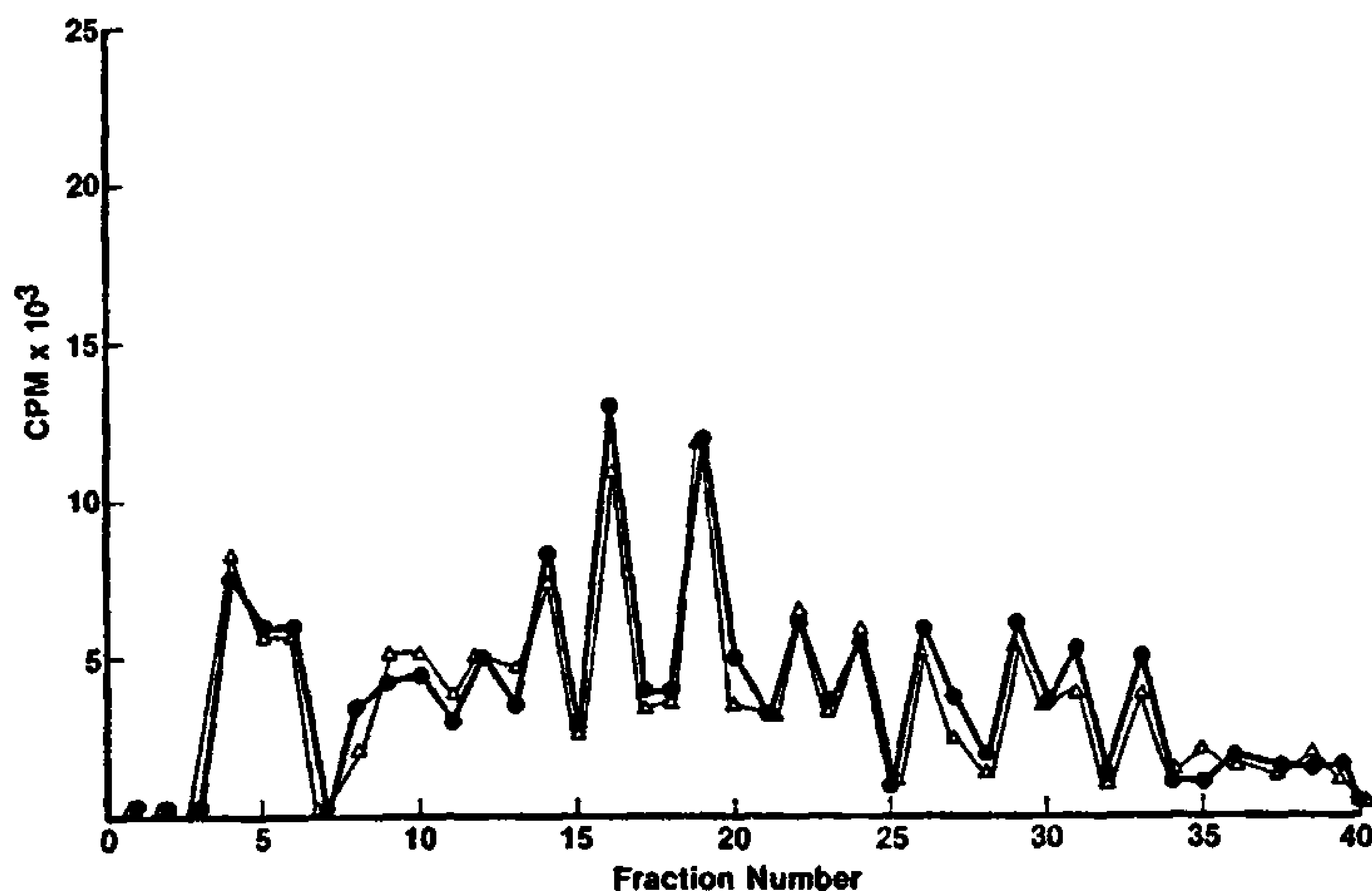

Figure 7. SDS-polyacrylamide gel electrophoresis profile of polypeptides synthesized in the absence of microwave exposure. Heat damaged or undamaged cells were incubated under normal growth conditions in the presence of radioactive methionine for 16 h and then polypeptides were analysed on gel. Symbols: (–△–), cells without heat damage; (–●–), heat damaged cells.

DISCUSSION

These studies were the follow up studies of our earlier observations with cancerous cell line (neuroblastoma NG108)[1] where it was observed that: (i) In normal NG108 cells and in heat damaged NG108 cells enhanced synthesis of RNA and protein was observed after exposure to 915 MHz, CW at 0.05 W/kg; (ii) Heat treatment induced at least two HSP; (iii) Exposure alone does not alter the arrangement of the polypeptides, and (iv) When heat treated NG108 cells were exposed an alteration in the polypeptide arrangement was observed (all the polypeptides except 4 were synthesized in large amount). In the present report we have observed that *N. crassa* cells do not synthesize any HSP. Our future aim will be to analyse those polypeptides which are repressed after heat and exposure treatment. These studies will help us understand the mechanism of interaction of low power level radiation with biological system.

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