

## EVIDENCE FOR THE ALTERED TRANSLATION IN THE NEUROBLASTOMA CELL LINE NG108 AS A RESULT OF EXPOSURE TO LOW LEVEL MICROWAVE RADIATIONS

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### ABSTRACT

Low power level microwaves (915 MHz, CW at 0.05 W/kg SAR) induce polypeptide rearrangement in a heat-damaged (up to 90% lethality) neuroblastoma cell line NG108. Transcription of total RNA (as measured by the incorporation of  $^3\text{H}$ -uridine), RNA polymerase I activity, and total protein synthesis (as measured by the incorporation of  $^{35}\text{S}$ -methionine) were also enhanced under exposure conditions. In cells not receiving heat treatment, exposure to microwaves enhanced the synthesis of both RNA polymerase I and proteins. Heat treatment alone induced at least two heat shocked polypeptides, and heat plus microwave exposure altered protein rearrangement. The translation of four low molecular weight (between 18,000 and 68,000 daltons) polypeptides was repressed almost completely, whereas synthesis of most polypeptides was induced. It was concluded that heat damaged neuroblastoma cells were more sensitive to microwave exposure in inducing alterations in protein rearrangements.

### INTRODUCTION

THE effect of electromagnetic radiation (EMR) on biological systems (especially transcription and translation) has been studied in human lymphocytes, oncogenes, *Drosophila*, and *Sciara coprophila*, and extensive reviews of these studies have been published<sup>1-6</sup>. Low level microwave radiation does not have any observable heating effect, but it alters the translation and polypeptide arrangement of the system under exposure, either by inducing or by suppressing some polypeptides, as compared with controls. Goodman *et al*<sup>7</sup> demonstrated that the use of increased pulsed electromagnetic fields could enhance cellular transcription; more recently<sup>4</sup>, they have shown that sine waves induced cellular transcription in *Sciara coprophila* salivary glands. ( $^3\text{H}$ -labelled uridine incorporation was used as the basis of the measurement of the level of transcription). They observed that repetitive pulsed electromagnetic radiation caused an increase in specific activity after 45 min exposure in all RNA size classes.

In the present report, we have submitted evidence that low level microwave radiation (915 MHz, CW with SAR of 0.05 W/kg) alters the profile of incorporation of  $^3\text{H}$ -uridine (a precursor for RNA synthesis) and  $^{35}\text{S}$ -methionine (a precursor for protein synthesis) in the heat damaged neuroblastoma cell line NG108. We chose to damage the cells by

heat treatment for two reasons. First, to see if cells and their macromolecules (RNA and protein) could be recovered by microwave exposure and secondly, to know if heating induced some polypeptides, thus enhancing the arrangement of polypeptides and recovery of macromolecules. Furthermore, since translation is directly related to transcription (as transcribed RNA is utilized to translate proteins), we measured the incorporation of  $^3\text{H}$ -uridine and  $^{35}\text{S}$ -methionine in NG108 cells. The logic of keeping one batch of cells at 4°C during some of the experiments is as follows. Since the basic metabolic rate of most organisms is very slow at low temperatures (e.g., 4°C), almost no incorporation of  $^3\text{H}$ -uridine and  $^{35}\text{S}$ -methionine is expected to occur in NG108 at 4°C. Any recovery of macromolecules and/or rearrangement in heat damaged cells occurs at 37°C and under exposure conditions. We tested this phenomenon by keeping one sample at 4°C, another at 37°C but without any exposure, and third at 37°C with exposure.

### MATERIALS AND METHODS

#### *Culturing conditions and maintenance of neuroblastoma cell line NG108*

The source and maintenance of the cultured neuroblastoma cells (NG108) have been described earlier<sup>8</sup>. NG108 cells were grown in Dulbecco's

modified eagle medium supplemented with thymidine (38.5 mg/100 ml) and hypoxanthine (136 mg/100 ml) in 25 cm<sup>3</sup> tissue culture flasks at 37°C. Cells grow to confluency in 5 days.

#### Chemicals and media

For culturing of the NG108 cells, the growth medium and supplemented materials were obtained from M.A. Bioproducts, Walkersville, Maryland, USA. <sup>3</sup>H-uridine (0.50 uCi/0.00174 μmol) and <sup>35</sup>S-methionine (11.8 Ci/mmol) were obtained from the New England Nuclear, Inc., Boston, USA. Acrylamide, bisacrylamide, ammonium persulphate, TEMED, 2-mercaptoethanol, glycine, Tris, sodium dodecylsulphate, coomassie blue, protein molecular weight markers, trichloroacetic acid, and acetic acid were the products of the Sigma Chemicals, and all other chemicals were of analytical grade. The composition of the cocktail mixture used for counting was described earlier<sup>9</sup>.

#### Instrumentation and dosimetry

All exposures were performed in a Crawford cell (Instruments for Industry, model BC-110) energized with a Hewlett Packard power source at 915 MHz, CW and the details of the equipment and conditions of exposure are described elsewhere<sup>9</sup>. The specific absorption rate (SAR) used was 0.05 W/kg, and this value was measured for different samples by detecting forward, reflected, and transmitted powers via a 20 dB bidirectional coupler (Narda Microwave, model 3020A) as described<sup>9</sup>.

#### Conditions for heat damage of NG108 cells

To establish the conditions for heat damage of neuroblastoma cells, constant number of cells incubated simultaneously in several batches at different temperatures (37, 38, 39, 45°C) for a fixed time (30 min). These cells were previously labelled with <sup>35</sup>S-methionine; after the heat treatment, the per cent radioactivity remaining (measured by trichloroacetic acid precipitable counts) was determined. It was concluded that a treatment at 39°C for 30 min is enough to damage neuroblastoma cells. The incorporation of the radioactive precursors (<sup>3</sup>H-uridine and <sup>35</sup>S-methionine) during exposure was measured in these heat damaged cells (<sup>3</sup>H-uridine, specific activity 0.50 uCi/0.00175 μmol, and <sup>35</sup>S-methionine, specific activity 11.8 Ci/mmol, were the products of the New England Nuclear, Inc., USA). The composition of the cocktail mixture used for counting was described earlier<sup>9</sup>.

#### Incorporation of <sup>3</sup>H-uridine and <sup>35</sup>S-methionine in NG108 cells during exposure to microwaves

Several batches of NG108 cells (with a constant number of cells in 5 ml medium) were heat-damaged as described above. Four such flasks were kept for exposure in a Crawford chamber at 0.05 W/kg SAR at 915 MHz, CW. Just before exposure was started, 2.5 uCi<sup>3</sup>H-uridine or 2.5 uCi<sup>35</sup>S-methionine were added to each flask. (These two radioactive materials were added separately in different flasks of different batches.) Therefore, the radioactive material was present in the medium only during exposure when the temperature of the Crawford cell was 37°C. The cells were exposed for different time intervals, and the reaction was stopped by adding an equal volume of 10% cold trichloroacetic acid and collecting the cells by centrifugation (2,000 g for 10 min at 4°C). Cells were washed thrice with 5% cold trichloroacetic acid. The pellet was then counted using a universal scintillation fluid in a liquid scintillation counter.

The procedure described by Grumnt *et al*<sup>10</sup> was followed for the assay of RNA polymerase I.

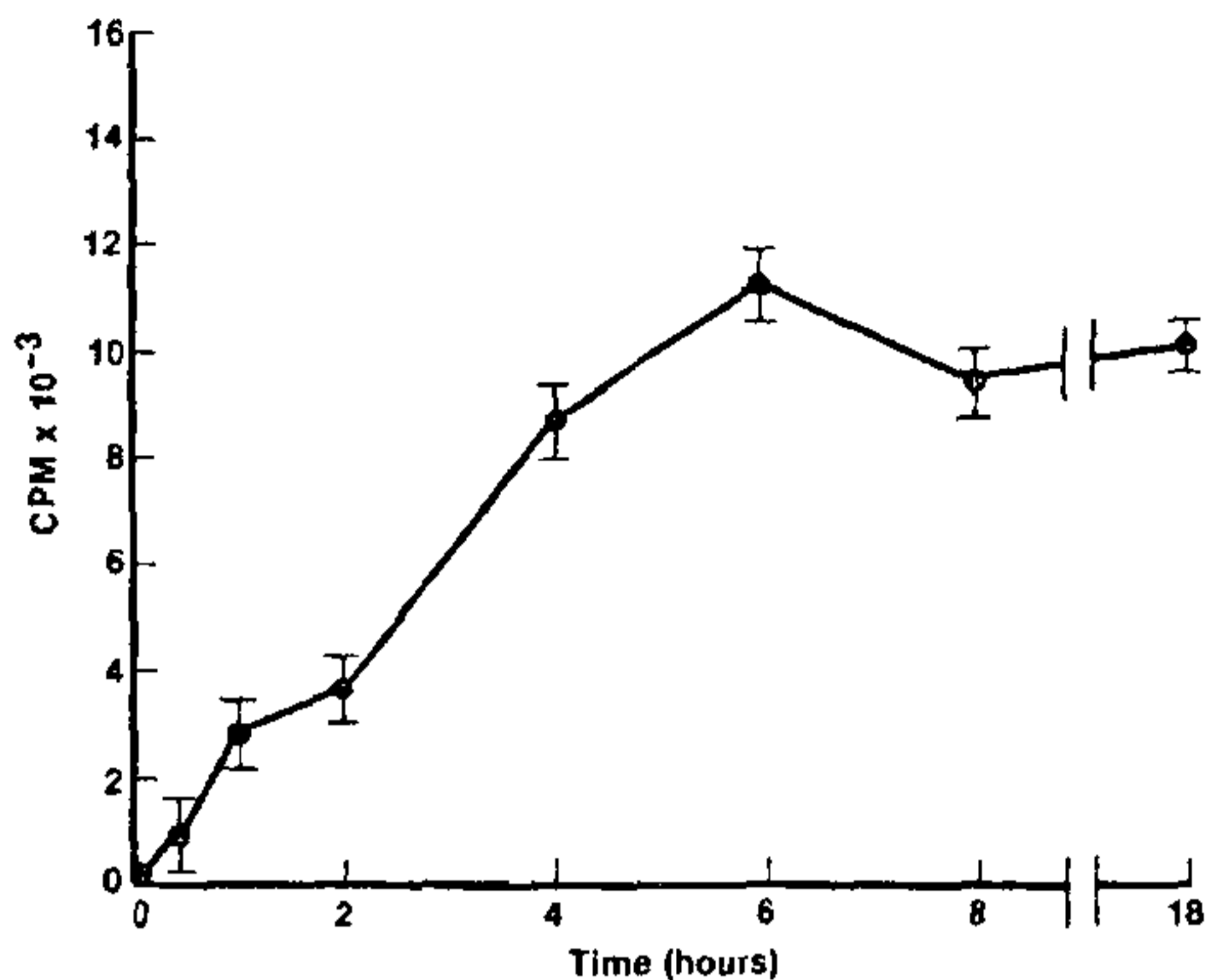
#### Sodium dodecyl sulphate polyacrylamide gel electrophoresis of <sup>35</sup>S-methionine labelled polypeptides

From a constant amount of cells (labelled with <sup>35</sup>S-methionine as described earlier) the proteins were isolated by a phenol extraction procedure<sup>11</sup>. The electrophoresis of polypeptides and counting of gel fractions were done according to Russell *et al*<sup>12</sup> using an automatic gel slicer (Fisher Scientific Co., USA). Shortly after electrophoresis, the gel was cut into 2 mm thick slices and each slice was counted in a liquid scintillation counter using a toluene base scintillation solution. To determine the molecular weights of polypeptides synthesized, standard molecular weight markers (bovine serum albumin, egg white lysozyme, DNase, and protease) obtained from the Bethesda Research Laboratories, MD. were used.

## RESULTS

#### Incorporation of <sup>35</sup>S-methionine into neuroblastoma cells

The profile of the incorporation of <sup>35</sup>S-methionine in heat damaged NG108 cells is presented in figure 1 which shows that the incorporation of <sup>35</sup>S-methionine is proportional to the duration of the time of splitting the cells. The peak is seen about 6 h of the splitting of cells.



**Figure 1.** Incorporation of  $^{35}\text{S}$ -methionine in NG108 cells.

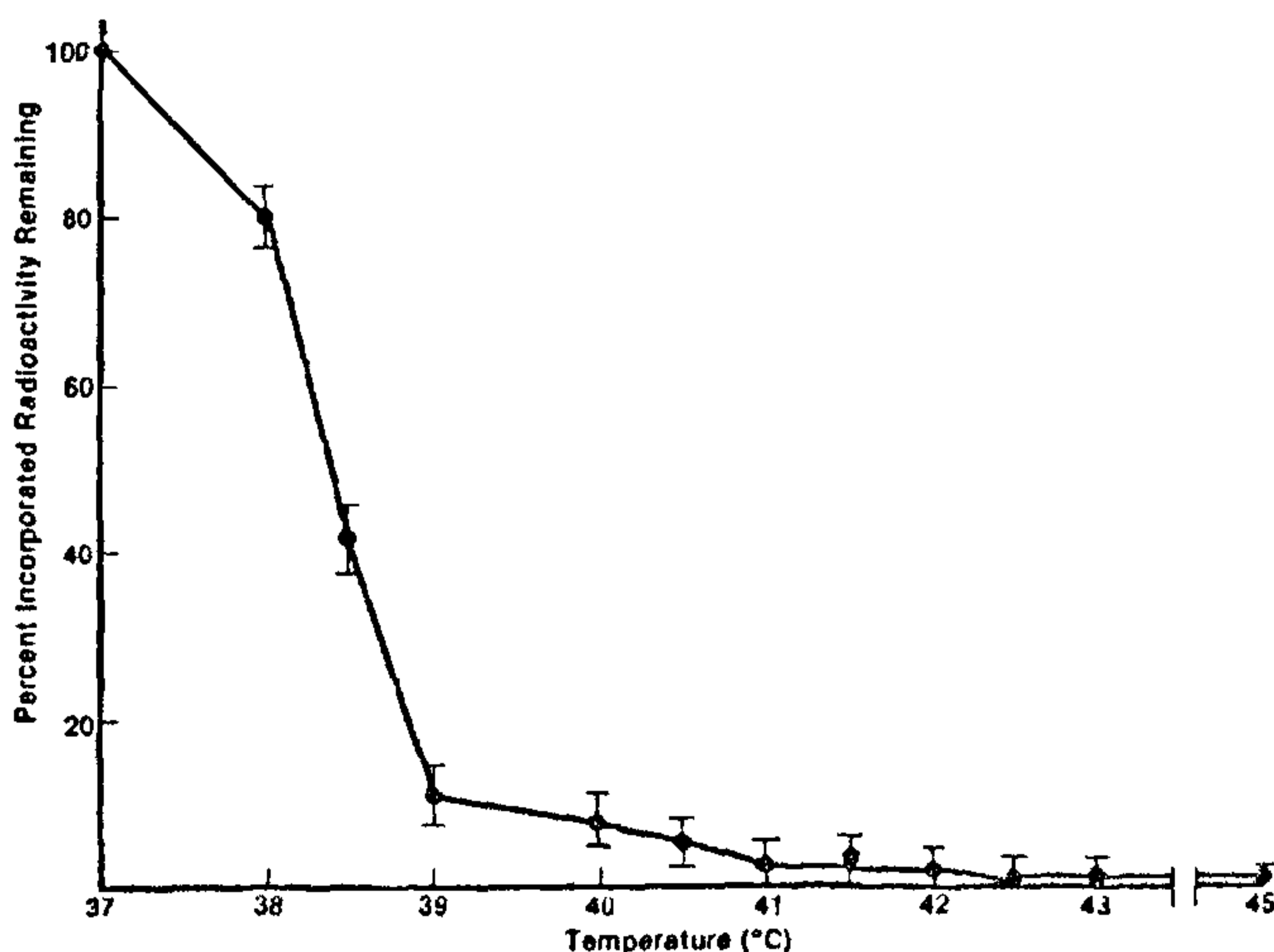
*Degradation of  $^{35}\text{S}$ -methionine labelled polypeptides of NG108 cells by heat*

To investigate the time involved in the degradation of polypeptides of neuroblastoma cells (NG108) by heat, the following experiment was performed. Neuroblastoma cells were labelled with  $^{35}\text{S}$ -methionine as described above. These cells were treated for 30 min at different temperatures as shown in figure 2. The reaction was stopped by adding an equal volume (i.e. 5 ml) of cold 10% trichloroacetic acid to the heat treated cells, incubating them on ice for 15 min, and then washing them

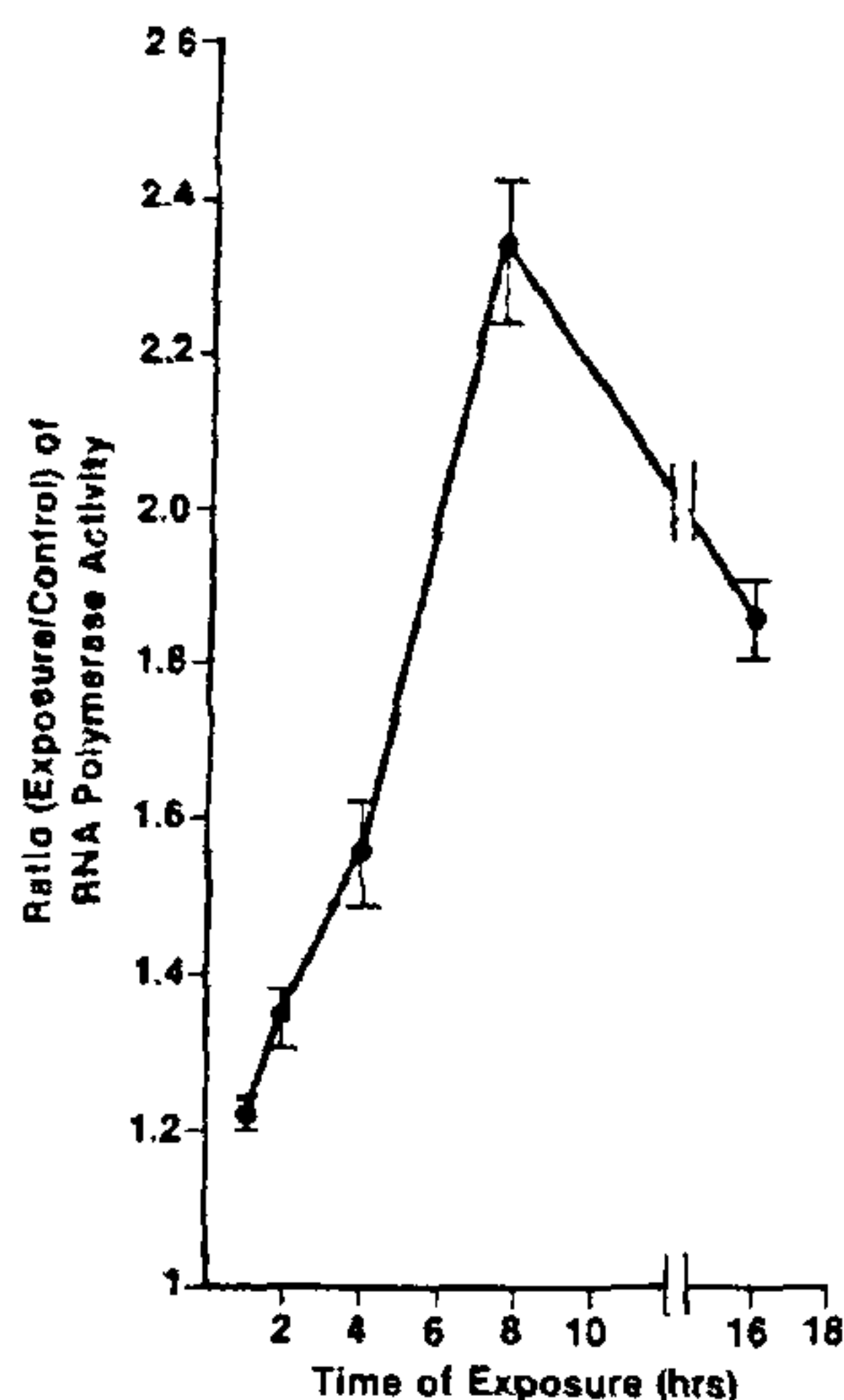
twice with cold 5% trichloroacetic acid. (Each time, the cell pellet was collected by centrifugation at low speed at 4°C). Trichloroacetic acid precipitable counts were determined and the results are shown in figure 2. The figure illustrates that about 20% polypeptides are degraded at 38°C, whereas about 85% polypeptides are degraded at 39°C for 30 min. At still higher temperatures, the polypeptides continue to degrade. For further experimentation, a 30 min exposure at 39°C was chosen for the heat damage of cells (considering that degradation of polypeptides reflects the damage of cells).

*Incorporation of  $^{35}\text{S}$ -methionine and  $^3\text{H}$ -uridine and RNA polymerase I activity in heat damaged NG108 cells during exposure to microwave*

Table 1 shows that microwave radiation induced enhanced transcription and translation. The ratio of exposed to control is 1.95 and 2.81 for  $^3\text{H}$ -uridine and  $^{35}\text{S}$ -methionine incorporation, respectively, after 4 h of exposure. The peak values (exposed/control) were seen after 7.5 h of exposure of cells i.e. 2.50 (for  $^3\text{H}$ -uridine) and 2.86 (for  $^{35}\text{S}$ -methionine). The incorporation of  $^3\text{H}$ -uridine and  $^{35}\text{S}$ -methionine directly reflects the level of transcription and translation in NG108 cells. Furthermore, almost no incorporation of the radioactive precursor is observed at 4°C. This is not surprising because we do not expect a high rate of metabolism in NG108 cells at such a low temperature.



**Figure 2.** Degradation of  $^{35}\text{S}$ -methionine labelled polypeptides of NG108 cells by incubation at different temperatures.



**Figure 3.** Effect of time of exposure of radiofrequency radiation on the activity of RNA polymerase I. The time of exposure was as indicated in the figure, and the conditions of exposure were as described<sup>9</sup>. The assay procedure for RNA polymerase I has been described elsewhere<sup>10</sup>.

When the RNA polymerase I activity was measured, the peak value was observed at about 7–8 h of exposure (figure 3).

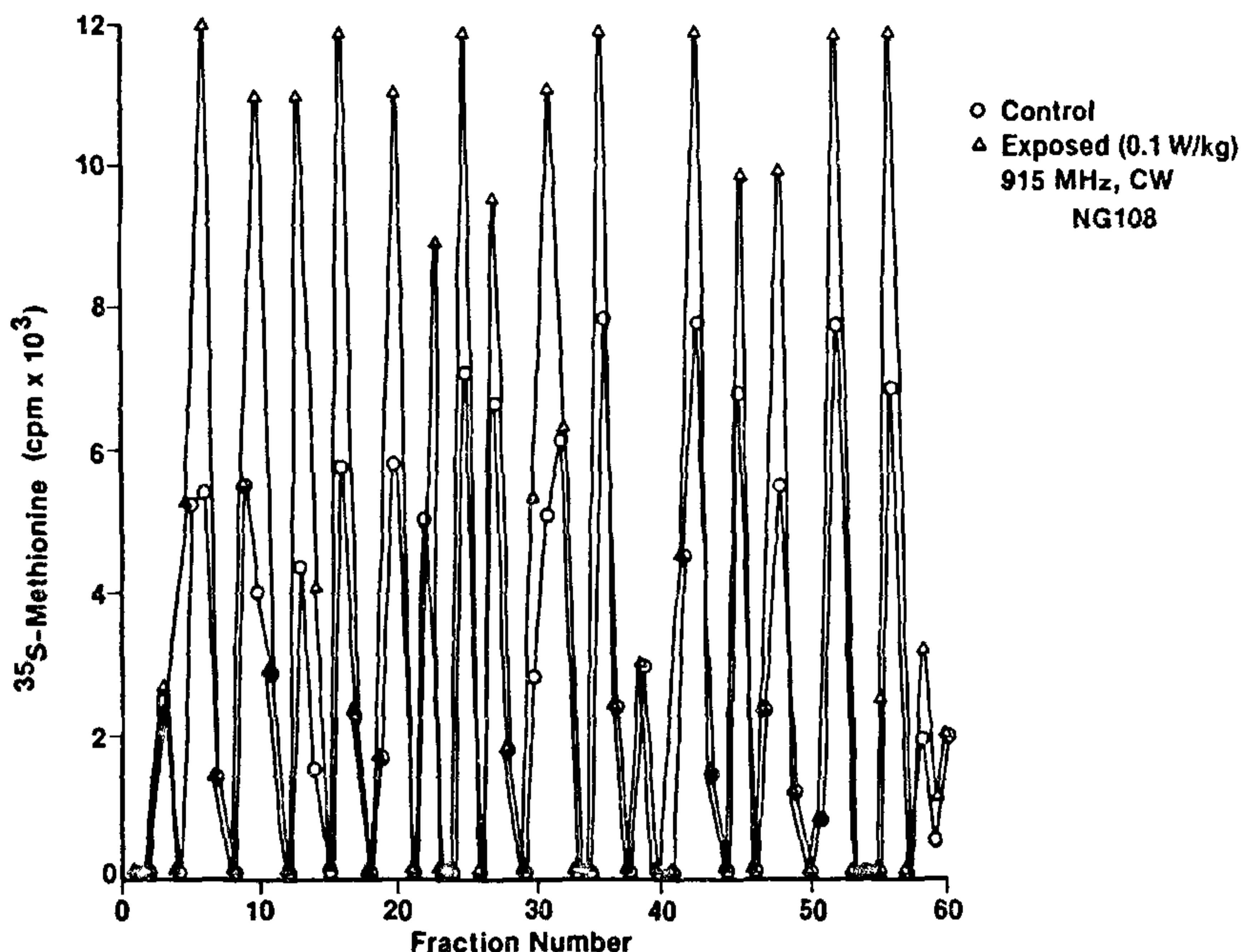
#### *Alteration in translation products due to exposure of microwaves*

Three batches, each containing a constant number of cells, received heat treatment (39°C for 30 min) as described earlier. <sup>35</sup>S-methionine (2.5 uCi/5 ml cells suspension) was added to all batches. One batch of

cell was exposed to microwaves, a second was maintained at 37°C (without exposure), and a third was maintained at 4°C, for 7.5 h (i.e., the time at which the incorporation of radioactivity is optimum). After the incorporation, proteins were isolated from each batch and polypeptides were analysed as described under "Materials and Methods". Interesting results were observed when we used sodium dodecyl sulphate polyacrylamide electrophoresis as the method of analysing polypeptides, with or without exposure to microwaves (figure 4). Cells without heat treatment show enhanced protein synthesis but no detectable change in the pattern (arrangement) of polypeptides. From a constant number of cells, the total amount of proteins synthesized after exposure was much higher than their unexposed counterparts (controls). Low level (SAR 0.05 mW/g) microwave radiation augmented translation of most of the polypeptides (figure 4). However, diminution of at least four polypeptides (molecular weights 20,000 to 75,000 daltons) occurred when heat-treated cells were used (figure 5). To determine if some heat shock polypeptides are synthesized under these conditions, the following experiment was performed. Two batches containing a constant number of cells were prepared as described earlier. Cells of one batch were heat-damaged (39°C for 30 min), whereas cells of the other batch were maintained at 37°C. Later, the recovery of polypeptides was observed for 6 h at 37°C. Then proteins were isolated from cells of each batch and analysed by SDS-polyacrylamide gel electrophoresis. The results are shown in figure 6. Two heat shock polypeptides of molecular weight 25,000 and 42,000 daltons (figure 6) are seen in the sample of cells which was heat damaged followed by recovery under normal growth conditions (i.e. at 37°C) in the presence of <sup>35</sup>S-methionine. A comparison of figures 5 and 6 seems to indicate quite clearly

**Table 1** Incorporation of <sup>35</sup>S-methionine and <sup>3</sup>H-uridine in heat damaged NG108 cells after exposure to microwaves

| Time of exposure (h) | CPM incorporated (Mean ± SE) |            |             |                            |            |              | Ratio of exposed to control (E/C) |                            |
|----------------------|------------------------------|------------|-------------|----------------------------|------------|--------------|-----------------------------------|----------------------------|
|                      | <sup>3</sup> H-uridine       |            |             | <sup>35</sup> S-methionine |            |              | <sup>3</sup> H-uridine            | <sup>35</sup> S-methionine |
|                      | 4°C                          | 37°C (C)   | Exposed (E) | 4°C                        | 37°C (C)   | Exposed (E)  |                                   |                            |
| 1                    | 13 ± 2                       | 316 ± 30   | 434 ± 36    | 20 ± 3                     | 1109 ± 44  | 1633 ± 521   | 1.37                              | 1.78                       |
| 2                    | 17 ± 1                       | 1028 ± 180 | 1487 ± 73   | 33 ± 2                     | 3321 ± 144 | 6191 ± 252   | 1.45                              | 1.80                       |
| 4                    | 13 ± 3                       | 2152 ± 275 | 3925 ± 355  | 23 ± 1                     | 3001 ± 521 | 8447 ± 284   | 1.95                              | 2.81                       |
| 7½                   | 23 ± 3                       | 3100 ± 280 | 7750 ± 410  | 25 ± 3                     | 4876 ± 95  | 13964 ± 507  | 2.50                              | 2.86                       |
| 16                   | 49 ± 15                      | 3991 ± 324 | 9865 ± 671  | 19 ± 3                     | 6321 ± 848 | 11023 ± 1049 | 2.47                              | 1.74                       |



**Figure 4.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis profile of <sup>35</sup>S-methionine labelled polypeptides of NG108 cells during exposure to radiofrequency radiation. In this experiment, no heat damage of cells was done. The detailed procedures are described under "Materials and Methods".

that heat shock polypeptide A (figure 6) corresponds to radiation repressible polypeptide B (figure 5) and heat shock polypeptide B (figure 6) corresponds to radiation repressible polypeptide C (figure 5). This suggests that the major effects of radiation may be on heat induced polypeptides.

## DISCUSSION

The effects of microwave radiation have been experimentally related to changes in cellular activity as diverse ion flux, enzymatic activity, DNA synthesis, and neurotransmitter release (see Ref. 13 and references therein). In our laboratory, we have previously demonstrated the enhancement of cell growth and augmented transcription in the yeast *Saccharomyces cerevisiae* as a result of exposure at 915 MHz, CW and 0.05 W/kg<sup>14</sup>. We have also obtained augmentation of DNA, RNA, and proteins in *Neurospora crassa* as a result of low power level microwave (915 MHz, CW) radiation (Dutta and Verma, unpublished, 1987). The increased

DNA synthesis and RNA synthesis were measured by measuring the incorporation of <sup>3</sup>H-thymidine and DNA polymerase I activity (in the case of DNA synthesis) and by incorporation of <sup>3</sup>H-uridine and RNA polymerase activity as well as isolation of DNA and RNA synthesized followed by electrophoresis. The present results strongly suggest that in neuroblastoma cells low level microwave radiation interacts with the two vital and related processes of the biological systems (transcription and translation); similar results have been shown in other organisms<sup>7,13,14</sup>. At present, we cannot say if the affected polypeptides, as a result of exposure to low power level electromagnetic radiation, are regulatory proteins and if these polypeptides are membrane bound. However, it is significant to know that the protein arrangement is altered due to the radiation itself. These proteins may be RNA polymerases themselves (or their subunits) or factors involved in protein initiation, elongation, or termination; or it may be just the change in the conformation of some of the polypeptides that

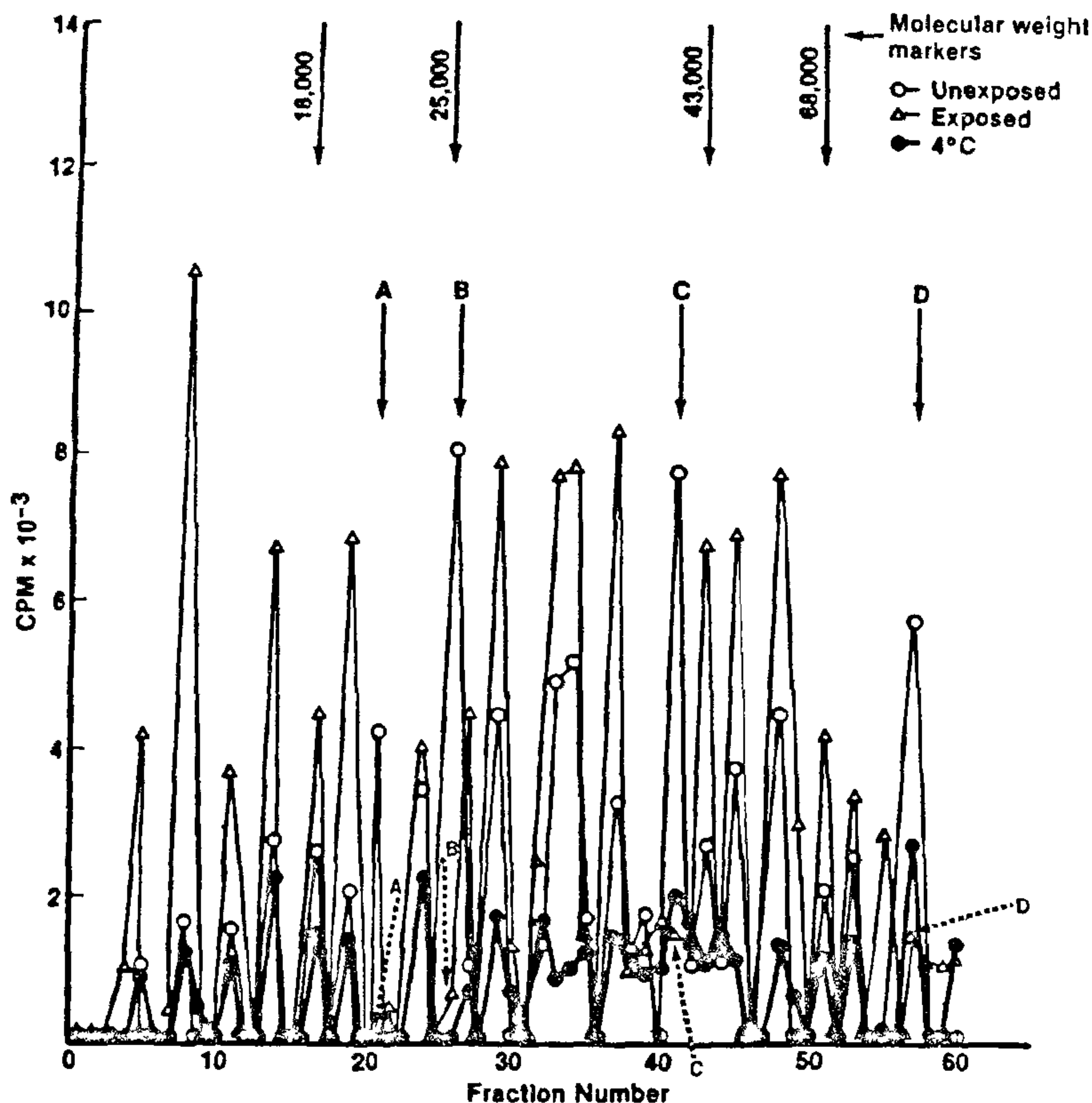


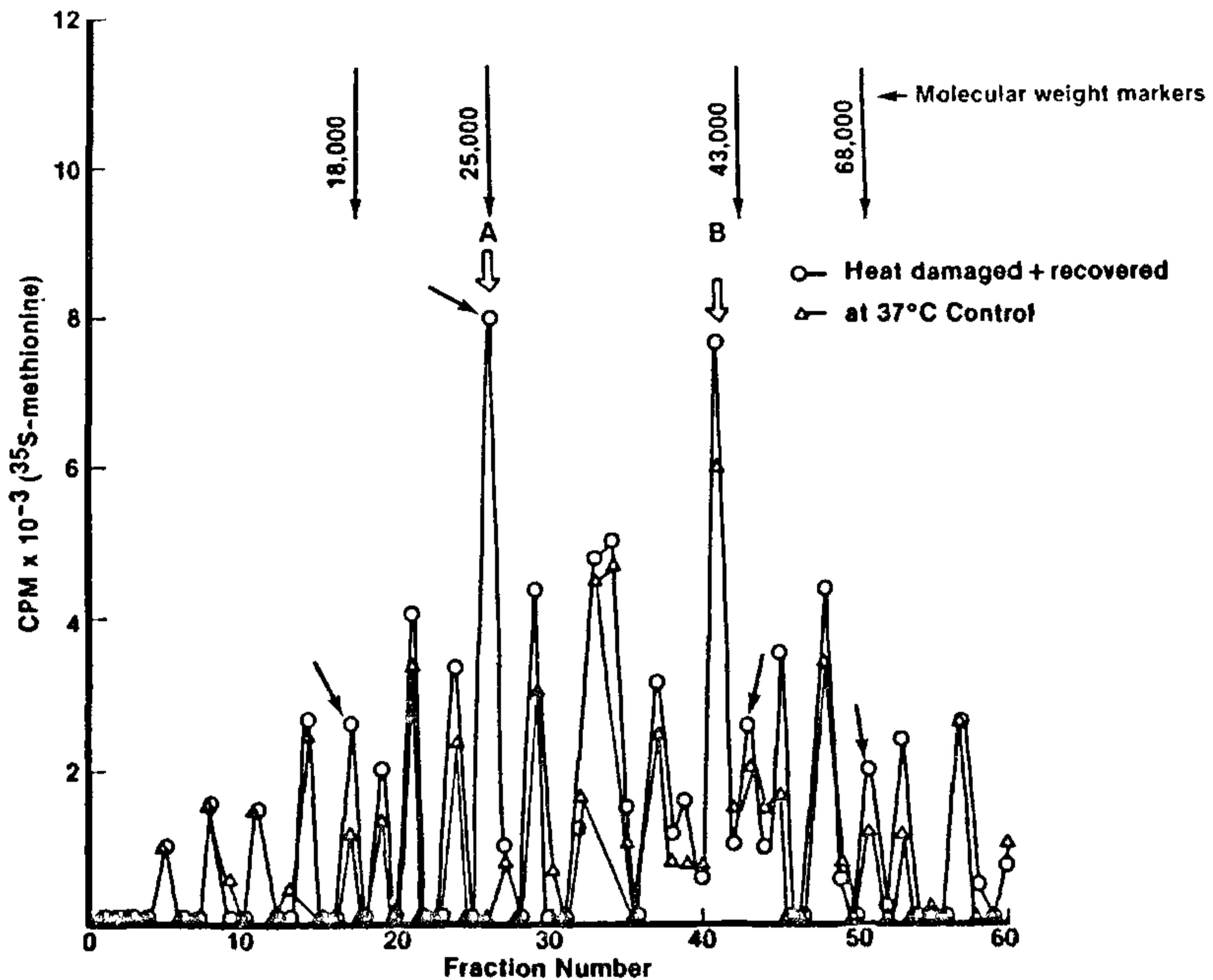
Figure 5. Sodium dodecyl sulphate polyacrylamide gel electrophoresis profile of  $^{35}\text{S}$ -methionine labelled polypeptides of NG108 cells during exposure to microwaves. Three batches of NG108 cells (with equal numbers of cells in each case) were heat damaged simultaneously. The incorporation of  $^{35}\text{S}$ -methionine was measured in (i) exposed cells incubated at  $37^\circ\text{C}$  ( $-\Delta-$ ), (ii) in unexposed cells maintained at  $37^\circ\text{C}$  ( $-\text{O}-$ ), and (iii) in unexposed cells maintained at  $4^\circ\text{C}$  ( $-\bullet-$ ). The molecular weights (dalton) of the protein markers are shown by different numbers at the top of the figure, and four solid arrows (pointing towards 4 different polypeptides) represent four polypeptides which were diminished (as also shown by broken arrows at the bottom of the figure) at the time of exposure.

suppresses or induces some of the possibly useful gene products. Research in these directions is urgently needed.

When the polypeptide profile of undamaged NG108 cells was observed with and without radiation, an increase in the synthesis of polypeptides was observed, but the arrangement of polypeptides was not changed (figure 4). This suggests that heat treatment is crucial for causing the alteration in the arrangement of polypeptides and that heating

induces protein rearrangement (figures 5 and 6). We have made similar observations in another eucaryotic organism, *Neurospora crassa* (unpublished results); therefore, it seems that this phenomenon may well be shown to be widespread. We believe that the present observations will help us to understand the interaction of heat and radiation with biological processes. This knowledge is very essential in radiation oncology and radiotherapy treatment.

It is known that heat is a most effective cell killing



**Figure 6.** Sodium dodecyl sulphate polyacrylamide gel electrophoresis profile of heat damaged and undamaged cells of NG108 without exposure. Symbols ( $\Delta$ ) cells without heat damage and maintained at 37°C throughout; (O), heat damaged cells maintained at 37°C but without any exposure to microwaves. The hollow arrows (A and B) represent two heat-shock polypeptides whereas solid arrows represent the molecular weight markers.

agent during the synthesis 'S' phase of the cells' mitotic cycle<sup>15</sup>. Radiation, on the other hand, is most effective during the mitosis 'M' phase of the cell cycle. It is quite possible that, in our experiments, the cells that were heat-treated were in the S phase (DNA synthesis phase) and after the treatment these were exposed to microwaves that induced the alteration in the translation patterns of the polypeptides. However, it is suggestive that detailed experiments should be conducted to understand the mechanism involved in these processes.

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## NEWS

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### CLEANING UP A CHEMICAL DUMP

...Resource Engineering (Houston, Texas) has "used naturally occurring micro-organisms to help turn waste into water and carbon dioxide. It has just finished the largest-ever field trial using microbes to help clean up part of French Limited, an improbably named lagoon near Houston that several companies have used as a chemical dump. Resource Engineering helped natural garbage-eating microbes to flourish by pumping oxygen and nutrients into the lagoon and making sure that the resulting soup was stirred continuously. The company thinks it would

cost \$ 40-50 million and take three-four years to clean the whole lagoon, which contains 80,000 cubic yards of contaminated sludge and 70,000 cubic yards of contaminated soil and sand. This compares with a cost of \$ 140 million and four-six years using a standard incinerator." [In *Economist* (London) 13 February 88, p. 88. Reproduced with permission from Press Digest, *Current Contents*®, No. 21, May 23, 1988, p. 13. (Published by the Institute for Scientific Information®, Philadelphia, USA.)]