PROTEIN SYNTHESIS DURING THE TOXIC EFFECT OF PLANT LATEX ON *ASPERGILLUS JAPONICUS*

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EXTRACTS of *Calotropis* sp.[1,2] and *Euphorbia* sp.[3] are known to have potent antimicrobial activity. A hydrocarbon degrading strain of *Aspergillus japonicus*[4-6] could degrade *Euphorbia nerifolia* and *Calotropis gigantea* latex as a sole source of carbon and energy inspite of their toxicity[2,7-9]. Similarly, another strain of *A. japonicus* independently isolated by Milstein *et al*[10] could degrade hydrocarbons and lignin related compounds[11]. Two important sets of proteins may have a fundamental role to play in the degradation process i.e. (i) protein(s) involved in the detoxification, and (ii) protein(s) involved in the bio-degradation. The aim of the present investigation was to study the above processes.

Latex from *E. nerifolia* and *C. gigantea* was tapped from the stem in the early hours of the morning. *A. japonicus* was cultivated as described earlier[5,6]. Spores of *A. japonicus* (1×10^6) were inoculated into the medium containing 4% w/v of glucose in 100 ml medium in 250 ml conical flasks on a 150 rpm rotary shaker. After 24 h of growth the cells were transferred to a fresh basal medium and the latex was supplemented as a sole source of carbon. This time interval was designated as 0 h. Degradation was carried out at 30°C.

Incorporation of radioactive precursors into proteins

Cells of *A. japonicus* were harvested after 24 h of growth on *C. gigantea* and *E. nerifolia* latex. Cells grown on glucose were taken as control. These cells on equal wet weight basis were resuspended in the same basal medium with the respective carbon sources. These were pulsed with a mixture of 5 μCi of (Carboxyl-^{14}C) L-leucine (0.1 Ci/mol, Bhabha Atomic Research Centre, Bombay, India) and 5×10^{-4} L-leucine. This was a slight modification of the procedure of Sturani *et al*[12]. The cells were homogenized in a Braum homogenizer using glass microbeads (0.5 mm diameter) for 4 min at 4°C ± 2 in the presence of 50 mM tris-HCl buffer (pH 7.2) and the homogenate was centrifuged at 800 *g* for 10 min at 4°C to remove the nuclei and the unbroken cells.

The supernatant obtained after recentrifuging (5000 *g* for 30 min) was termed as the soluble fraction. The pellet was washed thrice with cold 50 mM tris-HCl (pH 7.2) and resuspended in the above buffer containing 0.1% v/v triton X-100 for 1 h at 4°C. The extract was centrifuged (15,000 *g* for 30 min) and this supernatant was termed as the membrane or the particulate fraction. The incorporation of the radioactive leucine into the TCA precipitable material was determined as reported earlier[13]. Radio-activity was determined on a scintillation counter (Packard Tricarb) using the standard liquid scintillation counting solution (toluene, POP and POPOP). Cyclohexamide was added 1 h before supplementing the radioactive leucine.

Extraction and estimation of polyribosomes

Polysomes from the fungal system were extracted and estimated according to Christoffersen and Latice[14]. Polysomal RNA was analysed on a double beam spectrophotometer (Uvikon) by absorbance at 260 nm. Yeast RNA was used as a standard to convert the optical density values to the amount of RNA present.

Polyacrylamide gel electrophoresis

Proteins were separated on 7.5% w/v polyacrylamide gels. A 3 mA/gel power supply was provided with 0.05 M tris-glycin (pH 8.5) as the electrode buffer. All the samples were run simultaneously under identical conditions. Proteins (150 μg) after the run were fixed and stained with Coomassie Brilliant Blue.

A one-to-one and half-fold increase in the incorporation of ^{14}C-leucine into trichloroacetic acid (TCA) precipitable proteins was obtained in the cells metabolizing the latex of *C. gigantea* and *E. nerifolia* respectively, when compared to those grown on glucose at the fourth hour (figure 1). A linear increase in the incorporation of ^{14}C-leucine into both the fractions was seen in the cells metabolizing the plant latices when compared to those grown on glucose. The incorporation of the ^{14}C-leucine into the cells metabolizing the latex of *E. nerifolia* was greater than that observed with *C. gigantea* (figure 2).

More than one- and three-fold increase in the total polyribosomal content was observed in the cells grown on *C. gigantea* and *E. nerifolia* latex

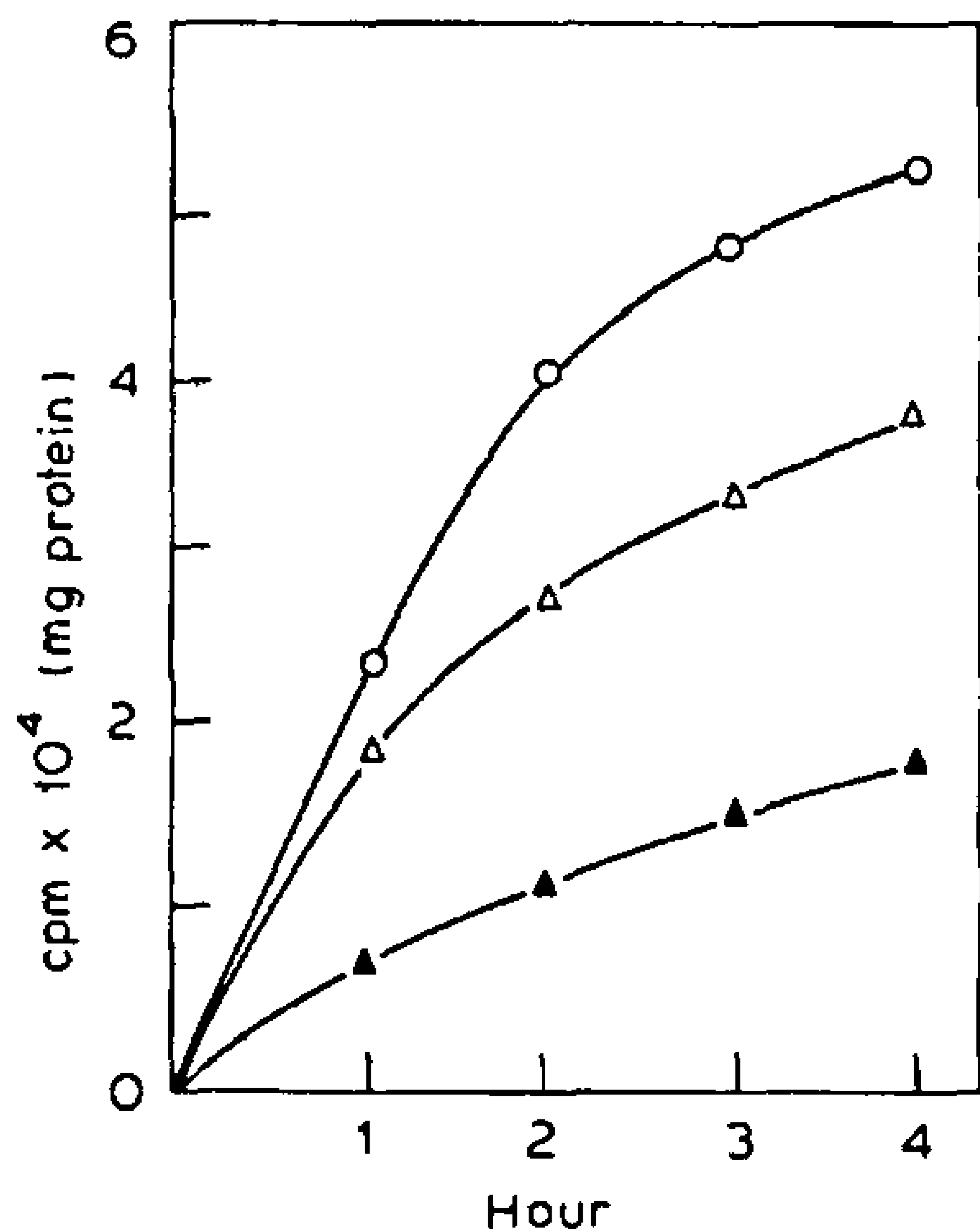

Figure 1. Incorporation of ^{14}C-leucine in the total protein of cells of *A. japonicus* degrading *C. gigantea* (△——△), *E. nerifolia* latex (○——○) and glucose (▲——▲) at different time intervals.

respectively when compared to those grown on glucose (figure 3).

Gravimetric analysis of the total solids carried out after freeze-drying the spent medium without mycelia at 24 h showed that 7% and 18% of the solids could be recovered in *C. gigantea* and *E. nerifolia* latex degrading flasks respectively, when compared to those at 0 h[15]. Similarly, 68% and 74% of the total solids were recovered from the respective flasks supplemented with cyclohexamide.

A decrease in the g/dry wt of the cells was observed in cells growing on glucose in the presence of cyclohexamide when compared to those grown without cyclohexamide. However, there was a significant decrease in the g/dry wt of cells growing on the plant latices in the presence of cyclohexamide when compared to that initially added at 0 h (table 1).

The electrophoretic mobilities of various proteins were different in cells grown on glucose, *C. gigantea* and *E. nerifolia* latex at 24 h. No significant difference in the total protein profiles was seen when cells grown on each of the above carbon sources were supplemented with cyclohexamide, except for a few bands in cells grown on glucose. This change in the protein profile due to protein degradation cannot be ruled out. Earlier studies with cells of *S. cerevisiae* showed a two-fold increase in the intracellular

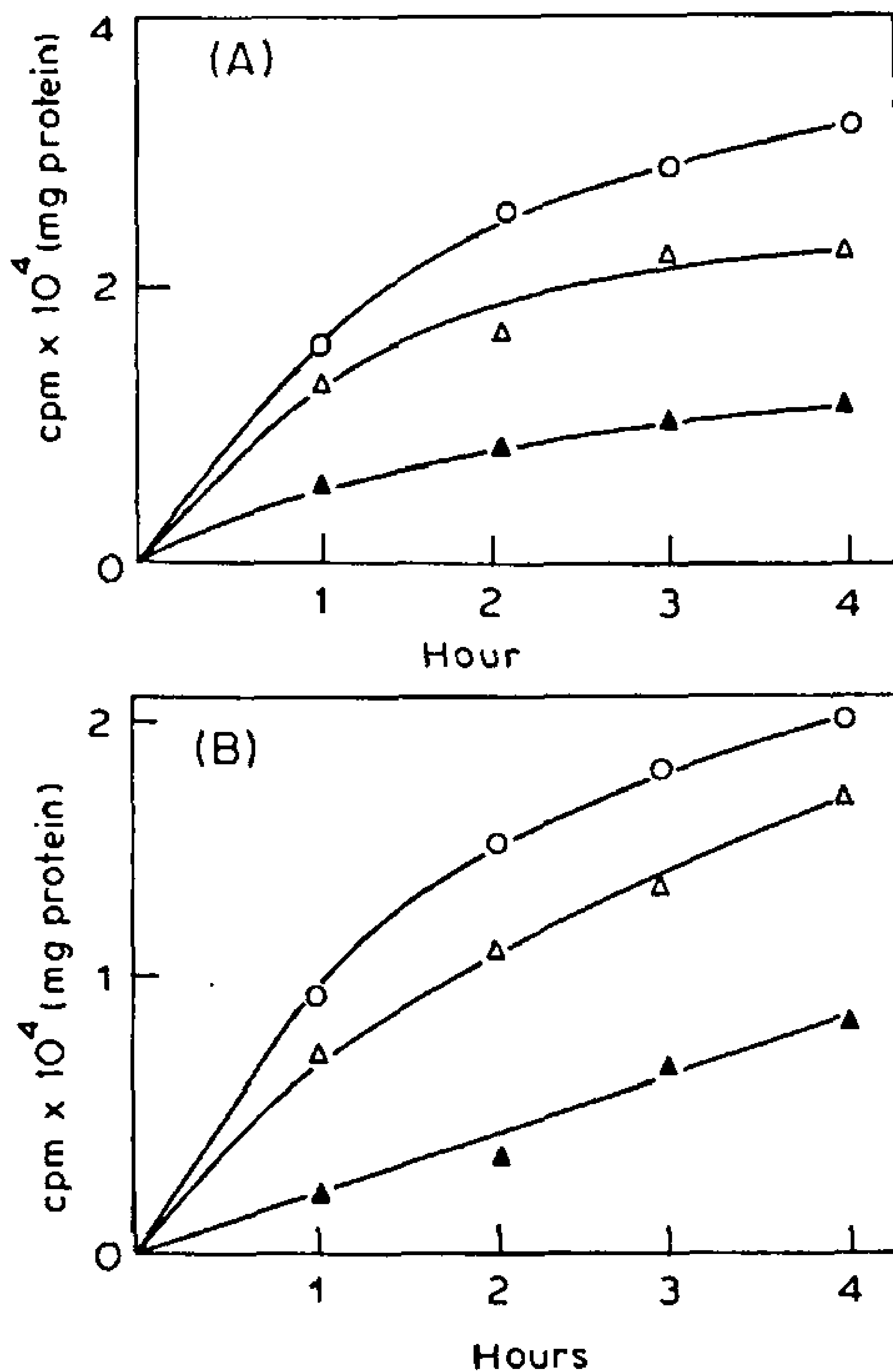

Figure 2A-B. Incorporation of ^{14}C-leucine in the proteins of the soluble (A) and the membrane (B) fractions of cells of *A. japonicus* grown on glucose (▲——▲), *C. gigantea* (△——△), and *E. nerifolia* latex (○——○) at different time intervals.

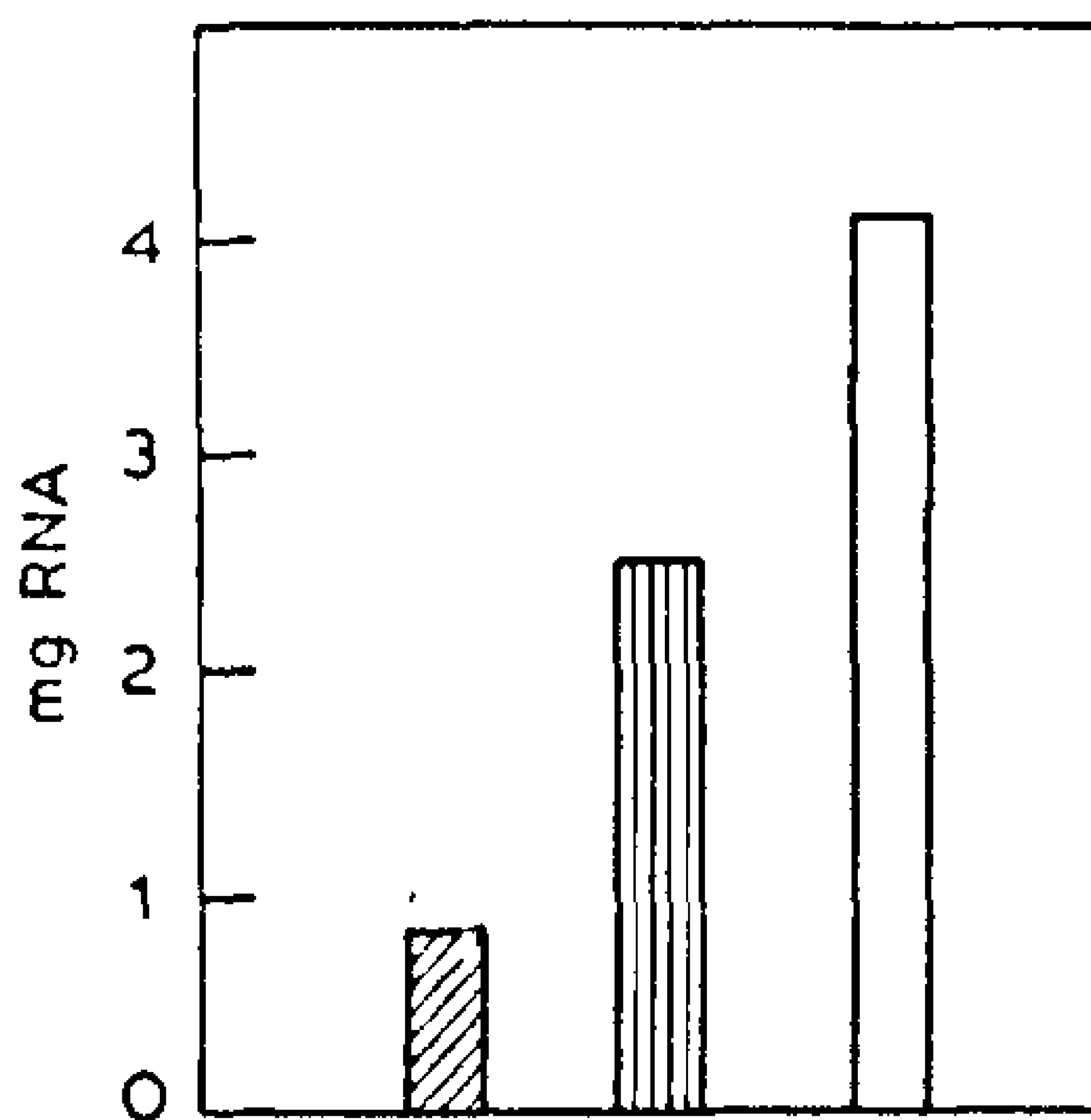

Figure 3. Polyribosome content of *A. japonicus* at 24 h during degradation of *C. gigantea* (▥), *E. nerifolia* latex (□) and glucose (▨).

Table 1 Changes in g dry wt of *A. japonicus* during degradation of plant latices in the presence and absence of cyclohexamide at different time intervals

Substrate	Time (h) 0	24	48	72
Glucose	2 3	18 6	26.4	29.9
Glucose + cyclohexamide	2.3	3 7	3 4	3.2
C. gigantea latex	2 3	6.6	7 2	6.8
C. gigantea latex + cyclohexamide	2.3	2.8	1.6	0.3
E. nerifolia latex	2.2	4.1	4 4	4.9
E. nerifolia latex + cyclohexamide	2.3	2.2	1 2	0 1

protein degradation when supplemented with cyclohexamide[16].

Cells of *A. japonicus* transferred from glucose to a fresh medium containing the same carbon source showed a linear increase in protein synthesis. These results indicate that the progressive increase in protein synthesis was due to the increase in the cell mass as reported in other fungal systems such as *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*[17].

However, cells of *A. japonicus* growing on the latices showed a three- and four-fold decrease in the cell mass in *C. gigantea* and *E. nerifolia* respectively, when compared to those metabolizing glucose at 24 h. These results indicate that the increase in protein synthesis was due to the synthesis of some specific gene product(s) involved in the degradation of plant latices and not exclusively due to the normal cell cycle. The gene product(s) are induc ıle in nature and are suppressed in the presence of cyclohexamide, as the total incorporation of the labelled amino acid in the cyclohexamide-treated cells was found to be between 400 and 500 cpm after 4 h of incubation. These inducible proteins are localized both in the membrane and the soluble fractions.

These results were substantiated by the increase in the polyribosome content involved in protein synthesis in cells metabolizing the plant latices when compared to those metabolizing glucose. These results indicate that an increase in the rate of protein synthesis was required for the metabolism of the plant latices when compared to those metabolizing glucose in *A. japonicus*.

Extracts from *Euphorbia* sp. were tested for their toxicity on cells from tissue culture. An increase in the protein synthesis[18], polyribosome content, glandular endoplasmic reticulum[19,20], tRNA and rRNA[21] was seen. These results indicate that the toxicity induced by these extracts from the plant latices show similar types of macromolecular changes in other eukaryotic systems also.

Abdulla and Omer[3] reported that latex containing sap of *Euphorbia granwata* remarkably inhibited the growth of submerged cultures of *Aspergillus flavus*, *Aspergillus nidulans*, *Fusarium* sp., *Curvularia* sp., *Helminthosporium* sp and *Candida* sp. We have recently shown the involvement of lipids from the latex of *C. gigantea* that are involved in the cell toxicity towards *A. japonicus*[2]. Our results indicate that the inhibition of *de-novo* protein synthesis in the latex metabolizing cells leads to rapid lysis of the cells, which was not observed in cells metabolizing glucose. Hence *de-novo* protein synthesis in *A. japonicus* was one of the essential factors required for the cells to survive for a longer period in the presence of plant latices. At this stage it is not clear as to how these proteins are involved in the degradation of specific components of plant latices, but one could speculate that these may be *de-novo* proteins localized in the membrane fraction involved in the transport of latex components subsequently metabolizing in the soluble fraction together with other *de-novo* proteins.

Christman *et al*[22] showed that more than 30 proteins were induced due to toxicity (oxidation stress) of H_2O_2 in *Salmonella typhimurium*. Similar proteins of uncertain functions, designated as heat-shock-proteins, have also been identified in several eukaryotic systems[23-25].

These sets of inducible proteins in *A. japonicus* whose functions are not fully elucidated are essentially required for adaptation to adverse conditions and they may be subsequently involved in the biodegradation of the plant latices. On the basis of the above preliminary results, we postulate that the collective involvement and transient appearance of several *de-novo* proteins which we designate as 'stress proteins' are essential for detoxification and possibly the degradation of complex substrates in the latex. Further work to elucidate the functions of these proteins is in progress.

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EFFECT OF CHEMICALS ON THE MORPHOLOGY OF *SCLEROTIUM ROLFSII* SACC CAUSING FOOT-ROT DISEASE OF BARLEY

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Morphological changes in fungi occur generally during unfavourable conditions. Fungi change their original structure to resist the action of chemicals and very little work has been done on this aspect[1-3]. In the present communication, two phenolic compounds (picric acid and 2, 4-dinitrophenol), one vitamin (riboflavin) and two amino acids (tryptophan and proline) causing considerable morphological changes in *Sclerotium rolfsii* Sacc., a foot-rot pathogen of barley at higher concentrations, are reported.

Required quantities of chemicals were mixed separately in Erlenmeyer flasks (250 ml), containing 100 ml autoclaved molten potato dextrose agar (PDA) to obtain the desired concentrations (100, 500, 1000, 5000, 10,000 ppm). Twenty ml of such amended medium was poured on each sterilized plate (90 mm diam) and allowed to solidify. Fresh mycelial discs (3 mm diam) were cut from actively growing margin of the fungus and transferred singly at the centre of the plates. The control sets did not receive any such chemical. Plates were incubated at 25 ± 1°C for 2 weeks. Changes in hyphal morphology, growth pattern and sclerotial morphology of the pathogen were observed.

Application of riboflavin, tryptophan and proline at 100 ppm and picric acid and 2, 4-dinitrophenol at 1000 ppm resulted in spare growth of the fungal colonies with stranded radiating hyphae appearing from the central inoculum. The number, shape and the size of sclerotia were also slightly reduced.

With 500 ppm riboflavin, tryptophan and proline and 5000 ppm picric acid and 2, 4-dinitrophenol, zonation appeared and the growth of the colonies were markedly arrested with irregular margin.