provides an excellent coverage in X-ray spectral region, an exposure of an order of millisecond is usually required to achieve the necessary dose. This may result in degradation of spatial resolution on account of thermal vibrations in the sample or blurring caused by expansion of the sample due to heating during the long exposure period[1,2]. Laser-produced plasmas, on account of their high peak brightness, can provide sufficient exposure in a single pulse of short duration (few nanoseconds or smaller), thereby avoiding the above problems. Further, smaller size and much lower capital cost of a laser-produced plasma source are obvious advantages for a moderate size laboratory.

In conclusion, X-ray contact microscopic imaging is performed in keV X-ray spectral region using a laser-produced plasma X-ray source. Images of unit magnification are obtained for ~5 μm thick yeast cells in single shot exposures of ~10 mJ/cm^2 with an estimated spatial resolution of ~120 nm. These images are observed with high magnification under a scanning electron microscope and an atomic force microscope. X-ray images show internal structures which are not seen under a conventional optical microscope due to low contrast in the visible region. Height profile of the developed photoresist observed under AFM correspond to a two-dimensional map of thickness integrated X-ray attenuation of the sample. This can be useful for potential application of elemental mapping of biological objects.

1. Richardson, M., Shinohara, K., Tanaka, K. A., Kinjo, Y., Ikeda, N. and Kado, M., *Soc. Photo-Opt. Instrum. Eng.*, 1992, **1741**, 133–141.
2. Stead, A. D., Cotton, R. A., Duckett, J. G., Goode, J. A., Page, A. M. and Ford, T. W., *J. X-ray Sci. Technol.*, 1995, **5**, 52–64.
3. Tomie, T., Shimizu, H., Majima, T., Kanayama, T., Yamada, M. and Miura, E., *Soc. Photo-Opt. Instrum. Eng.*, 1992, **1741**, 118–128.
4. Bollanti, S., Lazzaro, P. D., Flora, F., Giordano, G., Letardi, T., Schina, G., Zheng, C. E., Filippi, L., Palladino, L., Reale, A., Taglieri, G., Batani, D., Mauri, A., Belli, M., Scafati, A., Reale, L., Albertano, P., Grilli, A., Faenov, A., Pikuz, T. and Cotton, R., *J. X-ray Sci. Technol.*, 1995, **5**, 261–277.
5. Tomie, T., Shimizu, H., Majima, T., Yamada, M., Kanayama, T., Kondo, H., Yano, M. and Ono, M., *Science*, 1991, **252**, 691–693.
6. Eason, R. W., Cheng, P. C., Feder, R., Michette, A. G., Rosser, R. J., O'Neill, F., Owadano, Y., Rumsby, P. T., Shaw, M. J. and Turcu, I. C. E., *Opt. Acta*, 1986, **33**, 501–516.
7. Fletcher, J., Cotton, R. and Webb, C., *Soc. Photo-Opt. Instrum. Eng.*, 1992, **1741**, 142–153.
8. Bijkerk, F., Louis, E., Vander Wiel, M. J., Turcu, E. C. I., Tallents, G. J. and Batani, D., *J. X-ray Sci. Technol.*, 1992, **3**, 133–151.
9. Avtonomov, V. P., Vragov, K. M., Duda, V. I., Geondgian, Yu. G., Korol, V. Yu., Kuznecov, V. Ya. and Sorokin, V. V., *FIAN reprint*, 1991, No. 38, Moscow.
10. Chakera, J. A., Kumbhare, S. R. and Gupta, P. D., Proceedings of the National Laser Symposium, Indore, India, Feb 1997, pp. 286–287.

ACKNOWLEDGEMENTS. We thank Dr D. D. Bhawalkar and Academician N. G. Bosov for their keen interest and encouragement during the course of this work. The work is performed under ILTP collaboration project supported by Department of Science and Technology, India, and Russian Academy of Sciences, Moscow, Russia.

Received 22 August 1997; revised accepted 3 December 1997

Effect of 45 MeV ^{7}Li and 68 MeV ^{16}O charged particles on the microsomal membrane fluidity

M. Srivastava, D. Choudhary, A. Sarma* and R. K. Kale†

School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India
*Nuclear Science Centre, New Delhi 110 067, India

Microsomes prepared from liver of male Sprague Dawley rats were irradiated with various fluences of 45 MeV ^{7}Li and 68 MeV ^{16}O charged particles. The change in fluidity and lipid peroxidation was measured in terms of fluorescence polarization and MDA formation respectively. The fluidity of membrane was found to decrease with increase in fluence of both the particle radiations and could be ascribed to peroxidative damage. This effect persisted in post-irradiation period. 68 MeV ^{16}O ions were found to be more detrimental compared to 45 MeV ^{7}Li. Since membranes are also considered to be the critical targets of radiation action, these findings may have significance in understanding the radiobiological effect of high linear energy transfer radiation.

IMPORTANCE of high linear energy transfer (LET) radiations in the cancer therapy and estimating risks, especially in the space flights, has generated lot of interest in their radiobiological studies. Extensive work has been carried out using various biological endpoints. However, very little information is available on the effect of high LET radiations on biological membranes, which apart from DNA, are considered to be the critical targets of the detrimental actions of ionizing radiations. Therefore, an attempt was made to study the effect of 45 MeV ^{7}Li and 68 MeV ^{16}O ions in microsomal membranes in terms of fluorescence polarization using 1,6-diphenyl-1,3,5-hexatriene (DPH) probe which localizes in the fatty acyl side chain region of lipid bilayer[1].

Microsomes were prepared from the liver of Sprague Dawley rats (200–250 g body weight) as described by Varshney and Kale[2]. The animals were maintained in

†For correspondence.

the University Animal House. Water and food were provided *ad libitum*. 20 ml of microsomes (0.6 mg protein/ml) was taken in specially made sample holders which were kept on a magnetic stirrer. It was irradiated at atmospheric pressure with different fluences of 45 MeV ^{7}Li and 68 MeV ^{16}O ions at the Radiation Biology beam line of the 15 UD Pelletron at the Nuclear Science Centre, New Delhi. The energies were measured at the sample site using silicon surface barrier detectors. The LET values were found by TRIM calculations using water as model system. Theoretically derived depth–LET profiles for both the ions are shown in Figure 1 *a* and *b*. The Bragg peak is seen at 436 µm and 86 µm for 45 MeV ^{7}Li and 68 MeV ^{16}O ions respectively.

2 mM DPH was prepared in tetrahydrofuran. 100 µl of it was added to the 100 ml of rapidly stirring Tris-HCl buffer (10 mM, pH 7.4). Irradiated microsomes (0.6 mg protein/ml) were incubated with 2 µM DPH for 30 min at room temperature (25°C). Unirradiated microsomes served as control. Fluorescence polarization was measured by excitation with vertically polarized monochromatic light at 365 nm and the emission intensity was measured at 432 nm (slit 10 mm each) through a polarizer oriented either parallel or perpendicular to the direction of polarized excitation light. The degree of fluorescence polarization was calculated according to Haggerty *et al.*[3].

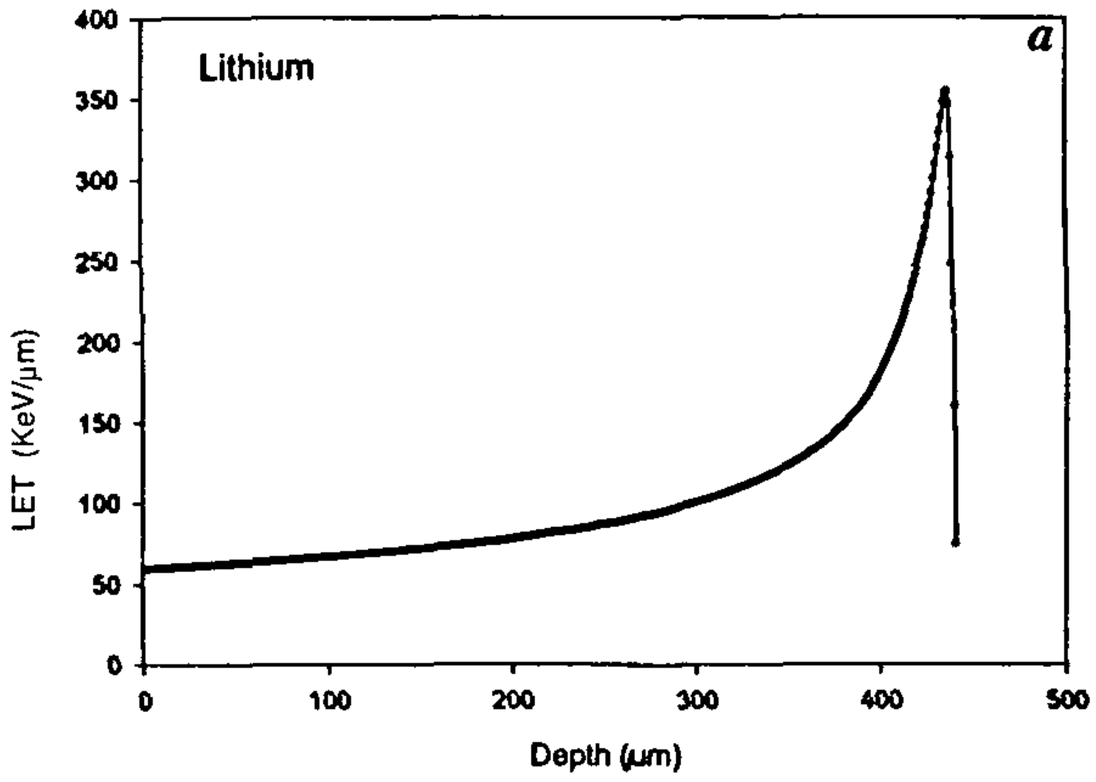

Figure 1. Depth (µm)–LET (keV/µm) profile of (*a*) 45 MeV ^{7}Li and (*b*) 68 MeV ^{16}O charged particles.

Lipid peroxidation was determined by the thiobarbituric acid (TBA) method and expressed in terms of malondialdehyde (MDA) formed per mg protein[2]. Microsomes containing 0.6 mg protein in 2 ml were mixed with 0.5 ml of 30% TCA and 0.5 ml of 52 mM TBA, and incubated in water bath for 45 min at 80°C. They were cooled and centrifuged at 3,000 rpm. The absorbance of clear supernatant was measured at 531.8 nm. Microsomal protein concentration was determined by Lowry *et al.*'s method[4] using bovine serum albumin as standard. The significance of difference in the quantitative data was statistically evaluated using the Student's *t*-test.

The fluorescence polarization has proven to be one of the most sensitive, reproducible and convenient means of probing the fluidity and the organization of membrane[5]. The results are shown in the Table 1. When microsomes irradiated with 45 MeV ^{7}Li and 68 MeV ^{16}O charged particles ($8 \times 10^{6} - 3 \times 10^{7}$ p/cm^{2}), the fluorescence polarization was found to increase significantly with fluence of both the particle radiations. Since, the fluorescence polarization is inversely proportional to the fluidity of membranes, our findings suggested that the high LET radiation caused the more close packing of membranes making it more rigid probably as a result of peroxidative damage which is known to shorten the fatty acyl chain, induce peroxides and aldehydes in the hydrocarbon region, remove unsaturation and induce cross linking among the lipid and protein molecules. To confirm this possibility, we have studied the lipid peroxidation under similar irradiation conditions. 45 MeV ^{7}Li and 68 MeV ^{16}O ions ($8 \times 10^{6} - 3 \times 10^{7}$ p/cm^{2}) were also found to increase lipid peroxidation significantly with fluence (Table 2). It was observed that 68 MeV ^{16}O charged particles induced more change in the membrane characteristics than 45 MeV ^{7}Li particles. More effectiveness of ^{16}O particles than ^{7}Li could be attributed to their differences in the energy distribution profiles, which showed that LET of 68 MeV ^{16}O ions was three times more than 45 MeV ^{7}Li. It is important to note that the fluences used in the present study are comparable to those used in the radiation therapy of cancer (e.g. ^{12}C is used in the range of 10^{6}–10^{8} ions/spill at SIS, GSI, Darmstadt)[6]. Although exact values are not available, it is estimated that the total fluence of the protons in space (which are the most abundant charged particles) is 10^{8} to 10^{9} per year which is close to the above-mentioned range[7]. However, it is clear that in terms of flux, values used in this experiment and those used for therapy are in the same range.

Table 1. Effect of charged particles on fluorescence polarization in microsomes

Fluence (p/cm^2)	Time (h) 0	3	6	12
		45 MeV ^{7}Li		
Control	0.145 ± 0.0023 (100 ± 1.58)	0.162 ± 0.0014^c (112 ± 0.96)	0.197 ± 0.0037^c (135 ± 2.55)	0.200 ± 0.0008^d (138 ± 0.55)
8×10^6	0.154 ± 0.0023^a (106 ± 1.58)	0.180 ± 0.0017^{a,c} (124 ± 1.17)	0.200 ± 0.0035^c (138 ± 2.41)	0.210 ± 0.0012^{a,d} (145 ± 0.82)
1×10^7	0.155 ± 0.0008^a (107 ± 0.55)	0.185 ± 0.0026^{a,c} (127 ± 1.79)	0.206 ± 0.0026^d (142 ± 1.79)	0.217 ± 0.0006^{b,d} (149 ± 0.41)
2×10^7	0.160 ± 0.0008^a (111 ± 0.55)	0.189 ± 0.0012^{b,d} (130 ± 0.82)	0.205 ± 0.0017^d (141 ± 1.17)	0.218 ± 0.0014^{a,d} (150 ± 0.96)
3×10^7	0.165 ± 0.0023^a (114 ± 1.58)	0.190 ± 0.0011^{b,c} (131 ± 0.76)	0.205 ± 0.0023^c (141 ± 1.58)	0.218 ± 0.0008^{b,d} (150 ± 0.55)
		68 MeV ^{16}O		
8×10^6	0.201 ± 0.0023^b (138 ± 1.58)	0.206 ± 0.0020^b (142 ± 1.38)	0.210 ± 0.0023^a (144 ± 1.58)	0.263 ± 0.0077^{b,d} (181 ± 5.31)
1×10^7	0.207 ± 0.0014^b (142 ± 0.96)	0.214 ± 0.0026^b (147 ± 1.79)	0.216 ± 0.0017^{a,c} (148 ± 1.17)	0.271 ± 0.0020^{b,d} (186 ± 1.38)
2×10^7	0.208 ± 0.0014^b (143 ± 0.96)	0.217 ± 0.0011^{b,c} (149 ± 0.76)	0.218 ± 0.0017^{a,c} (150 ± 1.17)	0.275 ± 0.0023^{b,d} (189 ± 1.58)
3×10^7	0.217 ± 0.0023^b (149 ± 1.58)	0.232 ± 0.0023^{b,c} (160 ± 1..58)	0.226 ± 0.0028^{a,c} (155 ± 1.93)	0.279 ± 0.0014^{b,d} (192 ± 0.96)

Each value is a mean ± SE of three separate experiments. The value in parentheses is the percentage of the absolute value with respect to the control unirradiated value at zero hour. Values indicated by $^a P < 0.05$ and $^b P < 0.001$ are significantly different from respective unirradiated controls; $^c P < 0.05$ and $^d P < 0.001$ are significantly different from respective unirradiated/irradiated values at zero hour. SE for fluence measurements range is between 0.7 and 1.7%.

Table 2. Effect of charged particles on lipid peroxidation in microsomes

Fluence (p/cm^2)	Time (h) 0	3	6	12
		45 MeV ^{7}Li		
Control	3.15 ± 0.057 (100 ± 1.80)	6.74 ± 0.046^d (213 ± 1.46)	9.08 ± 0.069^d (288 ± 2.19)	9.68 ± 0.098^d (307 ± 3.11)
8×10^6	3.54 ± 0.052^a (112 ± 1.65)	7.42 ± 0.115^{a,d} (235 ± 3.65)	9.01 ± 0.155^d (286 ± 4.92)	9.74 ± 0.080^d (309 ± 2.53)
1×10^7	3.85 ± 0.069^a (122 ± 2.19)	7.81 ± 0.098^{a,d} (247 ± 3.11)	9.25 ± 0.127^d (293 ± 4.03)	9.93 ± 0.115^d (315 ± 3.65)
2×10^7	4.34 ± 0.052^b (137 ± 1.65)	8.53 ± 0.151^{a,d} (270 ± 4.79)	9.57 ± 0.063^{a,d} (303 ± 2.00)	9.97 ± 0.144^d (316 ± 4.57)
3×10^7	4.73 ± 0.127^a (150 ± 4.03)	9.05 ± 0.138^{b,d} (287 ± 4.38)	9.78 ± 0.086^{a,d} (310 ± 2.73)	10.25 ± 0.161^{a,d} (325 ± 5.11)
		68 MeV ^{16}O		
8×10^6	3.72 ± 0.046^a (118 ± 1.46)	7.77 ± 0.103^{a,d} (246 ± 3.26)	9.35 ± 0.023^{a,d} (296 ± 0.73)	9.90 ± 0.144^d (314 ± 4.57)
1×10^7	4.01 ± 0.034^a (127 ± 1.07)	8.26 ± 0.075^{b,d} (262 ± 2.38)	9.45 ± 0.063^{a,d} (300 ± 2.00)	10.12 ± 0.173^d (321 ± 5.49)
2×10^7	4.63 ± 0.069^b (146 ± 2.19)	9.12 ± 0.092^{b,d} (289 ± 2.92)	9.72 ± 0.063^{a,d} (308 ± 2.00)	10.16 ± 0.115^{a,d} (322 ± 3.65)
3×10^7	5.07 ± 0.150^a (160 ± 4.76)	9.42 ± 0.109^{b,d} (299 ± 3.46)	10.01 ± 0.092^{a,d} (317 ± 2.92)	10.47 ± 0.132^{a,d} (332 ± 4.19)

Each value is a mean ± SE of three separate experiments. The value in parentheses is the percentage of the absolute value with respect to the control unirradiated value at zero hour. Values indicated by $^a P < 0.05$ and $^b P < 0.001$ are significantly different from respective unirradiated controls; $^c P < 0.05$ and $^d P < 0.001$ are significantly different from respective unirradiated/irradiated values at zero hour. SE for fluence measurements range is between 0.7 and 1.7%.

The importance of post-irradiation effect is well recognized. Moreover, due to physiological significance it is also important to know whether the effect of radiation in the membranes persists or not. Therefore, we also studied the changes in the fluorescence polarization and lipid peroxidation of microsomes (incubated at 37°C) at 3, 6 and 12 h of post-irradiation period (Tables 1 and 2). 45 MeV ^{7}Li and 68 MeV ^{16}O induced change in the fluidity was seen to increase further with time which was suggestive of persistence of the radiation effect in the post-irradiation period. It might be mentioned that the post-irradiation effect was also more in case of ^{16}O than ^{7}Li. It may be mentioned that a substantial increase in polarization occurred over 12 h for both irradiated and unirradiated membranes. However, there was small but consistent lower rate of change in polarization with 45 MeV ^{7}Li and 68 MeV ^{16}O ions in post-irradiation period. Similar pattern was also seen in lipid peroxidation. Being a chain reaction, lipid peroxidation is continued mainly due to its propagation. Since initiation of lipid peroxidation is faster than propagation, free radicals involved in propagation get accumulated. An accumulation of these free radicals in irradiated membranes is expected to be more, leading to enhance the possibility of their recombinations which makes them non-available for their propagation[8]. Therefore, the rate of lipid peroxidation and in turn polarization in post-irradiation period is likely to slow down slightly in irradiated microsomes.

In conclusion, our results suggest that exposure to the 45 MeV ^{7}Li and 68 MeV ^{16}O charged particles lead to the change in the fluidity. This change in the membrane property increased with post-irradiation time. It is quite possible that these high LET radiations might have induced close packing and highly constrained motions of hydrophobic interior of membrane leading to decrease in its fluidity. As importance of high LET radiation in cancer therapy and estimating the risks in the space flight, these findings may have significance from the radiobiological studies point of view.

1. Patel, J. M. and Block, E. R., *Free Rad. Biol. Med.*, 1986, **4**, 121–134.
2. Varshney, R. and Kale, R. K., *Int. J. Rad. Biol.*, 1990, **58**, 733–743.
3. Haggerty, D. F., Kalra, V. K., Popjak, G., Reynold, E. F. and Chiapelle, F., *Arch. Biochem. Biophys.*, 1976, **189**, 51–62.
4. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265–275.
5. Freifelder, D., *Physical Biochemistry*, W. H. Freeman and Company, New York, 1982, pp. 537–572.
6. Eickhoff, H., Franczak, B., Krause, U., Riedel, C., Rosch, W. and Steiner, R., *GSI Scientific Report*, 1995, pp. 163–164.
7. Feynman, J. and Gabriel, S. B., *IEEE Trans. Nucl. Sci.*, 1996, **43**, 344–352.
8. Kale, R. K. and Sitasawad, S. L., *Radiat. Phys. Chem.*, 1990, **36**, 361–364.

ACKNOWLEDGEMENTS. We thank Prof. G. K. Mehta, Director, Nuclear Science Centre, New Delhi and Prof. Asis Datta, Jawaharlal Nehru University, New Delhi for their keen interest in this work. M.S. and D.C. thank UGC for financial support.

Received 5 May 1997; revised accepted 6 November 1997

Regeneration of late leaf spot-resistant groundnut plants from *Cercosporidium personatum* culture filtrate-treated callus

P. Venkatachalam, K. Sankara Rao*†, P. B. Kavi Kishor‡ and N. Jayabalan§

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012, India
*Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India
‡Department of Genetics, Osmania University, Hyderabad 500 007, India
§Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, India

Cotyledon callus cultures of groundnut (*Arachis hypogaea* L.) derived from tikka late leaf spot disease-susceptible and resistant genotypes were exposed to various concentrations of fungal culture filtrate (FCF) of *Cercosporidium personatum*, the causal fungal agent of tikka late leaf spot disease. Fresh weight and cell viability of calli were determined after exposure to various concentrations of FCF. Sensitive calli have failed to increase in fresh weight and lost viability after exposure to media containing the FCF whereas insensitive calli retained growth and maintained viability similar to controls, viz. calli not exposed to FCF. Insensitive calli were selected by culturing on growth medium containing various concentrations of the FCF. Resistant calli obtained by selection survived three subcultures under the same conditions and were used for plantlet regeneration. Regenerated plants when transferred to soil–sand mixture in plastic cups and subsequently shifted to field conditions, set few viable seeds. Plants of R_2 generation exhibited enhanced resistance to tikka late leaf spot disease.

THE plant tissue culture technology has potential application in the development of disease-resistant plants in various crops. Further, the establishment of a host–pathogen interaction *in vitro* permits screening and selection for disease resistance at cellular level[1]. In recent years, pathotoxins (fungal elicitors) have been

†For correspondence.