ELSEVIER

Effect of vitamin E and taurine treatment on lipid peroxidation and antioxidant defense in perchloroethylene-induced cytotoxicity in mice

Abdul Shukkur Ebrahim and Dhanapal Sakthisekaran

Department of Medical Biochemistry, University of Madras, Taramani Campus, Madras, 600 113, India

Lipid peroxidation is thought to be an important event contributing to the toxicity of a variety of compounds like perchloroethylene (PER). PER is known to produce membrane damage through increased lipid peroxidation. An investigation of the relative importance of vitamin E and taurine in rendering protection to liver and kidney against PER induced cellular damage was performed. PER administered (3000 mg/kg body weight/day) mice were subjected to vitamin E (400 mg/kg body weight/day) and taurine (100 mg/kg body weight/day) treatment respectively for 15 days to study their individual effect on lipid peroxidative changes. A defective antioxidant defense system in PER administered mice was evidenced by the low level of enzymic antioxidants (SOD, CAT, GPx) and non-enzymic antioxidants (glutathione, ascorbic acid, total thiols, non-protein thiols, and vitamin E), with a simultaneous increase in lipid peroxidation (LPO) level. Vitamin E and taurine supplemented mice showed a marked reversal of these metabolic changes related to cellular damage caused by PER. These results suggest that PER induced cellular damage may be associated with lipid peroxidation and that can be effectively prevented by both vitamin E and taurine. (J. Nutr. Biochem. 8:270–274, 1997) © *Elsevier Science Inc. 1997*

Keywords: perchloroethylene; vitamin E; taurine; lipid peroxidation; antioxidants

Introduction

One of the most attractive approaches to disease prevention involves the use of specific nutrients to protect tissue against toxic and carcinogenic injury and degenerative diseases.[1]

Oxygen-centered free radicals are the important factors in several pathological conditions.[2] These free radicals can react with polyunsaturated fatty acids (PUFAs) to cause lipid peroxidation.[3] Lipid peroxidation seems to play a major role in the hepatotoxicity of halogenated hydrocarbons like perchloroethylene (PER).[4] Perchloroethylene is a solvent with wide spread use, particularly as a metal degreaser. PER has increasing potential to be a health risk to humans and an environmental problem as the major part of annual world production in released into the environment. On repeated and single exposure to PER, it has been shown that the largest affected organs were the liver, and to a lesser extent, the kidney.[5]

The endogenous defenses against the peroxidation of membrane lipids remain an area of continuous interest. Some xenobiotics are known to alter the activity of antioxidants and thereby decrease their ability to destroy the active oxygen species.[6]

The most widely studied protective agents are the antioxidant nutrients, which include vitamin E, vitamin C, taurine, carotenoids, and numerous polyphenolic compounds. Because these compounds directly scavenge reactive oxidants, they are hypothesized to constitute a vital endogenous defense against oxidative cell and tissue injury caused by toxic and carcinogenic chemicals.[7]

Vitamin E is a fat-soluble molecule and so enriched in

Address reprint requests to Dr. Dhanapal Sakthisekaran, University of Madras, Professor of Biochemistry, Taramani Campus, Madras, 600 113, India.
Received April 29, 1996; accepted January 21, 1997.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(97)00011-9

the interior of membranes is believed to function as an important cellular antioxidant.[8] It quenches and reacts with singlet oxygen, and, therefore, protects the membranes against this species. The important function of vitamin E is that it reacts with lipid peroxy radicals to form vitamin E radicals. It thus interrupts the chain reaction of lipid peroxidation by acting as a chain terminator.[9] Our earlier studies also proved the cytoprotective effect of vitamin E on glycoprotein changes in PER induced cytotoxicity.[10]

Taurine, a derived sulfur-containing amino acid has recently attracted greater attention as it is present in high amounts in various tissues, and it is considered to play various important physiological functions in each organ. For example, it has been demonstrated that taurine might have a modulating action on the function of the central nervous system, a protective effect in pancreatic cells against pancreatotoxins and a membrane-stabilizing action on the sarcoplasmic reticulum.[11] Taurine has also been used clinically as a therapeutic agent for liver diseases.[12]

Considering the cytoprotective efficacious of vitamin E and taurine, the present study was aimed to investigate the modulating effect of both vitamin E and taurine on lipid peroxidation and antioxidant defense in PER induced cytotoxicity in mice.

Methods and materials

Animals and treatment

Healthy male and female albino Swiss mice (25 to 30 g) were used throughout the study. The mice were maintained on commercial rodent chow, manufactured by M/s. Hindustan Lever Ltd., Bombay, India, in a light and temperature controlled room. Clean drinking water was provide ad libitum. The mice were divided into six groups of six animals each.

Group I: Control animals treated with sesame oil (3000 mg/kg bw/day) orally by intubation for 15 days.
Group II: Perchloroethylene treated animals (3000 mg/kg bw/day) orally by intubation for 15 days.
Group III: PER treated animals (as in group II) followed immediately by the administration of vitamin E (400 mg/kg bw/day) orally by intubation once a day for 15 days.
Group IV: PER treated animals (as in group II) followed immediately by the administration of taurine (100 mg/kg bw/day) orally by intubation once a day for 15 days.
Group V: Vitamin E alone treated animals (as in group III).
Group VI: Taurine alone treated animals (as in group IV).

At the end of the experimental period, the animals were fasted overnight and killed by cervical dislocation. The liver and kidney were quickly dissected out into ice-cold saline. Then 10% (wt/vol) tissue homogenates (liver and kidney) were prepared in Tris-HCl buffer, 0.1 M, pH 7.4 by using a high-speed Teflon homogenizer.

The following parameters were assayed in tissue homogenates. Reduced glutathione (GSH) was determined by the method of Moron et al.[13] (using Ellman's method[14]) based on the reaction with 5, 5′-dithiobis (2-nitro benzoic acid) to produce a compound with maximum absorption at 412 nm. Total and nonprotein thiol contents were determined by the method of Sedlak and Lindsay.[15] Ascorbic acid was measured by the method of Omaye et al.[16]

The activity of catalase was measured as the amount of H_2O_2 consumed per minute per mg protein by the method of Sinha.[17] Glutathione peroxidase (GPx) activity was assayed by the method of Rotruck et al.[18] The activity was expressed as μg of GSH utilized per minute per mg protein. The superoxide dismutase activity was measured by the method of Marklund and Marklund.[19] Lipid peroxidation was estimated by the method of Devasagayam.[20] Vitamin E was extracted, saponified and estimated by following the reduction of ferric iron to ferrous iron using bathophenanthroline as the chromogen by the method of Desai.[21] Protein was estimated by the method of Lowry et al.[22]

Statistical analysis

For statistical analysis, one-way analysis of variance (ANOVA) was used, followed by the Newman–Keuls multiple comparison test.

Results

Table 1 shows the levels of enzymatic and non-enzymatic antioxidants in the liver of perchloroethylene-treated mice in comparison with those of the age-matched group of control-, vitamin E-, and taurine-administered groups.

The level of catalase ($P < 0.001$), GSH peroxidase ($P < 0.001$), and superoxide dismutase ($P < 0.001$) were significantly lowered in PER-treated groups. Nonenzymic antioxidants such as ascorbic acid ($P < 0.001$) total thiols ($P < 0.001$), nonprotein thiols ($P < 0.001$), reduced GSH ($P < 0.001$), and vitamin E ($P < 0.001$) were also significantly decreased. PER-administered mice, however, when subjected to vitamin E and taurine supplementation, the decreased enzymic and nonenzymic antioxidant levels in liver showed a tendency to reach the levels seen in control animals.

Table 2 depicts the levels of lipid peroxides, cellular antioxidant defense enzymes such as superoxide dismutase, catalase, and GPx and nonenzymic antioxidants ascorbic acid, total thiols, nonprotein thiols, reduced GSH, and vitamin E obtained in kidney tissues of the various experimental groups. Administration of PER for 15 days resulted in the reduced levels of scavenging enzymes, nonenzymic antioxidants, and increased level of lipid peroxides, respectively. Surprisingly, after the co-administration of vitamin E and taurine the levels got concordantly increased in group III and group IV animals. Group V and VI, however, did not show any significant change when compared with control animals.

Discussion

Peroxidation of unsaturated membrane lipids by free radicals is one process which contribute to the toxic side effects observed following administration of, or exposure to a wide range of chemicals including PER. Green et al. suggested that the cytotoxic action of PER may lead to hepatic necrosis and kidney damage[23] and the results obtained in the present study demonstrate these actions.

The increase in lipid peroxidation observed in PER-administered animals may be a consequence of higher levels of superoxide radicals, which are produced in significant amounts in response to PER exposure or inhibition of free radical scavenging enzymes. Generally, cytochrome P_{450} is involved in the activation as well as in the detoxification processes of xenobiotics. Cytochrome P_{450}-dependent monooxygenases could convert halogenated hydrocarbons

Table 1 *Effect of vitamin E and taurine on the enzymic and nonenzymic antioxidants defense system in the mice liver of control and experimental groups*

Particulars	Group I (Control)	Group II (PER administered)	Group III (PER + vitamin E)	Group IV (PER + taurine)	Group V (Vitamin E alone)	Group VI (Taurine alone)
LPO (nmol/mg protein)	0.67 ± 0.07	0.97 ± 0.08^{a*}	0.71 ± 0.08^{b*}	0.78 ± 0.06$^{a\#b*c}$	0.61 ± 0.06$^{b*cd@}$	0.64 ± 0.07$^{b*cd\#e}$
SOD (U/min/mg protein)	5.47 ± 0.46	4.07 ± 0.25^{a*}	5.66 ± 0.57^{b*}	4.96 ± 0.50$^{b@}$	5.90 ± 0.61$^{b*d\#}$	5.56 ± 0.49^{b*}
CAT (μmol of H_2O_2 consumed/min/mg protein)	335 ± 37	206 ± 28$^{a@}$	293 ± 32^{b*}	307 ± 35^{b*}	323 ± 39^{b*}	328 ± 36^{b*}
GPx (μg of GSH consumed/min/mg protein)	43.6 ± 4.8	24.8 ± 3.1^{a*}	36.5 ± 3.0$^{a\#b*@}$c	32.4 ± 3.6$^{a*b@c}$	39.3 ± 3.6$^{b*cd\#}$	40.4 ± 4.2$^{b*cd\#e}$
GSH (μg/mg protein)	1.84 ± 0.20	0.82 ± 0.09^{a*}	1.12 ± 0.15^{a*b*}	1.87 ± 0.18$^{a*b\#c\#}$	1.76 ± 0.15^{a*b*c*d*}	1.75 ± 0.13$^{b*c*d*e@}$
TSH (μg/mg protein)	8.23 ± 0.42	5.32 ± 0.39$^{a\#}$	6.97 ± 0.64	6.37 ± 0.51	7.91 ± 0.60$^{b\#}$	7.75 ± 0.59$^{b@}$
NPSH (μg/mg protein)	1.40 ± 0.10	0.73 ± 0.08^{a*}	0.98 ± 0.07$^{b@}$	1.00 ± 0.07$^{a*b@}$	1.34 ± 0.11^{b*c*d*}	1.38 ± 0.07^{b*c*d*}
Ascorbate (μg/mg protein)	0.49 ± 0.05	0.26 ± 0.03^{a*}	0.37 ± 0.02^{b*}	0.46 ± 0.05$^{b\#}$	0.47 ± 0.06$^{b*c\#d@}$	0.52 ± 0.05$^{b*c\#d@}$
Vitamin E (μg/mg protein)	0.65 ± 0.07	0.39 ± 0.04$^{a\#}$	0.72 ± 0.07$^{b\#}$	0.53 ± 0.04$^{b@c@}$	0.81 ± 0.09$^{a@b*c*d@}$	0.67 ± 0.05$^{b\#d\#}$

Values are expressed as mean ± S.D. for six mice in each group.
*As compared with group I; [b]as compared with group II; [c]as compared with group III; [d]as compared with group IV; [e]as compared with group V.
Statistical significance, $^{*}P < 0.001$; $^{@}P < 0.01$; $^{\#}P < 0.05$.

Table 2 Effect of vitamin E and taurine on the enzymic and nonenzymic antioxidants defense system in the mice kidney of control and experimental groups

Particulars	Group I (Control)	Group II (PER administered)	Group III (PER + vitamin E)	Group IV (PER + taurine)	Group V (Vitamin E alone)	Group VI (Taurine alone)
LPO (nmol/mg protein)	1.39 ± 0.16	1.97 ± 0.12^{a*}	1.61 ± 0.13$^{b@}$	1.66 ± 0.17$^{b@}$	1.23 ± 0.11$^{b@c@d@}$	1.43 ± 0.16^{b*}
SOD (U/min/mg protein)	8.07 ± 0.68	4.60 ± 0.77^{a*}	7.16 ± 0.61^{b*}	7.13 ± 0.63^{b*}	8.58 ± 1.05$^{b*c\#d\#}$	8.88 ± 0.98$^{b*c\#d\#}$
CAT (μmol of H_2O_2 consumed/min/mg protein)	330 ± 31	219 ± 27^{a*}	296 ± 32$^{b@}$	274 ± 31$^{a\#b\#}$	332 ± 35^{b*d*}	345 ± 36$^{b*d\#}$
GPx (μg of GSH consumed/min/mg protein)	27.3 ± 2.4	15.3 ± 2.1^{a*}	22.6 ± 1.9$^{a\#b*}$	23.9 ± 2.6^{b*}	24.5 ± 1.8^{b*}	25.2 ± 1.9^{b*}
GSH (μg/mg protein)	1.08 ± 0.09	0.55 ± 0.06^{a*}	0.82 ± 0.08^{a*b*}	0.81 ± 0.09^{a*b*}	0.99 ± 0.13$^{a\#b*c*d@}$	1.07 ± 0.11$^{b*c*d*a\#}$
TSH (μg/mg protein)	9.17 ± 1.01	5.42 ± 0.72^{a*}	8.73 ± 0.74^{b*}	8.27 ± 0.80^{b*}	8.76 ± 0.77^{b*}	8.70 ± 0.78^{b*}
NPSH (μg/mg protein)	1.31 ± 0.10	0.72 ± 0.06^{a*}	1.09 ± 0.09$^{a\#b*}$	1.47 ± 0.13$^{b*c@}$	1.37 ± 0.17$^{b*c@}$	1.47 ± 0.18^{b*c*}
Ascorbate (μg/mg protein)	0.46 ± 0.05	0.21 ± 0.02^{a*}	0.34 ± 0.03^{a*b*}	0.43 ± 0.04$^{a\#b*c\#}$	0.50 ± 0.05$^{b*c*d@}$	0.49 ± 0.05$^{b*c*d@}$
Vitamin E (μg/mg protein)	0.48 ± 0.05	0.29 ± 0.02$^{a\#}$	0.57 ± 0.05$^{b\#}$	0.38 ± 0.04	0.61 ± 0.06$^{b\#}$	0.51 ± 0.04$^{b\#}$

Values are expressed as mean ± S.D. for six mice in each group.
*As compared with group I; [b]as compared with group II; [c]as compared with group III; [d]as compared with group IV; [e]as compared with group V.
Statistical significance, $^{*}P < 0.001$; $^{@}P < 0.01$; $^{\#}P < 0.05$.

into reactive metabolites that can either initiate LPO and/or bind covalently to macromolecules.[24] In previous studies, it has been suggested that acute liver toxicity of different chloroethylenes is probably caused by reactive intermediates that can bind to the liver tissues.[25] Trichloroethylene, one such compound causes destruction of drug-metabolizing enzymes, including cytochrome P_{450} which is covalently bound to microsomal and nuclear membrane proteins, and induces changes in membrane fatty acids.[26]

The initiation of LPO starts as a free radical chain reaction producing reactive chemical species such as lipid hydroperoxides, which can then initiate further LPO.[27] Vitamin E inhibits LPO by terminating free radical chain reactions.[28] If enough vitamin E is present in the membrane, the chain reaction is rapidly terminated and few chain branching initiation sites are produced.

It is well known that taurine is conjugated with bile acids in the liver and is excreted into bile as taurine conjugated bile acids. The suppressive action of taurine on hepatic lipid peroxidation that was found in vivo experiments suggests that taurine may have a direct effect on microsomal membranes and thus may decrease the susceptibility of the membranes to lipid peroxides.[29] The present study also clearly indicated that oral administration of taurine significantly suppresses lipid peroxide formation in the liver and kidney associated with PER administration.

Free radical production in cells is relatively low in normal conditions, given the various and very active defense systems including enzymic and nonenzymic antioxidant enzymes. The present data indicate that administration of PER caused a significant inhibition of glutathione peroxidase (GPx) in tissues (*Tables 1* and *2*). Decrease in GPx activity in PER-administered mice may also be a consequence of interaction of PER with selenium. PER metabolites (trichloroacetic acid and dichlorovinyl cysteine) with selenide associated with proteins of specific molecular weights and make these PER-Se protein complexes inactive, resulting in a decrease in enzyme activity.

Most of the SOD in tissues of cytoplasmic origin and contains Cu and Zn an essential prosthetic groups.[30] The decrease in SOD after PER administration may be attributable to an interaction between Cu and Zn with high levels of PER in tissues.

Catalase and GPx seem to have complementary catalytic activity. Decrease in catalase activity alone, therefore, may not be of great significance, but its simultaneous decrease with other antioxidant enzymes (GPx and SOD) might be of greater significance in cellular damage in organs like liver and kidney.[31]

GSH is endogenously synthesized in the liver[32] and is the first line of defense against prooxidant stress.[33] The reduced level may be attributable to two reasons: (1) GSH being involved in the formation of conjugates with PER-derived byproducts and (2) GSH acting as an intracellular e^- donor for the activity of GPx. The metabolism of PER produce trichloroacetic acid, which conjugates with GSH either non-enzymatically or by an reaction catalysed by glutathione-*S*-transferase.[34] The increased lipid peroxidation caused by PER administration also leads to the formation of hydroperoxides that are removed by GPx. Both of these reactions lead to a depletion of GSH levels.

The reduced GSH in tissues keeps up the cellular levels of the active forms of vitamin C and vitamin E. These vitamins also exist in interconvertible form and participating in neutralizing the free radicals. When there is a reduction in the level of GSH, cellular level of ascorbic acid level is lowered. This may be the reason for the decreased vitamin C in PER-intoxicated mice.

The level of sulphydryl groups were decreased in PER-induced cytotoxic conditions. Protein sulphydryl groups have also been suggested to contribute significantly to the antioxidant capacity of plasma although these oxidation could also be considered as a oxidative damage. The decrease in liver and kidney-SH group is because of GSH depletion through oxidation of free radicals.[35]

Vitamin E and taurine treatment maintains the GSH level and increases the activity of GPx. Thiriot et al.[36] have shown that vitamin E acts as a free radical scavenger and its antioxidant function occurs before that of GSH. Taurine and vitamin E increases the level of SOD and catalase. Similar results have also been reported by Manjula et al.[37] in experimental myocardial infarction. Taurine and vitamin E directly scavenges superoxide radicals and reduces the cellular damage caused by free radicals.

Conclusion

The results suggest that the biochemical lesions because of the activation of lipid peroxidation and decrease in the antioxidant status are significantly implicated in cellular damage induced by PER. The protective role of vitamin E and taurine is affected by decreasing the peroxide concentration and by normalizing the antioxidant defense system.

Acknowledgment

One of the authors (A.S.E.) gratefully acknowledges the Council of Scientific and Industrial Research, India for the financial assistance given to him in the form of a senior research fellowship.

References

1 Pryor, W.A. (1991). The antioxidant nutrients and disease prevention—what do we know and what do we need to find out. *Am. J. Clin. Nutr.* **53,** S391–S398

2 Bulkley, G.B. (1983). The role of oxygen free radicals in human disease processes. *Surgery* **94,** 407–411

3 Dumelin, E.E., and Tappel, A.L. (1977). Hydrocarbon gases produced during in vivo peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. *Lipids* **12,** 894–900

4 Kappus, H., Koster, U., Koster-Albrecht, D., Kieczka, H., and Remmer, H. (1978). In *Functions of Glutathione in Liver and Kidney* (H. Sies, and A. Wendel, eds.). p. 176–182, Springer-Verlag, New York, NY USA

5 Commission of the European Communities (1986). Trichloroethylene. In *Organochlorine Solvents, Health Risk to Workers*, p. 93–130, Royal Society of Chemistry, Luxembourg, Germany

6 Sandy, M.S., Dimonte, D., and Smith, M.T. (1988). Relationships between intracellular vitamin E, lipid peroxidation and chemical toxicity in hepatocytes. *Toxicol. Appl. Pharmacol.* **93,** 288–297

7 Pascoe, G.A. and Reed, D.J. (1989). Cell calcium, vitamin E, and the thiol redox system in cytotoxicity. *Free Radic. Biol. Med.* **6,** 209–216

8 Ohki, K., Takamura, T., and Nozawa, Y. (1984). Effect of α-tocopherol on lipid peroxidation and acyl chain mobility of liver micro-

somes from vitamin E—deficient rat. *J. Nutr. Sci. Vitaminol.* **30,** 221–231
9 Pascoe, G.A., Fariss, G.M.W., Olafsdottir, K., and Reed, D.J. (1987). A role of vitamin E in protection against cell injury: maintenance of intracellular glutathione precursors and biosynthesis. *Eur. J. Biochem.* **166,** 241–247
10 Ebrahim, A.S., Gopalakrishnan, R., Murugesan, A., and Sakthisekaran, D. (1995). *In vivo* effect of vitamin E on serum and tissue glycoprotein levels in perchloroethylene induced cytotoxicity. *Mol. Cell. Biochem.* **144,** 13–18
11 Zelikovic, I. and Chesnay, R.W. (1989). *Taurine, New Protective Roles for Selected Nutrients*, p. 253, Alan R. Liss, New York, USA
12 Masuda, M., Takino, T., Takamori, S., Harada, Y., Owgawara, Y., Tokura, Y., Kato, S., Adachi, H., Tanimura, M., Tabuchi, S., Hosokawa, K., and Tsuji, S. (1973). Clinical study on effect of taurine on hepatitis using double blind method. *The Clinical Report.* **7,** 151–173
13 Moron, M.S., Defieree, J.W., and Mannervik, K.B. (1979). Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat lung and liver. *Biochem. Biophys. Acta* **582,** 67–68
14 Ellman, G.L. (1959). Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82,** 70–77
15 Sedlak, J. and Lindsay, R.H. (1968). Estimation of total protein bound and non protein bound sulfhydryl groups in the tissue with Ellman's reagent. *Anal. Biochem.* **25,** 192–205
16 Omaye, S.T., Turnball, J.D., and Sauberlich, H.E. (1971). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Med. Enzymol.* **62,** 1–11
17 Sinha, A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem.* **11,** 469–474
18 Rotruck, J.T., Pope, A.L., and Ganther, H.E. (1973). Selenium: biochemical role as a component of glutathione peroxidase purification and assay. *Science* **179,** 588–590
19 Marklund, S. and Marklund, G. (1974). Involvement of superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* **47,** 469–474
20 Devasagayam, T.P.A., and Tarachand, V. (1987). Decreased LPO in the rat kidney during gestation. *Biochem. Biophys. Res. Commun.* **145,** 134–138
21 Desai, I.D. (1984). Vitamin E analysis methods for animal tissues. *Med. Enzymol.* **105,** 138–143
22 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folins—phenol reagent. *J. Biol. Chem.* **193,** 265–276
23 Green, T., Odum, J., Nash, J.A., and Foster, J.R. (1990). Perchloroethylene-induced rat kidney tumors: an investigation of the mechanisms involved and their relevance to humans. *Toxicol. Appl. Pharmacol.* **103,** 77–89
24 Bonse, G., Urban, Th., Reichert, D., and Henschler, D. (1975). Chemical activity, metabolic oxirane formation and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. *Biochem. Pharmacol.* **24,** 1829–1834
25 Rocisse, L. and Chakrabarti, S.K. (1986). Dose dependent metabolism of trichloroethylene and its relevance to hepatotoxicity in rats. *Environ. Res.* **40,** 450–454
26 Kriklund, T., Kjellstrand, P., and Haglid, K.G. (1986). Fatty acid changes in rat brain ethanolamine phosphoglycerides during and following chronic exposure to trichloroethylene. *Toxicol. Appl. Pharmacol.* **85,** 145–149
27 Fukuzawa, K., Takase, S., and Tsukatani, H. (1985). The effect of concentration on the antioxidant effectiveness of α-tocopherol in lipid peroxidation induced by superoxide free radicals. *Arch. Biochem. Biophys.* **240,** 117–120
28 Liebler, D.C., Kling, D.S., and Reed, D.J. (1986). Antioxidant protection of phospholipid bilayers by α-tocopherol. *J. Biol. Chem.* **261,** 12114–12119
29 Dianzani, M.U. and Ugazio, G. (1978). Lipid peroxidation. In: Biochemical Mechanisms of liver injury. Edited by Slater, T.E., pp. 669–707. Academic Press, London UK
30 Bauer, R., Demeter, I., Hasemann, V., and Johansen, J.T. (1980). Structural properties of zinc sites in cu, zn superoxide dismutase, perturbed angular correlation on the CD-superoxide dismutase derivative. *Biochem. Biophys. Res. Commun.* **94,** 1296–1302
31 Whanger, P.D. (1979). Cadmium effects in rats on tissue iron, selenium and blood pressure. Blood and cadmium in some Oregon resident. *Environ. Health Perspect.* **28,** 115–121
32 Lauterberg, B.H., Smith, C.V., and Mitchell, J.R. (1984). Regulation of hepatic glutathione homeostasis. In *Drug Metabolism and Drug Toxicity*, (J.R. Mitchell, M.G. Horning, eds), p. 321–330, Raven Press, New York, NY USA
33 Nicotera, P. and Orrenius, S. (1986). Role of thiols in protection against biological reactive intermediates. *Adv. Exp. Med. Biol.* **187,** 41–51
34 Raveendran, M., Thanislass, J., Uma Maheswari, G., and Devaraj, H. (1993). Induction of prooxidant state by the food flavour cinnamaldehyde in rat liver. *J. Nutr. Biochem.* **4,** 181–183
35 Ahmed, S., Singh, V., and Rao, G.S. (1994). Antioxidant potential in serum and liver of albino rats exposed to benzene. *Indian J. Exp. Biol.* **32,** 203–206
36 Thiriot, C., Durand, P., Jasseron, M.P., Kergonou, J.F., and Ducousso, R. (1987). Radiosensitive antioxidant membrane bound factors in rat liver microsomes: 1. The roles of glutathione and vitamin E. *Biochem. Int.* **14,** 1–8
37 Manjula, T.S., Deepa, R., and Shyamala Devi, C.S. (1994). Effect of aspirin on lipid peroxidation in experimental myocardial infarction in rats. *J. Nutrn. Biochem.* **5,** 95–98