Polish Journal of Environmental Studies Vol. 9, No. 5 (2000), 385-390

Effects of Whole-Body γ-Irradiation on Lipid Peroxidation and Anti-oxidant Enzymes in the Liver of *N*-nitrosodiethylamine-treated Mice

I.P. Grudziński, A. Frankiewicz-Jozko, J. Gajewska**, M. Szczypka**, A. Szymanski*

Department of Applied Physiology * Department of
Food Studies and Nutrition Physiology
Military Institute of Hygiene and Epidemiology, 01-163, Warsaw, Kozielska 4, Poland **
Department of Clinical Biochemistry, National Research Institute of Mother and Child, Warsaw, Poland

Received: December 14, 1999
Accepted: June 15, 2000

Abstract

B6C3F1 mice were treated *per os* with either normal saline or Af-nitrosodiethylamine (NDEA) (0.01, 0.1, 1.0 or 5.0 mg/kg body weight) daily for 21 days. On day 22nd of the experiment, the animals were whole-body γ-irradiated (10 Gy) and examined at 3.5 days post-radiation exposure. Pretreatment of mice with NDEA at the lowest dosage (0.01 and 0.1 mg/kg) increased thiobarbituric acid-reactive substances (TBARS) and catalase (CAT) activity in the liver. Since the agent at the highest doses (1.0 and 5.0 mg/kg) did not have any effect(s) on TBARS, it was associated with the selective increase of thiol (SH) groups and GSH-linked anti-oxidant enzyme activities such as glutathione peroxidase (GPX), transferase (GST) and reductase (GR). γ-irradiation decreased TBARS and increased superoxide dismutase (SOD) and GPX activity in NDEA-treated mice. Simultaneously, γ-rays did not have any effect(s) on GST and GR enzymes, and it slightly decreased SH groups and CAT activity. Results of the present study indicate that NDEA can promote lipid peroxidation in mice liver, γ-irradiation of mice at a dose of 10 Gy modifes the activity of hepatic anti-oxidant enzymes, which in turn can lead to the reduction of NDEA-induced lipid peroxidation and/or pro-oxidant shift(s). The anti-oxidant enzymes such as SOD and GPX are suggested to be mainly involved in this process(s).

Keywords: /V-nitrosodiethylamine, gamma radiation, lipid peroxidation, anti-oxidant enzymes, liver

Introduction

During the last decade, a large body of experimental evidence to demonstrate a relationship between radical oxygen species and ionizing radiation has been completed [1, 2]. However, an increased number of some chemical carcinogens in human food and water e.g. iV-nitroso compounds [3] emphasized the problem of assessing the adverse side effects such as chemical-induced oxidative stress in γ-irradiated tissues. It was not until the late '90s when the first N-nitroso agent that modified the cellular response to ionizing radiation was described [4].

Correspondence to: I.P. Grudzinski. tel. (022) 685-31-45; Fax (022) 838-10-69

In this paper, the authors showed that iV-nitrosodiethylamine (NDEA), a hepatotoxic and carcinogenic substance, increased lipid peroxidation in UV-irradiated lung fibroblasts [4]. As evidenced previously [5], ionizing radiation enhanced the production of some N-nitrosamines from their endogenous precursors such as nitrite and amines. Interestingly, γ-irradiation has been shown to decrease carbon tetrachloride-induced lipid peroxidation in mice liver [6]. It should be noted that electromagnetic fields were also found to change NDEA metabolism in mice [7].

Despite many years of active research, the biochemical mechanism(s) by which γ-radiation may affect the pro-/anti-oxidant balance of liver is not well characterized in animals pretreated with N-nitroso compounds. The principle objective of the present experiment was to elucidate the effects of a single dose of whole-body γ-irradiation on lipid peroxidation and anti-oxidant enzymes in the liver of NDEA-treated mice.

Materials and Methods

Animals and treatments. Male B6C3F1 mice aged 6-8 weeks were used in the studies. Throughout the experiment, the animals were given a standard diet (Murigran pellet, Motycz, Poland) and water *ad libitum.* The mice were divided into 5 groups of 10 animals in each group, and orally treated with an aqueous solution of NDEA (0.01, 0.1, 1.0 or 5.0 mg/kg body weight) daily for 21 days. Control mice received saline only. The half of randomly selected mice in each group were immobilized in plastic boxes at 12 hrs post-treatment (day 22), and immediately exposed to whole-body γ-irradiation (10 Gy). γ-ray(s) was delivered from a ^{60}Co source (1.5 Gy/min). The animals were sacrificed by cervical dislocation at 3.5 days post-radiation exposure, and the liver was removed from the mice and prepared for analysis.

Assays **and statistics.** Thiobarbituric acid-reactive substances (TBARS) were determined as markers of the lipid peroxidation in mice liver using the method of Ohkawa et al. [8]. Superoxide dismutase (EC **1.15.1.1)** was assayed as described by McCord and Fridovich [9]. Catalase (EC 1.11.1.6) activity was determined using the method of Beers and Sizer [10]. Glutathione peroxidase (EC 1.11.1.9) was assayed by the method of Paglia and Valantine [11]. Glutathione S-transferase (EC 3.1.2.7) was determined in accordance with the method of Habit et al. [12]. Glutathion reductase (EC 1.6.4.2) was assayed by the method of Bayoumi and Rosalki [13]. Thiol group (SH) level was determined using the procedure of Saville [14]. Protein content was measured by the method of Lowry et al. [15]. All results are shown as mean ± SD and analysis of variance with Dunnett's test was used for statistical analysis of the data. Differences were considered significant when probability *(p)* values were less than 0.05.

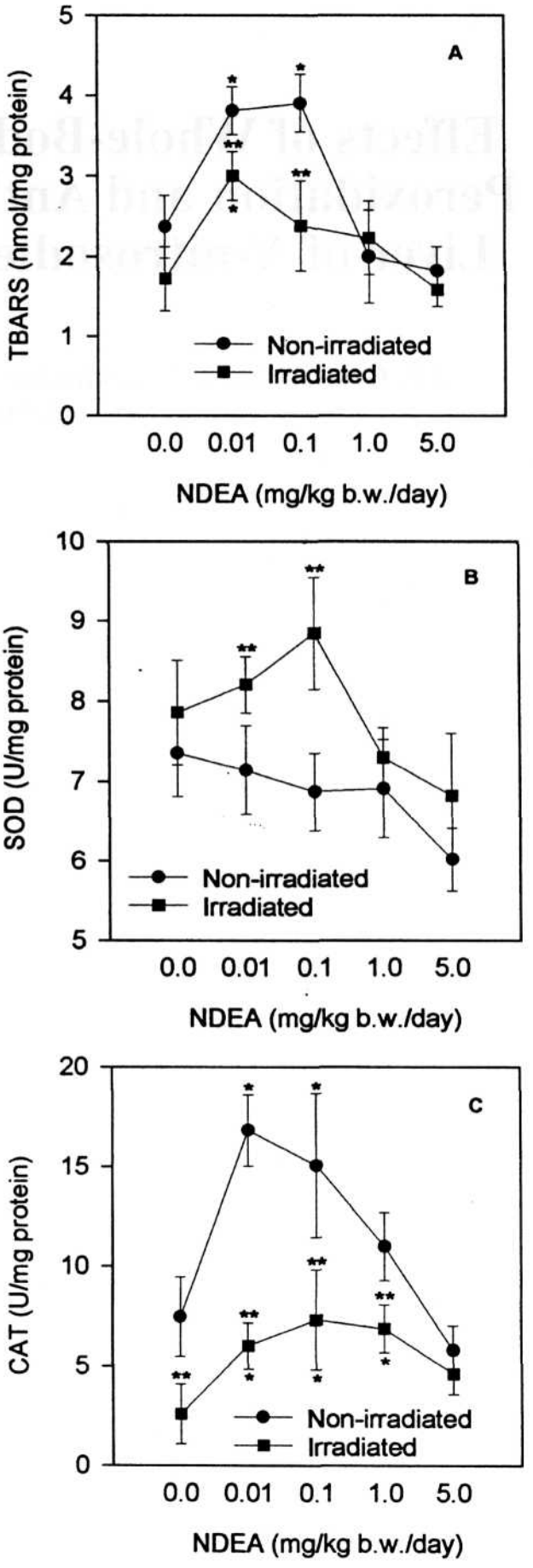

Fig. 1. Effect of γ-rays on thiobarbituric acid-reactive substances (TBARS) (A), and activities of superoxide dismutase (SOD) (B) and catalase (CAT) (C) in the liver of NDEA-treated mice. Values are mean ± SD, n = 5. * $P < 0.05$, versus the control (saline) group, * * P < 0.05, versus the corresponding non-irradiated group.

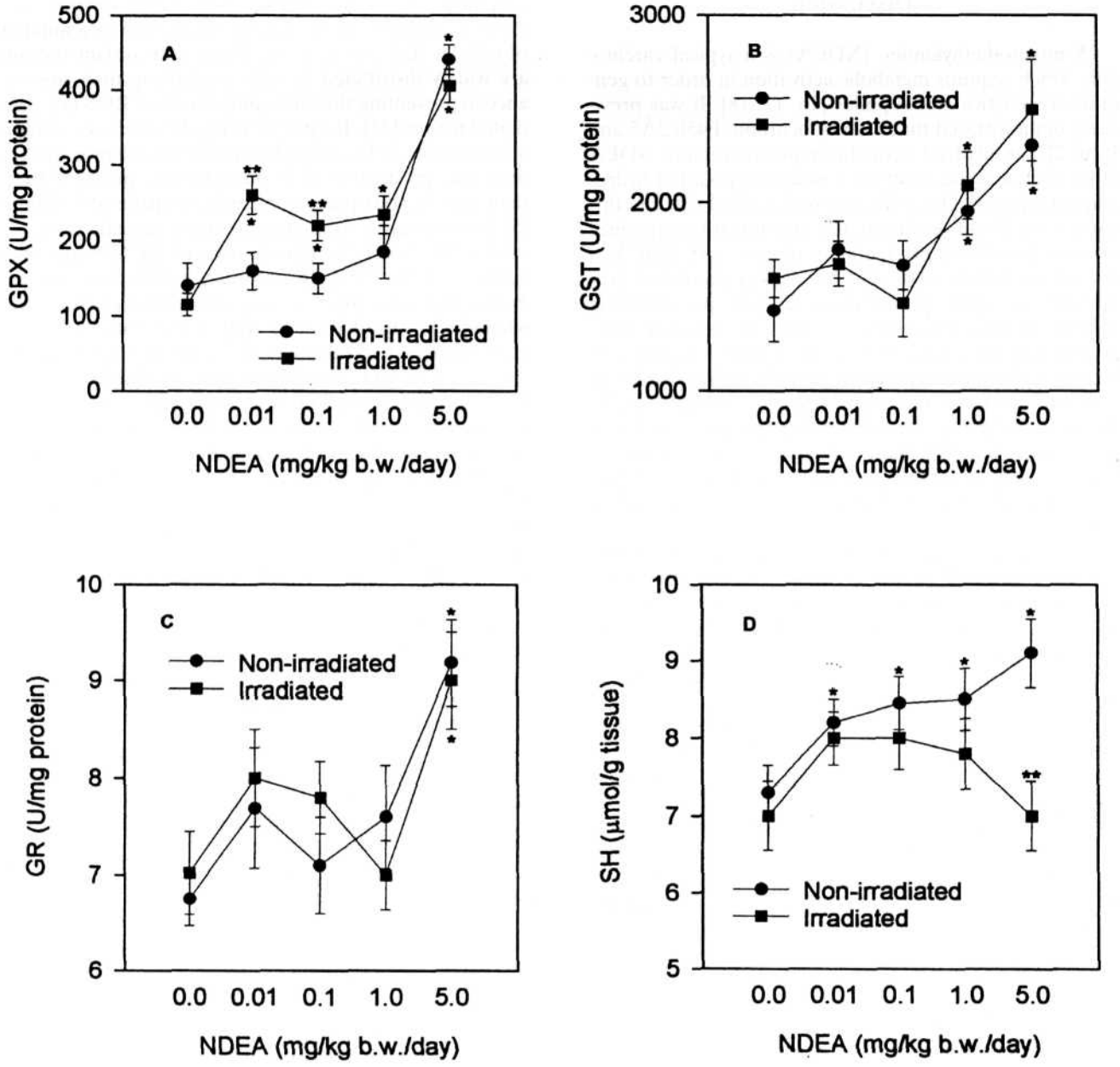

Fig. 2. Effect of γ-rays on activities of glutathione peroxidase (GPX) (A), glutathione S-transferase (GST) (B), glutathione reductase (GR) (C) and levels of thiol group (SH) (D) in the liver of NDEA-treated mice.
Values are mean ± SD, n = 5. * $P < 0.05$, versus the control (saline) group. ** $P < 0.05$, versus the corresponding non-irradiated group.

Results

Pretreatment of mice with NDEA at the lowest doses (0.01 or 0.1 mg/kg) increased TBARS level in the liver; however the agent at the highest dosage (1.0 and 5.0 mg/kg) did not have any effect(s) on TBARS in this tissue (Fig. 1A). As shown in Figures IB and 1C, hepatic superoxide dismutase (SOD) activity was unchanged and catalase (CAT) was increased in mice pretreated with a daily dose of 0.01 or 0.1 mg/kg NDEA. In contrast, when mice were pretreated with NDEA at the highest doses, both SOD and CAT activities were found to be unchanged from those of the control (Figs. IB, 1C). In the present study, the significant increase of GPX, GST and GR activities was mainly observed in mice pretreated with highest NDEA doses (Figs. 2A, 2B, 2C). Both the lowest and highest NDEA doses caused the increase of thiol groups (SH) in mice liver (Fig. 2D).

γ-irradiation decreased the amount of TBARS in the liver of NDEA-treated mice (Fig. 1A). As shown in figures IB and 2A, the activity of SOD and GPX was increased in mice pretreated with NDEA at the lowest dosage. In these groups, however, γ-irradiation did not have any effect(s) on GST and GR enzymes, and it lowered CAT activity and non-protein thiol (SH) levels in mice (Figs. 1C, 2B, 2C, 2D).

Discussion

N-nitrosodiethylamine (NDEA) is a typical carcinogen, which requires metabolic activation in order to generate its reactive electrophiles [16, 17, 18]. It was previously demonstrated that both cytochrome P450 2A5 and P450 2E1 is involved in the biotransformation of NDEA in rat liver, and the agent increased the amount of hydrogen peroxide (H_2O_2) and superoxide radical (O_2^-) in this tissue [19]. These results are not in contrast to ours since we have found that pretreatment of mice with NDEA at the lowest dosage (0.01 and 0.1 mg/kg) promoted production of lipid peroxidation and/or pro-oxidation shift(s). In accordance with the present data, the compound was also found to increase lipid peroxidation in human erythrocytes and rat liver mitochondria [20, 21]. It is of interest that N-nitrosodimethylamine (NDMA) also elevated TBARS in the sub-cellular fraction of rat liver [22]. Taken together these studies and the present results, we have suggested that NDEA induced lipid peroxidation and/or pro-oxidant shift(s) in a dose-dependent manner.

The overproduction of reactive oxygen species (ROS) is a risk factor of hepatocarcinogenesis [23], however, little is known about the role of some anti-oxidant enzymes in the liver of NDEA-treated animals. Song and co-workers have recently showed that NDEA increased the expression of the placental form of glutathione S-transferase (GST-P), which was found to be a sensitive marker of preneoplastic hepatocellular foci [24]. Similarly, Williams and colleagues noted that pretreatment of rats with NDEA increased the expression of GST-P in the liver [25]. In other resent studies [20], two anti-oxidant enzymes, i.e. catalase (CAT) and glutathione reductase (GR), but not SOD activity were found to be decreased in rat liver mitochondria pre-incubated with NDEA *in vitro*. In the present report, neither the highest nor lowest NDEA doses caused changes in SOD activity (Fig. IB). On the other hand, an increase in the production of hydrogen peroxide (H_2O_2), a substrate for catalase ($2H_2O_2 = H_2O + O_2$) or glutathione peroxidase (GPX) (ROOH + 2GSH = GSSG + ROH) was shown to elevate CAT activity in NDEA-treated mice (Fig. 1C). Since the agent at the highest dosage has been also found to induce GPX in mice liver (Fig. 2A), the activity of this enzyme was probably maintained *via* the reduction of the oxidized form of glutathione ($2GSH + H_2O_2 = GSSG + 2H_2O$). As shown in Figure 2D, the level of non-protein thiol groups (SH) was increased in this process(s). It should be noted that regeneration of the cellular GSH from its oxidized form (GSSG) requires enzyme, glutathione reductase (GR) and NADPH as the electron donor species [26]. Therefore, we hypothesized that NDEA-mediated increase in SH levels and/or GPX activity might be a significant anti-oxidant defense(s) in the liver to protect itself against additional oxidative injury. It seems plausible that any interference with CAT and GSH-linked pathway substantially reduces the ability of cells to inactivate H_2O_2 and other reactive oxygen species [27].

Exposure to ionizing radiation results in a complex set of responses whose onset, nature, and severity is a function of both total radiation dose and radiation quality [28]. In general, direct damage to tissues by ionizing radiation yields ROS, which may be diminished by a number of cellular defenses [29, 30]. These anti-oxidant systems are widely distributed in cells, underlying their importance in preventing the damaging effects of ROS in γ-irradiated tissues [31]. In spite of many direct effects of radiation in cells, it has been also found to enhance the endogenous production of iV-nitrosamines, plausibly from their endogenous precursors such as nitrite and amines [5]. Surprisingly, γ-irradiation has been recently shown to decrease carbon tetrachloride-induced TBARS and liver cirrhosis in mice [6]. In accordance with Nomura and Yamaoka's data [6], we have also evidenced that the whole-body γ-irradiation of NDEA-pretreated mice significantly ($p < 0.05$) decreased the amount of TBARS in the liver (Fig. 1A). These findings render further support to our preliminary results that γ-ray(s) at a dose level of 10 Gy diminished the NDEA-linked elevation of lipid peroxidation and/or pro-oxidant shift(s) due to increase in some anti-oxidant defenses, plausibly SOD and/or GPX activities (Figs. IB, 2A). It should be noted that many conflicting findings of dose- and/or time-related changes in CAT, SOD and other anti-oxidant enzyme activities have been recently published. For example, there are reports that SOD activity was decreased [32] or increased [29] in the liver of irradiated rats [29]. In the reported studies, the authors noted that the activity of CAT was decreased; however, the amount of hepatic TBARS was unchanged and decreased at 1 and 7 days post-X-irradiation exposure, respectively [29]. Since the induction of mitochondrial SOD was previously noted to protect against radiation-induced pro-oxidant states [33], an increased activity of both SOD and GPX was hypothesized to play a major anti-oxidative role in the liver of γ-irradiated mice (Figs. 1A, IB, 2A). It is generally known that cells exhibiting highs levels of SOD and/or GPX activity are relatively less vulnerable to the effects of γ-irradiation [27]. Interestingly, in the present experiment, the opposite directions between the activities of SOD and CAT were detected in γ-irradiated mice (Figs. IB, 1C). Furthermore, γ-radiation did not have any effects) on the activity of GST and GR enzymes, and it increased the activity of GPX in mice pretreated with NDEA (Fig. 2A). Since the normal activity of GPX depends on the availability of the reduced form of GSH [26], it was hypothesized that GR might assist in the regeneration of GSH in γ-irradiated mice (Fig. 2D). It should be emphasized that γ-irradiation at a dose level of 2.5 and 5.0 Gy did note have any effect(s) on SH groups in NDEA-treated mice (data not shown).

γ-irradiation induces various stimulating outcomes in both normal and pre-neoplastic cells, such as an increase in resistance to lipid peroxidation and/or oxygen-induced toxicity [34]. Therefore, with precaution it should be noted that the effect(s) of γ-rays on the formation of lipid peroxides and/or pro-oxidant shift(s) in mice pretreated with NDEA for three weeks was plausibly associated with one of the earliest stages in NDEA-induced toxicity. A more decisive long-term experiment(s) must be developed to illustrate the consequence of NDEA-induced oxidative stress on γ-radiation-mediated effects in the liver of mice. In addition, our findings require further investigation to ascertain the molecular reasons for the

change in activities of some anti-oxidant enzymes in NDEA-induced hepatocarcinogenesis. A more thorough approach may also include the determination of the expression pattern of each anti-oxidant enzyme at the gene level.

Conclusion

Our results revealed N-nitrosodiethylamine (NDEA) as a potent lipid peroxidation producer in mice liver. The dose levels of NDEA causing lipid peroxidation significantly increased catalase activity and thiol groups (SH) level, and the agent did not have any effect on hepatic superoxide dismutese. γ-irradiation has been shown to decrease lipid peroxidation in the liver of NDEA-treated mice. The effect(s) of γ-rays was mainly associated with the increased activities of two anti-oxidant enzymes, superoxide dismutase and glutathione peroxidase in mice liver.

Acknowledgements

The authors wish to thank Ms. Ewa Piotrowska and Ms. Ewa Grabowska for excellent technical assistance.

References

1. MAISIN J.R., Chemical radioprotection: past, present and future prospects. Int. J. Radiat. Biol., **73,** 443, **1998.**
2. ZMYSLONY M, JAJTE J, RAJKOWSKA E, SZMIGIELSKI S., Weak (5 mT) static magnetic field stimu lates lipid peroxidation in isolated rat liver microsomes in vitro. Electro- and Magnetobiology **17,** 109, **1998.**
3. LIJINSKY W., N-nitrosocompounds in the diet. Mutat. Res, **443,** 129, **1999.**
4. YAMASHITA Y, SUMI N, ARIMOTO S, HAYATSU H, Enhancement of the clostagenicity of N-nitrosodialkylamines plus near-ultraviolet irradiation by ethanol in Chinese hamster lung fibroblasts. Environ. Mol. Mutagen, 29, 296, 1997.
5. SVIATCHENKO VV, MALENCHENKO AF, The effect of ionizing radiation on nitrosamine formation from an thropogenic precursors. Radiobiologia, **32,** 546,1992.
6. NOMURA T, YAMAOKA K, Low-dose γ-ray irradiation reduces oxidative damage induced by $CC1_4$ in mouse liver. Free Rad. Biol. Med, **27,** 1324, **1999.**
7. SINGH S, KHANDUJA K.L, MITTAL P.K, Effect of 50 Hz sinusoidal electromagnetic field on the kinetics of $^{14}CO_2$ exhalation after [^{14}C]-N-nitrosodiethylamine administration in mice. Bioelectromagnetics, **20, 1, 1999.**
8. OHKAWA H, OHISHI N, YAGI K, Assay for lipid perox ides in animal tissues by thiobarbituric acid reaction. Anal. Biochem, 95, 351, **1979.**
9. MC CORD J.M, FRIDOVICH J, Superoxide dismutase: an enzymatic function of erythrocuprein (Hemocuprein). J. Biol. Chem, **244,** 6049, **1969.**
10. BEERS R.F, SIZER I.W., A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem, **195,** 133, **1952.**
11. PAGLIA D.E, VALANTINE W.N, Studies on the quanti tative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med, **70,** 158, **1967.**
12. HABIT W.H, PABST M.J, JACOBY W.B, Glutation S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem, **249,** 7130, **1974.**
13. BAYOUMI R.A., ROSALKI S.B, Evaluation of methods of coenzyme activation of erythrocyte enzymes for detection of deficiency of vitamins Bl, B2 and B6. Clin. Chem, **22,** 327, **1976.**
14. SAVILLE B, A scheme for the colorimetric determination of microgram amounts of thiols. Analyst, **83,** 670, 1958.
15. LOWRY O.H, ROSENBROUGH N.J, FARR A.L, RAN DALL R.J, Protein measurement with the Folin phenol re agent. J. Biol. Chem, **193,** 265, **1951.**
16. SINGH R, SWEETMAN G.A, FARMER P.B, SHUKER D.G., RICH K.J, Detection and characterization of two ma jor ethylated deoxyguanosine adducts by high performance liquid chromatography, electrospray mass spectrometry, and (32)P postlabeling. Development of an approach for detec tion of phosphotriesters. Chem. Res. Toxicol, **10,** 70, **1997.**
17. CAMUS A.M., GENESTE O, HONKOKSOKI P, BEREZIAT J.C, HENDERSON C.J, WOLF C.R, BARTSCH H, LANG M.A, High variability of nitrosamine metabolism among individuals: Role of cytochrome P450 2A6 and 2E1 in the dealkylation of N-nitrosodimethylamine and N-nitrosodiethylamine in mice and humans. Mol. Car cinogen, **7,** 268, **1993.**
18. WASTL U.M., ROSSMANITH W, LANG M.A, CAMUS-RANDON A.M., GRASL-KRAUPP B, BURSCH W, SCHULTE-HERMANN R, Expression of cytochrome P450 2A5 in preneoplastic and neoplastic mouse liver lesions. Mol. Carcinogen, 22, 229, **1998.**
19. HIETANEN E, BARTSCH H, AHOTUPA M.A, BEREZIAT J.C, BUSSACCHINI-GRIOT V, CABRAL J.R, CAMUS A.M., LAITINEN M, WILID H, Mechanism of fat-related modulation of N-nitrosodiethylamine-induced tu mors in rats: organ distribution, blood lipids, enzymes and pro-oxidant state. Carcinogenesis, **12,** 591, **1991.**
20. BANSAL A.K, BHATNAGAR D, SONI G.L, Effect of N-nitrosodiethylamine on lipid peroxidation and antioxidant enzymes in rat liver mitochondria: Protective role of anti oxidants. Bull. Environ. Contam. Toxicol, **59,** 254, **1997.**
21. BANSAL A.K, BHATNAGAR D, SONI G.L, In vitro ef fect of N-nitrosodiethylamine on lipid peroxidation and anti oxidant system in human erythrocytes. Toxicol. Vitro, **10,** 649, **1996.**
22. AHOTUPA M, BUSSACCHINI-GRIOT V, BEREZIAT J.C, CAMUS A.M., BARTSCH H, Rapid oxidative stress induced by N-nitrosoamines. Biochem. Biophys. Res. Com**mun, 146,** 1047, **1987.**
23. PUNNONEN R, KUDO R, PUNNONEN K, HIETANEN E, KUPPOALLA T, KAINULAINEN T, SATO H, AHOTUPA K, Activities of antioxidant enzymes and lipid peroxidation in endometrial cancer. Eur. J. Cancer, **29,** 266, **1993**
24. SONG K.Y, LIM I.K, PARK S.C, LEE S.O, PARK H.S, CHOI Y.K, HYUN B.H, Effect of nodularin on the ex pression of glutathione S-transferase placental form and proliferating cell nuclear antigen in N-nitrosodiethylamine initiated hepatocarcinogenesis in the male Fischer 344 rat. Carcinogenesis 20, 1541, 1999.
25. WILLIAMS GM, IATROPOULOS MJ, JEFFREY AM, LUO FQ, WANG CH, PITTMAN B, Diethylnitrosamine exposure-responses for DNA ethylation, hepatocellular pro liferation, and initiation of carcinogenesis in rat liver display non-linearities and thresholds. Arch. Toxicol, **73,** 394, **1999.**

26. MEISER A, ANDERSON M.E., Glutathione. Ann. Rev. Biochem., **52,** 711, **1993.**
27. JONAS SK, RILEY PA., Modification of the in vitro cytotoxicity of hydrogen peroxide by iron complexes. Free Rad. Res. Comraun, **17,** 407, **1992.**
28. BARCELLOS-HOFF MH., How do tissue respond to dam age at the cellular level? The role of cytokines in irradiated tissues. Rad. Res., **150,** S109, **1999.**
29. PELTOLA V, PARVINEN M, HUHTANIEMI I, KUL-MALA J, AHOTUPA M., Comparison of effects of 0.5 and 3.0 Gy X-irradiation on lipid peroxidation and antioxidant enzyme function in rat testis and liver. J. Androl., **14,** 267, **1993.**
30. GOMI F, MATSSUTO M., Effects of aging and food restriction on the antioxidant enzyme activity of rat livers. J. Gerontol. Biol. Sci., **53A,** 161, **1998.**
31. RILEY PA., Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int. J. Radiat. Biol. 65, 27, **1994**
32. KERGONOU JF, BRAQUET M, ROCQUET G., Influ ence of whole-body gamma irradiation upon rat liver mitochondria fractions. Radiat. Res. **88,** 377, **1981.**
33. WONG G.H., Protective role of cytokines against radiation: Induction of mitochobdrial MnSOD. Biochim. Biophys. Acta, **1271,** 205, **1995.**
34. LEE Y.J., DUCOFF H.S., Radiation-enhanced resistance to oxygen: a possible relationship to radiation-enhanced lon gevity. Mech. Age. Develop., **27,** 101, **1984.**