Biochemical Pharmacology, Vol. 44, No. 8, pp. 1495–1500, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

EFFECTS OF CHRONIC ETHANOL ADMINISTRATION ON FREE RADICAL DEFENCE IN RAT MYOCARDIUM

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(*Received* 9 *March* 1992; *accepted* 27 *July* 1992)

Abstract—Cellular protection against free radical reactions was measured in myocardium from ethanol-fed rats using ethanol administration in drinking water as a model of moderate alcohol intoxication. The activities of Cu,Zn-superoxide dismutase (SOD) and glutathione-*S*-transferase were higher in ethanol-fed rats than in controls, whereas Mn-SOD, catalase and glutathione peroxidase activities were not altered by ethanol treatment. Myocardial zinc was higher and selenium concentration lower in ethanol-fed rats than in controls. Ethanol consumption, which failed to modify the myocardial vitamin E level, did not result in increased lipid peroxidation, but decreased cytosolic and membraneous protein thiols.

The contribution of free radicals to heart disease has been emphasized and oxidative stress has been suggested to represent a fundamental mechanism in the production of myocardial injury [1].

Whereas the association between chronic ethanol consumption and myocardial disturbances is well recognised [2, 3], the pathogenesis of these disturbances has not been established fully. However, enhanced lipid peroxidation resulting from ethanol administration could play an important role in the ethanol-induced impairment of myocardial function [4–6].

Ethanol-induced changes in the myocardial antioxidant defence could be responsible, at least partly, for such disturbances in lipid peroxidation. Studying the ethanol-induced changes in the activity of rat heart antioxidant enzymes, Antonenkov and Panchenko [7] reported that chronic ethanol administration resulted in an increase in catalase activity without significant changes in the activity of the other enzymes studied [7]. Overt cardiomyopathy being apparent only when the rats were administered 3-amino-1,2,4-triazole, a catalase inhibitor, together with ethanol, the probable role of catalase in the pathogenesis of alcoholic cardiomyopathy was suggested [8, 9]. This however appears questionable, since catalase appears to play a minor role as compared to glutathione peroxidase in the detoxification of hydrogen peroxide in the rat heart [10, 11].

The present study was initiated to determine the effects of chronic ethanol administration on the various enzymes involved in the antioxidant defence [Cu,Zn- and Mn-superoxide dismutases (SODs†) (EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione peroxidase (EC 1.11.1.9) and glutathione *S*-transferase (EC 2.5.1.18)], as well as on α-tocopherol, the main membraneous lipid soluble chain-breaking antioxidant. Due to their possible involvement in free radical mechanisms, iron, copper, zinc, manganese and selenium levels have also been studied. Furthermore, changes in lipid peroxidation and protein thiol oxidation, which may be linked to an ethanol-induced oxidative stress, have been considered.

This study was undertaken using ethanol administration in drinking water as described by Prasad *et al.* [12]. According to these authors, the rat on 10% (v/v) ethanol in the drinking water is a reasonable model for studying the effects of moderate alcohol consumption on specific biochemical pathways.

MATERIALS AND METHODS

Experimental animals. Male Sprague–Dawley rats weighing approximately 100 g at the start of the experiment were maintained on a standard laboratory diet (Iffa-Credo, U.A.R., France) containing 64% of energy as carbohydrate, 11% as lipid and 25% as protein. The vitamin E content of the diet was 150 mg/kg. Control rats had tap water as drinking fluid, whereas experimental animals received as sole drinking fluid an aqueous ethanol solution (10% v/v) for 4 weeks. The ethanol intake of these animals amounted to 7–9 g/kg body weight/day, representing about 18% of total energy intake. The body weight gains were similar in the control and experimental groups.

At the end of the experimental period, rats were fasted for 16 hr. However, access to the ethanol drinking fluid was maintained to prevent any possible withdrawal stress. The animals were killed by decapitation and the hearts removed immediately and washed in ice-cold 0.15 M NaCl. A sample of the heart was frozen in liquid nitrogen until used for the analysis of the non-heme iron and trace element contents. The remaining heart was homogenized and used for the determinations of enzymatic activities.

Tissue homogenization and preparation of subcellular fractions. A 10% (w/v) homogenate in

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† Abbreviations: SOD, superoxide dismutase; t-BOOH, *t*-butylhydroperoxide; TBARS, thiobarbituric acid reactive substances.

Table 1. Effects of chronic ethanol administration on enzymatic free radical defence

	Control rats	Ethanol-fed rats
Cu,Zn-SOD (U/mg protein)	19.39 ± 1.73 (12)	23.38 ± 1.95† (12)
Mn-SOD (U/mg protein)	16.32 ± 1.62 (12)	16.77 ± 1.91* (12)
Catalase (mU/mg protein)	19.14 ± 2.58 (12)	18.61 ± 2.16* (11)
Glutathione peroxidase (H_2O_2) (nmol/min/mg protein)	183.84 ± 13.32 (12)	176.78 ± 17.12* (12)
Glutathione peroxidase (*t*-BOOH) (nmol/min/mg protein)	148.99 ± 6.66 (12)	141.63 ± 8.67* (12)
Glutathione *S*-transferase (CDNB) (nmol/min/mg protein)	48.55 ± 1.78 (12)	53.78 ± 2.99† (12)

Ethanol-treated rats were given 10% ethanol (v/v) as sole drinking fluid for 4 weeks. Results are expressed as means ± SEM. The number of animals is shown in parentheses.
* $P > 0.05$; † $P < 0.01$ vs control value.
CDNB, 1-chloro-2-4-dinitro benzene.

Table 2. Non-heme iron and trace elements in rat myocardium after chronic ethanol administration

	Control rats	Ethanol-fed rats
Non-heme iron (μg/g)	78 ± 5 (12)	79 ± 6* (12)
Copper (μg/g)	5.50 ± 0.34 (12)	5.69 ± 0.40* (12)
Zinc (μg/g)	16.55 ± 1.46 (12)	19.65 ± 1.01† (12)
Manganese (μg/g)	0.39 ± 0.03 (6)	0.41 ± 0.03* (6)
Selenium (μg/g)	1.55 ± 0.15 (12)	1.25 ± 0.08† (12)

Results are expressed as means ± SEM. The number of animals is shown in parentheses.
* $P > 0.05$; † $P < 0.01$ vs control value.

0.25 M sucrose was prepared for the determination of catalase activity or in a solution containing KCl (25 mM), $MgCl_2$ (5 mM), EDTA (1 mM), dithiothreitol (0.1 mM) and Tris (50 mM) pH 7.2 for that of glutathione peroxidase and *S*-transferase activities. Homogenates were then centrifuged at 3000 *g* for 10 min and supernatants were used for the determination of enzyme activities.

Cytosolic, mitochondrial and microsomal fractions were obtained by differential centrifugation as in Ref. 13 and used for the determination of SOD activities and vitamin E content.

Assay of enzymatic activities. Cu, Zn-SOD and Mn-SOD were assayed following McCord and Fridovich [14] as described in Ref. 15. Catalase activity was determined spectrophotometrically by measuring the decrease in absorbance of H_2O_2 at 240 nm [16]. Glutathione peroxidase was assayed using H_2O_2 (0.25 mM) or *t*-butylhydroperoxide (*t*-BOOH) (0.20 mM) as described in Ref. 17. Glutathione *S*-transferase activity was measured using 1-chloro-2-4-dinitro benzene (1 mM) by the method described by Habig *et al.* [18].

Vitamin E. α-Tocopherol was determined by HPLC following Vatassery and Hagen [19] as described in Ref. 20.

Trace elements. Copper, zinc and manganese were determined by flame atomic absorption spectrometry after acidic extraction and selenium was measured by furnace graphite atomic spectrometry with Zeemann correction [21]. Non-heme iron was determined after trichloracetic/hydrochloric acid extraction by inductively coupled plasma atomic spectrometry [21].

Assay of thiobarbituric acid reactive substances (TBARS). Lipid peroxidation was evaluated from the formation of thiobarbituric acid reactive substances (TBARS) as described in Ref. 22 after 4 or 8 weeks of ethanol feeding.

Determination of sulfhydryl contents in heart proteins. Protein thiols were determined following Sedlak and Lindsay [23] separately in two heart fractions, one corresponding to the cytosol (105,000 *g* supernatant), the other including all cell membraneous structures together (150,000 *g* pellet) as described by Pompella *et al.* [24].

Other experimental procedures. Protein concentration was determined by the method of Lowry *et al.* [25]. Ethanol concentrations were determined as described by Polkins and Mackell [26].

Expression of results. Results were expressed as means ± SEM. Values obtained in the ethanol-fed and control groups were compared by Student's *t*-test.

RESULTS

In accordance with Ref. 12, the consumption of

Table 3. Effects of chronic ethanol feeding on TBARS in rat heart homogenates

Treatment	TBARS (nmol malonaldialdehyde/mg protein) Group I (N = 6)	Group II (N = 6)	Group III (N = 12)
Controls	0.29 ± 0.03	0.25 ± 0.03	0.27 ± 0.07
Ethanol-treated	0.32 ± 0.03*	0.25 ± 0.03*	0.18 ± 0.02†

Group I: animals fed ethanol (10% v/v) for 4 weeks.
Group II: animals fed ethanol (10% v/v) for 4 weeks and injected with ethanol (25 mmol/kg, i.p.) 2 hr before killing. The control rats received only ethanol (25 mmol/kg, i.p.) 2 hr before killing.
Group III: animals fed ethanol (10% v/v) for 8 weeks.
Results are expressed as means ± SEM. The number of animals is shown in parentheses.
* $P > 0.05$; † $0.02 > P > 0.01$ vs control value.

10% ethanol in drinking water had no significant effect on the weight gain or total caloric intake when compared to that of control animals. Blood level of ethanol measured at the end of the ethanol-feeding period (4 weeks) was 7.8 ± 0.5 mM.

The activities of cytosolic Cu,Zn-SOD were significantly increased, whereas mitochondrial Mn-SOD activities were unchanged after chronic ethanol administration (Table 1). Catalase and Se-glutathione peroxidase activities (measured with H_2O_2 or *t*-BOOH as substrates) were unaffected by chronic ethanol feeding, whereas glutathione *S*-transferase activities were significantly higher in the hearts of ethanol-fed rats (Table 1).

The myocardial content of non-heme iron was not modified by ethanol feeding (Table 2). However, ethanol-fed rats had significantly higher levels of heart zinc, whereas the concentration of copper and manganese did not differ from the controls. In contrast, the selenium content was significantly decreased after chronic ethanol administration (Table 2).

No enhanced production of TBARS was induced in the heart by chronic ethanol feeding either alone or followed by an acute ethanol load (25 mmol/kg, i.p.) 2 hr before killing (Table 3). When ethanol feeding was prolonged for 8 weeks, TBARS were significantly lower in the heart homogenate from ethanol-fed rats as compared to the controls.

Chronic ethanol administration did not modify α-tocopherol levels of the whole heart homogenates (36.59 ± 2.23 vs 36.88 ± 2.53 μg/g heart in 11 controls and 11 ethanol-treated rats, respectively; $P > 0.05$). The α-tocopherol content of the mitochondrial fraction (0.28 ± 0.02 vs 0.26 ± 0.01 μg/mg protein) and the microsomal fraction (0.65 ± 0.05 vs 0.64 ± 0.07 μg/mg protein) were also not statistically different in the same controls and ethanol-treated rats. In contrast, protein thiols were decreased in both the soluble and the membraneous fraction of homogenates from ethanol-fed rats (Fig. 1).

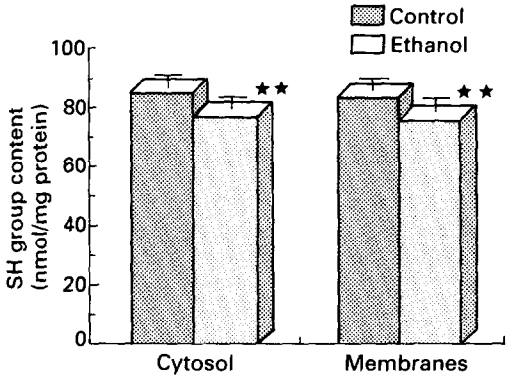

Fig. 1. Protein thiols in the soluble and membraneous fractions of rat myocardial homogenates after chronic ethanol administration. Results are expressed as means ± SEM with six rats in each group. **$P < 0.01$ vs control value.

DISCUSSION

The observed significant increase in Cu,Zn-SOD activity was unexpected, since ethanol administration has been reported to decrease the activity of this enzyme in various rat tissues, such as the liver [15, 27–29] and brain [30, 31]. However, enhanced Cu,Zn-SOD activity following chronic ethanol administration has been reported previously in the lung [32], a tissue which shares with the myocardium the characteristic of high oxygen utilization. The activity of the heart enzyme has also been reported to be elevated after an acute exercise bout as compared to the resting values [33]. This last finding led to the suggestion that the increased Cu,Zn-SOD activity may be related to an enhanced free radical generation inside the cardiac cell [33]. Such a mechanism may also account for the ethanol-induced increase in myocardial Cu,Zn-SOD activity observed in the present study.

Contrasting with this enhancement, no significant changes were observed here in catalase and glutathione peroxidase activities. The resulting disturbances in the ratio of antioxidant enzymes

could induce an increased generation of reactive species damaging for the cell. A decrease in the ratio (glutathione peroxidase + catalase) to SOD has thus been reported during the progressive development of cardiomyopathy in hamsters with a genetic predisposition to a fatal cardiomyopathy [34]. The lack of changes in catalase activity appears in contradiction with previous reports of enhanced activity of this enzyme in alcohol-treated rats [7, 8, 35, 36]. Since lipids or lipid metabolism appear to play a primary role in the changes in catalase activity [36] it may be suggested that the discrepancies between the data previously reported and the present data concerning ethanol-induced changes in heart catalase activity are linked to the much higher dietary lipid level in the previous reports as compared to the present report. The decrease in heart selenium content did not affect the Se-dependent glutathione peroxidase which plays a major role in myocardial hydrogen peroxide detoxification [10, 11]. However, it appears that the decrease in the tissular selenium content could disturb selenoproteins acting as antioxidants, but different from glutathione peroxidase [37]. One may recall that selenium bioavailability may be an important factor in heart diseases [38].

Contrasting with the lack of changes in heart glutathione peroxidase activity, we observed a significant increase in the activity of glutathione *S*-transferase in the ethanol-fed rats. Since glutathione *S*-transferase can be activated by zinc [39], the enhanced activity observed here in the hearts of ethanol-fed rats may be linked to the ethanol-induced increase in myocardial zinc concentration. This enhancement in glutathione *S*-transferase activity may represent an adaptative response alleviating the toxic effects of ethanol on the heart. Glutathione *S*-transferases are actually multifunctional proteins which are involved in the protective mechanisms against the deleterious actions of toxic substances and/or of their metabolites. However, glutathione transferases could also be involved in rat myocardium in a non-oxidative pathway of ethanol metabolism leading, as in human heart, to the generation of fatty acid ethyl esters [40]. Such esters may contribute to the myocardial disturbances in ethanol-fed rats. Nevertheless, fatty acid ethyl ester synthesis could involve enzymes different from glutathione *S*-transferase [41].

Besides contributing possibly to the enhanced glutathione *S*-transferase activity, the increase in myocardial zinc concentration may play a role in the antioxidant defence by decreasing the susceptibility of specific sulfhydryl groups to oxidation and/or by competing with prooxidant metals for binding sites, thus decreasing their ability to transfer electrons in a particular environment [42]. It has been shown recently that tissular zinc and metallothionin levels are increased by a wide range of radical-generating agents [43]. It can therefore be suggested that the increase in the myocardial zinc level reported here in alcohol-fed rats is induced by a prooxidative effect of ethanol administration in the heart and may represent an adaptative mechanism acting against this prooxidative effect. Furthermore, zinc being implicated in the stabilization of Cu,Zn-SOD could contribute to the increase in this enzyme activity.

The lack of enhanced lipid peroxidation observed here in the heart of ethanol-fed rats may be linked to the maintenance of a normal α-tocopherol level in the tissular homogenates, as well as in the microsomal and mitochondrial subcellular fractions. A lack of α-tocopherol disturbances in the heart of ethanol-treated rats had been reported previously by Bjørneboe *et al.* [44]. The increased myocardial zinc content reported here could also be involved in the absence of lipid peroxidation disturbances since a drastic enhanced lipid peroxidation after chronic ethanol administration was observed by Coudray *et al.* [45] in zinc-deficient rats only. The increase in myocardial lipid peroxidation reported previously by other investigators after chronic ethanol feeding may be linked to the high lipid content of the diets [5, 6]. It was thus reported that the generation of carbon-centered lipid radicals in the hearts of ethanol-fed rats was much greater in extent in rats fed ethanol together with a lipid-rich diet [46]. Whereas no significant changes in heart lipid peroxidation were apparent in the present study after 4 weeks of ethanol administration, a significant reduction in TBARS formation was observed in rats receiving ethanol for 8 weeks. As observed in the liver of ethanol-treated animals [29], changes in the membraneous fatty acid composition could be involved in the decrease in lipid peroxidation following sustained ethanol administration for 8 weeks under our experimental conditions. Such changes in fatty acid composition have also been suggested [47] to play a prominent role in the pathogenesis of alcoholic cardiomyopathy in turkeys, in which ethanol produces an impairment in left ventricle function and disturbances in the antioxidant defence systems [48]. However the decrease in TBARS observed in these rats could also result, at least partly, from an ethanol-induced increase in myocardial aldehyde dehydrogenase activity [49], an enzyme which uses malonaldehyde as substrate.

The decrease in myocardial protein sulfhydryl groups observed here in the cytosol as well as in the membranes from ethanol-fed rats appears to be a prominent finding. It has been shown previously that oxidation of protein thiols represents a more sensitive and specific marker of free radical mechanisms than lipid changes, at least in the vascular compartment [50]. It was furthermore suggested by Remmer *et al.* [51] that ethanol promotes oxygen radical attack on proteins rather than on lipids. The putative role of oxidative stress in toxicity from long-term exposure to ethanol is controversial because of contradictory evidence regarding the occurrence of lipid peroxidation in tissues of ethanol-treated rats [52, 53]. The ethanol-induced decrease in cardiac protein sulfhydryl groups reported here is probably linked to an oxidative stress and may play an important role in the disturbed activity of important myocardial enzymes, such as creatine kinase, that we reported recently in such ethanol-fed rats [54].

Acknowledgements—This work was supported by grants from INSERM (CRE 90207), Fondation de France and IREB (92-09).

REFERENCES

1. McCord JM, Free radicals and heart disease. In: *Nutritional Impact of Food Processing* (Eds. Somogyi JC and Müller HR), pp. 327–337. Karger, Basel, 1986.
2. Rubin E, Alcoholic myopathy in heart and skeletal muscle. *N Engl J Med* **301**: 28–33, 1979.
3. Segel LD, The development of alcohol-induced cardiac dysfunction in the rat. *Alcohol Alcoholism* **23**: 391–401, 1988.
4. Garcia-Bunuel L, Lipid peroxidation in alcoholic myopathy and cardiomyopathy. *Med Hypotheses* **13**: 217–231, 1984.
5. Edès I, Toszegi A, Csanady M and Bozoky B, Myocardial lipid peroxidation in rats after chronic alcohol ingestion and the effects of different antioxidants. *Cardiovasc Res* **20**: 542–548, 1986.
6. Antonenkov VD, Pirozhkov SV, Popova SV and Panchenko LF, Effect of chronic ethanol, catalase inhibitor 3-amino-1,2,4-triazole and clofibrate treatment on lipid peroxidation in rat myocardium. *Int J Biochem* **21**: 1313–1318, 1989.
7. Antonenkov VD and Panchenko LF, Effect of chronic ethanol treatment under partial catalase inhibition on the activity of enzymes related to peroxide metabolism in rat liver and heart. *Int J Biochem* **20**: 823–828, 1988.
8. Kino M, Chronic effects of ethanol under partial inhibition of catalase activity in the rat heart: Light and electron microscopic observations. *J Mol Cell Cardiol* **13**: 5–21, 1981.
9. Tsiplenkova VG, Vikhert AM and Cherpachenko NM, Ultrastructural and histochemical observations in human and experimental alcoholic cardiomyopathy. *J Am Coll Cardiol* **8**: 22A–32A, 1986.
10. Doroshow JH, Locker GY and Myers CE, Enzymatic defenses of the mouse heart against reactive oxygen metabolites. *J Clin Invest* **65**: 128–135, 1980.
11. Simmons TW and Jamall IS, Relative importance of intracellular glutathione peroxidase and catalase *in vivo* for prevention of peroxidation to the heart. *Cardiovasc Res* **23**: 774–779, 1989.
12. Prasad JS, Daune L, Crankshaw DL, Erickson RR, Elliott CE, Husby AD and Holtzman JL, Studies on the effect of chronic consumption of moderate amounts of ethanol on male rat hepatic microsomal drug-metabolizing activity. *Biochem Pharmacol* **34**: 3427–3431, 1985.
13. Rouach H, Houzé P, Orfanelli MT, Gentil M, Bourdon R and Nordmann R, Effect of acute ethanol administration on the subcellular distribution of iron in rat liver and cerebellum. *Biochem Pharmacol* **39**: 1095–1100, 1990.
14. McCord JM and Fridovich I, Superoxide dismutase: an enzymic function for erythrocuprein. *J Biol Chem* **244**: 6049–6055, 1969.
15. Ribière C, Sinaceur J, Nordmann J and Nordmann R, Liver superoxide dismutases and catalase during ethanol inhalation and withdrawal. *Pharmacol Biochem Behav* **18**: 263–266, 1983.
16. Aebi H, Catalase. In: *Methods of Enzymatic Analysis* (Eds. Bergmeyer HV), pp. 673–684. Academic Press, New York, 1974.
17. Vertechy M, Cooper MB, Ghirardi O and Ramacci MT, Antioxidant enzyme activities in heart and skeletal muscle of rats of different ages. *Exp Gerontol* **24**: 211–218, 1989.
18. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
19. Vatassery GT and Hagen DF, A liquid chromatographic method for quantitative determination of α-tocopherol in rat brain. *Anal Biochem* **79**: 129–134, 1977.
20. Rouach H, Park MK, Orfanelli MT, Janvier B and Nordmann R, Ethanol-induced oxidative stress in the rat cerebellum. *Alcohol Alcoholism* Suppl 1: 207–211, 1987.
21. Houzé P, Rouach H, Gentil M, Orfanelli MT and Nordmann R, Effect of allopurinol on the hepatic and cerebellar iron, selenium, zinc and copper status following acute ethanol administration to rats. *Free Rad Res Commun* **12–13**: 663–668, 1991.
22. Mihara B, Uchiyama M and Fukuzawa K, Thiobarbituric acid value on fresh homogenate of rat as a parameter of lipid peroxidation in aging CCl_4 intoxication, and vitamin E deficiency. *Biochem Med* **23**: 302–311, 1980.
23. Sedlak J and Lindsay RH, Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* **25**: 192–205, 1968.
24. Pompella A, Romani A, Benedetti A and Comporti M, Loss of membrane protein thiols and lipid peroxidation in allyl alcohol hepatotoxicity. *Biochem Pharmacol* **41**: 1255–1259, 1991.
25. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
26. Poklins A and Mackell MA, Evaluation of a modified alcohol deshydrogenase assay for the determination of ethanol in blood. *Clin Chem* **28**: 2125–2127, 1982.
27. Ribière C, Sinaceur J, Nordmann J and Nordmann R, Discrepancy between the different subcellular activities of rat liver catalase and superoxide dismutases in response to acute ethanol administration. *Alcohol Alcoholism* **20**: 13–18, 1985.
28. Schisler NJ and Singh SM, Effect of ethanol *in vivo* on enzymes which detoxify oxygen free radicals. *Free Rad Biol Med* **7**: 117–123, 1989.
29. Zidenberg-Cherr S, Olin KL, Villanueva J, Tang A, Phinney SD, Halsted CH and Keen CL, Ethanol-induced changes in hepatic free radical defense mechanisms and fatty-acid composition in the miniature pig. *Hepatology* **13**: 1185–1192, 1991.
30. Ledig M, M'Paria JR and Mandel P, Superoxide dismutase activity in rat brain during acute and chronic alcohol intoxication. *Neurochem Res* **6**: 385–391, 1981.
31. Ledig M, Kopp P and Mandel P, Alcohol effect on superoxide dismutase activity in nervous tissue. In: *Alcohol Toxicity and Free Radical Mechanisms. Advances in the Biosciences* (Eds. Nordmann R, Ribière C and Rouach H), Vol. 71, pp. 79–86. Pergamon Press, Oxford, 1988.
32. Rikans LE and Gonzalez LP, Antioxidant protection systems of rat lung after chronic ethanol inhalation. *Alcoholism Clin Exp Res* **14**: 872–877, 1990.
33. Ji LL, Dillon D and Wu E, Myocardial aging: antioxidant enzyme systems and related biochemical properties. *Am J Physiol* **261** (*Regul Integrative Comp Physiol* **30**): R386–R392, 1991.
34. Mailler K, Macleod I and Morris W, Age related changes in anti-oxidative enzymes in cardiomyopathic hamster hearts. *Mech Ageing Dev* **59**: 37–45, 1991.
35. Fahimi HD, Kino M, Hicks L, Thorp KA and Abelman WH, Increased myocardial catalase in rats fed ethanol. *Am J Pathol* **96**: 373–390, 1979.
36. DeMaster EG, Kaplan E and Chesler E, The differential response of tissue catalase activity to chronic alcohol administration in the rat. *Alcoholism Clin Exp Res* **5**: 45–48, 1981.
37. Burk RF, Recent developments in trace element metabolism and function: newer roles of selenium in nutrition. *J Nutr* **119**: 1051–1054, 1989.
38. Shamberger RJ, Selenium in the drinking water and cardiovascular disease. *J Environ Pathol Toxicol* **4**: 305–311, 1980.
39. Cho CH and Fong LYY, The interaction of ethanol and zinc on hepatic glutathione and glutathione

transferase activity in mice. *Agents Actions* **29**: 382–385, 1990.
40. Bora PS, Spilburg CA and Lange LG, Metabolism of ethanol and carcinogens by glutathione transferases. *Proc Natl Acad Sci USA* **86**: 4470–4473, 1989.
41. Sharman R, Gupta S, Singhal SS, Ahmad H, Haque A and Awasthi YC, Independent segregation of glutathione S-transferase and fatty acid ethyl ester synthase from pancreas and other human tissues. *Biochem J* **275**: 507–513, 1991.
42. Bray TM and Bettger WJ, The physiological role of zinc as an antioxidant. *Free Rad Biol Med* **8**: 281–291, 1990.
43. Sato M and Sasaki M, Enhanced lipid peroxidation is not necessary for induction of metallothionein-I by oxidative stress. *Chem Biol Interact* **78**: 143–154, 1991.
44. Bjørneboe GEA, Bjørneboe A, Hagen BF, Mørland J and Drevon CA, Reduced hepatic α-tocopherol content after long-term administration of ethanol to rats. *Biochim Biophys Acta* **918**: 236–241, 1987.
45. Coudray C, Boucher F, Richard MJ, Arnaud D, De Leiris J and Favier A, Zinc deficiency, ethanol, and myocardial ischemia affect lipoperoxidation in rats. *Biol Trace Element Res* **30**: 103–118, 1991.
46. Reinke LA, Lai EK, Du Bose CM and Mac Cay PB, Reactive free radical generation *in vivo* in heart and liver of ethanol-fed rats: correlation with radical formation *in vitro*. *Proc Natl Acad Sci USA* **84**: 9223–9227, 1987.
47. Soos I, Facsko A, Edes I, Kiss E and Csanady M, Effects of chronic alcohol ingestion on myocardial lipid and fatty acid composition in adult turkeys. *Cardiovasc Res* **25**: 881–884, 1991.
48. Edes I, Piros G, Forster T and Csanady M, Alcohol-induced congestive cardiomyopathy in adult turkeys: effects on myocardial antioxidant defence systems. *Basic Res Cardiol* **82**: 551–556, 1987.
49. Remla A, Menon PVG, Kurup PA, Kumari S and Saravarghese, Effect of ethanol administration on metabolism of lipids in heart and aorta in isoproterenol induced myocardial infarction in rats. *Indian J Exp Biol* **29**: 244–248, 1991.
50. Radi R, Bush KM, Cosgrove TP and Freeman BA, Reaction of xanthine oxidase-derived oxidants with lipid and protein of human plasma. *Arch Biochem Biophys* **286**: 117–125, 1991.
51. Remmer H, Kessler W, Einsele H, Hintze TH, Diaz de Toranzo G, Gharaibeh AM and Frank H, Ethanol promotes oxygen-radical attack on proteins but not on lipids. *Drug Metab Rev* **20**: 219–232, 1989.
52. Nordmann R, Ribière C and Rouach H, Ethanol-induced lipid peroxidation and oxidative stress in extrahepatic tissues. *Alcohol Alcoholism* **25**: 231–237, 1990.
53. Nordmann R, Ribière C and Rouach H, Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Rad Biol Med* **12**: 219–240, 1992.
54. Hininger I, Ribière C and Nordmann R, Disturbances in myocardial creatine kinase following ethanol administration to rats—trials of prevention by allopurinol, desferrioxamine and propranolol. *Alcohol Alcoholism* **26**: 303–307, 1991.