J. Pharm. Pharmacol. 2000, 52: 547–552
Received
Accepted

Protective Effect of *N*-Acetylcysteine on Rat Liver Cell Membrane During Methanol Intoxication

I. DOBRZYÑSKA*, E. SKRZYDLEWSKA†, I. KASACKA‡ AND Z. FIGASZEWSKI*§

Institute of Chemistry, University in Bialystok, Al. Pilsudskiego 11/4, 15-443 Bialystok, Departments of †Analytical Chemistry and ‡Histology, Medical University, 15-230 Bialystok 8, and §Faculty of Chemistry, University of Warsaw, ul. Pastera 1, 02-093 Warsaw, Poland

Abstract

Methanol is oxidized in-vivo to formaldehyde and then to formate, and these processes are accompanied by the generation of free radicals. We have studied the effect of *N*-acetylcysteine on liver cell membrane from rats intoxicated with methanol ($3{\cdot}0\,g\,kg^{-1}$).

Evaluation of the effect was achieved by several methods. Lipid peroxidation and surface charge density were measured. An ultrastructural study of the liver cells was undertaken. The concentration of marker enzymes of liver damage (alanine aminotransferase and aspartate aminotransferase) in blood serum was measured.

Methanol administration caused an increase in lipid peroxidation products (approximately 30%) as well as in surface charge density (approximately 60%). This might have resulted in the membrane liver cell damage visible under electron microscopy and a leak of alanine aminotransferase and aspartate aminotransferase into the blood (increase of approximately 70 and 50%, respectively). Ingestion of *N*-acetylcysteine with methanol partially prevented these methanol-induced changes. Compared with the control group, lipid peroxidation was increased by approximately 3% and surface charge density by approximately 30%. Alanine aminotransferase and aspartate aminotransferase activity increased by 9 and 8%, respectively, compared with the control group.

The results suggested that *N*-acetylcysteine was an effective antioxidant in methanol intoxication. It may have efficacy in protecting free radical damage to liver cells following methanol intoxication.

Methanol toxicity is a significant problem in the area of drug abuse. The accidental intoxication of this compound in man still takes place as it is mistakenly ingested instead of ethanol. The toxicity of methanol is connected with its metabolism to formaldehyde and then to formate, which occurs in the liver (Liesivuori & Savolainen 1991). These processes are accompanied by the generation of free radicals (Kato et al 1990). Therefore, the severe derangement of the subcellular metabolism and structural alterations of liver cells characterize acute or chronic methanol toxicity.

Formaldehyde and the free radicals generated during methanol metabolism are very reactive compounds and they result in liver cell damage.

Correspondence: I. Dobrzyñska, Institute of Chemistry, University in Bialystok, Al. Pilsudskiego 11/4, 15-443 Bialystok, Poland.

The condition of the cell may be estimated by measuring the value of the cell membrane charge (Skrzydlewska et al 1997). Under physiological conditions the membrane cell has total negative electric charge (Benga & Holmes 1984). Its value is determined by structural positive charge carriers i.e. by free amino groups of proteins and aminophospholipids as well as by negative charge carriers such as phosphatidylserine of phospholipids, sialic acid of glycophorine and free carboxyl groups of polypeptide chains (Benga & Holmes 1984). During pathological processes different changes can be observed e.g. the development of tumours is accompanied by an increase in the membrane potential or the necrosis of the cell is accompanied by a decrease in the surface charge (Dolowy 1984). The surface charge density can also be influenced by active exogenic compounds such as methanol or its oxidation products, especially free radicals.

Therefore, much interest has been focused on antioxidant therapies. One of the compounds which possesses antioxidative and cytoprotective properties is *N*-acetylcysteine. It also forms disulphide bridges with protein sulphydryl groups and protects membrane proteins against oxidative and destructive effects (Sochman 1994).

The aim of this study was to investigate the effects of *N*-acetylcysteine on the disturbances in the structure of the liver cell membrane, and the leak of cytosolic enzymes (alanine aminotransferase and aspartate aminotransferase) into the blood serum during methanol intoxication in rats.

Materials and Methods

Chemicals

Methanol and *N*-acetylcysteine were purchased from Sigma (USA). Methanol was prepared as a 50% solution in isotonic saline and rats were administered one 3·0 g kg^{-1} dose intragastrically. *N*-Acetylcysteine was dissolved in saline and administered intraperitoneally as a 150 mg kg^{-1} dose.

Animals

Male Wistar rats (approximately 230 g) were housed in groups under a normal light–dark cycle. The rats had free access to a granular standard diet and water. All procedures were in accordance with the guide for care and use of laboratory animals. The local Animal Care Committee approved the protocol.

The rats were divided into four main groups. The control group received 1·0 mL saline intragastrically (n = 42). A second group received 150 mg kg^{-1} *N*-acetylcysteine intraperitoneally (n = 42). After 12 and 24 h the rats were administered another 150 mg kg^{-1} dose. The methanol group received 3·0 g kg^{-1} methanol as a 50% solution in isotonic saline intragastrically (n = 42). The final group was treated with 150 mg kg^{-1} *N*-acetylcysteine (i.p.) with subsequent administration of 3·0 g kg^{-1} methanol as for the methanol group. After 12 and 24 h intoxication a further dose of *N*-acetylcysteine (150 mg kg^{-1}) was administered.

Blood and liver preparation

The rats were killed with ether anaesthesia 6, 12, 24 h, 2, 5, or 7 days after the beginning of the experiment (six animals in each group). The blood was taken by puncture of the heart and the liver was removed quickly and placed in iced 0·15 M NaCl solution.

The livers were perfused with the 0·15 M NaCl solution to remove blood cells, blotted on filter paper, quickly weighed and then homogenized in 9 mL ice-cold 0·25 M sucrose and 0·15 M NaCl, containing 6 μL 250 mM BHT (butylated hydroxy toluenel) in ethanol to stop peroxide formation during the assay. The homogenization procedure was performed as quickly as possible under fully standardized conditions; 10% homogenates were centrifuged at 10 000 *g* for 15 min at 4°C (McCord & Fridovich 1972), and the supernatant was kept on ice until assayed.

Biochemical analysis

The extent of lipid peroxidation in livers was assayed with thiobarbituric acid (TBA). Chromogenous condensation product of thiobarbituric acid with malondialdehyde (thiobarbituric acid-reactive substances; TBA-rs) was extracted from the aqueous phase into butanol and then absorption at 532 nm was monitored (Buege & Aust 1978). The concentration of malondialdehyde was expressed in nmol TBA-rs (mL liver homogenate)$^{-1}$.

The cytotoxicity of methanol for liver cells was evaluated by measuring serum activities of alanine aminotransferase and aspartate aminotransferase using the Diagnostic Cormway test (Bergmayer et al 1986a, 1986b).

Electrochemical analysis

The surface charge density of the hepatocyte membrane was measured by the electrophoresis method (Krysinski & Tien 1986; Thonart & Paquot 1987). Liver cells were suspended in phosphate-buffered saline (PBS) in a measuring container. These studies were performed according to a method described by Minc & Krysinski (1978). The velocity of hepatocyte membrane movement was measured by electric field intensity from 0·4 to 0·2 V m^{-1}. Electrophoretic mobility was calculated from the formula u = v/E, where v is the speed of the cell movement, and E is the potential gradient.

The electrophoretic mobility depends on the charge density, shape and size of the object. For a spherical cell of the radius r, the mobility is related to the surface charge density by the equation: $\delta = 3\eta u\ (2r)^{-1}$, where η is the viscosity of solution, u is the electrophoretic mobility, and r is the radius of the cell (Skrzydlewska et al 1997).

Ultrastructural analysis

Liver sections (1 mm^3, five from each animal) were collected for ultrastructural examination. They

were immediately fixed in 3·6% glutaraldehyde, then in 2% osmium tetroxide, dehydrated in an alcoholic series and propylene oxide, and then finally embedded in Epon 812. Ultrathin sections were contrasted with lead citrate and uranyl acetate and evaluated under a transmission electron microscope OPTON 900 PC.

Statistical analysis

The data obtained were expressed as mean ± s.d. Analysis was by two-way standard statistical analyses, with Tukey's test for multiple comparisons to determine significance between different groups. $P < 0{\cdot}05$ was considered significant.

Results

Lipid peroxidation in liver of rats administered methanol or methanol with N-*acetylcysteine*

It has been shown that intragastric treatment of rats with methanol causes an increase in the amount of lipid peroxidation products, measured as thiobarbituric reactive substances (TBA-rs), during the seven days after intoxication. This enhancement was significant for five days after intoxication, with the highest increase of 30% 24 h after methanol administration (Table 1). Intraperitoneal administration of *N*-acetylcysteine (150 mg kg^{-1}; the first administration before saline, and then after 12 and 24 h) did not influence the lipid peroxidation products. The treatment of intoxicated rats with *N*-acetylcysteine significantly prevented changes in the quantity of TBA-rs caused by methanol (increase of approximately 3% only).

Surface charge density of liver cells from rats administered methanol or methanol with N-*acetylcysteine*

The change in the surface charge density of liver cells from methanol-treated rats was similar to that for the lipid peroxidation products (Table 1). Methanol administration increased the surface charge density and the highest value was reached 24 h after administration (approximately 60% increase compared with control). *N*-Acetylcysteine ingestion did not significantly change the surface charge density. In rats administered methanol and *N*-acetylcysteine, the increase in surface charge density was significantly smaller compared with the methanol group; the highest increase was 30%.

Ultrastructural evaluation of liver from rats administered methanol or methanol with N-*acetylcysteine*

Microscopic-electronic examinations have proved that changes in the structure of cell organelles (focal vacuolization of intraplasmic reticulum, blurred structure of mitochondrial crests) and in the membranes restricting these organelles appeared 24 and 48 h after intoxication (Figure 1A). Particularly clear changes were visible in a cell membrane on a surface directed towards the Disse space, where smoothing of hepatocyte surface was observed, occasionally accompanied by fragmental impairment of the cell membrane. Intensification of degeneration change in liver cells was diminished after administration of *N*-acetylcysteine to methanol intoxicated rats (Figure 1B). Significant improvement was observed after 24 and 48 h following methanol intoxication. Interruption of cell membrane in the perivascular space and leakage of

Table 1. Effect of *N*-acetylcysteine on the lipid peroxidation products measured as TBA-rs (nmol g^{-1}) and on the surface charge density (10^{-8} C m^{-2}) in the liver of rats intoxicated with methanol.

Treatment group	Intoxication time						
	Control	6 h	12 h	24 h	2 days	5 days	7 days
	TBA-rs (nmol g^{-1})						
Methanol	85·2 ± 6·2	92·6 ± 7·9*	102·0 ± 8·9*	109·0 ± 7·1*	101·8 ± 7·8*	93·5 ± 8·2*	86·4 ± 7·1
N-Acetylcysteine	85·2 ± 6·2	85·0 ± 7·2	84·7 ± 7·4	83·1 ± 7·2	81·4 ± 6·9	83·5 ± 7·2	84·5 ± 7·1
Methanol + *N*-acetylcysteine	85·2 ± 6·2	85·5 ± 7·5	87·7 ± 7·4†	88·0 ± 7·7†	86·6 ± 7·5†	85·5 ± 7·2	85·3 ± 7·0
	Surface charge density (10^{-8} C m^{-2})						
Methanol	5·93 ± 0·37	7·76 ± 0·33*	8·37 ± 0·40*	9·74 ± 0·59*	9·11 ± 0·21*	7·08 ± 0·22*	6·49 ± 0·55
N-Acetylcysteine	5·93 ± 0·37	6·28 ± 0·29	6·84 ± 0·36	6·59 ± 0·33	6·23 ± 0·10	5·77 ± 0·21	5·73 ± 0·25
Methanol + *N*-acetylcysteine	5·93 ± 0·37	6·53 ± 0·25†	6·79 ± 0·54*†	7·61 ± 0·41*†	7·08 ± 0·29*†	6·01 ± 0·45†	5·72 ± 0·10†

Methanol (3·0 g kg^{-1}) was administered intragastrically to rats. The rats were then killed 6, 12, 24 h, 2, 5, or 7 days after intoxication. Control rats received 0·15 M NaCl. *N*-Acetylcysteine (150 mg kg^{-1}) was given intraperitoneally before and at 12 and 24 h after methanol or NaCl administration. Values are mean ± s.d.; n = 6; *$P < 0{\cdot}05$ compared with control; †$P < 0{\cdot}05$ compared with methanol group.

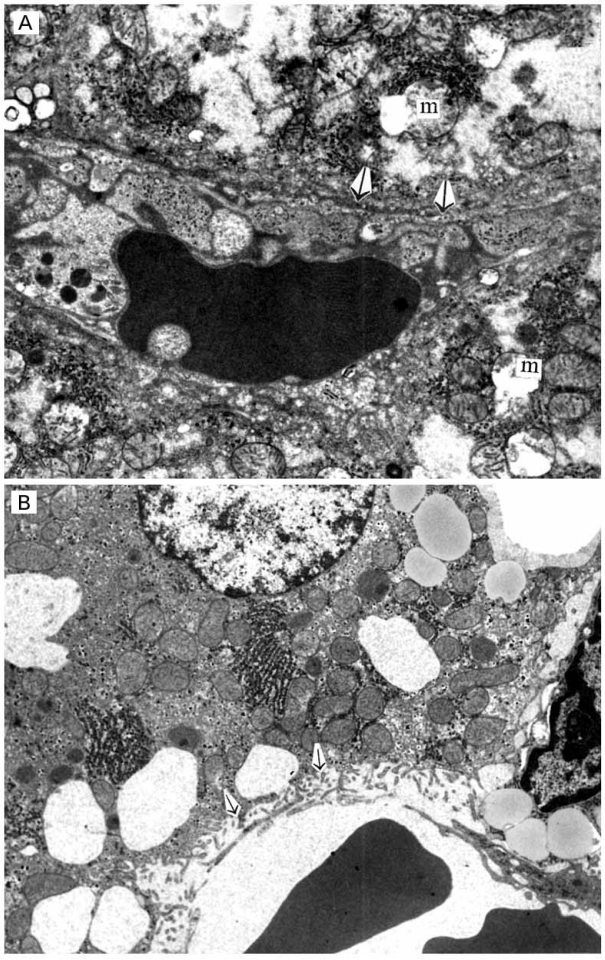

Figure 1. A. Rat liver 24 h following methanol intoxication (3·0 g methanol kg^{-1}, i.g.). Microscopic-electronic picture of the vascular pole and the vascular sinus. Smoothing of cell membrane (arrows). Mitochondria (m) with damaged surrounding membrane and blurred internal structure. Vacuolization of intraplasmic reticulum. TEM ×4400. B. Ultrastructural picture of a fragment of the vascular sinus and the vascular pole of parenchymatous cell of the rat liver 24 h following methanol (3·0 g methanol kg^{-1}, i.g.) and *N*-acetylcysteine (i.p., before and 12 and 24 h after methanol treatment) administration. TEM ×3000.

organelle to the extracellular space were not observed in the animals from these groups.

Aminotransferase activity in the serum of rats administered methanol or methanol with N-*acetylcysteine*

The activity of alanine and aspartate aminotransferases in the serum was significantly increased from 12 h to 5 days after methanol intoxication. The highest increase was 2 days after intoxication by approximately 70% for aspartate aminotransferase and 50% for alanine aminotransferase (Table 2). *N*-Acetylcysteine alone did not influence enzyme activity.

N-Acetylcysteine treatment of intoxicated rats resulted in a smaller increase in the activity of the enzymes compared with the methanol-only-treated rats, by approximately 9 and 8% for aspartate and alanine aminotransferases, respectively.

Discussion

Methanol influences the protein–lipid structure of the hepatocyte membrane (Klemm & Yurttas 1992). In physiological conditions the protein–lipid structure is retained by water molecules. Methanol takes over the function of water but it enables binding of membrane proteins and lipids, and disturbs the membrane structure (Klemm & Yurttas 1992).

Formaldehyde does not occur in the free state in biological systems (McMartin et al 1979). It is a very reactive compound, reacting easily with most protein amino acid residues (Tome et al 1985; Skrzydlewska 1994). Formed hydroxymethyl derivatives may react further with nucleophilic groups and form methylene bridges (Tome et al 1985). As a result, intra- and intermolecular bonds are formed (Tome et al 1979). These bonds cause changes in the structural and physicochemical properties of the proteins as well as their function (Tome et al 1979).

Free radical generation and a decrease in antioxidant potential of the liver observed in methanol intoxication may be the reason for the membrane peroxidation (Skrzydlewska & Farbiszewski 1997a). The reactions of free radicals with lipids result in the formation of small molecular aldehydes measured as thiobarbituric reactive substances, observed in this study. The maximum concentration of lipid peroxidation products corresponds with maximum surface charge density. The reactive oxygen species and the small molecular aldehydes can react with membrane proteins and modify their structure (Esterbauer et al 1991). As a result, the surface membrane hepatocyte positive charge of the protein is decreased and a negative charge occurs.

Changes in the membrane structure and charge could also be caused by impairment in the structure of the hepatocyte membrane skeleton. It seems that the alterations in the distribution of phosphatidylserine, which is the component of the skeleton, could cause an increase in negative charge density (Chiu et al 1989). It is possible that a component of the membrane skeleton-spectrin tetramers can be subject to deformation and in this way it can affect the interaction with membrane phospholipids (Chiu et al 1989). The ultrastructural picture of the liver cells confirmed the biochemical and electrochemical results.

Ultrastructural examination indicated that methanol intoxication caused damage to the cell

Table 2. Effect of *N*-acetylcysteine on aspartate aminotransferase and alanine aminotransferase activity in serum of rats intoxicated with methanol.

Treatment group	Intoxication time						
	Control	6 h	12 h	24 h	2 days	5 days	7 days
	Aspartate aminotransferase (IUL^{-1})						
Methanol	155 ± 10	162 ± 12	189 ± 14*	214 ± 14*	214 ± 13*	209 ± 13*	157 ± 12*
N-Acetylcysteine	155 ± 10	160 ± 11	156 ± 12	158 ± 13	152 ± 13	148 ± 12	159 ± 12
Methanol + *N*-acetylcysteine	155 ± 10	162 ± 12	164 ± 12†	164 ± 13†	168 ± 13*†	162 ± 13†	153 ± 13†
	Alanine aminotransferase (IUL^{-1})						
Methanol	32·3 ± 2·0	33·7 ± 2·9	41·2 ± 3·2*	49·4 ± 3·3*	54·3 ± 3·1*	47·3 ± 3·0*	37·8 ± 2·8*
N-Acetylcysteine	32·3 ± 2·0	32·2 ± 2·6	31·3 ± 2·5	30·0 ± 2·5	35·1 ± 2·8*‡†	27·2 ± 2·2*	30·4 ± 2·4
Methanol + *N*-acetylcysteine	32·3 ± 2·0	32·0 ± 2·5	32·7 ± 2·6†	33·8 ± 2·7‡†	28·9 ± 2·5*	34·7 ± 2·7‡†	32·3 ± 2·3

Methanol ($3{\cdot}0\ g\ kg^{-1}$) was administered intragastrically to rats. The rats were then killed 6, 12, 24 h, 2, 5, or 7 days after intoxication. Control rats received 0·15 M NaCl. *N*-Acetylcysteine ($150\ mg\ kg^{-1}$) was administered intraperitoneally before and at 12 and 24 h after methanol or NaCl. Values are mean ± s.d.; n = 6. $^*P < 0{\cdot}05$ compared with control, $‡P < 0{\cdot}05$ compared with *N*-acetylcysteine group, $†P < 0{\cdot}05$ compared with methanol group.

membrane of the hepatocyte, mostly near the vascular pole. In the initial period, this damage was observed as disturbances in the shape and microvillus distribution. The next stage was the insertion of cytoplasm into the lumen of sinuses until segmental damage of the cell membrane occurred and cell contents poured out into the lumen of sinuses. These changes are not characteristic and they are accompanied by cell damage agents such as hypoxia and toxic damage (David 1978). The most intensive ultrastructural changes were observed 24 h and 2 days following methanol ingestion. The time of these changes was consistent with the time of an increase in liver lipid peroxidation products. This was the reason for the increase in cell membrane permeability and leakage of enzymes (alanine and asparagine aminotransferases) from liver cells into the intercellular space and into the blood.

Skrzydlewska & Farbiszewski (1997b) showed that methanol intoxication also led to a decrease in glutathione. The relevant decrease in liver glutathione is particularly harmful because it is a basic cytosolic antioxidant and a cofactor of formaldehyde dehydrogenase, and as a consequence the toxic action of formaldehyde is enhanced. Under such conditions interest has been focused on compounds that act as antioxidants and are capable of stimulating glutathione synthesis. One of those compounds which possesses cytoprotective and antioxidative properties is *N*-acetylcysteine (Prescott et al 1979; Harrison et al 1990). *N*-Acetylcysteine is a sulphydryl group donor serving as a precursor of glutathione synthesis and inhibiting the formation of extracellular reactive oxygen intermediates. Moreover it may directly react with electrophilic compounds such as formaldehyde and free radicals (Lauteburg et al 1983; Nakano et al 1995). Although glutathione depletion may be insufficient to cause lipid peroxidation, it is agreed that it may favour the peroxidation produced by other factors. Since *N*-acetylcysteine metabolism is related to reduced glutathione, at least part of the beneficial effect of *N*-acetylcysteine may be ascribed to the inhibition of lipoperoxidative processes. Restoration of membrane properties would further contribute to the protective effect of *N*-acetylcysteine. Pascale et al (1989) have shown that *N*-acetylcysteine prevents the inhibition of rat liver plasma membrane Na/K ATPase by ethanol, a fact that has been related to the maintenance of the glutathione pool.

Our results suggest that *N*-acetylcysteine may be useful in protection against hepatocyte damage due to the generation of free radicals during methanol metabolism and consequently to subsequent peroxidative disintegration of cell membranes. These therapeutic effects may be linked to the antioxidant effect and the free radical and formaldehyde scavenging action of *N*-acetylcysteine. This suggests that *N*-acetylcysteine may be considered as an adjunctive compound during therapy for methanol intoxication. It is especially important because ethanol, used as an antidote in methanol intoxication, also generates free radicals and disturbs membrane integration (Reinke et al 1997).

Acknowledgement

This work was supported by a grant from Polish Committee of Scientific Research No 4P05B04011.

References

Benga, G., Holmes, R. P. (1984) Interactions between components in biological membranes and their implications for membrane function. Prog. Biophys. Molec. Biol. 43: 195–257

Benga, G., Holmes, R. P. (1984) Interactions between components in biological membranes and their implications for membrane function. Prog. Biophys. Molec. Biol. 43: 195–257

Bergmayer, H. U., Horder, M., Rej, R. (1986a) International Federation of Clinical Chemistry (IFCC) Scientific Committee. Analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). J. Clin. Chem. Clin. Biochem. 24: 481–495

Bergmayer, H. U., Horder, M., Rej, R. (1986b) International Federation of Clinical Chemistry (IFCC) Scientific Committee. Analytical section: Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 2. IFCC method for aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) J. Clin. Chem. Clin. Biochem. 24: 497–510

Buege, J. A., Aust, S. D. (1978) Microsomal lipid peroxidation. Methods Enzymol. 52: 302–310

Chiu, D., Kuypers, F., Lubin, B. (1989) Lipid peroxidation in human red cell. Seminars Hematol. 26: 257–276

David, H. (1978) Cellular pathobiology. In: Johannesses, J. V. (ed) Electron Microscopy in Human Medicine, Vol.2. McGraw-Hill Interm. Book Company, pp 35–39

Dolowy, K. (1984) Bioelectrochemistry of cell surfaces. Prog. Surf. Sci. 15: 245–368

Esterbauer, H., Schaur, R. J., Zoller, J. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. Free Radic. Biol. Med. 11: 81–128

Harrison, P. M., Keas, R., Bray, G. P., Alexander, G. J., Williams, R. (1990) Improved outcome of paracetamol-induced fulminant hepatic failure by late administration of acetylcysteine. Lancet 335: 1572–1573

Kato, S., Kawase, T., Alderman, J., Inatori, N., Lieber, C. S. (1990) Role of xanthine oxidase in ethanol-induced lipid peroxidation. Gastroenterology 98: 203–210

Klemm, W. R., Yurttas, L. (1992) The dehydration theory of alcohol intoxication. In: Watson, R. R. (ed) Drug and Alcohol Abuse Reviews, Vol. 3. The Human Press Inc., pp 169–185

Krysinski, P., Tien, H. T. (1986) Membrane electrochemistry. Prog. Surf. Sci. 23: 317–412

Lauteburg, B. H., Corcoran, G. B., Mitchell, J. R. (1983) Mechanism of action of N-acetylcysteine in the protection against the hepatotoxicity of acetaminophen in rats in vivo. J. Clin. Invest. 71: 980–991

Liesivuori, J., Savolainen, H. (1991) Methanol and formic acid toxicity: biochemical mechanisms. Pharmacol. Toxicol. 69: 157–163

McCord, J. M., Fridovich, I. (1972) The generation of superoxide radicals during the autooxidation of hemoglobin. J. Biol. Chem. 247: 6960–6962

McMartin, K. E., Martin-Amat, G., Noker, P. E., Lack, T. R. (1979) Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem. Pharmacol. 28: 645–649

Minc, S., Krysinski, P. (1978) Electrophoretic studies on adsorption of tetramethylammonium cation (TMA^+) on human erythrocytes in vitro. Bioelectrochem. Bioenerg. 5: 247–251

Nakano, H., Boudjema, K., Alexandre, E., Imbs, P., Chenard, M. P., Wolf, P. (1995) Protective effects of N-acetylcysteine on hypothermic ischemia-reperfusion injury of rat liver. Hepatology 22: 539–545

Pascale, R., Daino, L., Garcca, R., Frasetto, S., Ruggiu, M. E., Vannini, M. G. (1989) Inhibition by ethanol of rat liver plasma membrane Na^+K^+ATPase: protective effect of S-adenosylmethionin, L-methionine and N-acetylcystine. Toxicol. Appl. Pharmacol. 97: 216–229

Prescott, L. F., Illingworth, R. N., Critchley, J. A., Stewart, M. J., Adams, R. D., Proudfoot, A. T. (1979) Intravenous N-acetylcysteine: the treatment of choice for paracetamol poisoning. Br. Med. J. 2: 1097–1100

Reinke, A., Moore, D. R., McCay, P. B. (1997) Free radical formation in livers of rats treated acutely and chronically with alcohol. Alcoholism: Clin. Exp. Res. 21: 642–646

Skrzydlewska, E. (1994) Interaction of formaldehyde with amino acids, peptides and proteins. Pol. J. Environ. Stud. 3: 13–20

Skrzydlewska, E., Farbiszewski, R. (1997a) Antioxidant status of liver, erythrocytes and blood serum of rats in acute methanol intoxication. Alcohol 14: 431–437

Skrzydlewska, E., Farbiszewski, R. (1997b) Glutathione consumption and glutathione related enzymes inactivation in the liver, erythrocytes and serum of rats after methanol intoxication. Arch. Toxicol. 71: 741–745

Skrzydlewska, E., Dobrzynska, I., Figaszewski, Z. (1997) Oxidative processes and changes of surface charge density of erythrocytes in rat during methanol intoxication. Cell. Mol. Biol. Lett. 2: 345–353

Sochman, J. (1994) Regional ischemic and reperfusion injury, free oxygen radicals and the possible role of N-acetylcysteine in acute myocardial infarction. Core Vasa 36: 269–279

Thonart, Ph., Paquot, M. (1987) Zeta potential measurements for biotechnology applications. In Wise, D. L. (ed), Bioenvironmental Systems, Vol. 4. CRC Press, Inc., Boca Raton, Florida, pp 75–98

Tome, D., Bertrand, D., Viroben, G., Delort-Laval, J. (1979) Tanage par le formd de diments proteiqes du ruminant. I. Etude preliminarire du mode d'action du formol sur un substrat proteiqe modele. Ann. Technol. Agric. 29: 299–318

Tome, D., Naulet, N., Kozlowski, A. (1985) C^{13}-n.m.r. characterization of formaldehyde bonds in model mixtures and proteins containing lysine. Int. J. Peptide Protein Res. 25: 258–266