The effect of cysteine oxidation on isolated hepatocytes

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1. Isolated hepatocytes incubated with 4 mM-cysteine lose reduced glutathione, adenine nucleotides and intracellular enzymes, thus showing extensive membrane damage. 2. The toxic effects of cysteine are enhanced by NH_4Cl . 3. Lactate, ethanol and unsaturated fatty acids afford significant protection against cysteine-induced cytotoxicity. 4. Addition of catalase to the incubation medium also protected against cysteine toxicity, indicating that H_2O_2 formed during the oxidation of cysteine is involved in the toxic effects observed. 5. Under anaerobic conditions cysteine did not cause leakage of lactate dehydrogenase from cells, confirming that rapid autoxidation is an essential condition for development of the toxic effects of cysteine.

This work was started as a result of a previous observation that cysteine (above 0.2 mM) causes a depletion of glutathione in isolated hepatocytes (Viña *et al.*, 1978). Since GSH plays an important role in maintaining normal cell metabolism (see Högberg *et al.*, 1978) we studied the effect of cysteine on some of the properties of isolated hepatocytes, namely adenine nucleotide content and leakage of lactate dehydrogenase to the suspension medium. Factors which modify the effect of cysteine are presented. We propose that the cytotoxicity of cysteine may be due to the generation of H_2O_2 , $\text{OH}^\bullet$ and $\text{O}_2^{\bullet-}$ during the rapid oxidation of cysteine to the corresponding disulphide. Since cysteine or its derivatives, i.e. *N*-acetylcysteine (Piperno & Berssenbruegge, 1976; Prescott *et al.*, 1977) or cysteamine are used in clinical practice the facts reported here may be of practical importance.

Materials and methods

Animals

Female rats of the Wistar strain were fed on a standard Oxoid laboratory diet (Oxoid, London S.E.1, U.K.). Fed rats had access to food *ad libitum*. Starved rats were without food for 48 h but had free access to water. Isolated hepatocytes were prepared according to Berry & Friend (1969) with the modifications (Method 1) described in Viña *et al.* (1978).

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Abbreviation used: GSH, reduced glutathione.

Reagents

Cysteine hydrochloride was obtained from BDH. Solutions were prepared immediately before use by dissolving the hydrochloride in ice-cold water and neutralizing with the calculated amount of 1 M-NaOH. Since cysteine is oxidized at great speed (see Fig. 1) it is important to add the amino acid to the incubation medium immediately before the cells. Enzymes and coenzymes were obtained from Boehringer Corp. (London). Methylglyoxal was obtained from Sigma. All other reagents were analytical grade.

Analytical methods

GSH was measured by the glyoxalase method of Racker (1951). The conditions of the assay were those described by Bernt & Bergmeyer (1974) with the modification of Viña *et al.* (1978). ATP was measured according to Lamprecht & Trautschold (1963) and ADP and AMP by the method of Adam (1963).

Lactate dehydrogenase activity was measured at pH 7.0 in 0.1 M-phosphate buffer at 25°C as described by Bergmeyer *et al.* (1974).

Results

Effect of cysteine and NH_4Cl on [ATP] and lactate dehydrogenase leakage in isolated hepatocytes

Examination of the metabolic consequences of GSH loss in hepatocytes incubated in the presence of 4 mM-cysteine led to the observation that urea synthesis from NH_4Cl was markedly depressed. This

Table 1. *Effect of cysteine on ATP content and loss of lactate dehydrogenase from isolated hepatocytes from fed or 48 h starved rats*

Each flask contained about 80mg fresh wt. of hepatocytes in 4ml of suspension containing 2.5% (w/v) albumin. Incubation was for 30min at 38°C. ATP is expressed as $\mu\text{mol/g}$. Lactate dehydrogenase is expressed as i.u./ml of cell-free supernatant. The concentrations of substrates shown were 4mM-cysteine and 10mM- NH_4Cl . Values are means $\pm$ S.E.M. for the number of experiments shown in parentheses. The total lactate dehydrogenase released to the medium when digitonin was added to the suspension of hepatocytes was about 15i.u./ml. The effect of NH_4Cl gave *P* values: * < 0.0005; ** < 0.005.

Additions	Fed rats ATP	48 h starved rats	
		ATP	Lactate dehydrogenase
None	2.58 $\pm$ 0.10 (3)	2.24 $\pm$ 0.24 (4)	1.83 $\pm$ 0.44 (6)
Cysteine	2.41 $\pm$ 0.05 (3)	1.74 $\pm$ 0.17 (4)	3.18 $\pm$ 0.17 (4)
NH_4Cl	2.10; 2.89	2.07 $\pm$ 0.20 (6)	—
NH_4Cl + cysteine	1.93 $\pm$ 0.11 (3)	0.43 $\pm$ 0.10 (5)*	11.69 $\pm$ 2.48 (5)**

was found to be due to enhancement of the toxic effects of cysteine by NH_4^+ . $(\text{NH}_4)_2\text{SO}_4$ could substitute for NH_4Cl , indicating that the cation was responsible for the observed effect. Table 1 shows a fall of 22% in [ATP] when hepatocytes were incubated with 4mM-cysteine. Addition of 10mM- NH_4Cl increased the loss of ATP to 80%. This finding was not modified by the addition of ornithine (results not shown). Loss of ATP was also accompanied by leakage of lactate dehydrogenase into the suspending medium. Comparison of the leakage of lactate dehydrogenase with the total released with digitonin (Zuurendonk & Tager, 1974), showed that in the presence of cysteine and NH_4Cl the leakage of lactate dehydrogenase is 78% of the total. We have at present no explanation for the mechanism of the enhancement by NH_4^+ of the toxicity of cysteine. Hepatocytes from well-fed rats are not as susceptible to damage. Indeed ATP concentrations in isolated hepatocytes from well-fed rats were significantly lower than in controls only when they were incubated in the presence of cysteine and NH_4Cl . However, cysteine (4mM) alone was sufficient to promote significant falls in ATP concentration in hepatocytes from starved rats (Table 1). The addition of NH_4Cl as sole substrate resulted in only minor changes in the ATP content of both fed and starved hepatocytes.

Effect of glucogenic substrates on the cysteine-mediated loss of adenine nucleotides in starved hepatocytes

Unlike urea synthesis, gluconeogenesis with lactate as the main substrate was unaffected by addition of cysteine. The experiments in Table 2 show that lactate and oleate can protect hepatocytes from starved rats against the toxic effects of cysteine plus NH_4Cl . It also demonstrates that the loss of ATP in the presence of cysteine and NH_4Cl is accompanied by a significant loss of total adenine

nucleotides. Stimulators of the rate of gluconeogenesis, i.e. lysine and cyclic AMP, were without effect. Ethanol (10mM), which inhibits gluconeogenesis from lactate (Krebs *et al.*, 1969), enhanced the protection of the adenine nucleotides. Thus the effect of cysteine was independent on the rate of gluconeogenesis.

Factors affecting the toxicity of cysteine in isolated hepatocytes from starved rats

It was clear that certain substrates were able to protect isolated hepatocytes, to some extent, from the toxic effect of added cysteine and NH_4Cl ; among these substances ethanol was the most effective (Table 3). The effect of ethanol was independent of its metabolism. Pyrazole, a potent inhibitor of alcohol dehydrogenase (Theorell & Yonetani, 1963; Theorell, 1967), was without effect on the protective action of ethanol. In addition to oleate (see Table 2), other unsaturated fatty acids lowered the loss of adenine nucleotides (Table 3). The effect of fatty acids plus ethanol was additive.

Effect of catalase and superoxide dismutase on cysteine toxicity

When hepatocytes were incubated with cysteine or cysteine and NH_4Cl in the presence of catalase the cytotoxic effects of cysteine, i.e. lactate dehydrogenase leakage or GSH depletion, were greatly diminished (Table 4). Peroxidase was also effective in preventing loss of ATP and leakage of lactate dehydrogenase when added together with aminophenazone as substrate (results not shown). Cu/Zn-superoxide dismutase prepared from erythrocytes was without effect but Mn-superoxide dismutase prepared from *Bacillus steorothermophilus* was effective in preserving ATP and showed partial protection against leakage of lactate dehydrogenase. It is probable that cysteine effectively inhibits the copper-containing superoxide dismutase but is

Table 2. *Effect of lactate and stimulators of gluconeogenesis on depletion by cysteine and NH₄Cl of adenine nucleotides in hepatocytes from 48 h starved rats*

Flasks were incubated in duplicate in each experiment. The mean cell wt. was 77.4 mg. Each flask contained 4 ml of suspension, including 1 ml of undialysed 10% (w/v) bovine serum albumin. Concentrations of the substrates were: cysteine, 4 mM; NH₄Cl, 10 mM; lactate, 10 mM; lysine, 2 mM; dibutyryl cyclic AMP, 0.2 mM; oleate, 1 mM; ethanol, 10 mM. Values are means $\pm$ S.E.M. for three determinations; *. $P < 0.0005$ for the enhancement by NH₄Cl of the toxic effect of cysteine and the protection by lactate, the glucogenic substrates and ethanol. Undialysed albumin is known to contain bound fatty acid and our analysis shows the presence of Cu²⁺ was sufficient to cause the rapid oxidation of cysteine.

Additions	[Adenine nucleotides] (μ mol/g)			
	ATP	ADP	AMP	Total
None	2.04 $\pm$ 0.18	0.99 $\pm$ 0.15	0.21 $\pm$ 0.01	3.32 $\pm$ 0.32
Cysteine	1.47 $\pm$ 0.20	0.62 $\pm$ 0.05	0.35 $\pm$ 0.05	2.44 $\pm$ 0.28
Cysteine + NH ₄ Cl	0.28 $\pm$ 0.05*	0.60 $\pm$ 0.10	0.32 $\pm$ 0.03	1.20 $\pm$ 0.08
Cysteine + NH ₄ Cl + lactate	1.66 $\pm$ 0.08*	0.68 $\pm$ 0.09	0.27 $\pm$ 0.07	2.61 $\pm$ 0.12
Cysteine + NH ₄ Cl + lysine	0.28 $\pm$ 0.03	0.53 $\pm$ 0.12	0.29 $\pm$ 0.03	1.10 $\pm$ 0.17
Cysteine + NH ₄ Cl + dibutyryl cyclic AMP	0.42 $\pm$ 0.13	0.61 $\pm$ 0.11	0.46 $\pm$ 0.02	1.35 $\pm$ 0.23
Cysteine + NH ₄ Cl + oleate	0.67 $\pm$ 0.20	0.45 $\pm$ 0.07	0.38 $\pm$ 0.05	1.51 $\pm$ 0.27
Cysteine + NH ₄ Cl + lactate + lysine + dibutyryl cyclic AMP + oleate	2.43 $\pm$ 0.04*	0.65 $\pm$ 0.10	0.38 $\pm$ 0.05	3.37 $\pm$ 0.10
Cysteine + NH ₄ Cl + lactate + lysine + dibutyryl cyclic AMP + oleate + ethanol	2.74 $\pm$ 0.10*	0.53 $\pm$ 0.07	0.57 $\pm$ 0.03	3.84 $\pm$ 0.16
Cysteine + lactate + lysine + dibutyryl cyclic AMP + oleate	3.10 $\pm$ 0.10*	0.70 $\pm$ 0.06	0.22 $\pm$ 0.02	4.02 $\pm$ 0.18

Table 3. *Effect of unsaturated fatty acids and ethanol on depletion of adenine nucleotides by cysteine and NH₄Cl in hepatocytes from 48 h starved rats*

Each flask contained 4 ml of suspension of hepatocytes, containing 2.5% (w/v) bovine albumin fraction V. The concentrations of added substrates were: cysteine, 4 mM; NH₄Cl, 10 mM; ethanol, 10 mM; fatty acids, 1 mM. Incubation was for 30 min at 38°C. The values are means $\pm$ S.E.M. for three determinations; * $P < 0.005$, ** $P < 0.0025$, for the enhancement by NH₄Cl of the toxic effect of cysteine or the protection by ethanol or ethanol plus fatty acids.

Additions	[Adenine nucleotides] (μ mol/g)			
	ATP	ADP	AMP	Total
None	2.35 $\pm$ 0.06	0.80 $\pm$ 0.21	0.22 $\pm$ 0.02	3.37 $\pm$ 0.25
Cysteine	0.44 $\pm$ 0.16	0.61 $\pm$ 0.18	0.42 $\pm$ 0.11	1.47 $\pm$ 0.08
Cysteine + NH ₄ Cl	0.14 $\pm$ 0.08*	0.53 $\pm$ 0.15	0.38 $\pm$ 0.12	1.05 $\pm$ 0.13
Cysteine + NH ₄ Cl + ethanol	1.42 $\pm$ 0.18**	0.73 $\pm$ 0.19	0.41 $\pm$ 0.04	2.56 $\pm$ 0.07*
Cysteine + NH ₄ Cl + linoleate	1.08 $\pm$ 0.03	0.65 $\pm$ 0.17	0.45 $\pm$ 0.07	2.37 $\pm$ 0.24
Cysteine + NH ₄ Cl + linolenate	0.80 $\pm$ 0.10	0.63 $\pm$ 0.21	0.49 $\pm$ 0.09	1.92 $\pm$ 0.18
Cysteine + NH ₄ Cl + arachidonate	0.75 $\pm$ 0.04	0.58 $\pm$ 0.21	0.51 $\pm$ 0.11	1.88 $\pm$ 0.27
Cysteine + NH ₄ Cl + ethanol + linoleate	1.52 $\pm$ 0.09*	0.60 $\pm$ 0.20	0.45 $\pm$ 0.03	2.67 $\pm$ 0.17*
Cysteine + NH ₄ Cl + ethanol + linolenate	1.90 $\pm$ 0.17*	0.64 $\pm$ 0.20	0.58 $\pm$ 0.02	3.12 $\pm$ 0.21*
Cysteine + NH ₄ Cl + ethanol + arachidonate	1.88 $\pm$ 0.18*	0.62 $\pm$ 0.22	0.55 $\pm$ 0.04	3.05 $\pm$ 0.25*

less effective against the manganese-containing enzyme.

Effect of cysteine on [ATP] and lactate dehydrogenase leakage from hepatocytes under anaerobic conditions

The concentration of ATP in hepatocytes incubated under anaerobic conditions was very low, as expected. The presence of cysteine and NH₄Cl caused

a further depletion of ATP. This was unaffected by the addition of catalytic amounts of CuSO₄ or catalase (Table 5). However, cysteine, NH₄Cl and CuSO₄ did not increase significantly the leakage of lactate dehydrogenase from the cell under these conditions. As expected, hepatocytes incubated with 4 mM-cysteine plus 10 mM-NH₄Cl under strict anaerobic conditions showed substantial loss of ATP (Table 5). However there was no significant increase in the leakage of lactate dehydrogenase.

Table 4. *Effect of catalase on cysteine-mediated loss of lactate and GSH depletion in hepatocytes from 48 h starved rats*

Hepatocytes were incubated in 4 ml of suspension containing 2.5% (w/v) bovine albumin fraction V in Krebs–Hensleit (1932) saline for 30 min at 38°C. The concentrations of added substrates were: cysteine, 4 mM; NH_4Cl , 10 mM; catalase, about 1000 i.u. per flask. Lactate dehydrogenase was measured in the cell-free supernatant at pH 7.0. The initial values before incubation were: lactate dehydrogenase, 1.37 i.u./ml; GSH, 3.23 ± 0.32 (4) $\mu\text{mol/g}$ fresh wt. Values are means $\pm$ S.E.M. for the number of experiments shown in parentheses. *P* values for the effect of catalase are: * < 0.0125; *** < 0.005, and for the effect of NH_4Cl ** < 0.01.

Additions	Lactate dehydrogenase in supernatant (i.u./ml)	GSH ($\mu\text{mol/g}$ fresh wt. of cells)
None	1.83 ± 0.44 (6)	1.61 ± 0.22 (7)
Catalase	1.58 ± 0.73 (3)	2.24 ± 0.27 (5)
Cysteine	3.18 ± 1.07 (4)	0.15 ± 0.08 (6)
Cysteine + catalase	1.27 ± 0.14 (4)	0.84 ± 0.25 (6)*
Cysteine + NH_4Cl	11.69 ± 2.48 (5)**	0.17 ± 0.10 (4)
Cysteine + NH_4Cl + catalase	1.28 ± 0.12 (3)***	0.89 ± 0.22 (4)*

Table 5. *Effect of cysteine and NH_4Cl on [ATP] and loss of lactate dehydrogenase under anaerobic conditions by rat hepatocytes*

Each flask contained 4 ml of suspension containing 78.6 mg wet wt. of hepatocytes from 48 h starved rats and 1 ml undialysed albumin (10%, w/v). The concentrations of the substrates shown were: cysteine, 4 mM; NH_4Cl , 10 mM; catalase, about 1000 i.u./flask; CuSO_4 , 20 μM . Flasks were gassed with CO_2/N_2 (1:19) and contained yellow phosphorus in the centre well. Incubation was at 38°C for 10 min before addition of cysteine. Incubation continued for a further 20 min. The cysteine solution was shaken under N_2 and with phosphorus present for 10 min before use; it was added by syringe through Suba Seal caps. A typical experiment is shown.

	[ATP] ($\mu\text{mol/g}$ of cells)	Lactate dehydrogenase (i.u./ml of medium)
Initial zero time value	2.50	0.38
No substrate incubated	0.73	0.60
Cysteine	0.21	0.62
Cysteine + NH_4Cl	0.19	0.56
Cysteine + CuSO_4	0.44	0.60
Cysteine + NH_4Cl + catalase	0.28	0.60

These findings were unaffected by the addition either of catalytic amounts of Cu^{2+} as CuSO_4 or of catalase. A representative experiment is shown. Table 2 shows values for ATP loss which are

Fig. 1. *Effect of catalase on the autooxidation of cysteine (50 μmol) in the presence of 1.75 μM - Cu^{2+} (O) without catalase and (●) with 1000 i.u. of catalase*
The expected oxygen consumption was 12.5 μmol .

comparable with the values in hepatocytes from starved rats in Table 1. Cysteine oxidizes readily in the presence of catalytic amounts of divalent metal ions, e.g. Cu^{2+} (Warburg, 1949; Tsen & Tappel, 1958), with the formation of H_2O_2 . Fig. 1 shows the time course of oxygen consumption and the effect of catalase on this reaction. The final end point is the same both with and without catalase, but in the presence of catalase the initial rate of the reaction is about half the rate without catalase. Incubation of hepatocytes with H_2O_2 at or in excess of concentrations expected from the oxidation of 4 mM-cysteine failed to reproduce the loss of adenine nucleotides or leakage of lactate dehydrogenase.

Discussion

The observation that cysteine in high concentration causes the loss of GSH in isolated hepatocytes (Viña *et al.*, 1978) has been confirmed. Further evidence of cytotoxicity is the finding that cysteine also causes a decrease in the concentration of ATP and leakage of lactate dehydrogenase and that this effect of cysteine can be markedly enhanced by the presence of high concentrations of NH_4^+ (Table 1). No satisfactory explanation for the effect of NH_4^+ can be offered. It is tempting to propose that urea synthesis makes heavy demands on the supply of ATP and that, in a situation where cytotoxicity prevails, ATP replacement fails to keep pace with its loss. However urea formation from NH_4Cl as sole substrate is fairly low. The failure of ornithine, which

stimulates urea synthesis in isolated starved hepatocytes (Krebs *et al.*, 1974), to enhance the effect of NH_4Cl suggests that this does not provide an acceptable explanation. The finding that ATP loss is much greater in hepatocytes from starved rats than in hepatocytes from well-fed rats (Table 1) may in part explain the differences in our findings compared to those of Beatty & Reed (1981), who did not observe a loss of GSH in cells from fed rats incubated under somewhat different conditions.

The loss of ATP is accompanied, in the most toxic situation (i.e. high cysteine plus NH_4Cl), by a loss of total adenine nucleotides (Table 2). Gluconeogenesis, unlike urea synthesis, is much less affected by cysteine and this is shown to result from the capacity of lactate and oleate to afford some protection of the adenine nucleotide pool even in the presence of $10\text{mM-NH}_4\text{Cl}$. Inhibition of gluconeogenesis by the addition of ethanol resulted in normal concentrations of ATP and of total adenine nucleotides. Ethanol alone did not protect fully against the effect of cysteine plus NH_4Cl (see Table 3, line 4), whereas ethanol in the presence of the glucogenic substrates (Table 2, line 9), gave full protection, indicating that the substrates *per se* rather than their metabolism are responsible for these effects.

The outstanding effect of added ethanol led us to examine ethanol as a potential protecting agent against loss of ATP. Table 3 shows that ethanol is able to protect the cells against cysteine plus NH_4Cl . The fact that pyrazole, a potent inhibitor of alcohol dehydrogenase (Theorell & Yonetani, 1963; Theorell, 1967) did not modify the protective effect of ethanol on cysteine cytotoxicity (results not shown) indicates that the protection afforded by ethanol is due to the ethanol and not to its metabolic products. The effect of oleate as a protective agent was also shared by other unsaturated fatty acids. Linoleate, linolenate and arachidonate caused increases of 8-, 6- and 5-fold in the [ATP] compared with cysteine plus NH_4Cl . This is in comparison with the 10-fold effect of ethanol. Ethanol plus a fatty acid was better than either the fatty acid or ethanol alone (Table 3).

It is known that cysteine undergoes metal-catalysed autoxidation (Warburg & Negelein, 1921), and that H_2O_2 is formed. Taking leakage of lactate dehydrogenase as a measure of severe cell damage, the effect of adding catalase to hepatocytes incubated with high concentrations of cysteine and NH_4Cl showed that the enzyme gave complete protection (Table 4). Catalase also gave a significant measure of protection to GSH (Table 4). Cysteine is oxidized readily in the presence of catalytic amounts of divalent metal ions, i.e. Cu^{2+} (Fig. 1). During the autoxidation of cysteine H_2O_2 may be formed according to the following reaction:

However, when hepatocytes were incubated with the concentrations of H_2O_2 expected to arise due to the oxidation of 4mM-cysteine , no changes in ATP concentrations or lactate dehydrogenase leakage appeared. The effectiveness of catalase added to the incubation medium in protecting against cysteine-mediated cytotoxicity while the intracellular peroxide-removing enzymes seemed unable to protect the cells suggested that the peroxide formed in the medium during cysteine oxidation entered into secondary reactions which resulted in cell damage. The effectiveness of ethanol, a potent scavenger of free hydroxyl radicals (Beauchamp & Fridovich, 1970; Fridovich, 1974) points to the formation of this highly reactive chemical species. We have recently shown the formation of free radicals during the autoxidation of cysteine (Saez *et al.*, 1983). Table 5 shows that cell damage, as measured by leakage of lactate dehydrogenase, is entirely dependent on cysteine oxidation. Thus, in the anaerobic situation neither the loss of ATP (due to anoxia) nor the presence of cysteine, NH_4Cl or Cu^{2+} caused any leakage of lactate dehydrogenase.

We conclude that the critical event leading to the cytotoxicity of cysteine is its rapid oxidation resulting first in the formation of H_2O_2 and then of free radicals. Thus in situations where cysteine undergoes rapid oxidation and suitable radical scavengers are absent, hepatocytes from starved rats are sufficiently damaged as to result in substantial loss of cytoplasmic lactate dehydrogenase.

Loss of GSH and cell viability during metabolism of bromobenzene has been reported by Thor *et al.* (1978, 1979). There was no evidence, however, that free radical damage was involved.

Beatty & Reed (1981) did not observe a depletion of GSH or other signs of cell damage in isolated hepatocytes incubated with 3mM-cysteine . This can be explained, at least in part, by the different conditions used. We have shown that several compounds can protect against cysteine-induced toxicity. In their work a complex incubation medium containing 33 ingredients was used. Furthermore, foetal calf serum, which we have found to contain up to 17mM-lactate , was added. Table 2 shows that lactate protects against cysteine toxicity. These conditions may result in a very slow rate of oxidation of cysteine. Indeed Beatty & Reed (1981) reported a rate of oxidation of cysteine of 3nmol/min . However, it can be calculated from Fig. 1 that we obtained a rate of oxidation of cysteine of more than 2000nmol/min . Samples of bovine albumin fraction V contained copper [$17\mu\text{mol/ml}$ of a 10% (w/v) solution] which was decreased to only $10\mu\text{mol}$ after extensive dialysis. Thus in the present experiments, conditions prevailed which allowed for a rate of oxidation of cysteine more than 60 times faster than the rate reported by Beatty & Reed (1981),

which may explain their failure to observe any toxic effect of cysteine. It is known that relatively low doses of cysteine can be toxic to rats (Birnbaum *et al.*, 1957; Olney *et al.*, 1971) and to cultured cells (Nishiuch *et al.*, 1976). We observed that intraperitoneal injections of cysteine or *N*-acetylcysteine cause a depletion of liver GSH in rats (Viña *et al.*, 1980).

The results presented here provide further insight into the mechanism of cysteine-induced cytotoxicity. Since cysteine or its derivatives, i.e. *N*-acetylcysteine, are used as therapeutic agents, the facts reported here may be of both theoretical and practical importance.

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