Biochem. J. (1980) **188**, 549–552
Printed in Great Britain

Effect of Ethanol on Glutathione Concentration in Isolated Hepatocytes

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(*Received 31 December 1979*)

1. Ethanol induces a decrease in GSH (reduced glutathione) concentration in isolated hepatocytes. Maximal effects appear at 20mM-ethanol. The concentration-dependence of this decrease is paralleled by the concentration-dependence of the activity of alcohol dehydrogenase. 2. Pyrazole, a specific inhibitor of alcohol dehydrogenase, prevents the ethanol-induced GSH depletion. 3. Acetaldehyde, above 0.05mM, also promotes a decrease in GSH concentration in hepatocytes. 4. Disulfiram (0.05 mM), an inhibitor of aldehyde dehydrogenase, potentiates the fall in GSH concentration caused by acetaldehyde. 5. The findings support the hypothesis that acetaldehyde is responsible for the depletion of GSH induced by ethanol. 6. Methionine prevents the effect of alcohol or acetaldehyde on GSH concentration in hepatocytes.

Glutathione is present in high concentrations in liver, in both the mitochondrial and cytosolic compartments (Vignais & Vignais, 1973; Jocelyn, 1975; Wahlländer *et al.*, 1979). Its importance in detoxification mechanisms has been reviewed (Sies & Wendel, 1978). Since liver is a major target organ of the toxic effects of ethanol, we decided to study the effect of ethanol on GSH concentration in isolated liver cells. In a previous study (Guerri & Grisolia, 1978) it was shown that hepatic GSH concentration was decreased in chronic-ethanol-treated rats. In the present paper we report that ethanol decreases GSH concentration in isolated hepatocytes. We also show that this effect is due to acetaldehyde and that the effect of both compounds on GSH concentration can be prevented by simultaneous incubation with methionine.

Materials and Methods

Animals

Wistar rats were fed on a standard diet for rats and mice (Sandersa Industrial, Pinto, Madrid, Spain). They had free access to food and water.

Chemicals

Amino acids, ethanol, acetaldehyde and pyrazole (1,2-diazole) were from Merck, Darmstadt, Germany. Glyoxalase I and collagenase (grade II) were from Boehringer, Mannheim, Germany. Methylglyoxal and disulfiram (tetraethylthiuram disulphide) were obtained from Sigma. All other chemicals were of the highest purity available.

Abbreviation used: GSH, reduced glutathione.

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Preparation of liver cells

Hepatocytes were isolated by the method of Berry & Friend (1969) and incubated under the conditions established by Viña *et al.* (1978) to maintain physiological GSH concentration.

Cell viability as judged by Trypan Blue exclusion or by the method of Mapes & Harris (1975) was 80–95%. In all cases the cells were incubated for 60min.

Determination of GSH

GSH was determined by the glyoxalase method of Racker (1951) as described by Bernt & Bergmeyer (1974). All results are expressed as means ± S.D. for the numbers of observations in parentheses.

Results

Effect of ethanol and acetaldehyde on GSH concentration in liver cells

As shown in Table 1, GSH concentration was lower in liver cells incubated with ethanol than in cells incubated without it. Maximal effects were observed at 20mM-ethanol. Higher concentrations of the drug caused smaller effects on GSH concentration. This seems to parallel the effect of ethanol concentration on the activity of alcohol dehydrogenase, which is inhibited at high concentrations of ethanol (Theorell & Bonnichsen, 1951; Dalziel &

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Table 1. *Effect of ethanol on GSH concentration in isolated hepatocytes, and of pyrazole on ethanol-induced decrease in GSH concentration*

For details see the text. Initial GSH concentration was 4.0 ± 0.1 (4) μmol/g wet wt.

Additions (mM)	Concn. of GSH after 60 min incubation (μmol/g wet wt.)	(% of control)
0 (control)	2.5 ± 0.3 (7)	100
Ethanol (10)	2.0 ± 0.1 (3)	80
Ethanol (20)	1.5 ± 0.2 (3)	60
Ethanol (40)	1.9 ± 0.1 (3)	76
Ethanol (20) + pyrazole (0.05)	2.6 ± 0.3 (3)	104
Ethanol (20) + pyrazole (0.1)	2.7 ± 0.3 (3)	108

Table 2. *Effect of acetaldehyde on GSH content in isolated hepatocytes*

For details see the text. Initial GSH concentration was 4.0 ± 0.1 (4) μmol/g wet wt.

Concn. of acetaldehyde (mM)	GSH concn. after 60 min incubation (μmol/g wet wt.)	(% of control)
0	2.5 ± 0.3 (7)	100
0.05	1.8 ± 0.4 (3)	72
0.10	1.6 ± 0.5 (3)	64
1.00	1.4 (2)	57

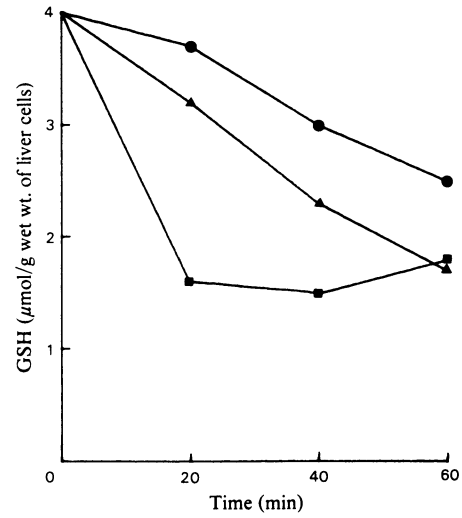

Fig. 1. *Time course of GSH depletion in hepatocytes induced by ethanol or acetaldehyde*

For details see the text. ●, Control; ▲, +ethanol (20 mM); ■, +acetaldehyde (0.1 mM).

Dickinson, 1966). This finding would be consistent with acetaldehyde being the true toxic agent with respect to GSH depletion. In fact GSH prevents inactivation of enzymes by acetaldehyde *in vitro* (Guerri & Grisolia, 1978). Thus the effect of this aldehyde on the GSH content of liver cells was also tested.

It is shown in Table 2 that acetaldehyde was much more effective than ethanol in decreasing the GSH content of hepatocytes, i.e. 0.05 mM-acetaldehyde decreased GSH concentration to about 70% of the control value. In contrast with the concentration effect of ethanol (see Table 1), higher concentrations of acetaldehyde caused larger decreases in GSH concentration (Table 2).

The time course of GSH depletion caused by ethanol or acetaldehyde is shown in Fig. 1.

Removal of ethanol effect by pyrazole

It is well established that pyrazole (1,2-diazole) is a very effective inhibitor of alcohol dehydrogenase (Theorell & Yonetani, 1963). Addition of 0.05 mM-pyrazole completely prevented the fall in GSH concentration in liver cells incubated with ethanol (Table 1). These results show that the ethanol effect is not due to the presence of ethanol itself, but to acetaldehyde, which arises as a product of the interaction of ethanol with alcohol dehydrogenase.

Effect of ethanol on GSH concentration in cells lacking alcohol dehydrogenase

The lack of effect of ethanol on GSH concentration of hepatocytes when ethanol dehydrogenase was inhibited led us to investigate the effect of ethanol on GSH concentration of fresh human erythrocytes, which do not contain alcohol dehydrogenase (Von Wartburg, 1971).

When washed erythrocytes were incubated with ethanol, GSH content was not significantly different

from that of the controls. However, when they were incubated with acetaldehyde (1mM), GSH concentration after 30min of incubation was 59% of the control. This confirms the hypothesis that alcohol dehydrogenase activity is essential for the GSH depletion caused by ethanol.

Effect of disulfiram (Antabuse) on the acetaldehyde induced GSH depletion

In order to clarify whether the acetaldehyde-induced GSH depletion is due to the aldehyde itself or to its metabolic products, we incubated hepatocytes with acetaldehyde in the presence of disulfiram, a specific inhibitor of aldehyde dehydrogenase (Erwin & Deitrich, 1966). As shown in Table 3, 0.05mM-acetaldehyde decreased GSH concentration to 80% of that in the controls. However, when cells were incubated with acetaldehyde (0.05mM) and disulfiram (0.1mM), GSH concentration was 50% of the control value (Table 3). These results confirmed the hypothesis that the acetaldehyde-induced GSH depletion is due to acetaldehyde itself and not to its oxidative products.

Reaction of GSH and acetaldehyde in vitro. When GSH (0.1mM) and acetaldehyde (0.1mM) were incubated at 37°C in Krebs–Henseleit saline (Krebs & Henseleit, 1932) for 20 and 40min, GSH concentration was respectively 61 and 55% of the value obtained when GSH was incubated under the same conditions in the absence of acetaldehyde. However, when hydrazine (0.1mM) was added to a mixture of GSH (0.1mM) and acetaldehyde (0.1mM) that had been incubated for 20min and the incubation was continued for 20min more, GSH concentration returned to values not statistically different from controls. This shows that the spontaneous reaction of GSH and acetaldehyde is reversible.

Reversal of the effects of ethanol and acetaldehyde by methionine

We have previously reported that methionine is essential to maintain GSH concentration in liver cells on incubation (Viña *et al.*, 1978). Thus, to investigate if methionine could prevent the decrease in GSH of cells incubated with ethanol or acetaldehyde, we incubated hepatocytes with these substances in the presence and absence of methionine. Methionine (2mM) completely reversed the GSH depletion caused by ethanol (40mM) or acetaldehyde (0.1mM). This effect may be due to the active synthesis of GSH, since methionine increases the synthesis of cysteine, the limiting factor for the synthesis of GSH (Tateishi *et al.*, 1974), via the cystathionine pathway (Reed & Orrehnius, 1977).

Discussion

The maintenance of physiological concentration of GSH is essential for a large number of cellular functions (Kossower & Kossower, 1979). It was previously shown that the hepatic GSH concentration was decreased after the administration of an acute dose of ethanol to rats (Macdonald *et al.*, 1977). However, the effect of acetaldehyde was not tested. In this study we have shown that incubation with ethanol or acetaldehyde promotes a depletion of GSH in hepatocytes and that maximal effects appear when ethanol concentration is about 20mM. The law in several countries, e.g. United Kingdom and Spain, does not consider drivers unduly incapacitated until their ethanol concentration in blood is 18mM. Krebs *et al.* (1969) also observed in the perfused rat liver that inhibition of gluconeogenesis by ethanol was decreased at high ethanol concentrations.

The removal of the ethanol effect by an inhibitor of alcohol dehydrogenase (pyrazole) and the potentiation of the acetaldehyde effect by an inhibitor of aldehyde dehydrogenase (disulfiram) show that the effect of ethanol is due to its conversion into acetaldehyde and not to the presence of ethanol itself or to the other ethanol-induced metabolic alterations, i.e. changes in the [NAD^+]/[NADH] ratio (Williamson *et al.*, 1967).

There is now experimental evidence that acetaldehyde could be involved in many of the typical manifestations of alcohol abuse (Lindros, 1978). Cederbaum & Rubin (1976) showed that acetal-

Table 3. *Effect of disulfiram (Antabuse) on acetaldehyde-induced GSH depletion in hepatocytes*
For details see the text. Initial GSH concentration was 4.0 ± 0.1 (4) μmol/g wet wt.

Substrates added (mM)	GSH concn. after 60min incubation (μmol/g wet wt.)	(% of control)
None	3.1 ± 0.5 (3)	100
Acetaldehyde (0.05)	2.5 ± 0.2 (3)	80
Acetaldehyde (0.05) + disulfiram (0.05)	2.0 ± 0.1 (3)	66
Acetaldehyde (0.05) + disulfiram (0.1)	1.7 ± 0.3 (3)	54

dehyde inhibits several mitochondrial functions and that this inhibition can be reversed by cysteine and other thiols. However, we did not test cysteine, because this amino acid (above 0.3 mM) promotes a depletion of GSH in hepatocytes (Viña *et al.*, 1978). The possible explanation for this effect has been discussed elsewhere (Krebs *et al.*, 1978).

We have shown that GSH reacts with acetaldehyde in the absence of cells to form an adduct and that removal of acetaldehyde by reaction with hydrazine leads to the return of GSH concentration to control values. Thus, we suggest that when acetaldehyde is formed in the hepatocytes an equilibrium is established between the aldehyde, GSH and the adduct formed by the reaction of both and that GSH concentration may return to physiological values when free acetaldehyde is removed by the action of aldehyde dehydrogenase. The fact that the acetaldehyde-induced GSH depletion is increased when acetaldehyde dehydrogenase is inhibited is in accordance with this suggestion.

Methionine, which contributes to GSH synthesis in liver cells via the cystathionine pathway (Reed & Orrehnius, 1977), was very effective in reversing the GSH depletion caused by ethanol or acetaldehyde.

It has been previously described that death resulting from acute ethanol toxicity may be prevented by the administration of sulphur-containing compounds (Macdonald *et al.*, 1977). This, together with the fact that methionine reverses the ethanol-induced GSH depletion in hepatocytes, may be of both theoretical and practical importance.

We thank Mrs. J. Belloch for the preparation of liver cells and Dr. V. Rubio and Dr. F. Pallardó for revising the manuscript.

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