when dihydroergocryptine was added. These data suggest the existence of α-adrenergic receptors inhibiting the stimulation of thyroid adenylate cyclase by isoproterenol.

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Institut de Recherche Interdisciplinaire (LMN), School of Medicine, University of Brussels, 1000 Brussels, Belgium

DANIEL H. DESMEDT

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Effect of acetaminophen (paracetamol) and its antagonists on glutathione (GSH) content in rat liver

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Acetaminophen (paracetamol) overdose may be fatal due to hepatic centrilobular necrosis [1, 2]. Mitchell and his colleages showed that liver necrosis was due to covalent binding to macromolecules of a reactive metabolite [3] and proposed a protective role for reduced glutathione (GSH)* in mice [4] and man [5].

It has been shown that acetaminophen toxicity can be decreased by oral administration of charcoal which slows the absorption of the drug from the intestinal tract [6]. Other treatments are based on the administration of a variety of substances, including methionine [7] and *N*-acetyl cysteine [8, 9], to counteract the toxic effects of acetaminophen. According to the GSH protective hypothesis of Mitchell *et al.* [4, 5], these compounds may act by preventing GSH depletion. Other authors have proposed a direct binding of the antagonists of acetaminophen to a reactive metabolite of the drug [10].

In this communication we report the effect of methionine and of *N*-acetyl cysteine on GSH depletion due to acetaminophen overdose and also that high doses of *N*-acetyl cysteine alone decrease hepatic GSH content in rats.

Animals. Wistar rats were fed on a standard diet for rats and mice. (Sandersa Industrial S.A. Pinto, Madrid, Spain). They always had free access to food and water. Although some previous studies on acetaminophen toxicity in rats have been carried out in fasted animals [10] we used well fed rats because fasting significantly decreases hepatic GSH content in rats [11].

Acetaminophen and aminoacids were dissolved in a small volume of physiological saline and injected intraperitoneally (i.p.). *N*-acetyl cysteine was injected i.p. as a 10% aqueous solution. It was tested that injection of similar amounts of both solvents did not affect hepatic GSH content. The dose of acetaminophen injected was 0.5 g/kg body wt (3.3 mmoles/kg). A similar dose had been previously used by other authors [10].

Chemicals. Acetaminophen was a gift of the Department of Pharmacy of the Faculty of Medicine (Valencia, Spain). Amino acids and other chemicals were of the highest purity available.

Determination of GSH and GSSG. Reduced glutathione (GSH) was determined by the glyoxalase method of Racker [12].

Rats were killed by cervical dislocation and livers removed, weighed and homogenized in 2% perchloric acid in physiological saline. Analysis of GSH in rapidly frozen livers showed that the above procedure did not affect the hepatic GSH content. Normal GSH concentration was 5.40 ± 0.37 μmoles/g (four observations). Oxidized glutathione (GSSG) was determined as previously described [13].

* Abbreviations used: GSH, reduced glutathione; GSSG, oxidized glutathione.

Table 1. Effect of methionine on the maintenance of hepatic glutathione (GSH) after acetaminophen overdose*

Dose of methionine injected (g/kg body wt [mmoles/kg])	Hepatic GSH content (micromoles/g fresh wt)	Per cent of normal
0 [0]	2.26 ± 0.96 (4)†	41
0.1 [0.6]	3.93 ± 0.40 (3)	73
0.5 [3.3]	4.41 ± 0.41 (3)	81
1.5 [10]	4.71 ± 0.12 (3)	87

* Rats were injected with 0.5 g/kg body wt (3.3 mmoles/kg body wt) of acetaminophen and killed 10 hr after the injection. Methionine was injected together with acetaminophen.

† Numbers in parentheses indicate number of observations.

Comparison of the effects of N*-acetyl cysteine and methionine on hepatic GSH content after administration of acetaminophen.* Figure 1 shows the time course of the depletion of liver GSH content after administration of acetaminophen (0.5 g/kg = 3.3 mmoles/kg) alone, acetaminophen (0.5 g/kg = 3.3 mmoles/kg) plus methionine (1.0 g/kg = 6.0 mmoles/kg) and acetaminophen (0.5 g/kg = 3.3 mmoles/kg) plus *N*-acetyl cysteine (1.0 g/kg = 5.0 mmoles/kg). It is also shown that hepatic GSH content 10 hr after injection of acetaminophen is not significantly different from the value obtained for acetaminophen plus *N*-acetyl cysteine, but that in the case of acetaminophen and methionine the value is similar to the physiological content.

When *N*-acetyl cysteine was injected to rats intoxicated with paracetamol (0.5 g/kg = 3.3 mmoles/kg), in five successive doses (1, 3, 5, 7 and 9 hr after injection of paracetamol) but injecting each time 0.2 g/kg (1.0 mmoles/kg) (i.e. a fifth of the total *N*-acetyl cysteine used previously in one single dose), and the rats were killed 10 hr after paracetamol administration, hepatic GSH content was 2.53 ± 0.60 μmoles/g (three observations), a value not different from that obtained in rats injected with paracetamol alone.

In view of the protective effect of methionine on hepatic GSH, we tested the effects of various doses of methionine injected together with 0.5 g/kg (3.3 mmoles/kg) acetaminophen (Table 1). Even when methionine at a dose of 0.1 g/kg (0.6 mmoles/kg) was injected 4 hr after acetaminophen (0.5 g/kg = 3.3 mmoles/kg) administration, GSH content 10 hr after injection of acetaminophen was maintained at 4.28 ± 0.12 μmoles/g fresh wt [3].

Mitchell *et al.* [4, 5] showed that GSH plays a key role in protecting liver against acetaminophen toxicity. We have shown that methionine is effective in minimizing GSH depletion after acetaminophen overdose. It is significant that in rats injected with 0.5 g/kg (3.3 mmoles/kg) of acetaminophen, a dose of 0.1 g/kg (0.6 mmoles/kg) of methionine, i.e. a fifth of the amount of acetaminophen injected, was sufficient to maintain high concentrations of hepatic GSH, both when the amino acid was injected together with acetaminophen and when the amino acid was injected 4 hr after the acetaminophen injection. This provides further experimental support for the suggestion of McLean [14] that it might be useful to include methionine in proprietary tablets of acetaminophen or to treat acetaminophen overdose with methionine within the first few hours after the ingestion of the drug.

The finding that methionine is more effective than *N*-acetyl cysteine is in keeping with a report by Thor *et al.* [15] who found that methionine was a potent protective agent against bromobenzene toxicity whereas cysteine did not prevent a decrease in GSH content induced by bromobenzene in isolated hepatocytes.

McLean and Day [14] observed, in rats, that methionine was effective at lower doses than cysteine–HCl at protecting against acetaminophen-induced liver injury, as judged by measurement of plasma isocitrate dehydrogenase or by histological examination. However, they did not measure GSH in livers of rats injected with acetaminophen and cysteine or acetaminophen and methionine.

Thor *et al.* [16] observed recently in isolated hepatocytes incubated in the presence of *N*-acetyl cysteine and acetaminophen that only the GSH conjugate of acetaminophen was formed. However, our results show that injection of *N*-acetyl cysteine did not accelerate the recovery of hepatic GSH content in rats intoxicated with acetaminophen (see Fig. 1). Since *N*-acetyl cysteine has proved to be a good antagonist for acetaminophen toxicity [8, 9], the hepatoprotective role of liver GSH against acetaminophen intoxication has to be reconsidered. A similar conclusion was suggested by Wendel *et al.* [17] studying acetaminophen-induced lipid peroxidation.

A possible explanation for the failure of *N*-acetyl cysteine to accelerate GSH recovery after paracetamol-induced GSH depletion is that *N*-acetyl cysteine is rapidly deacetylated in liver and that the cysteine moiety is then oxidized

Table 2. Effect of *N*-acetyl cysteine alone on hepatic glutathione (GSH)*

Dose of *N*-acetyl cysteine injected (g/kg body wt [mmoles/kg])	Hepatic GSH content (μmoles/g fresh wt)	Per cent of normal
0.25 [1.5]	4.97 ± 0.12 (2)†	92
0.50 [3.0]	4.46 ± 0.21 (3)	82
1.00 [6.0]	4.23 ± 0.21 (4)	78
1.50 [9.0]	3.05 ± 0.31 (4)	56

* Rats were killed 2 hr after the injection.

† Numbers in parentheses indicate number of observations.

Table 3. Time course of GSH depletion induced by *N*-acetyl cysteine 1.0 g/kg (5.0 mmoles/kg) body wt*

Time after injection (hr)	Hepatic GSH content (μmoles/g fresh wt)	Per cent of control
2	4.23 ± 0.21 (4)†	78
4	2.31 ± 0.18 (3)	42
6	2.52 ± 0.16 (3)	46
10	2.95 ± 0.33 (3)	54
24	5.12 ± 0.57 (3)	94

* Normal GSH content was 5.40 ± 0.37 μmoles/g fresh wt (four observations).

† Numbers in parentheses indicate number of observations.

to cystine which is not a good precursor for GSH synthesis in liver [16].

Effect of N-acetyl cysteine (and cysteine) on liver GSH. Since hepatic GSH content of rats injected with acetaminophen (0.5 g/kg = 3.3 mmoles/kg) and *N*-acetyl cysteine was lower than that of rats injected with acetaminophen (0.5 g/kg = 3.3 mmoles/kg) alone, both when the dose of *N*-acetyl cysteine used was 1.0 g/kg (5.0 mmoles/kg) (see Fig. 1) and when it was 0.2 g/kg (0.5 mmoles/kg) (GSH content 2 hr after injection: 1.01 ± 0.28 μmoles/g fresh wt, for three observations), we decided to test the effect of *N*-acetyl cysteine alone on hepatic GSH content and found a decrease in the GSH content that was dependent on the dose of *N*-acetyl cysteine administered (Table 2). We found no significant changes in hepatic GSSG content in rats injected with *N*-acetyl cysteine. Injection of large doses of methionine alone did not significantly affect hepatic GSH or GSSG content.

The time course of GSH depletion after injection of *N*-acetyl cysteine (1 g/kg = 5.0 mmoles/kg) is shown in Table 3.

N-acetyl cysteine is used as a mucolytic and as a protective agent in acetaminophen overdose [8, 9]. However, the recommended dose of *N*-acetyl cysteine in man is about 0.3 g/kg (1.5 mmoles/kg) body wt. Our results (Table 2) show that in the rat this would not cause a significant decrease in GSH content.

N-acetyl cysteine is readily taken up by liver and hydrolysed to cysteine [5]. When free cysteine (0.5 g/kg = 4.1 mmoles/kg) was injected into rats, hepatic GSH content fell to 2.60 ± 0.30 μmoles/g wet wt. We showed previously the depletion of GSH induced by cysteine in isolated hepatocytes [18] and suggested a possible explanation for the GSH depletion induced by high concentrations of cysteine in isolated hepatocytes [19].

The relevant points reported in this communication are: (1) methionine showed a protective effect on hepatic GSH depletion due to acetaminophen overdose even when administered 4 hr after the injection of the drug; (2) *N*-acetyl cysteine did not decrease hepatic GSH depletion caused by acetaminophen overdose; (3) large doses of *N*-acetyl cysteine or cysteine alone decrease hepatic GSH content in rats.

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Cátedra de Bioquímica y Fisiología, Facultad de Medicina, Av. Blasco Ibañez 17, Valencia 10, Spain

JOSÉ VIÑA
FRANCISCO J. ROMERO
JOSÉ M. ESTRELA
JUAN R. VIÑA

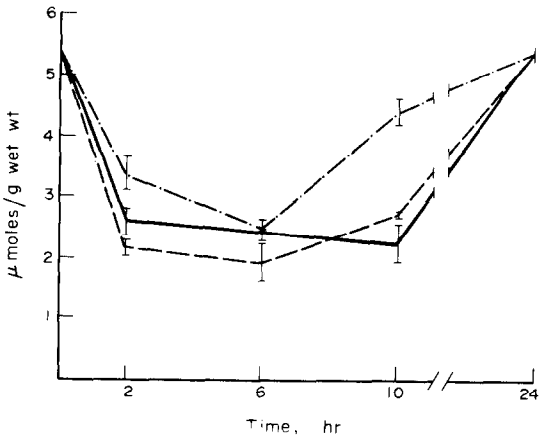

Fig. 1. Time course of hepatic GSH depletion after injection of acetaminophen 0.5 g/kg (3.3 mmoles/kg) (———), acetaminophen 0.5 g/kg (3.3 mmoles/kg) and methionine 1.0 g/kg (6.0 mmoles/kg) (–·–·–·–), and acetaminophen 0.5 g/kg (3.3 mmoles/kg) plus *N*-acetyl cysteine 1.0 g/kg (5.0 mmoles/kg) (– – – –).

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