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The effect of some carbonyl compounds on rat liver glutathione levels

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SOME foreign compounds that are metabolized to mercapturic acids after administration to animals depress liver GSH levels,[1–4] and an initial stage in mercapturic acid biosynthesis is the enzyme catalysed conjugation of these compounds with GSH.[1, 5] The reactions of GSH with many $\alpha\beta$-unsaturated carbonyl compounds are catalysed by glutathione S-alkenetransferases that are present in liver preparations from several animal species,[6, 7] suggesting that these compounds would be partly metabolized to mercapturic acids. Further evidence is provided by results reported in this paper which show that certain $\alpha\beta$-unsaturated carbonyl compounds lower liver non-protein thiol levels (mainly GSH[8]) after administration to rats.

Experimental

The carbonyl compounds, which were all liquids, were administered by intraperitoneal injection to female rats (Chester Beatty strain) weighing between 200 g and 380 g. For most of these compounds, published toxicity data refer to oral administration only, and for intraperitoneal injection a quarter or less of the oral LD_{50} was given. Where the doses were small, the compound was dissolved in arachis oil or in 0·1 M-orthophosphate buffer, pH 7·4. Control rats were dosed with arachis oil or with buffer. Of the treated rats, two were killed after 30 min and four after 2 hr; the controls (usually three) after 2 hr. The livers were immediately removed, homogenized in 5 vol. of 0·1 M-orthophosphate buffer, pH 7·4, an equal volume of 4% (w/v) sulphosalicylic acid was added, and the mixture centrifuged at approx. 2000 *g* for 30 min. All operations were carried out below 10°. The supernatant was assayed for GSH by the 5,5′-dithiobis-(2-nitrobenzoic acid) method.[9] Recoveries of GSH added to the whole homogenate ranged between 87–101 per cent (mean 96 per cent). Mean control GSH (forty-four rats) was 155 mg/100 g liver with values ranging between 96–220 mg/100 g liver, but the majority were within 20 per cent of the mean value. Johnson[2] reported 151 mg/100 g liver by a similar assay method, and Woodward[10] obtained 172 mg/100 g liver by the glyoxalase method. In cases where GSH levels fell to about 90 per cent of the control value, the result is not considered significant.

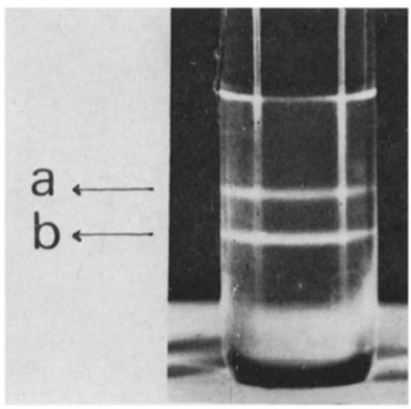

FIG. 1. Kinetoplastic DNA after centrifugation in a Cs-Cl-EB density gradient. Tube was examined in a darkened room with 366 mμ light from a Desaga Uvis lamp and photographed.

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Results and discussion

Compounds containing activated double bonds, that are substrates for glutathione S-alkenetransferases[7, 11] depressed liver GSH levels markedly after administration to rats, except for the naturally occurring compounds[12, 13] hex-2-en-1-al and non-2-en-1-al (Table 1). The enzyme-catalysed reaction of GSH with both these compounds is rapid,[11] and the former reacts readily nonenzymically with GSH.[7] However, two rats that received hex-2-en-1-al at a higher dose of 0·32 ml/kg died within 5 min, and GSH levels were 26 per cent of the control value. These unsaturated aldehydes are probably rapidly converted to the corresponding acids and alcohols in the liver.

TABLE 1. EFFECT OF CARBONYL COMPOUNDS ON RAT LIVER GLUTATHIONE LEVELS

Compound	Dose (ml/kg)	GSH level (% of controls)	
		After 30 min	After 2 hr
Non-2-en-1-al	0·128	88	97
Hex-2-en-1-al	0·08	78	82
Cyclohex-2-en-1-one	0·125	17‡	—
Cinnamaldehyde dimethylaceta	0·4	80	75
Cinnamaldehyde	0·5	53†	35
Parasorbic acid	0·132	11	26
Diethyl maleate	0·6	6†	10†
Diethyl fumarate	0·68	54	24
Vinyl acetate	0·8	77†	149*
Ethyl cinnamate	0·8	95	87
Methyl methacrylate	0·87	92	68†
Cyclohexanone	0·4	88	72
Diethyl succinate	1·0	98	98

The compounds are listed in approx. order of enzymic reactivity with GSH.[11] Rats weighing between 200 g and 380 g were used, but for each compound, control and treated rats weighed almost the same.
* Mean of 2 values.
† Mean of 3 values.
‡ Mean of 4 values.

Nonenzymic and enzymic rates of reaction of GSH with cinnamaldehyde and the corresponding dimethylacetal are similar,[7] but the former depressed rat liver GSH more. This effect is unlikely to be entirely due to the higher dose used.

Cyclohex-2-en-1-one caused severe erythema in the rats: these were all killed after 30 min for this reason. Parasorbic acid (δ-lactone of 5-hydroxyhex-2-en-1-oic acid), a naturally occurring carcinogenic compound,[14] depressed rat liver GSH to levels comparable with those produced by cyclohex-2-en-1-one which it structurally resembles.

Vinyl acetate and ethyl cinnamate are slowly reacting substrates for enzyme-catalysed conjugation with GSH.[6] The former produced an apparent elevation of rat liver GSH levels 2 hr after administration.

Methyl methacrylate does not react with GSH over several hours at pH 7·0 and 25°, and depression of liver GSH by this compound was unexpected. However, it may be activated *in vivo* by conversion to 2,3-epoxypropyl methacrylate which is a substrate for a glutathione S-epoxidetransferase type of enzyme.[15]

Diethyl succinate and cyclohexanone do not contain activated double bonds and were not expected to depress rat liver GSH levels. However, the latter depressed GSH levels, and since no reaction of this compound with GSH was detected over 1 hr at pH 7·4 and 37°, it may require activation *in vivo*, possibly to cyclohex-2-en-1-one.

Intraperitoneal administration of diethyl maleate (0·69 ml/kg) to male rats[11] depressed liver GSH to similar levels as found in female rats (Table 1).

Results in this paper generally show that compounds that are good substrates for glutathione S-transferases also lower liver GSH levels soon after administration to the rat. Failure to react with GSH nonenzymically or enzymically is not absolute proof that a compound may not be metabolized to a mercapturic acid since further bioactivation (such as epoxidation) may be required.

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Quinazoline antifolates as inhibitors of dihydrofolate reductase from human leukemia cells

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SIGNIFICANT antileukemic activity of a series of 2,4-diaminoquinazolines in L1210 mouse leukemia test systems has recently been reported by Hutchinson and Shimoyama.[1, 2] Several compounds of the series were found to be more effective in these test systems than was methotrexate (MTX), the dihydrofolate reductase inhibitor presently used clinically. *In vitro*, the 2,4-diaminoquinazolines were found to be potent inhibitors of the enzyme dihydrofolate reductase from mouse L1210 leukemia cells.[3]

The effectiveness of the 2,4-diaminoquinazolines antifolates as inhibitors of human dihydrofolate reductase has not yet been reported. The purpose of the present communication is to describe the activity of the 2,4-diaminoquinazoline antifolates as inhibitors of dihydrofolate reductase from human leukemia cells, and to present some additional studies concerning the mode of action of these compounds.