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SHORT COMMUNICATION

The influence of some aliphatic compounds on rat liver glutathione levels*

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ORAL administration of bromobutane (1·6 m. moles/kg) to rats has been shown to cause a moderate depression of liver GSH levels[1] (Table 1). Iodomethane exerts a much greater effect at moderate doses[2, 3] (Table 1), and it has been shown to S-methylate GSH under influence of a liver enzyme (glutathione S-alkyl transferase) both *in vivo* and *in vitro*.[2–4] Examination of the liver enzyme showed that it had a fairly wide specificity for the substrate other than GSH[4] and this communication reports the effect on rat liver GSH of some aliphatic compounds administered orally. Some discrepancies between effects seen *in vivo* and *in vitro* are discussed.

TABLE 1. EFFECT ON RAT LIVER GLUTATHIONE OF ORAL DOSES OF VARIOUS COMPOUNDS

200 g female rats were dosed by stomach tube with compound dissolved in 0·2 ml arachis oil, glycerol formal or normal saline and killed 2 hr later. GSH was assayed in alcoholic or metaphosphoric/salt extracts[6] by method of Beutler, Duron and Kelly[7]; mean control GSH (10 rats) was 4·9 μmoles/g.

Compound	Dose (m.moles/kg)	GSH level (% of controls)
Iodomethane	0·35	58 (mean of many)
	0·53	17 (mean of many)
Iodoethane	0·81	65, 42
Bromoethane	1·16	52
1-bromobutane†	1·6	50
Methylene dichloride	11·8	106, 99
Chlorobromomethane	15·4	101, 106, 109, 144*
1,2-dichloroethane	4·0	53, 81, 40, 34
Chloroform	0·84	93, 111
	8·4	55, 50
Trichloroethylene	15·2	107,101
Carbon Tetrachloride	1·3	96, 116
	13·0	*117
2-chloroethanol	0·68	18, 16
Chloroacetaldehyde	0·53	54, 55
Chloroacetonitrile	0·53	19, 30
Chloroacetamide	0·53	20, 50
Iodoacetamide	0·53	87, 90
2-Bromoethanol	0·68	28, 46
2·3-Dibromopropanol	0·92	86
	1·85	6, 22
	3·7	8, 8
Tri-(2,3-dibromopropyl) phosphate	0·72	112, 114
Tri-(2-chloroethyl) phosphate	0·70	98, 80
ß-Propiolacetone	0·53	97, 105

* Values are uncertain owing to very high assay blanks caused by lipid material in tissue extract supernatant.

† Data of Barnes, James & Wood (1959): compound administered in aqueous suspension.

Choice of dose was determined by published toxicity data where available; about half the LD_{50} was chosen to be as high as possible with little risk of terminal illness effecting any change. Compounds in solution (0·2 ml/200g rat) were administered orally at about 10 a.m. and the rats killed at noon (the uniform procedure obviated the confusion brought about by known diurnal variations in liver GSH levels[5]). Liver GSH levels are shown in Table 1: no measurement of oxidized glutathione was

made. The possibility that the depressions of liver GSH reported here are due to transformation to GSSG is unlikely since Barnes, James and Wood found with all their compounds that GSSG levels fell roughly in proportion to the GSH depression[1] and with the present extraction technique paper chromatography shows very little GSSG in extracts of livers from rats dosed with iodomethane or 2-chloroethanol or from controls.

Table 1 shows that the simple mono-halogeno-paraffins were effective at comparatively low doses: these are probably all substrates for glutathione S-alkyl transferase,[1] although problems of physical solubility prevent the higher compounds from being tested easily *in vitro*, Insolubility also prevented satisfactory *in vitro* tests in the case of the multi-halogeno-compounds listed, but the pronounced effect of 1,2-dichloroethane at 4 m. moles/kg is remarkable for a compound considered relatively inert under normal chemical reaction conditions; the same comment could be made concerning the high dose of chloroform although the effect may well be non-specific at such a large molar dose. The most interesting results are those with the halogeno-alcohols, particularly the 2-halogeno-ethanols. These are not substrates for glutathione S-alkyl transferase[4] and yet are almost as effective as iodomethane in lowering both liver and kidney GSH levels: besides the effect shown in Table 1, 0·68 m. moles/kg of 2-chloroethanol depressed kidney GSH to 60 per cent after two hours. A possible explanation is that the 2-chloroethanol is converted *in vivo* by the liver and kidney to chloro-acetaldehyde which reacts rapidly with GSH *in vitro* even without any enzymic catalysis.[4] In some recent preliminary experiments I have shown that chloroethanol reduces NAD *in vitro* in the presence of concentrated rat liver supernatant: the slow rate (about 0·07 μmole NAD/min/g liver with optimum substrate at pH 7·5 and 20)[5] approaches that needed to account for the measured loss of GSH *in vivo*. I could not show whether the catalyst was distinct from ethanol dehydrogenase, but Treble has shown that horse-liver contains an enzyme distinct from alcohol dehydrogenase which would dehydrogenate 2-fluoroethanol with NAD as cofactor.[8]

TABLE 2. INFLUENCE OF ORAL ADMINISTRATION OF ETHANOL ON SOME METABOLIC EFFECTS OF 2-CHLOROETHANOL

200 g rats were dosed by stomach tube with 0·3 ml glycerol formal containing compound and killed after 2 hr. Dose included ethanol, 2-chloroethanol, both or neither (control). Mean control rat liver GSH (determined as in Table 1) was 5·2 μmoles/g.

Dose of 2-chloroethanol (mg/kg)		0	52	65	83	102
Deaths in 24 hr	(A) Without Ethanol	Nil	0/4	0/4	3/4	4/4
	(B) With Ethanol	*Nil	0/4	0/4	2/4	0/4
Liver GSH after 2 hr (% Normal)	(A) Without Ethanol	100	16, 18, 18	—	—	—
	(B) With ethanol	100	24, 12, (14†)	—	—	—

* Not measured. Ethanol LD_{50} = 13·6 g/kg.[10]

† Ethanol given 45 min before 2-chloroethanol

Some parallel experiments were performed with 2-chloroethanol in which the influence of ethanol administration on its toxicity and its effect on liver glutathione was investigated (Table 2). The oral LD_{50} of 2-chloroethanol was 77 mg/kg (determined according to Weil[9]) and was greater than 100 mg/kg with the same dose schedule but with 500 mg/kg ethanol included in the dose. Not only were there fewer deaths among the animals given ethanol but the survivors in the group looked much healthier throughout the experiment when compared with the non-ethanol group. It is clear from Table 2 that ethanol prevented neither the entry of 2-chloroethanol into the liver nor its reaction there with glutathione although this reaction may be slower in presence of ethanol. The experiment does show, however, that the toxic action of 2-chloroethanol is inhibited by ethanol and this could well be by means of protective competition at a sensitive site beyond the liver.

* Most of the results reported here have already appeared in a thesis for the PhD. degree of the University of London.

Further investigation of the toxicity and metabolism of 2-chloroethanol both *in vitro* and *in vivo* is contemplated: a derivative of glutathione has been shown to be formed in the liver and should be fairly easily identifiable.

It is interesting that, in contrast to the unreactive chloroethanol, iodoacetamide, which is highly reactive chemically and is a substrate for glutathione S-alkyl transferase, does not significantly affect liver glutathione when given orally. Presumably it reacts vigorously with stomach tissue constituents and never reaches the liver.

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REFERENCES

1. M. M. BARNES, S. P. JAMES and P. B. WOOD, *Biochem. J.* **71**, 680 (1959).
2. M. K. JOHNSON, *Biochem J.* **87**, 9 P. (1963).
3. M. K. JOHNSON, *Biochem. J.* Submitted for Publication.
4. M. K. JOHNSON, *Biochem. J.* Submitted for Publication.
5. L. V. BECK, V. D. RIECK and B. DUNCAN, *Proc. Soc. Exp. Biol. Med.* **97**, 229 (1958).
6. M. K. JOHNSON, *Anal. Biochem.* Submitted for Publication.
7. E. BEUTLER, O. DURON and B. M. KELLY, J. *Lab. Clin. Med.* **61**, 882 (1963).
8. D. H. TREBLE, *Biochem. J.* **82**, 129 (1962).
9. C. S. WEIL, *Biometrics*, **8**, 249 (1952).
10. H. F. SMYTH, JR., J. SEATON and L. FISCHER, *J. industr. Hyg.* **23**, 259 (1941).