

Biotransformation in rats of vinyl chloride vis-à-vis its oncogenicity

Early warning that one year's intermittent inhalational exposure of Wistar rats to 3% (v/v) of vinyl chloride elicited epidermoid and mucoepidermoid carcinomas in the para-auricular region of surviving animals¹ has been confirmed in more detailed and extensive investigations², which revealed, in addition to Zymbal gland carcinomas, liver angiosarcomas and nephroblastomas in Sprague-Dawley rats that had been exposed to atmospheric concentrations as low as 250 ppm. Vinyl chloride also induced liver angiosarcomas in Swiss mice at 500 ppm². This latter work² established a direct relationship between the dose level and length of treatment with vinyl chloride and neoplastic response. Those observations and the world-wide record by June 1974 of 24 cases of liver angiosarcoma amongst workers, who had been engaged upon vinyl chloride/polyvinyl chloride manufacture, demand a better understanding of the biological fate of vinyl chloride in mammals in relation to its established oncogenicity. Moreover, angiosarcoma induction by chronic intraperitoneal (vinyl chloride) injections³ implies that vinyl chloride, and not a product of its photolysis, is the causative agent.

Excretion data showed that the main eliminative route for [¹⁴C] vinyl chloride after oral, intravenous or intraperitoneal administration to rats is pulmonary (Table 1) (see also ref 4); both unchanged vinyl chloride and vinyl chloride-related CO₂ are excreted through the lungs and other [¹⁴C] metabolites via the kidneys. A marked change in

excretion pattern that accompanies a nearly two thousand-fold difference in intragastric dose level is due to a saturable drug metabolism and to an highly efficient pulmonary excretion of original substance that leaves the relatively low concentration of vinyl chloride material remaining in circulating blood for hepatic biotransformation. For groups of animals that had been orally dosed vinyl chloride within a dose-level range of 1-450 mgkg⁻¹, the pulmonary output of unchanged substance (expressed in terms of % of the dose) is proportional to the logarithm of the reciprocal dose (Fig 1). Excretion patterns after i.v. and i.p. injections are predictable from the characteristics of excretion after oral administration (Table 1). Whilst pulmonary excretion of unchanged vinyl chloride after oral dosing is complete within 3-5 h, pulmonary elimination of CO₂ and renal excretion of vinyl chloride metabolites occupies 3 days. In comparison, 99% of a small i.v. dose was excreted unchanged within 1 h of injection; 80% within 2 min. Since the rate of elimination of single oral doses of [¹⁴C] vinyl chloride is unaffected by up to 60 days' chronic dosing with the unlabelled substance at 3, 30 and 300 mgkg⁻¹ day⁻¹, excretion data for an acute dose apply also to the chronic situation.

Distribution studies by whole-animal autoradiography agree with deductions made from the excretion data, but small localizations of ¹⁴C are visible in the para-auricular region of appropriate sections, possibly in the Zymbal glands.

Of the vinyl chloride in the circulating blood, which escapes the highly efficient pulmonary arterial-alveolar transfer and is metabolized, thiodiglycollic acid, S-(2-chloroethyl) cysteine, N-acetyl-S-(2-chloroethyl) cysteine and urea are major urinary metabolites, chloroacetic acid and glutamic acid comprise the minor metabolites, and methionine and serine are trace metabolites. Families of related [^{14}C] metabolites were separated from the urine of [^{14}C] vinyl chloride-treated rats by anion-exchange chromatography, and residues from successively displaced fractions were derivativized, purified by t.l.c. and preparative GC and examined by GC-mass spectrometry⁵. Accurate mass measurements of the principal ions and NMR spectroscopy aided allocations of structure, and where possible, mass spectra were compared with those of authentic substances.

The halogen atom of vinyl chloride is stable to nucleophilic reagents, and glutathione reacts directly in vivo with [^{14}C] vinyl chloride in an anti-Markownikov manner to give S-(2-chloroethyl) cysteine (a) (Fig 2), which is further metabolized into the mercapturic acid, N-acetyl-S-(2-chloroethyl) cysteine (b). Retention of chlorine in metabolites (a) and (b) would have been realized only through the reactions that have been mentioned. Associative reaction with molecular O_2 typically involves the bonding of both olefinic C atoms at the transition state. We envisage a singlet oxygen-bonded form⁶ in dynamic equilibrium with a cyclic peroxide form. Chloroacetic acid (c) would be formed through rearrangement of a singlet oxygen-bonded transition state, and (c) is metabolized further into thiodiglycollic acid (d) and glutamic acid (e), whereas

formaldehyde (f), and through it CO_2 (g) and urea (h), is formed by dismutation of a cyclic peroxide transition state. Evidence for formaldehyde formation rests on the production of $^{14}\text{CO}_2$ and $[^{14}\text{C}]$ urea, and on the detection of $[^{14}\text{C}]$ methionine (i) and $[^{14}\text{C}]$ serine (j), themselves established formaldehyde metabolites. Formation of formaldehyde and CO_2 along this metabolic pathway finds confirmation in their production in vitro amongst the reaction products of a known cyclic peroxide of vinyl chloride^{7,8}.

Another reaction that seemed feasible would involve addition of a thiol to vinyl chloride followed by addition of the resulting 2-chlorothioethane to another molecule of vinyl chloride to yield di-(2-chloroethyl) sulphide, which would hydrolyse to thiodiglycol, and which might be expected to yield thiodiglycollic acid by subsequent oxidation. Whilst present work does not exclude di-(2-chloroethyl) sulphide formation, it is unlikely that this intermediate is a major source of thiodiglycollic acid (see refs. 9,10). In comparison, our observation that thiodiglycollic acid is the hitherto undiscovered major metabolite (more than 60% of the dose) of chloroacetic acid in rats strongly supports its genesis from vinyl chloride by this metabolic pathway.

Although present work does not exclude the occurrence in vivo chloroethylene oxide, it provides no evidence for its formation, despite the fact that rearrangement of this substance to chloroacetaldehyde¹⁴ would account for chloroacetic acid production. However, if the epoxide were formed even as a bonded transition state, the ensuing reaction of the epoxide or its rearrangement product with glutathione would afford

products¹², which would differ structurally from the ones, which have been established rigorously for metabolites (a) and (b). Moreover, chloroethylene oxide is not prepared direct from vinyl chloride in vitro¹¹.

The metabolism of vinyl chloride is important to its oncogenicity. Thus, (i) formation of all three major urinary metabolites, thiodiglycollic acid S-(2-chloroethyl) cysteine and N-acetyl-S-(2-chloroethyl) cysteine, utilizes glutathione, and under certain circumstances, vinyl chloride exposure would deplete the glutathione pool seriously, despite compensating mechanisms. Besides significant blocking of non-protein sulphydryl groups in the blood of vinyl chloride operatives¹³, some decrease in the availability of hepatic non-protein sulphydryl groups has been found in treated rats¹⁴. Since a fundamental role for glutathione in the body may be to protect against electrophilic attack by drug metabolites and other alkylating agents¹⁵, chronic exposure to an high dose level of vinyl chloride would lower the body's protection against attack by reactive metabolites. (ii) Biotransformation of vinyl chloride involves intermediary metabolism, and formation of glutamic acid implies C₂-alkylation of an oxaloacetate intermediate, whilst thiodiglycollate production involves double C₂-alkylation of cysteine-derived hydrogen sulphide¹⁶. In addition, well-established metabolic pathways for formaldehyde afford very small amounts of methionine and serine¹⁷. The fact that this study provides ample evidence for C₁ and C₂ alkylation of intermediary metabolites suggests that this sort of chemical reaction may lead to the transcriptional changes of the RNA, DNA or other macromolecular cell constituents, which would appear to be responsible

for vinyl chloride-induced neoplasm. However, alternative reactive metabolites may contribute to the physico-chemical mechanism of carcinogenesis and for example, di-(2-chloroethyl) sulphide may be important.

It may be significant that vinyl chloride has been shown to react in vivo with the same sort of nucleophilic protein constituents, with which N-hydroxy-2-fluorenylacetamide sulphate (the ultimate carcinogen about which most is known) reacts in vitro and in vivo¹⁸. Those reactions are held to be especially important to the oncogenicity of 2-fluorenylacetamide through its reaction with nucleic acid.¹⁹

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- ¹ Viola, P.L., Proc. 10th int. Cancer Res. Congr., Houston Texas, May 22-29 (1970); Viola, P.L., Bigotti, A., and Caputo, A., Cancer Res., 31 516-522 (1971).
- ² Maltoni, C., Int. Congr. Ser. no. 322, Advances in tumour prevention, detection and characterization, vol. 2, 8pp. Cancer detection and prevention. Proc. 2nd int. Symp. on Cancer Detection and Prevention, Bologna, April 9-12 (Excerpta-Medica, Amsterdam 1973); Maltoni, C., and Lefemine, G., Rc. Accad. naz. Ser. 8, 56, 1-11 (1974); Maltoni, C., and Lefemine, G., Envir. Res., 7, 387-405 (1974).
- ³ Maltoni, C., in Wld. Hlth., Int. Agency Res. Cancer, Int. Techn. Rep. no. 74/005. Report of a Working Group on Vinyl chloride, Lyon, June 24, 25 (1974).
- ⁴ Schaumann, O., Medizin Chem., 2, 139-147 (1934).
- ⁵ Green, T., and Hathway, D.E., Chem.-Biol. Interactions, in the press
- ⁶ Alder, R. W., Baker, R., and Brown, J. M., 'Mechanism in Organic Chemistry' (Wiley-Interscience) London, 1971) p. 295 et seq.
- ⁷ Lederer, M., Angew. Chem., 71, 162 (1959)
- ⁸ Razuvaev, G.A., and Minsker, K.S., J. gen. Chem. U.S.S.R., 28, 957-964 (1958).
- ⁹ Roberts, J. J., and Warwick, G. P., Biochem. Pharmac., 12, 1329-1334 (1963)
- ¹⁰ Davison, C., Rozman, R. S., and Smith, P. K., Biochem. Pharmac., 7, 65-74 (1961).
- ¹¹ Zief, M., and Schramm, C. H., Chem. Ind., 660, 661 (1964).

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- ¹²Johnson, M. K., Biochem. Pharmac., 16, 185-199 (1967)
- ¹³Gabor, S., Radu, M., Preda, N., Abrudean, S., Ivanof, L., Anca, Z., and Valaczkay, C., Ig. Microbiol. Epidem., Buc., 13, 409-418 (1964)
- ¹⁴Hefner, R. E., Watanabe, P. G., and Gehring, P. J., Ann. N.Y. Acad. Sci. in the press
- ¹⁵Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R., and Brodie, B. B., J. Pharmac. exp. Ther., 187, 211-217 (1973).
- ¹⁶Kun, E., in 'Metabolic Pathways' (edit. by Greenberg, D.M.) Vol. 2, p. 248 et seq (Academic Press, New York, 1961).
- ¹⁷Hathway, D. E., 'Biotransformations', in 'Foreign Compound Metabolism in Mammals' (edit. by Hathway, D. E.) Vol. 2, p. 247 (The Chemical Society, London, 1972).
- ¹⁸Miller, J. A., and Miller, E. C., Jerusalem Symp. on Quantum Chem. & Biochem. (edit. by Bergmann, E. D., and Pullman, B.) vol. 1, 237-261 (The Israeli Academy of Sciences and Humanities, Jerusalem, 1969).
- ¹⁹Kriek, E., Biochim. biophys. Acta, 355, 177-203 (1974).

TABLE 1

Excretion of radioactivity in rats, given a single dose of [^{14}C] vinyl chloride

Four rats were each dosed i.g. with 250 μg of [^{14}C] vinyl chloride per kg in corn oil solution, and another 4 rats were each dosed similarly with 450 mg of [^{14}C] vinyl chloride per kg. Four rats were each injected in the femoral vein with 250 μg of [^{14}C] vinyl chloride per kg in N-(β -hydroxyethyl) lactamide. Four rats were each injected i.p. with 250 μg of [^{14}C] vinyl chloride per kg in N-(β -hydroxyethyl) lactamide, and another 4 animals were each injected similarly with 450 mg of [^{14}C] vinyl chloride.

Size of dose	Time (h)	Radioactivity Excreted (% of dose) ^a											
		Intragastric				Intravenous				Intraperitoneal			
		Exhaled air		Urine	Faeces	Exhaled air		Urine	Faeces	Exhaled air		Urine	Faeces
		Vinyl chloride	CO ₂			Vinyl chloride	CO ₂			Vinyl chloride	CO ₂		
250 $\mu\text{g kg}^{-1}$	0-24	3.7 $\pm$ 1.2	12.6 $\pm$ 1.1	71.5 $\pm$ 5.0	2.8 $\pm$ 2.5	99.0 $\pm$ 0.8	0.1	0.5	0.1	43.2 $\pm$ 4.6	10.3 $\pm$ 2.2	41.5 $\pm$ 4.8	1.6
	24-48		0.9	3.3	1.6						0.7	1.6	0.2
	48-72			0.3	0.2								
	Total	3.7 $\pm$ 1.2	13.5 $\pm$ 1.3	75.1 $\pm$ 4.2	4.6 $\pm$ 3.0	99.0 $\pm$ 0.8	0.1	0.5	0.1	43.2 $\pm$ 4.6	11.0 $\pm$ 1.2	43.1 $\pm$ 5.7	1.8
450 mg kg ⁻¹	0-24	91.9 $\pm$ 2.5	0.6	4.5 $\pm$ 2.3	0.4					96.7 $\pm$ 4.1	0.7	2.5 $\pm$ 0.9	0.1
	24-48		0.1	0.8	0.3							0.1	
	48-72			0.1									
	Total	91.9 $\pm$ 2.5	0.7	5.4 $\pm$ 2.2	0.7					96.7 $\pm$ 4.1	0.7	2.6 $\pm$ 0.9	0.1

^aValues shown are the means $\pm$ S.D. of those means.

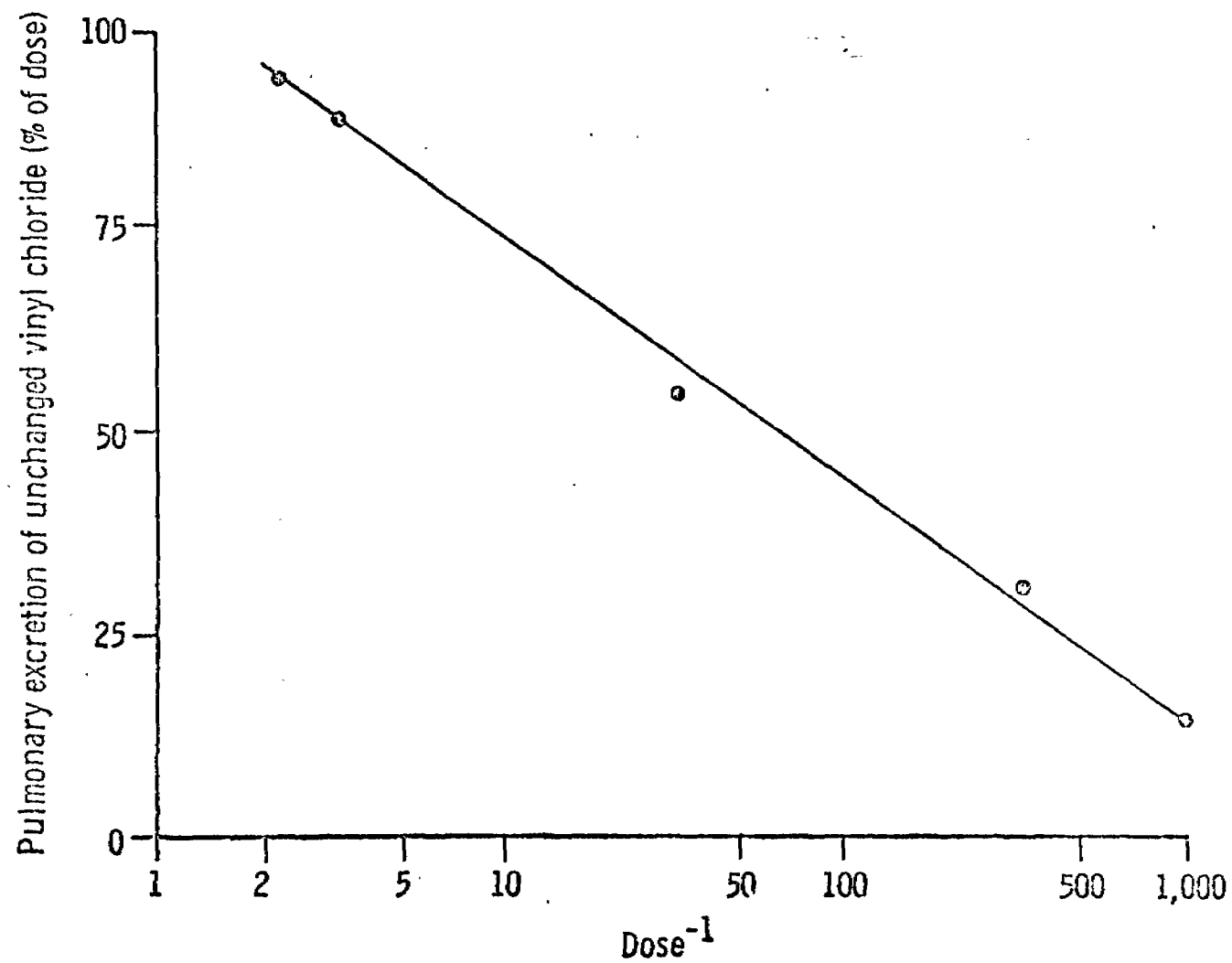
Pulmonary excretion of unchanged vinyl chloride
(% of dose)

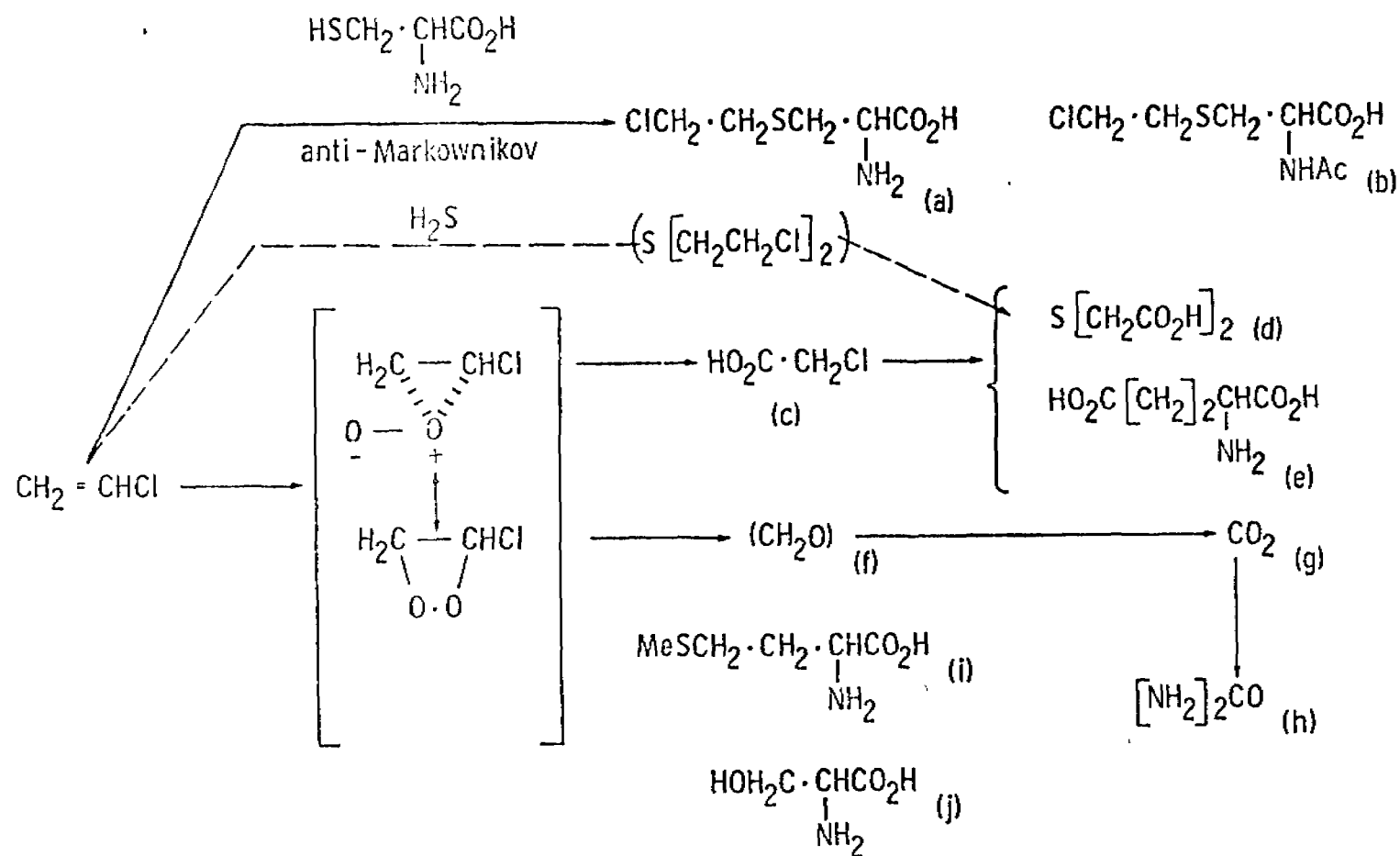
Dose⁻¹

Fig. 1 Linear-log plot of the pulmonary excretion of unchanged vinyl chloride against the reciprocal doses

Five groups of 3 rats were dosed i.g. with vinyl chloride at dose levels in the range of 1 to 450 mgkg⁻¹; each point represents 3 independent value

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Fig. 2 Scheme for the metabolism of vinyl chloride in rats