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Comparative Mammalian Metabolism of Vinyl Chloride and Vinylidene Chloride in Relation to Oncogenic Potential

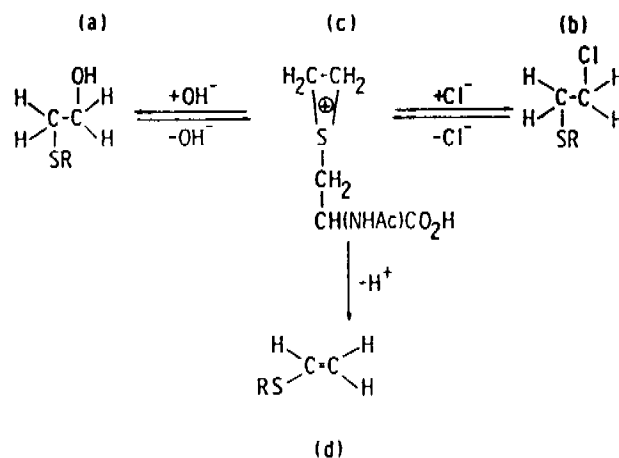
by David E. Hathway*

Elucidation of the role of vinyl chloride metabolites in the various reaction sequences which comprise the metabolic pathway, including the interaction of reactive metabolites with some purine and pyrimidine residues of target-organ DNA, provides some explanation for the (oncogenic) properties associated with the original substance. Comparative investigation of the biological fate of vinylidene chloride reveals an agent of low oncogenic potential which is likely to be damaging only under special circumstances, and species differences which suggest that the mouse is more susceptible than the rat towards vinylidene chloride oncogenicity.

The research work with which this communication is concerned is based on the idea that knowledge of the biology of the reactive metabolites of chemical carcinogens in the mammal, including the precise nature of the chemical changes to the DNA of the nucleus, ought to give an insight into the (oncogenic) properties of the parent compounds.

In tracer studies, *N*-acetyl-*S*-(2-hydroxymethyl)-cysteine was shown to be a major vinyl chloride metabolite in rats, but according to the method of protective esterification that was used so a derivative either of *N*-acetyl-*S*-(2-chloroethyl)cysteine or of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine was isolated from body fluids (1, 2). Thus, by Fischer-Speier methylation, *N*-acetyl-*S*-(2-chloroethyl)cysteine was obtained, and with diazomethane, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine. It might be stated in passing that throughout the investigations described, mass spectrometry, involving electron impact (EI) and chemical ionization sources and multiple-ion detection and all combinations of these facilities, was used extensively both for product identification and analysis and for the purposes of detection. Treatment of the *O*-methyl ester of

N-acetyl-*S*-(2-hydroxyethyl)cysteine (a) with the methanol-HCl reagent gave a mixture of *N*-acetyl-*S*-(2-chloroethyl)cysteine (b), and *S*-(2-chloroethyl)cysteine, and conversely, the *O*-methyl ester of *N*-acetyl-*S*-(2-chloroethyl)-cysteine (b) was hydrolyzed rapidly by water to that of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine (a) (2). Hence, the reversible reaction processes connecting the two substances would seem to be modulated through the intermediacy of episulfonium ion (c) and formation of this ion would in fact be rate-limiting in respect of the hydrolysis of



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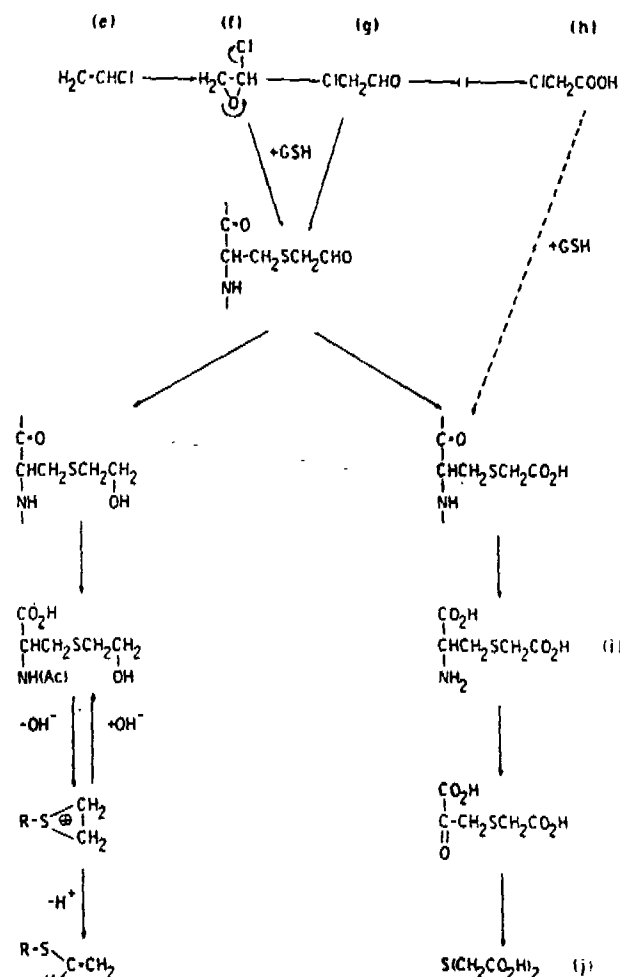
N-acetyl-*S*-(2-chloroethyl)cysteine. Nucleophilic attack of OH⁻ on the episulfonium ion would be expected to give olefin (3), and in fact, *N*-acetyl-*S*-vinylcysteine (d) (2) was recovered from the urine of vinyl chloride-treated animals whenever diazomethane esterification was used to protect *S*-containing metabolites.

Surprisingly, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine *O*-methyl ester was methylated with neutral methanol, and the *O*-methyl esters of *N*-acetyl-*S*-(2-methoxy[¹⁴C]ethyl)cysteine plus *N*-acetyl-*S*-[¹⁴C]vinylcysteine degrade to give the volatile [¹⁴C]*S*-(2-methoxyethyl) (prop-1 or 2-enyl) sulfide. Although the mechanism of formation was not investigated, we felt that acetaldehyde, a known dissociation product of *S*-vinylcysteine-derived *S*-vinylcysteine-*S*-oxide (4) might undergo concerted condensation with *N*-acetyl-*S*-(2-methoxyethyl)cysteine leading to elimination of thermodynamically stable glyoxylate. [There is an analogy for such a concerted condensation reaction in the work of Dabritz and Virtanen (4) on the tear-producing volatile components of the onion.]

The half-mustard *S*-containing metabolites of vinyl chloride did not behave as mutagens in the Ames test (2).

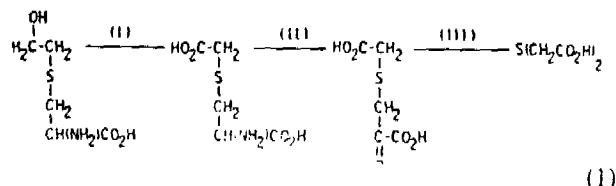
Thiodiglycolic acid is another major vinyl chloride metabolite (1).

In order to determine whether vinyl chloride yielded chloroethylene oxide *in vivo*, the biogenesis of several vinyl chloride metabolites and related compounds were investigated in rats (2). *S*-(2-Hydroxyethyl)cysteine gave 0.5% of the authentic thiodiglycolic acid, and this result was seen to be highly significant, because of the instability (*v. supra*) of the starting material under exceedingly mild conditions of reaction. The metabolic pathway concerned [Eq. (1)] appears to include endgroup oxidation (I), amino-acid transamination (II), and oxidative decarboxylation (III), and the results of the animal feeding experiments suggest that chloroacetaldehyde (g) chloroacetic acid (h), and *S*-(2-carboxymethyl)cysteine (i) might lie on a common pathway connecting vinyl chloride (e) with thiodiglycolic acid (j). However, other evidence implies that chloroacetic acid (h) does not belong to this metabolic pathway (e-j). Thus, < 0.1% has even been detected in the body fluids of any of our vinyl



chloride-treated animals. Either there is a high rate of turn-over or this compound is not a major vinyl chloride metabolite. The latter possibility seems more likely, since relatively large amounts are produced in vinylidene chloride metabolism, and those animals, thiodiglycolic acid accounts for even greater proportion of the dose than in previous experiments with vinyl chloride. A few metabolic pathways for thiodiglycolic acid chloroacetic acid and involving cysteine desulfurase is unacceptable. Experiments with unlabeled vinyl chloride in rats in which the cysteine-cysteine pools had been labeled adequately with ¹⁴C gave [¹⁴C]thiodiglycolic acid, showing that a part of the C-skeleton must be derived in fact from cysteine. In rats treated with chloroacetaldehyde, the presence of thiodiglycolic acid and *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, but not of chloroacetic acid, among the urinary metabolites was established by mass fragmentometry.

Thus, it is probable that *in vivo* chloroethylene



oxide (f) was formed (5) from vinyl chloride (e) and transformed spontaneously (6) into chloroacetaldehyde (g); there is supporting evidence (7-10) for vinyl chloride epoxidation *in vitro*. This supposition is supported by the facts that chloroacetaldehyde affords both *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycollic acid *in vivo* and that *S*-(2-carboxymethyl) cysteine has been identified by mass fragmentometry amongst the hydrolytic products of an hepatic extract prepared from vinyl chloride-treated animals. Since chloroacetaldehyde and chloroethylene oxide are mutagenic in the Ames test (11-13) and in Chinese hamster V79 cells (14), they may be relevant to vinyl chloride carcinogenicity.

Respective formation of 9 β -D-2'-deoxy ribofuranosylimidazo-[2,1-i]purine or 3 β -D-2'-deoxy ribofuranosyl-2-oxo-2,3-dihydroimidazo-[1,2-c]pyrimidine from deoxy adenosine or deoxy cytidine by reaction with chloroacetaldehyde (15) or chloroethylene oxide was readily confirmed. Recognition of the nucleoside units of DNA that were modified by reaction with active vinyl chloride metabolites *in vivo* provides opportunity for the construction from appropriate animal data of the corresponding dose-response, time-response relationships, in comparison with the ones for tumor incidence/occurrence in those animals. The presence of these two imidazonucleoside derivatives has now been established by mass fragmentometry (16) in the enzymic hydrolysate of modified rat-liver DNA, prepared from rats, which had been exposed chronically to vinyl chloride (250 ppm in their drinking water) for 1 year (Fig. 1). A smaller proportion of the 9 β -D-2'-deoxy ribofuranosylimidazo-[2,1-i]purine, than would have been expected to have been formed, was found both in the animal experiments with vinyl chloride and in model reactions between chloroacetaldehyde and calf thymus DNA (16). This observation is consistent with some degree of DNA depurination brought about by the reaction of vinyl chloride, and in our model experiments, we have found evidence for the presence of the detached purine, viz., imidazo-[2,1-i]purine. Hence, the alkylation that produces imidazo-derivative formation (with DNA) labilizes the N₉-purine β -glycoside linkage, which leads to depurination. The gap so produced might then be filled by various bases, resulting in "mispairing" during DNA replication. These results are very important, because in general, there is excellent agreement between the severe damaging effect of depurination to DNA and mutagenicity (17-19).

Thus, in retrospect, one would suspect vinyl chloride of being mutagenic/carcinogenic.

On the other hand, vinylidene chloride (k) metabolism in rats gave thiodiglycollic acid (r) and

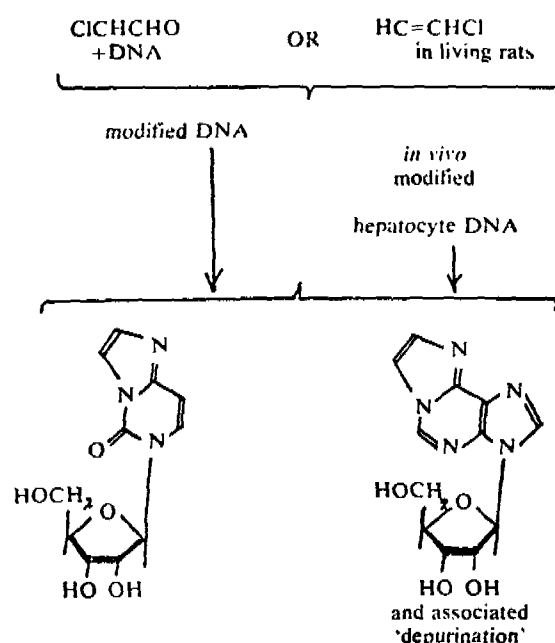


FIGURE 1. Scheme suggesting the model reaction of chloroacetaldehyde with (calf-thymus) DNA and the bio-transformation of hepatocyte DNA by vinyl chloride *in vivo*. Both reaction processes afford 3 β -D-2'-deoxy ribofuranosyl-3 β -oxo-2,3-dihydroimidazo-[1,2-c]pyrimidine (left-hand side) and 9 β -D-2'-deoxy ribofuranosylimidazo-[2,1-i]purine (right-hand side).

an *N*-acetyl-*S*-cysteinyl-acetyl derivative (p) as major urinary metabolites, plus substantial amounts of chloroacetic acid (l), dithioglycollic acid (t) and thiodiglycollic acid (s) (20). It is probable that chloroacetic acid (l), which is a vinylidene chloride metabolite *per se*, lies on a major metabolic pathway for vinylidene chloride, since it affords several metabolites in common with vinylidene chloride (20).

There is a strong supposition that detoxification of chloroacetic acid (l) is effected through a glutathione *S*-acyl transferase-catalyzed reaction process and ensuing degradative sequence for the resulting carboxymethylglutathione (n), and that this represents the principal metabolic pathway for chloroacetic acid and a major one for vinylidene chloride. Thiodiglycollic acid is the ultimate detoxification product, and previous work (2) established the biotransformation of *S*-(2-carboxymethyl) cysteine (g) into that substance. A feasible metabolic pathway to thiodiglycollic acid from chloroacetic acid and involving cysteine desulfhydrase is unacceptable. In experiments (rats) with unlabeled vinylidene chloride in which the cysteine-cystine pools had been labeled with ¹⁴C, labeled thiodiglycollic acid resulted, and a part of the C-skeleton of

that substance must be derived in fact from cysteine (20). Formation of a small amount of [^{14}C]thiodiglycollic acid (t) (and hence of the intermediate [^{14}C]thiodiglycollic acid) (s) is reconcilable with the action of Michaelis's (21) unspecific β -thionase, which would lyse a small proportion of the preponderating [^{14}C]thiodiglycollic acid.

Moreover, Kolbe electrolysis (22) of one molecular proportion of the [^{14}C]thiodiglycollic acid metabolite from [$1\text{-}^{14}\text{C}$]1,1-dichloroethylene or [$1\text{-}^{14}\text{C}$]chloroacetic acid gave one equivalent of $^{14}\text{CO}_2$ (23), and this evidence is consistent with the transformation of vinylidene chloride into chloroacetic acid by a mechanism involving migration of one Cl atom and the loss of the other one (20, 23). Hence, the metabolic pathway which was tentatively proposed for the biotransformation of vinylidene chloride into thiodiglycollic acid does in fact operate in rats.

It is equivocal whether the very small amounts of CO_2 and urea are produced by the action of epoxide hydratase on 1,1-dichloroethylene oxide or by a minor oxidative pathway for chloroacetic acid.

There is a strong supposition that the *N*-acetyl-*S*-cysteinylacetyl derivative (p), which is a metabolite of vinylidene chloride, but not of chloroacetic acid, may be formed in fact from 1,1-dichloroethylene oxide through the agency of glutathione *S*-epoxide transferase to afford *S*-glutathione acetyl chloride (m) and its subsequent reactions (20). This supposition is important, since the reactivity displayed by 1,1-dichloroethylene oxide appears to be relevant to the possible interaction of reactive vinylidene chloride metabolites with mouse kidney DNA (Fig. 2), which is a prerequisite of tumor initiation (24). Such interaction would be analogous to that of vinyl chloride with rat-liver DNA *in vivo*, which forms imidazo derivatives with some nucleoside residues (16). Further work is in progress to investigate this hypothesis.

Comparative studies (25) provide clues of differences between rats and mice in the processing of vinylidene chloride (Table 1). Thus, in mice, the production of thiodiglycollic acid is considerably reduced and the formation of the *N*-acetyl-*S*-cysteinylacetyl metabolite is increased. The higher β -thionase activity in mice than in rats accounts for the greater conversion of thiodiglycollic acid into dithioglycollic acid *via* thioglycollic acid in the former species of animal. Yllner's (26) mice excreted a proportion of a dose of chloroacetic acid as unchanged starting acid. Thus, in mice, the metabolic pathway from chloroacetic acid to thiodiglycollic acid seems to be readily saturable, possibly on account of an inadequacy in the reaction catalysed by glutathione *S*-acetyl transferase. Under these circumstances, detoxification of

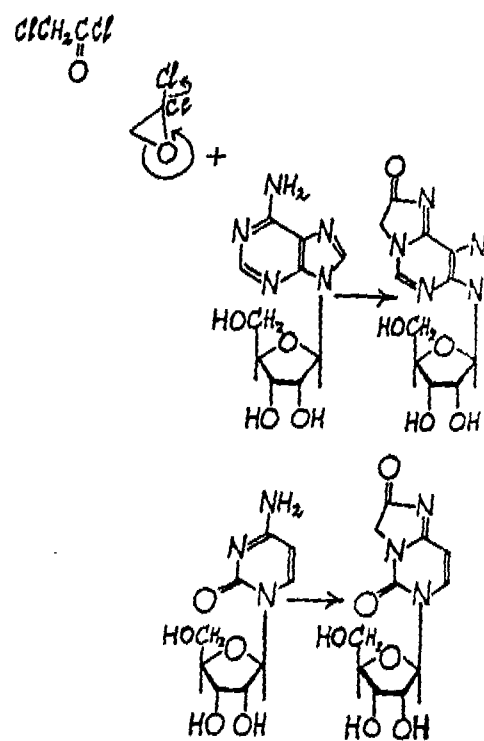


FIGURE 2. Scheme suggesting the feasible interaction of reactive vinylidene chloride metabolites, 1,1-dichloroethylene oxide and chloroacetyl chloride, with adenosine and cytosine respectively.

Table 1. Relative proportion of products from metabolism of chloroacetic acid and vinylidene chloride in rats and mice.

Substrate	Metabolite	Yield of metabolites, %		
		Rats	Mice	
Chloroacetic acid	Chloroacetic acid	~	6-22	-
	Thiodiglycollic acid	90	37	30-40
	<i>N</i> -Acetyl- <i>S</i> -(2-carboxymethyl) cysteine	2	40	40
Vinylidene chloride	Chloroacetic acid	3	-	-
	Thiodiglycollic acid	37	-	3
	Thioglycollic acid	3	-	5
	Dithioglycollic acid	5	-	20
	<i>N</i> -Acetyl- <i>S</i> -cysteinylacetyl derivative	48	-	70

1,1-dichloroethylene oxide by glutathione *S*-epoxide transferase and the modification of DNA by 1,1-dichloroethylene oxide or chloroacetyl chloride would be expected to be more significant in mice than in rats. This diagnosis of species susceptibility seems to accord with Maltoni's (24) discovery of vinylidene chloride oncogenicity in (the kidneys of) mice.

Vinylidene chloride emerges as an agent of low, perhaps very low, oncogenic potential, which can be damaging only in a special set of biological circumstances, which we have partially defined and on which work is continuing.

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REFERENCES

1. Green, T., and Hathway, D. E. The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem. Biol. Interact.* 11: 545 (1975).
2. Green, T., and Hathway, D. E. The chemistry and biogenesis of S-containing metabolites of vinyl chloride in rats. *Chem. Biol. Interact.* 7: 137 (1977).
3. Ogston, A. G., et al. The replacement reactions of β,β' -dichlorodiethyl sulphide and of some analogues in aqueous solution: the isolation of β -chloro- β' -hydroxy-diethylsulphide. *Trans. Faraday Soc.* 44: 45 (1948).
4. Dabritz, E., and Virtanen, A. I. S-Vinyl-cystein-S-oxid, ein Homologes zur Vorstufe der trärentreibenden Substanz der Zwiebel. *Chem. Ber.* 98: 781 (1965).
5. Van Duuren, B. L. On the possible mechanism of carcinogenic action of vinyl chloride. *Ann. N.Y. Acad. Sci.* 276: 258 (1975).
6. Gross, H., and Freiburg, J. Zur Existenz von Chloräthylenoxid. *J. Prakt. Chem.* 311: 506 (1969).
7. Rannig, U., et al. The mutagenicity of vinyl chloride after metabolic activation. *Ambio* 3: 194 (1974).
8. Batten, P. L., et al. Liver-microsome mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* 67: 596 (1975).
9. Gross, H., et al. Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic activation. *Biochem. Pharmacol.* 24: 2013 (1977).
10. Salmon, A. G. Cytochrome P450 and the metabolism of vinyl chloride. *Cancer Letters* 2: 109 (1976).
11. Bartsch, H., Malaveille, C., and Montesano, R. Human, rat and mouse liver-mediated mutagenicity of vinyl chloride in *S. typhimurium* strains. *Int. J. Cancer* 15: 429 (1975).
12. Malaveille, C., et al. Mutagenicity of vinyl chloride, chloroethylene oxide, chloroacetaldehyde and chloroethanol. *Biochem. Biophys. Res. Commun.* 65: 363 (1975).
13. McCann, J., et al. Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylene chlorohydrin), vinyl chloride and cyclophosphamide. *Proc. Nat. Acad. Sci. U.S.A.* 72: 3190 (1975).
14. Huberman, E., Bartsch, H., and Sachs, L. Mutation induction in Chinese hamster V79 cells by two vinyl chloride metabolites, chloroethylene oxide and chloroacetaldehyde. *Int. J. Cancer* 15: 539 (1975).
15. Barrio, J. R., Secrist, J. A., and Leonard, N. J. Fluorescent adenosine and cytidine derivatives. *Biochem. Biophys. Res. Commun.* 46: 597 (1972).
16. Green, T., and Hathway, D. E. Interactions of vinyl chloride with rat-liver DNA *in vivo*. *Chem. Biol. Interact.* In press.
17. Lawley, P. D., et al. Inactivation of bacteriophage T7 by mono- and di-functional sulphur mustards in relation to cross-linking and depurination of bacteriophage DNA. *J. Mol. Biol.* 39: 181 (1969).
18. Roberts, J. J. Nucleic acid modifications and cancer. In: *Biology of Cancer*, E. J. Ambrose and F. J. C. Roe, Eds., Halstead Press, Chichester, 2nd ed., 1975.
19. Loveless, A. *Genetic and Allied Effects of Alkylating Agents*. Butterworths, London, 1966.
20. Jones, B. K., and Hathway, D. E. The biological fate of vinylidene chloride in rats. *Chem. Biol. Interact.* In press.
21. Michaelis, L., and Schubert, M. P. The reaction of iodoacetic acid on mercaptans and amides. *J. Biol. Chem.* 106: 331 (1934).
22. Kolbe, H. Untersuchungen über die Elektrolyse organischer Verbindungen. *Justus Liebigs Ann. Chem.* 69: 257 (1849).
23. Walker, G. H., and Hathway, D. E. Electrochemical analysis of the [carboxy- ^{14}C]aliphatic carboxylic acid metabolites resulting from tracer molecules. *Biochem. J.* 167: 505 (1977).
24. Maltoni, C. Proceedings of the TAPPI International Conference, Hamburg, January 26, 1977.
25. Jones, B. K., and Hathway, D. E. Differences between mice and rats in the metabolism of vinylidene chloride. *Br. J. Cancer*. In press.
26. Yllner, S. Metabolism of chloroacetate-1- ^{14}C in the mouse. *Acta Pharmacol. Toxicol.* 30: 69 (1971).

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