

of the carbonic anhydrase activity in the testis was contributed by the erythrocytes in the circulating blood and how much was derived from the local tissues. From the findings, it was concluded that alterations in the carbonic anhydrase activity of the testes following Cd administration reflected changes in the blood content and therefore in erythrocyte carbonic anhydrase in the organ.

The exact role played by these and possibly other factors in the damaging effects exerted on the testis by Cd remains to be established. The problem continues to attract wide interest, however, and it may be that the placing of a few more key pieces will enable the rest of the jigsaw to be fitted into place.

[P. Cooper]

K-1711-(100)

VINYL CHLORIDE—PART 2: MUTAGENICITY

Last month, we reviewed the more important metabolic studies on vinyl chloride (VC). Now we turn to mutagenicity. This aspect of VC's biological profile has been extensively investigated, particularly in bacteria.

Mutagenicity in Salmonella typhimurium

The preliminary studies of mutagenicity involved the Ames test. When VC, at a concentration of 20% (v/v) in air, was incubated with *Salmonella typhimurium* strain TA1535 and a mammalian metabolizing system, namely a fortified post-mitochondrial rat-liver supernatant, the number of histidine revertants was increased to three times the spontaneous mutation rate (Rannug et al. AMBIO 1974, 3, 194). An increase over the spontaneous mutation rate also occurred in strains TA1535, TA1530 and G46 when they were exposed to a similar VC atmosphere in the presence of a 9000-g mouse-liver supernatant (S-9 fraction) fortified with an NADPH-generating system (Bartsch et al. Int. J. Cancer 1975, 15, 429). The greatest response was seen with TA1530, VC treatment (for 48 hr) being associated in this case with a 28-fold increase in the number of histidine revertants. The VC concentration of the incubation medium below the 20% atmosphere was found to be 0.004 M at equilibrium. Although exposure to 20% VC in air proved to be mutagenic towards TA1530 in the absence of metabolic activation, mutagenic activity was doubled by the addition of liver fractions from phenobarbital-treated mice. The highest mutagenic responses occurred in the presence of a 9000-g supernatant or with the recombined microsomal and soluble protein fraction in the presence of an NADPH-generating system. Purified microsomal fractions were less efficient activators, and soluble liver proteins (100,000-g supernatant) alone were almost without effect. The addition of alcohol dehydrogenase and NAD⁺ to either the fortified post-mitochondrial or the 100,000-g liver supernatant did not increase the mutation rate (Bartsch et al. loc.cit.).

In the TA1535 and TA100 strains, McCann et al. (Proc. natn. Acad. Sci. U.S.A. 1975, 72, 3190) found that although a VC atmosphere (20% v/v) gave

evidence of direct mutagenic activity, its activity was doubled by the addition of the S-9 fraction from a PCB-treated rat. The results of a similar study by Andrews et al. (Mutation Res. 1976, 40, 273) seem to be anomalous, in that VC at levels of up to 15% in air were shown to be directly mutagenic in TA1535 and an S-9 fraction from PCB-treated rats produced only a slight enhancement. However, the differences between these two sets of results may partly be explained by the different incubation times involved.

Vinyl chloride is not thought to be mutagenic *per se*. The increase in the number of histidine revertants seen when VC is incubated with strains TA1530, TA1535 or TA100 in the absence of any mammalian metabolizing system is thought to be a result of the non-enzymic breakdown products of VC or of compounds formed by the bacterial enzyme system (Bartsch et al. *loc.cit.*).

Aqueous solutions of VC (initial concentration 0.083 M) gave no evidence of mutagenicity when incubated with *S. typhimurium* strains TA1530, TA1535 or G46 together with a fortified 9000-g liver supernatant from phenobarbital-treated mice (Bartsch et al. *loc.cit.*). A similar lack of activity of VC in solution (at an initial concentration of 0.022 M) was reported by Elmore et al. (Biochim. biophys. Acta 1976, 442, 405) in TA100. It was thought that the inactivity of VC in these systems may have been caused by rapid diffusion of monomer from the liquid phase into the atmosphere.

S. typhimurium strains TA1530, TA1535, TA100 and G46 respond with varying sensitivity to monofunctional alkylating agents (base-pair substitutions or deletions). Strains TA1536, TA1537 and TA1538, which are specifically reverted by frameshift mutagens, were unaffected by 20% VC atmospheres even in the presence of liver fractions from rats or mice (Bartsch et al. *loc.cit.*; Rannug et al. *loc.cit.*).

The effects of the various liver fractions seen in the studies reviewed by Bartsch & Montesano (Mutation Res. 1975, 32, 93) indicate that the mixed-function oxidases are responsible for the conversion of VC to mutagenic metabolites in mammals. Garro and his colleagues (*ibid* 1976, 38, 81), however, did not believe that the hepatic fractions were exerting their effect by an enzymatic mechanism. In contrast to the results of Bartsch et al. (*loc.cit.*) who reported that 20% VC in air induced a mutagenic response in TA1530 only when an NADPH-generating system was present, Garro et al. (*loc.cit.*), testing 75% VC in air in the same *Salmonella* strain, found that the activating effect of the S-9 microsomal fraction on VC mutagenesis was independent of the NADPH-generating system (NADPH being a necessary co-factor for the mixed-function oxidases) and was largely unaffected by a thermal treatment that would be sufficient to inactivate all enzyme activity. The increases in VC-induced mutagenicity observed when the liver fractions were obtained from either untreated or PCB-treated animals were similar, although the PCB would be expected to increase the mixed function oxidase content of the liver. Garro et al. (*loc.cit.*) considered, therefore, that the liver fraction might have been increasing the mutagenicity of VC non-enzymatically, perhaps by promoting the formation of free radicals. A free-radical generating system, riboflavin irradiated with UV light, doubled the number of VC-induced revertants in the *S. typhimurium*

(TA1530) system, and this stimulation was further enhanced by the presence of a photopolymerization accelerator—*N,N,N',N'*-tetramethylethylenediamine.

Mutagenicity of VC metabolites

The two suspected VC metabolites, chloroethylene oxide and 2-chloroacetaldehyde each directly increased the number of histidine revertants in strain TA1530 of *S. typhimurium*. At initial concentrations of 0.4 $\mu\text{mol/ml}$ the oxide was about twice as active as the aldehyde with respect to mutagenicity, but chloroethylene oxide exhibited a lower toxicity; 11% of a cell population survived an oxide concentration of 0.4 $\mu\text{mol/ml}$ while less than 0.004% of the *Salmonella* cells exposed to the same concentration of the aldehyde survived (Malaveille et al. *Biochem. biophys. Res. Commun.* 1975, 63, 363).

Chloroethylene oxide was strongly mutagenic towards TA1535. At an initial concentration of 0.45 mM, the spontaneous mutation rate of three mutants/ 10^8 cells was increased to 96/ 10^8 surviving cells. Chloroacetaldehyde at an initial concentration of 0.5 mM only increased the spontaneous mutation rate approximately three-fold (Rannug et al. *Chemico-Biol. Interactions* 1976, 12, 251). It was calculated that chloroethylene oxide, with a half-life in solution of only a few minutes at 37°C, was some 450 times as mutagenic as the aldehyde on a mol:mol basis. The oxide (tested at 0.26 M) and 2-chloroacetaldehyde (0.1 M) were also shown to be mutagenic in *Salmonella* strain TA100 (Elmore et al. *loc.cit.*). Because of its instability, an increased number of histidine revertants were observed when the oxide was pre-incubated with the test micro-organism at 30°C. All of the four possible forms of 2-chloroacetaldehyde exhibited a mutagenic response against TA100; the pure compound was more active than the monomer hydrate which in turn was more active than the dimer hydrate and trimer, probably demonstrating the importance of the intact electrophilic carboxyl group in eliciting a mutagenic response (Elmore et al. *loc.cit.*).

In the presence of a post-mitochondrial mouse-liver fraction, 2-chloroethanol (108 $\mu\text{mol/plate}$) increased the number of histidine revertants in the TA1530 strain of *S. typhimurium* about ten times; distinct mutagenic activity, but of a lower order, was also observed in the absence of metabolic activation (Bartsch et al. *loc.cit.*). Chloroacetic acid induced neither a direct nor a tissue-mediated response in this strain. In the study by McCann et al. (*loc.cit.*) 2-chloroethanol was weakly mutagenic against TA100, inducing 0.6 revertant colonies/ μmol , and demonstrated a trace of activity in TA1535. Microsomes significantly increased the number of compound-induced histidine revertants in TA100 whilst horse-liver alcohol dehydrogenase together with NADH had no analogous effect. Elmore et al. (*loc.cit.*) observed no mutagenic response when 2-chloroethanol was incubated at 1 mM with strain TA100. When tested by Rannug et al. (1976, *loc.cit.*) 2-chloroethanol and chloroacetic acid were without effect on TA1535 at concentrations up to 1.5 mM; much higher concentrations of 2-chloroethanol (1 M) proved to be weakly mutagenic.

Mutagenicity in other systems

In *Bacillus subtilis*, chloroethylene oxide and 2-chloroacetaldehyde selectively inhibited strain MC-1, a mutant lacking recombination repair of DNA; chloroethanol, chloroacetic acid and VC itself (in solution) were

inactive in this system (Elmore et al. loc.cit.). Chloroethylene oxide, at concentrations of up to 25 μ M, and 2-chloroacetaldehyde, at concentrations up to 12.8 μ M, also produced a dose-dependent induction of 8-azaguanine- or ouabain-resistant mutants in Chinese hamster V79-4 cells *in vitro*, but 2-chloroethanol or chloroacetic acid were inactive at concentrations nearly 100 times higher (Huberman et al. Int. J. Cancer 1975, 16, 639).

In the presence of mouse-liver microsomes, vinyl chloride atmospheres (up to 50% v/v) significantly increased the forward mutation frequency of *Schizosaccharomyces pombe* and induced gene conversion in *Saccharomyces cerevisiae* (Loprieno et al. Mutation Res. 1976, 40, 85). No mutagenic activity was apparent in the absence of the microsomal fraction. Chloroethylene oxide was directly and highly mutagenic in these test systems (*idem*, Cancer Res. 1977, 36, 253). In either the presence or absence of microsomes, a commercial aqueous solution of 2-chloroacetaldehyde exhibited only feeble genetic activity, whilst 2-chloroethanol was totally inactive. Since a concentration of 0.05 mM chloroethylene oxide induced the same mutagenic frequency as 50 mM VC, it seems likely that all the activity of the monomer in this system could be ascribed to the oxide.

In a host-mediated assay in the mouse, VC given at 700 mg/kg by gavage significantly increased the mutation frequency of *Sch. pombe*, which had been injected into the peritoneal cavity during 12 hr of treatment (Loprieno et al. 1976, loc.cit.). Methyl methanesulphonate, used as a positive control, proved to be almost 200 times as active as VC. No significant increases in forward mutation were seen 3 or 6 hr after an ip injection of 250 mg 2-chloroacetaldehyde/kg (Loprieno et al. 1977, loc.cit.).

Either in ethanolic solution or in the gaseous state, VC induced no detectable mutagenic change in two strains of *Neurospora crassa*, a eukaryotic micro-organism (Drozdowicz & Huang, Mutation Res. 1977, 48, 43). The addition of an S-9 liver fraction from either control or phenobarbital-treated rats had no effect. The apparent lack of sensitivity of this system may have been due to the inability of VC and its metabolites to penetrate the conidia.

A significant increase in the frequency of recessive lethals over that in controls was observed in male *Drosophila melanogaster* exposed to VC at 850 ppm for 2 days and there was a slight increase in mutation rate on exposure to 30 ppm for 17 days (Verburgt & Vogel, *ibid* 1977, 48, 327). No further enhancement in the recessive lethal incidence was seen after 2-day exposures to VC levels in excess of 10,000 ppm. It was suggested that above this concentration the *Drosophila* enzymes were no longer capable of metabolizing (activating) additional VC. In contrast to the recessive lethal assay, negative results were obtained in tests on dominant lethals, translocations and entire and partial sex-chromosome loss in *Drosophila* exposed to VC at 30,000 ppm for 2 days (Verburgt & Vogel, loc.cit.).

VC has failed to produce dominant lethals when tested in a mammalian system. Male CD-1 mice, exposed to 3000, 10,000 or 30,000 ppm VC for 6 hr/day for 5 days, were housed for 5 days in each of the following 8 wk

with successive pairs of untreated females (Anderson et al. Mutation Res. 1976, 40, 359). Using the female mice as the indicator organism there was no evidence that VC had any mutagenic effects on any maturation stage of spermatogenesis. There was no significant increase in the number of post-implantation early foetal deaths, no evidence of pre-implantation egg loss (except at the highest VC dose, where the loss showed a low level of significance but could largely be attributed to a single female), nor any reduction in fertility. A dominant lethal effect was clearly demonstrated in the positive controls given a single ip injection of 200 mg cyclophosphamide/kg or five oral doses of 200 mg ethyl methane-sulphonate/kg.

In most of the Salmonella studies, VC demonstrated activity only in the presence of a microsomal liver fraction. The Ames test has given conflicting evidence on metabolism; whilst the studies of Bartsch et al. (loc.cit.), in line with the other *in vitro* studies, suggested that the microsomal fraction of the liver was acting enzymatically, the experiments of Garro et al. (loc.cit.) indicated, so far uniquely, that a free-radical mechanism was responsible for the conversion of VC to its active metabolites. VC elicited a mutagenic response in a simple eukaryotic system, demonstrating activity in *Sch. pombe* and *Saccharomyces cerevisiae* both *in vitro* in the presence of liver microsomes and in a host-mediated assay. The incidence of recessive lethal mutation was increased in *Drosophila* treated with 30 ppm VC for 17 days. Chloroethylene oxide and 2-chloroacetaldehyde have proved to be mutagens—the oxide particularly so—in prokaryotic micro-organisms, namely the strains of *Salmonella* susceptible to frameshift mutagens and *B. subtilis*. This evidence further implicates chloroethylene oxide as the active VC metabolite.

No evidence of activity was seen in the dominant lethal assay in mice. However, further investigations of mutagenicity in mammals are required before an assessment of the genetic hazards presented by VC to man will be possible.

[J. Hopkins]