5.2.0 Vinyl Chloride

VINYL CHLORIDE—PART II

The first instalment of this review (BIBRA Bull. 1976, 15, 6) was concerned principally with the development of acro-osteolysis and haemangiosarcoma in workers exposed industrially to vinyl chloride (VC). This part continues with a description of the clinical and histological characteristics of VC-induced angiosarcoma, with animal studies on the monomer and with its probable metabolism and mechanism of action.

Angiosarcoma and its clinical and histological precursors

On the basis of a study of pathological material from a group of VC-PVC workers, Thomas & Popper (Ann. N.Y. Acad. Sci. 1975, 246, 268) have reported that the livers of patients dying as a result of angiosarcoma were abnormally large, averaging over 4 kg, and were massively involved with cystic blood-filled tumours which replaced most of the liver tissue. In the larger specimens, some of the cystic spaces were several centimetres in diameter and were associated with large areas of fibrosis, haemorrhage and necrosis. The haemorrhage caused by the rupture of these cavernous cysts was the immediate anatomical cause of death in several patients.

Prior to tumour development, the histological alterations of the liver following VC exposure are in most cases relatively discrete and unspecific (Gedigk et al. ibid 1975, 246, 278). Degenerative lesions, adaptive responses and fibrosis occur, as in many other types of hepatotoxic damage, but the degenerative areas are sharply demarcated from the undamaged parenchyma; this pattern of degenerative lesion in conjunction with the activation and proliferation of the sinusoidal cells and hepatocytes may be considered characteristic of VC damage. The proliferation of sinusoidal cells is similar to that seen in arsenic poisoning.

The severity of the degenerative lesions, development of septal fibrosis and changes in the sinusoidal cells have all been found to be dependent on the time of exposure to VC. However, in contrast to the degenerative changes, which were found to be reversible upon removal of the patient from VC exposure, the proliferative activity does not appear to decrease and may finally converge into a tumour. From studies of the livers of workers exposed to VC, it has been suggested that the fibrosis may be a precursor in the development of angiosarcoma (Popper & Thomas, ibid 1975, 246, 172), a probability supported by findings in some animal studies (see following page). The persistence of proliferative activity was thought to occur only after severe exposure of sufficiently long (> 10 years) duration (Gedigk et al. loc. cit.). In a single case of lesser exposure (lasting 5·5 years) to high levels of VC, the activation of both hepatocytes and sinusoidal lining cells was virtually absent after cessation of exposure, although fibrosis persisted (Berk et al. ibid 1975, 246, 70).

Histological features associated specifically with angiosarcoma have suggested a continuous spectrum of changes initially manifest by multifocal areas of stimulated sinusoidal cells followed by increasing degrees of sinusoidal cell atypia and proliferation associated with sinusoidal dilatation and culminating in a progressively growing infiltrative angiosarcoma with a sinusoidal growth pattern. Further growth of the sinusoidal type of angiosarcoma results in the observed papillary and cavernous growth patterns (Thomas & Popper, loc. cit.). The series of changes in the liver appear to be multicentric, but only some of the lesions progress to fully developed angiosarcomas that become clinically evident. Because of the multicentricity of this development, however, surgical treatment is not possible and

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chemotherapy must be relied upon. In one patient deposits of angiosarcoma were observed in many other organs in addition to the liver and results of further investigations of this nature are awaited (Thomas & Popper, loc. cit.). These authors consider that at least some of these extrahepatic tumours were independent primary neoplasms, but this view has not yet been confirmed.

The pathway by which VC activates and stimulates the sinusoidal cells and the hepatocytes, and the correlation between the two, are still unknown (Gedigk, loc. cit.; Thomas & Popper, loc. cit.) but animal studies are increasing our understanding of the mechanisms involved.

Animal studies

The pioneer study on exposure of rats to VC (BIBRA Bull. 1972, 11, 41) involved exposure to high concentrations (30,000 ppm) for 4 hours daily on 5 days/week over a 12-month period. Subsequent interpretation (Maltoni & Lefemine, Ann. N.Y. Acad. Sci. 1975, 246, 195) suggested that the malignant skin tumours observed in this study were carcinomas arising from the Zymbal gland (a sebaceous gland of the exterior acoustic duct), which is responsive to a large number of carcinogens, and that the malignancies of the lung were metastases from Zymbal-gland tumours.

Preliminary results of experiments designed to study the effects of VC administered to animals by different routes and under different conditions have now been reported (Maltoni & Lefemine, loc. cit.). VC administered by inhalation produced a range of tumours in all the animal species studied (rats, mice and hamsters), liver angiosarcomas being observed in all three species. Repeated daily exposure comparable to that used in the early test had a carcinogenic effect in both rats and mice at levels down to 50 ppm, the incidence of angiosarcomas and nephroblastomas being dose related in the lower part of the dose range. Initial results in the rat also showed that the neoplastic response was affected by the length of exposure to VC, that it varied with different strains and that there was apparently a trans-placental effect. During the discussion arising from the presentation of his paper at a symposium on VC (Annals of the New York Academy of Sciences 1975, 246, 221), Professor Maltoni explained that fibrosis was observed only in some of the livers in which angiosarcomas were present and was thought to be a transitional stage in tumour development following exposure to the lower levels of VC. Administration of VC by oral intubation (BIBRA Bull. 1975, 14, 153) also resulted in the occurrence of angiosarcoma.

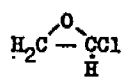
The effects of a single 6-hour exposure to 50,000 ppm VC in air on rats pretreated with phenobarbitone (PB) have been studied (Jaeger et al. Nature, Lond. 1974, 252, 724). Acute biochemical and histological changes were observed in these rats, but in rats that had not been pretreated, exposure to the same level of VC caused no abnormality. In PB-treated rats, marked vacuolization of the centrilobular parenchymal cells and focal necrosis of the mid-zonal parenchyma were observed. PB induces components of the hepatic mixed-function-oxidase system, an intracellular enzyme complex located within the membranes of the endoplasmic reticulum. The observation that PB enhanced injury from VC exposure, coupled with the fact that acute VC-induced liver lesions involve the endoplasmic reticulum, suggests that this organelle is the primary site of generation of toxic metabolites from VC, as it is known to be for other halogenated hydrocarbons. Ultrastructural observations (Reynolds et al. Envir. Hlth Perspect. 1975, 11, 227) have supported this hypothesis.

Rats pretreated with PB and exposed to VC at a level of 50,000 ppm for 6 hours on five consecutive days showed no biochemical abnormalities, and histological findings were not indicative of recurrent acute injury, suggesting that injury following the first exposure to VC protects against further injury for at least 5 days and allows healing of the initial lesion (Jaeger et al. loc. cit.).

Similar experiments were carried out involving pretreatment either with PB or with 3-methylcholanthrene, another inducer of the mixed-function oxidase system (Drew et al. Envir. Hlth Perspect. 1975, 11, 235). After exposure to 13,500 ppm VC for 6 hours/day for 10 days, morphological changes in the liver similar to those resulting from a single exposure, as described by Jaeger et al. (loc. cit.), were observed in only two rats, both from the group of four pretreated with PB. Of the other parameters investigated, including growth rate, organ weights and biochemical changes, only growth rates showed a significant difference from controls. On day 3 of the VC exposure, a marked decrease in growth rate was observed in PB-pretreated rats, but not in the other groups.

Metabolism and mechanism of action

Data on the structure-activity relationships of direct-acting alkylating carcinogens and on the mechanism of action of chemical carcinogens in general have been reviewed for their relevance to VC (Van Duuren, Ann. N.Y. Acad. Sci. 1975, 246, 258). Since VC is a small relatively unreactive molecule compared with known direct-acting carcinogens, it seems likely that metabolism to activated carcinogenic intermediates occurs. Likely intermediates, particularly in the liver, are epoxides, since although epoxidizing enzymes are present in many organs and tissues at low levels, they are particularly rich in the liver. A favoured structure for the active carcinogenic intermediate is chloroethylene oxide:



Since this is not only an epoxide but also an α-haloether, it belongs to two groups of compounds known to include carcinogens. A similar compound has been suggested as a major intermediate of trichloroethylene.

Like other α-haloethers, chloroethylene oxide has a strong alkylating potential and a very short half-life (1·6 minutes) in neutral aqueous solution, comparable with the half-life of less than 2 minutes for bis(chloromethyl) ether (Huberman et al. Int. J. Cancer 1975, 16, 639).

Preliminary studies (Hefner et al. Ann. N.Y. Acad. Sci. 1975, 246, 135) on the fate of VC inhaled by rats indicated that, when present at levels below 100 ppm in the inhaled air, it was metabolized mainly via the alcohol dehydrogenase pathway, involving sequential oxidation to 2-chloroethanol, chloroacetaldehyde and monochloracetic acid. At higher exposure levels, this pathway appeared to be saturated and it was speculated that one alternative pathway might involve the direct epoxidation of VC by microsomal oxidases, and subsequent rearrangement of the resulting chloroethylene oxide to chloroacetaldehyde.

Isolated chloroethylene oxide rearranges spontaneously to chloroacetaldehyde, which will react with glutathione to give S-carboxymethylglutathione as a final metabolic product (Van Duuren, loc. cit.). This is consistent with the reduction of non-protein sulphydryl levels in the livers of VC-exposed rats, and with the detection of metabolites conjugated

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If the inferences of Hefner et al. (loc. cit.) are correct, the metabolism of VC to carcinogenic intermediates only after saturation of a primary metabolic pathway, which produces no carcinogens, offers hope for the existence of a no-effect level for VC.

Since angiosarcoma of the liver is a rare disease in man, consideration of other chemicals that cause the same type of tumour is worthwhile. Arsenic causes liver effects similar to those of VC and the mechanism for its toxicity has been shown to be via its reaction with 6,8-dithiooctanoic acid (α-lipoic acid), in which arsenic forms a stable bridge between two sulphydryl groups. Chloroethylene oxide would react similarly with α-lipoic acid (Hefner et al. loc. cit.).

It is commonly accepted that many chemical carcinogens exert their effects by binding covalently to cellular macromolecules. Support for this in connexion with VC intermediates has been obtained by incubation of ^{14}C-labelled VC with rat-liver microsomes (Bolt et al. Lancet 1975, I, 1425). VC metabolites were observed to bind covalently to microsomal protein, and to other SH-containing proteins and also to RNA if these were added, but only in the presence of NADPH. Such covalent binding to protein was depressed by the addition of glutathione and by an inhibitor of the microsomal mixed-function oxidizing system (Kappus et al. Nature, Lond. 1975, 257, 134).

O_2^- radicals are known to be involved in epoxidation by the microsomal enzyme system and appear to convert VC to a metabolite which binds covalently to a protein like albumin (Kappus et al. loc. cit.). Experiments using the xanthine-oxidase model system, which generates H_2O_2 and the O_2^- radical, bound VC metabolites to albumin, whereas a control experiment with albumin and H_2O_2 did not, thus offering further support for the hypothesis of an epoxide intermediate.

Mutagenicity

Most chemical carcinogens show a mutagenic effect after metabolic activation (de Serres, Mutation Res. 1975, 33, 11). In vitro mutagenicity studies in strains of Salmonella typhimurium on VC incubated with the 9000 g supernatant of liver homogenates from rat, mouse and man indicated (Bartsch et al. Int. J. Cancer 1975, 15, 429) that the liver will efficiently convert VC into mutagenic metabolites. It is not clear, however, which VC metabolites are responsible, nor whether these are identical with those responsible for carcinogenicity. The results with livers of PB-pretreated animals again suggest that microsomal mixed-function oxidases play a role in VC biotransformation.

Mutagenicity studies with specific metabolites of VC showed chloroethylene oxide to cause a mutagenic response with a much lower toxicity than was exerted by an equimolar concentration of chloroacetaldehyde, thus demonstrating that chloroethylene oxide acts as a mutagen per se and not exclusively via its spontaneous rearrangement product, chloroacetaldehyde. Both of these compounds were strongly mutagenic compared with 2-chloroethanol and monochloracetic acid (Huberman et al. loc. cit.).

A study of 56 workers exposed to various levels of VC and compared with 24 not so exposed (Purchase et al. Lancet 1975, II, 410) suggested that VC might have a detectable effect on chromosomal aberrations in man. Lymphocyte cultures prepared from blood samples from both groups showed a

significantly ($P < 0.05$) increased percentage of B, Cu and Cs cells in the exposed workers.

In a dominant lethal study in mice, males exposed to 3000–30,000 ppm VC for 6 hours/day on five consecutive days were mated with non-exposed females (Purchase et al. loc. cit.). There was no significant increase in the number of early deaths per implantation compared with a control group not exposed to VC. Thus the mutagenic effects of VC appear not to occur in the germ cells, possibly because active metabolites responsible for the toxic effects do not reach the testis. A potential danger to the foetus from mutagenic effects transmitted via the sperm does not seem, therefore, to exist.

Exposure levels

Analytical methods for the detection of VC in beverages, vegetable oils and vinegars have been developed using gas-liquid chromatography with a flame-ionization detector, confirmation being possible by mass spectrometry (Williams & Miles, J. Ass. off. analyt. Chem. 1975, 58, 272). VC was only detected in foodstuffs packaged in such a way that there was contact with PVC. Levels in alcoholic beverages ranged from 0 to 1·6 μg/ml, in vinegar from 0 to 8·4 μg/ml and in peanut oil from 0·3 to 3·3 μg/ml. It was not possible to assess which foodstuffs were more susceptible to VC contamination, owing to the large variety of types of PVC used.

Controlled storage tests using PVC containers of known residual monomer content (Tester & Moffitt, Paper 4b, presented to the HPF/PRI Joint Conference on "Vinyl Chloride and Safety at Work", held at Hayes, Middx, on 28 May 1975) have shown that levels of extraction of VC into the contents are of the same order for a wide range of foodstuffs and that the rate of extraction increases with temperature. Maximum extraction levels after long periods of time indicate a partition between the PVC container and its contents, such that the concentration of VC is always much higher in the PVC. At 23°C, foodstuffs stored in containers made of PVC with a residual monomer content of 30 ppm gave maximum concentrations of VC in the contents as follows: water, 0·14 ppm; orange squash, 0·05 ppm; maize oil, 0·10 ppm. Margarine stored in containers with residual VC contents of about 80 ppm contained a maximum of 0·08 ppm VC at the container wall (0·05 ppm in the centre of the margarine) when stored at room temperature and 0·01 and 0·006 ppm, respectively, when stored at 5°C. Residual monomer concentrations in PVC bottles of current manufacture are 5 ppm or less and in PVC containers for margarine 10 ppm or less.

A higher exposure to VC is likely to occur through the use of aerosol sprays containing VC. Analysis of air concentrations (Gay et al. Ann. N.Y. Acad. Sci. 1975, 246, 286) showed that the user of such sprays is exposed to high peak concentrations of VC even with good room ventilation, and in smaller rooms the concentration persists for some time. A decrease in VC concentration with time appeared to be a dilution effect of room ventilation. The peak concentration recorded within the breathing zone was 380 ppm 1 minute after a 30-second release of spray.

Legislation and recommendations on VC

Current uncertainties about the effects of exposure to low levels of VC are reflected in recommended maximum working concentrations. Within the EEC countries, the maximum allowable concentration (MAC) for VC varies between 5 and 100 ppm (Official Journal of the European Communities 1975,

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Data on migration of VC from different types of PVC container to specific foodstuffs is lacking and hence regulations or recommendations are scarce. In Canada, it is proposed to prohibit the sale of food in packages that may yield any amount of VC to the food (ibid 1975, 14, 188). In the USA, testing of PVC products is in progress to comply with proposed restrictions, which will limit the use of PVC in contact with food to cases where potential migration of VC from container to food is negligible (ibid 1975, 14, 472). Such regulations, of course, depend upon the limits of detection of analytical techniques, which may be expected to decrease as methods improve.

Conclusion

In the 3-year period following the first reports of liver angiosarcoma in PVC-production workers, concern over VC toxicity has rapidly increased. The results of experimental and epidemiological studies are of necessity preliminary, but certain points have been established. The association of angiosarcoma incidence with VC exposure has been substantiated by the results of animal experiments, and available data point to the implication of chloroethylene oxide as a carcinogenic intermediate. Further results of such studies are awaited, but of greater urgency is the development of a test for the early diagnosis of angiosarcoma and of its precursor lesions in persons exposed to VC.

Industrial exposure to VC has, in general, been greatly reduced, but ultimate legislation will depend on whether a no-effect level for VC can be established. The major source of domestic exposure is VC-containing sprays. VC is also present in some foodstuffs, as a result of contact with PVC, but forthcoming legislation is likely to reduce the permitted levels to below analytical limits of detection.

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