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The Hepatic Role in Carcinogenesis and Its Early Detection—The Vinyl Chloride Model^{1,2}

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The liver's role in vinyl chloride toxicity and carcinogenicity is providing a better understanding of the chemical carcinogenesis mechanism. A variety of both malignant and benign hepatic tumors has been demonstrated with prolonged exposure to vinyl chloride. The multi-system involvement of this carcinogen and toxin has provided a model for the study of chemical carcinogenesis common to both man and animal. Clinical studies have shown the usefulness of biochemical, radioisotopic, and radiological studies in the detection of toxic and carcinogenic lesions. Animal studies have demonstrated the biochemical metabolism by the liver of vinyl chloride-produced intermediates which are mutagenic in bacterial systems and may be the ultimate carcinogens. Hepatic subcellular enzyme studies prove preliminary evidence of cellular adaptation and increased detoxification. Disruption of this oxidization and detoxification balance may be the key to the malignant transformation of cells. A working hypothesis is presented which may explain the metabolism of vinyl chloride into mutagenic intermediates by the liver cell and the development of malignant transformation by extra hepatic sinusoidal lining cells, lung cells, and brain tissue.

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INTRODUCTION

Currently there is a growing concern that chemical compounds may be responsible for most human cancer through environmental contact. Although 100,000 to 200,000 new chemicals are introduced into industry each year, little is known about their effects. These compounds are primarily synthetics and thus not natural to the environment. The view that industrial chemicals may be latent carcinogenic hazards has again been brought into sharp focus by the discovery of vinyl chloride-induced angiosarcoma. Vinyl chloride ($\text{CH}_2 = \text{CH}-\text{Cl}$ monochloroethylene, a gas) is the basic molecule or monomer of polyvinyl chloride and its co-polymers and one of the most important organic intermediates in the plastics industry. The resulting plastic resin, polyvinyl chloride, is used in innumerable consumer and industrial products, such as containers, wrapping film, electrical insulation, pipelines, credit cards, etc. Until recently (early 1970's) vinyl chloride was regarded as being relatively non-toxic [1,2]. Initially this opinion seemed to be supported by the facts that it was used transiently as an anaesthetic and had been used commercially in industry for many years [3].

The direct information on the toxicity of vinyl chloride to man was obtained from experiments by research workers on themselves, from the evaluation of its suit-

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²This article is the thirteenth in a series entitled, "Seminars on Liver Disease," that have been presented as part of the Training Program in Liver Disease at the Veterans Administration Hospital, West Haven, Connecticut. Dr. Harold O. Conn, Professor of Medicine, Yale University School of Medicine, and Director of the Training Program in Liver Disease, is guest editor.

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ability as an anaesthetic [4, 5], and from study of the cases of vinyl chloride poisoning contracted during industrial use [6, 7, 8, 9].

Sporadic studies with vinyl chloride polymerization workers demonstrated varying degrees of hepatic biochemical derangements, hematological abnormalities, and skin changes [9]. Acute short term exposures led to disturbances of the central nervous system, cardiac arrhythmias, severe irritation of the mucosal membrane of the eyes and the respiratory tract, and in some cases—severe pulmonary edema with obstruction of the liver and kidneys. Chronic inhalation trials in animals clearly showed that vinyl chloride was toxic to the liver and kidneys as well as irritating to the mucosal membranes and the lungs. Microscopic examination of liver sections showed degeneration of the central lobules while the damage to the kidneys chiefly involved the tubule and the interstitial lining somewhat like that of carbon tetrachloride damage [10, 11, 12].

BACKGROUND

The literature, however, contained virtually no information on damage to man after chronic exposure until Filatova et al. reported disturbances of the blood vessels and nerves in individuals exposed with 20–300 ppm of vinyl chloride on a continuous basis [13]. Cordier et al. [14] and Wilson et al. [15] were the first to report the hitherto unrecognized disorder termed occupational acroosteolysis (AOL) which included the symptoms of tenderness of the fingertips, gradual destruction of bony integrity of the fingers and a Raynaud's-like phenomena.

Studies were initiated in animals to reproduce the acroosteolysis. In 1971, Violi et al. [16] while exposing animals to 30,000 ppm to induce acroosteolysis, accidentally discovered cancer. Maltoni et al. [17] while studying various levels of exposure demonstrated that angiosarcoma occurred at 250 ppm; the Manufacturing Chemists Association's studies [18] demonstrated these liver cancers even at 50 ppm. At the same time, Dr. John Creech, at the B.F. Goodrich Chemical Company Plant in Louisville, Kentucky, discovered an hepatic angiosarcoma in one employee. Creech, recalling an earlier hepatic angiosarcoma at the plant, reviewed the medical histories of previous employees. Four additional angiosarcomas were found, further supporting the connection between vinyl chloride exposure and tumor development. Measures to control the levels of vinyl chloride exposure were then instituted following federal regulation.

THE CHEMICAL

Vinyl chloride's chemical structure is a double-bonded, 2-carbon halogenated hydrocarbon which has structural similarities to tri-chloroethylene, an inhalation anaesthetic. As previously noted, vinyl chloride was once considered as an anaesthetic but was discarded because it caused myocardial irritability. Some of its important physical properties include a low boiling point, a high specific gravity, a low solubility in water, and a half-life in air which ranges from 3–20 hours. Knowledge of these physical properties may be important in determining how this agent produces a cumulative effect in the environment which ultimately leads to cancer formation.

Vinyl chloride is both toxic and carcinogenic, as recognized by the wide variety of associated disorders which have been found among vinyl chloride polymerization workers and vinyl chloride-exposed animals. A yet incomplete list of these

TABLE 1
Occupational Vinyl Chloride
Exposure Associated Disorders

-
1. Thrombocytopenia
 2. Reticulocytosis
 3. Splenomegaly
 4. Hepatic fibrosis
 5. Scleroderma-like skin changes
 6. Acro-osteolysis
 7. Raynaud's phenomenon
 8. Leukopenia
 9. Hepatomegaly
 10. Pulmonary functional impairment
 11. Angiosarcoma
 12. Cardiac arrhythmia
 13. Nephroblastomas*
 14. Zymbal gland carcinomas*
 15. Large cell lung cancer
 16. Brain cancer
-

*In rats only

associated disorders is shown in Table 1. Animal and epidemiological studies indicate the probability that cancer induction at other sites is also directly attributed to prolonged and excessive vinyl chloride exposure [19].

In order to develop effective methods of prevention, accurate knowledge of the pathogenesis of this environmental chemical in ultimately producing its most destructive effect—cancer—is needed.

EXPERIMENTAL ANIMAL STUDIES—TUMOR FORMATION

The carcinogenicity of vinyl chloride has been demonstrated in a variety of animals as well as in humans at exposure levels that vary from 50–30,000 ppm. As illustrated in Table 2, a variety of tumor types have been found in rats, mice, and hamsters. An extensive list of benign tumors have also been reported in mice, rats, and hamsters exposed to vinyl chloride. Maltoni's group has now demonstrated primary liver cell cancers in exposed newborn rats.

Direct hepatocellular injury as well as pulmonary, mucosal and skin injuries have been shown in directly exposed animals. Pretreatment with many agents increases the toxicity of vinyl chloride; they include phenobarbital, ethanol, polychlorinated biphenyls and pesticides such as hexachlorobenzene [20]. This relationship to vinyl chloride's ability to induce cancer is under study, particularly in view of the industrial environment which allows exposure to many chemicals to occur concurrently.

HUMAN STUDIES

Epidemiological studies in the human strongly suggest that exposure beyond 10 years is associated with increased cancer mortality, mainly digestive system cancers, primarily hepatic [21, 22]. There also appears to be a higher incidence of large cell carcinomas of the lung, brain glioblastoma multiformis, and lymphomas [23, 24], although there is some disagreement as to the interpretation of this aspect of the epidemiological data.

TABLE 2
Carcinogenicity of Vinyl Chloride
(Exposure: 50-10,000 ppm)

Tumor Type	Species
1. Liver—angiosarcoma	Adult humans, rats, mice and hamsters
2. Liver—hepatocellular carcinoma	Newborn rats
3. Lung—adenocarcinoma	Rats and mice
4. Lung—large cell carcinoma	Humans*
5. Mammary adenocarcinoma	Mice
6. Zymbal gland tumors	Rats
7. Nephroblastoma	Rats
8. Osteochondromas	Rats
9. Skin epitheliomas	Hamsters
10. Melanomas	Hamsters
11. Glioblastoma multiforme	Humans*
12. Lymphoma	Humans*

*Strongly suggested epidemiologically

The multisystem involvement of this carcinogenic and toxic chemical is further illustrated in man. Early physical findings of vinyl chloride-injury include hepatomegaly, portal hypertension, possible mild systemic pulmonary hypertension, bilateral midzonal pleural thickening of the lung, and splenomegaly with and without increased portal pressure [25]. These anatomical and physiological findings most frequently occur in the absence of the traditional clinical biochemical derangement of the liver. Screening studies of vinyl chloride workers during the past two and a half years have clearly illustrated the irregular and often delayed appearance of abnormalities in aspartate and alanine aminotransferases (SGOT and SGPT), alkaline phosphatase as well as other hepatocellular enzymes. Gamma glutamic transpeptidase (GGTP), believed to be a more sensitive indicator of hepatocellular injury, has proven to have too high a false-positive rate to warrant its use in the screens for hepatocellular injury. Sorbitol dehydrogenase (SDH) studies indicate that this enzyme, which is liver tissue specific, is too insensitive for primary screening but is useful for confirmatory testing.

Anionic dye clearance studies (Indocyanine Green, ICG) have demonstrated the highest specificity and sensitivity of all the primary screening procedures tested when performed at the 5 mg/kg dose level. At the traditional 0.5 mg/kg level, it has the same effectiveness as the aminotransferases and alkaline phosphatase combined. The frequency of abnormal ICG dye clearances increases with prolonged exposure to vinyl chloride as illustrated in Fig. 1 and correlates well with the cumulative exposure to vinyl chloride as well as the histological evidence of hepatocellular injury. These functional studies for detecting hepatocellular injury do not, however, identify the cause.

Clinical studies have illustrated the usefulness of radioisotopic liver-spleen scans as a primary screening procedure for anatomical lesions of the liver and spleen. This procedure has proven to be the single most reliable method of tumor detection. Sixteen of the 19 individuals with anatomical lesions were detected by liver-spleen scan. In contrast, only 32 of 950 normal individuals had scan abnormalities which further diagnostic studies proved incorrect. This method provides an 84% sensitivity and 97% specificity, with only a 3% false-positive rate.

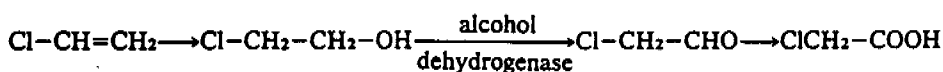
Diagnostic angiographic studies of these radioisotopic abnormalities in 80 individuals have demonstrated 3 major lesions. The first is peliosis hepatis, illustrated in Fig. 2. These lesions are usually numerous involving the entire liver, and have a diffuse stain throughout the nodules which persists into the late venous phase without central hypovascularity [26].

A second, similar lesion has been discovered in individuals with splenomegaly, and named lienal peliosis (Fig. 3). These splenic lesions demonstrate a shortened celiac artery to portal vein circulation time, normal portal vein diameters, and increased spleen size. This has been found only in individuals with long-term vinyl chloride exposure.

The final lesion is that of angiosarcoma (Fig. 4). This tumor has characteristic angiographic features of central hypovascularity, midarterial puddling, and a prolonged peripheral tumor stain which continues up to 30–36 seconds after injection. These characteristic findings have allowed differentiation from other primary hepatocellular cancers, benign tumors and benign vascular lesions [26]. These angiographic lesions have been pathologically confirmed with the additional histological finding including peliosis hepatis, sinusoidal dilatation, and activated sinusoidal cells with increased deposits of collagen in the sinusoidal space of Disse. Exploratory wedge biopsies have in addition demonstrated increased subcapsular fibrosis with subcapsular bile duct proliferation plus the often described portal fibrosis [27].

VINYL CHLORIDE METABOLISM AND CARCINOGENESIS

Present biochemical knowledge indicates that vinyl chloride is most likely metabolized by the liver in a three step process [28]. At concentrations less than 50 ppm, vinyl chloride is metabolized by the alcoholic dehydrogenase system into chloroacetaldehyde and monochloroacetic acid.



An alternative pathway which appears to become operative at 220 ppm is oxidation by the peroxidase-catalase system.

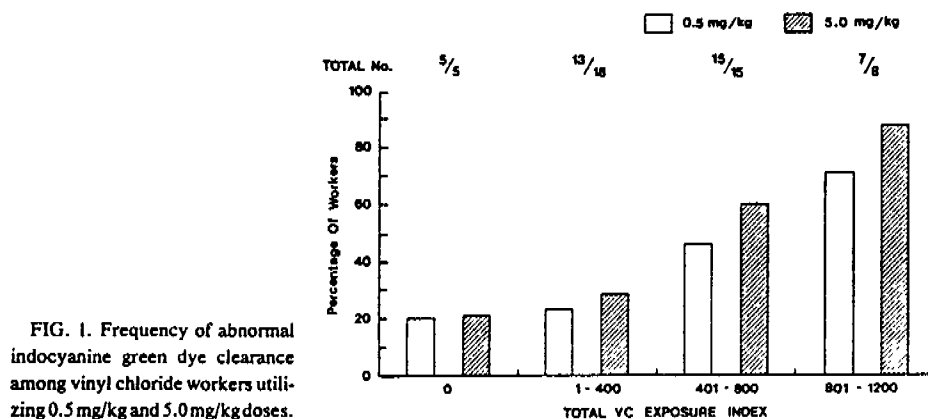


FIG. 1. Frequency of abnormal indocyanine green dye clearance among vinyl chloride workers utilizing 0.5 mg/kg and 5.0 mg/kg doses.



FIG. 2. Hepatic arteriogram: Venous phase. Changes of peliosis hepatis are present throughout the left lobe. The multiple nodular stains represent peliosis hepatis lesions (*arrows*) ranging from 2-3 mm to 2 cm in size.

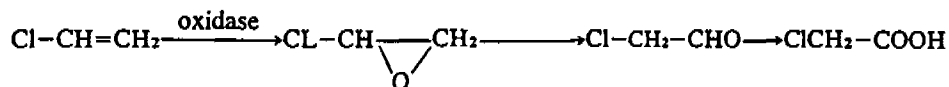


FIG. 3. Splenic arteriogram: Venous phase (15 seconds). There are 3-4 circular and oval stains (*arrows*) in the superior and inferior-lateral portions of the spleen. Normal pancreatic stain occurs just above the splenic vein.



FIG. 4. Hepatic arteriogram: Venous phase. At approximately 14–15 seconds the peripheral stain is identified (arrows), lasting through the entire phase. Scattered areas of puddling are also present in and around the area of central hypovascularity.

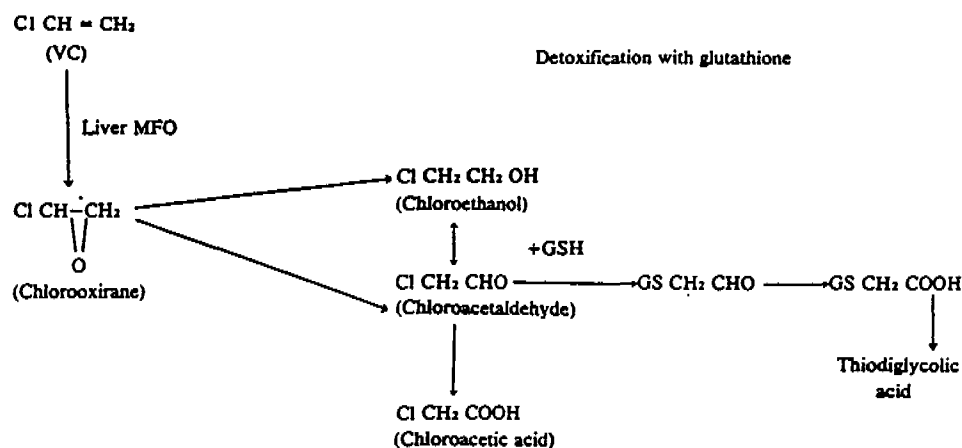
In this case chloroacetaldehyde is again formed. At higher levels oxidation appears to be by the mixed function oxidase system, forming chloroethylene oxide which spontaneously rearranges to form chloroacetaldehyde which then can be further oxidized to form monochloroacetic acid.



As illustrated in Table 3, vinyl chloride oxidation intermediates chloroethanol and chloroacetaldehyde, at low doses, are most likely detoxified via the glutathione-cysteine conjugation system. This system, however, is saturable and at higher levels vinyl chloride is excreted via the lungs [28]. It appears that at higher vinyl chloride levels increased amounts of chloroethanol and chloroacetaldehyde are further oxidized to chloroacetic acid which is excreted in the urine. This is further supported by the absence of chloroacetic acid in urines of rats exposed to low, short term levels of vinyl chloride but found in urine of rats exposed to 5,000 ppm for an extended time and reported in workers exposed to levels greater than 250 ppm for a prolonged time [29, 30].

Elmore et al., utilizing a modified Ames system and pure synthesized vinyl chloride intermediates, has demonstrated that vinyl chloride, chloroethanol, and

TABLE 3
Proposed Metabolic Fate of Vinyl Chloride



chloroacetic acid are not mutagenic in bacteriological systems [31]. This may indicate that the vinyl chloride monomer is neither hepatotoxic nor carcinogenic until it has been metabolized to its intermediate forms by the liver and/or other tissues.

Alternatively, chloroethanol—the most transportable of the vinyl chloride metabolites—may be transferred or diffused to adjacent cells, such as the sinusoidal lining cells, where it could be converted to chloroacetaldehyde but less likely to be detoxified or further oxidized. Since vinyl chloride appears to bind the serum albumin, it may, itself be transported to and oxidized by extra-hepatic cells which are unable to fully oxidize or completely detoxify its metabolites, and thereby lead to molecular DNA injury and cancer formation at distant tissue sites.

These quandaries led to further study of the hepatochemical changes in rats undergoing progressively increased exposure to vinyl chloride. Subcellular enzymes and metabolites were studied in animals exposed to from 10–20,000 ppm vinyl chloride, ranging from 14–137 hours. Microsomal enzymes, including P-450, NADPH cytochrome *c* reductase, and mixed function oxidases were studied. Determinations of cytochrome *c* oxidase as the mitochondrial, tritiated-leucine incorporation as the protein synthesis and glucose-6-phosphatase as the carbohydrate metabolism markers were also done. Glutathione and glutathione reductase as oxidative and detoxification markers were determined in addition to the conventional clinical biochemical studies which included the aspartate (SGOT) and alanine aminotransferase (SGPT), alkaline phosphatase, bilirubin, lactic acid dehydrogenase (LDH), total protein, albumin, cholesterol, and triglycerides.

During the entire 137 hours of exposure there were no significant changes in the mitochondrial and the microsomal enzymes, the tritiated-leucine incorporation, or in the glutathione content. There was however, after 71 hours, a rise in the glutathione reductase and a concomitant fall in glucose-6-phosphate. This occurred without any histologically discernible changes in the hepatocytes by light microscopy nor any significant changes in the conventional clinical biochemical studies.

The discovery of a decreased glucose-6-phosphatase after "simulated" chronic exposure led to the study of enzymes in the pentose phosphate shunt pathway. Weber and Lea [32] had found similar changes for primary hepatocellular neoplasms, demonstrating that in a rapidly developing primary hepatocellular tumor, there is decreased gluconeogenesis with a reduction in the glucose-6-phosphatase, followed by an increase in glucose-6-phosphate dehydrogenase and transaldolase. These biochemical changes were also followed by an increase in purine biosynthesis (increased phosphoribosylpyrophosphate aminotransferase (PRPP) and increases in the production of ATP and GTP leading to increased nucleic acid synthesis.

Vinyl chloride-exposed animals showed no significant changes in the glucose-6-phosphatase dehydrogenase activity during the initial 84 hours of exposure. However, after 103 hours, there was significant increase in glucose-6-phosphatase dehydrogenase. Studies of PRPP, at least up to 137 hours, have as yet shown no significant changes. Studies are now underway using animals exposed to 130 to 250 hours to determine if the biochemistry in vinyl chloride injury is similar to that in primary hepatocellular tumors.

This, however, does not explain why the hepatocyte, which is the primary cell for oxidizing and detoxifying vinyl chloride, is not the primary target for cancer transformation. How do the hepatocytic biochemical changes, seen in the early phase of high vinyl chloride exposure, relate to the later morphological changes that occur in the adjacent sinusoidal cells?

MORPHOLOGICAL FINDINGS

Electron microscopic examination of liver sections of mice exposed from 1 to 6 months to 2,500–6,000 ppm vinyl chloride, for 5 hours/day, 5 days/week—a level known to induce angiosarcoma [33]—have demonstrated hepatocellular changes as early as one month. These changes included hypertrophy of the smooth endoplasmic reticulum (believed to reflect vinyl chloride metabolism) and, plasma membrane loss of microvilli with invaginations—possibly reflecting the movement of injurious metabolites across the membrane and out of the cell, allowing the metabolites to be picked up by the sinusoidal cells [34].

The sinusoidal cell reactions were multicellular. Increasing numbers and sizes of lipocytes were seen with little fibrosis. Macrophages were seen filled with phagosomes, sometimes containing long needle-like crystals. Although there were many mononuclear cells present, the main abnormalities were seen in the endothelial lining cells. In the early stages they are larger and thicker—possibly swollen. Later, they became bulky and in places, multi-layered containing increased organelles, especially mitochondria and endoplasmic reticulum. Later disruptions in the sinusoidal walls seen were consistent with beginning peliosis hepatis. The lining cells, probably the precursors of angiosarcoma, often resembled fibroblasts. However, their endoplasmic reticulum did not contain any collagen components. These observations by Schaffner et al. [34] give support to the suggestion that metabolites of vinyl chloride produced in the hepatocytes may be transported through the plasma membrane and enter sinusoidal lining cells, eventually leading to angiosarcoma. Attempts at screening for vinyl chloride hepatic injury might be better aimed at the endothelial cells and the hepatic sinusoidal circulation rather than the hepatocytes.

Our work in humans has identified similar findings. One major difference at

present is an increased collagen deposition, characteristic of human vinyl chloride injury and likely species specific. Light microscopic studies utilizing special stains on hepatic tissue from individuals with extensive exposure to vinyl chloride but without clinical biochemical hepatic abnormalities have shown distinctive midzonal increased deposition of collagen in the space of Disse [35]. Routine light microscopic studies using hematoxylin and eosin failed to easily demonstrate this midzonal increased collagen. The increased deposition along the hepatic cell surface is associated with larger sinusoidal space and activation of the sinusoidal lining cells illustrated by increased nuclear size and cytoplasmic content. The increased collagen deposition, when studied electron microscopically, demonstrates compression of the hepatocytes by the collagen bundles which initially give the appearance of *intra*-hepatocellular collagen bundles as illustrated in Fig. 5. The strands of collagen appear to compress the hepatocytes causing cords of hepatocytes to be broken and to coalesce with adjoining sinusoids eventually leading to peliosis hepatis-like lesions.

These observations led us to the study of the proteroglycan role in collagen formation in vinyl chloride-exposed workers. It had been suggested in the literature that glycosaminoglycans in blood and/or urine might be useful as means of early cancer detection since a number of studies had demonstrated the production of sulfated glycosaminoglycans with malignant states. Pathologists have often used this feature as a diagnostic aid in characterizing malignant vascular tumors of the skin

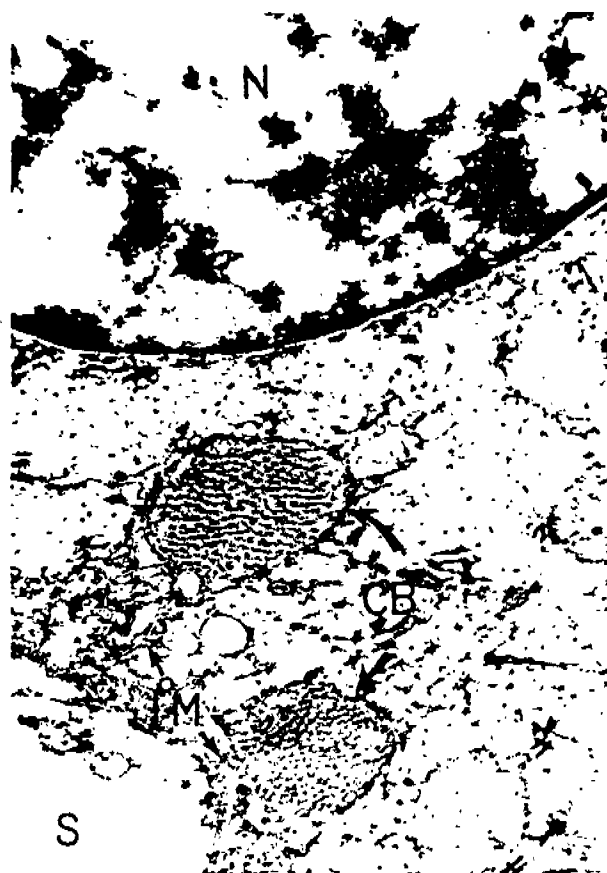


FIG. 5. Electron microscopy showing collagen (CB) bundles (arrows) invaginating into the hepatocyte, giving the appearance of inter-hepatocytic collagen. N = nucleus; S = sinusoidal space; IM = invagination into the cell membrane (small arrows).

[36]. Others have noted a strong positive Alcian blue glycosaminoglycan staining reaction in human angiosarcoma tissue [37]. This suggested that quantitative and qualitative determinations of glycosaminoglycan production in individuals with neoplasm, either by serum or urine, might be used to identify those at high risk or as an early indicator of neoplastic formation. The feasibility of glycosaminoglycan "spot test" for vinyl chloride production workers made this an attractive possibility for mass screening.

Urinary glycosaminoglycans, measured as uronic acid, were studied in individuals with alcoholic cirrhosis, viral hepatitis, secondary liver metastasis, hepatic angiosarcoma and normal controls.

The percentage of total glycosaminoglycans that was dialyzable and the percentage of unfractionated total that appeared in the hyaluronic acid, chondroitin sulfate, or in the heparin fractions was similar for all groups. However the distribution of positive fractions varied with the different groups studied.

Seven of the nine vinyl chloride-exposed individuals, other than those with angiosarcoma, had glycosaminoglycan positive chondroitin sulfate fractions with negative hyaluronic acid and heparin fractions whereas this occurred in only 3 of the 32 urines from other hepatic diseases [38].

In addition, study of the total tissue glycosaminoglycan levels in angiosarcoma tumors and fibrotic tissue adjacent to the tumor demonstrated that tumor tissue itself had higher levels of hyaluronic acid and heparin fractions as compared to the non-tumor adjacent tissue which had higher levels of chondroitin sulfate fractions. A similar relationship was found in cirrhotic liver tissue and normal controls. This data conforms to reports by others that both hepatic connective tissue disorder [39, 40, 41, 42, 43] and hepatic cancer [44] result in increased hepatic glycosaminoglycan levels. It may be significant that the angiosarcoma patient has half the urinary glycosaminoglycan excretion of patients with liver metastasis and that analysis of angiosarcoma tumor tissue exhibits half the glycosaminoglycan content reported by Kojima et al. [44] for hepatocellular carcinoma. The increases in liver and urinary glycosaminoglycans may well reflect the importance of these substances in the process of fibrogenesis and tumor growth. Although no significant differences were found in total glycosaminoglycans of vinyl chloride-exposed individuals with associated liver injury, there was a significant difference in the excretion patterns of these individuals. Seventy-eight percent of them had positive chondroitin sulfate fractions in contrast to only nine percent of the non-exposed liver injury cases. Thus the change in the glycosaminoglycan excretion pattern in individuals with pre-cancerous injuries may be of significant prognostic and diagnostic importance [45].

The urinary glycosaminoglycan excretion patterns in an angiosarcoma patient 3 months to 2 weeks prior to death demonstrated an increase in the urinary chondroitin sulfate fraction with a change in its composition as the disease progressed. During this time, the chondroitin sulfate composition showed a continuous increase in the ratio of the 1.25 M NaCl to the 1.5 M NaCl fractions. This was due to an increase in the 1.25 M eluate and a decrease in the 1.5 M eluate fraction and was 2.3 times greater than the controls. In the most advanced stage of the angiosarcoma the ratio increased to 13.7 times greater [46].

These very preliminary studies would suggest that alterations in the ratios of these fractions' compositions may be useful in evaluating the severity and subsequent progression of disease. Early lesions may produce small changes in the ratio which would become more pronounced as the disease worsened. The

determination of this ratio might also be useful in identifying those individuals with significant injury or continuing progression of disease despite changes in the environment. Finally therapeutic measures for intervention might be better evaluated for their effectiveness in arresting cancer development as reflected by the glycosaminoglycan changes which in turn reflect changes in collagen formation.

SUMMARY AND HYPOTHESIS

Vinyl chloride appears to enter the body through the respiratory tract, the skin, or by swallowing; it is absorbed and transported to the liver by the systemic and portal circulatory systems. In the liver, the primary site of metabolism, it is oxidized by various enzyme systems: alcohol dehydrogenase at lower exposure levels; the peroxidase-catalase system at intermediate levels; and mixed function oxidases at higher levels. At the higher levels, the mixed function oxidase system transforms vinyl chloride to chlorooxiranes which are then spontaneously transformed into chloroethanol and chloroacetaldehyde.

These two intermediate metabolites, chloroethanol and chloroacetaldehyde, are detoxified by conjugation with glutathione and cysteine-SH groups and are excreted in the urine. At even higher doses increasing amounts of the chloroacetaldehyde are further oxidized to chloroacetic acid and excreted as an end product in the urine. However, when chloroacetaldehyde and/or the chlorooxiranes exceed the detoxification threshold of the hepatocyte, this leads to hepatocellular toxicity and/or stimulation of the sinusoidal cells. This acute event in turn acts as a stimulating mechanism for increased collagen deposition in the space of Disse and sinusoidal areas as shown by electron and light microscopy studies. The increase in the collagen deposition in sinusoidal spaces then leads to disruption of hepatic cell surface function, disruption of the hepatic cords, coalition of the sinusoidal spaces and eventual peliosis hepatis. These lesions alternately lead to sufficient vascular dysfunction to add further to the biochemical manifestation of hepatic cellular injury.

Since it is highly unlikely that the unstable chlorooxiranes are able to be transported to adjacent cells and that the chloroacetaldehyde would most likely be conjugated or detoxified within the hepatocyte, an intermediate form, such as chloroethanol, which is transportable from the hepatocyte, may then move on to the adjacent sinusoidal lining cells, or possibly even further to other extrahepatic tissue. At these extrahepatic sites, an intermediate, such as chloroethanol, may then be converted to chloroacetaldehyde. The extrahepatic tissue sites are most likely unable to further convert the chloroacetaldehyde to chloroacetic acid nor to detoxify it sufficiently, if at all, by their own detoxification systems. This would allow a longer contact period with the cell's DNA. In addition many of the extrahepatic cells normally are regenerating at faster rates than hepatocytes, thus increasing the possibility of DNA derangement and ultimate carcinogenesis.

Alternatively, vinyl chloride itself may be taken up by extrahepatic tissue, oxidized but incompletely detoxified, allowing the cell itself to become susceptible to direct DNA injury. In this or similar manner, chemical metabolites may induce injury to the DNA in rapidly replicating cells at sites beyond the liver, thus accounting for other cancers developing with vinyl chloride.

The recent work by Maltoni's group, showing that exposure of newborn rats to the same dose of vinyl chloride as adult rats, results in primary hepatocellular carcinoma (40-45%) rather than in angiosarcoma (8-12%), lends further support to

the concept that the hepatocyte's ability to resist cancer transformation is dependent upon its ability to detoxify the mutagenic/carcinogenic metabolite of vinyl chloride.

This review of our present knowledge of vinyl chloride injury and cancer formation in man is, at best, a very rough hypothetical outline. With continued investigation and study it will allow us to more accurately and completely fill in the missing pieces of this fascinating puzzle, thus leading us to a better understanding of the pathogenesis of chemically induced cancer in the biologically complex human system.

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