BIO-MEDICAL RESEARCH
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Brief Summary

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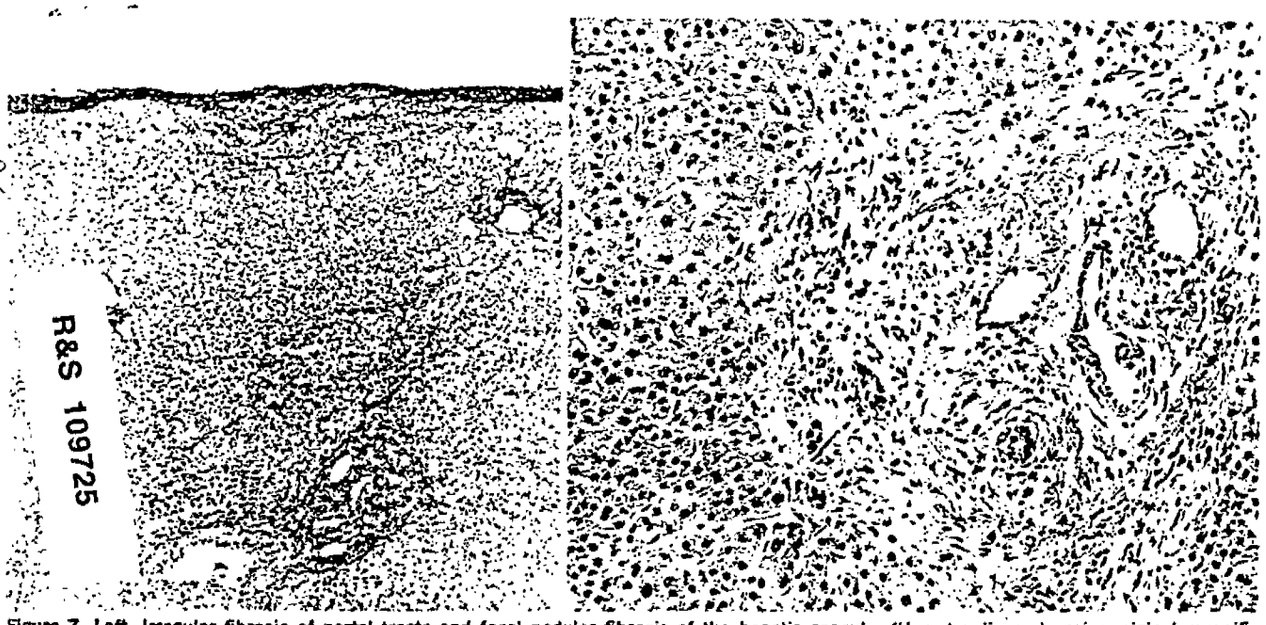

Figure 7. Left. Irregular fibrosis of portal tracts and focal nodular fibrosis of the hepatic capsule. (Hematoxylin and eosin; original magnification, x 45.) NIH accession number S74-411. Right. Portal tract from the same liver shown in Figure 1. The fibrosis interrupts the limiting plate and extends into surrounding hepatic parenchyma. There is a slight infiltrate of lymphocytes and minimal proliferation of bile ducts. (Hematoxylin and eosin; original magnification, x 160). NIH accession number S74-411. (Reproduced by permission, *Ann NY Acad Sci* 246: 174-194, 1975.)

react with other intracellular macromolecules (DNA, RNA, protein, lipids). Although Gillette and others have yet yet attempted to correlate the covalent binding of reactive metabolites to DNA with carcinogenesis, such a reaction has been speculated as a potential mechanism for carcinogenesis.

Recent work in our laboratory and elsewhere, much of still unpublished, has included studies in rats on the e of ingested ^{14}C-vinyl chloride monomer, and the isolation and identification of the major urinary metabolites this chemical. The ingestion study showed that vinyl loride monomer is metabolized to polar products that readily excreted in the urine. These results also support the hypothesis of dose-dependent metabolism. The atic nonprotein sulfhydryl content of rats exposed to l chloride monomer showed a progressive depression ted to the concentration and duration of exposure. sistent with the sulfhydryl depression, two of the three ary metabolites isolated thus far have been identified hiodiglycolic acid and *N*-acetyl-*S* (2-hydroxyethyl) eine. Thus it seems that vinyl chloride monomer or eactive metabolites covalently bind with hepatic glutaand are subsequently hydrolyzed and excreted in ine as conjugates of cysteine.

her studies on vinyl chloride monomer continue to rt the hypothesis that its carcinogenicity is related metabolic formation of reactive metabolites. Studies 9) have shown an enhanced positive mutagenic re- in certain strains of *Salmonella typhimurium* exto vinyl chloride monomer if microsomal enzymes ified liver homogenates are present. The metabolites monomer identified in the urine of rats exposed to inical indicate that the primary deactivating mechanism is by conjugation with the hepatic nonprotein sulfhydryl compounds, glutathione and cysteine. Our current research is directed toward further elucidating the metabolism of vinyl chloride monomer to reactive products and their potential to bind with intracellular macromolecules. Resolution of the metabolism and pharmacokinetics of vinyl chloride monomer will undoubtedly be of great help in resolving its hazards and will ultimately provide a scientific basis for guidelines concerning tolerable levels of exposure.

Histologic Features: Hepatic Fibrosis

Dr. Hans Popper*: Only little more than half a year ago, I became acutely aware of an effect of vinyl chloride exposure on the liver when, within 1 week, three persons called this connection to my attention. Doctor Lelbach from Bonn, Germany, sent a reprint about a peculiar intralobular fibrosis of the liver seen by peritoneoscopy and liver biopsy in workers exposed to gaseous vinyl chloride (7); Dr. Selikoff called me and asked what I knew about hepatic angiosarcoma, a rare tumor newly observed in the same type of workers in this country; and Dr. Thomas, here in Bethesda, showed me histologic slides from several such workers. Subsequently, I have had the privilege of studying, with Dr. Thomas, a large amount of histologic material that has been collected in the Laboratory of Pathology of the National Cancer Institute. The following report is based on this cooperation, and, I hope, shows the heuristic value of environmental pathology (40) in rapidly assembling information that might enlighten other areas of pathology and medicine.

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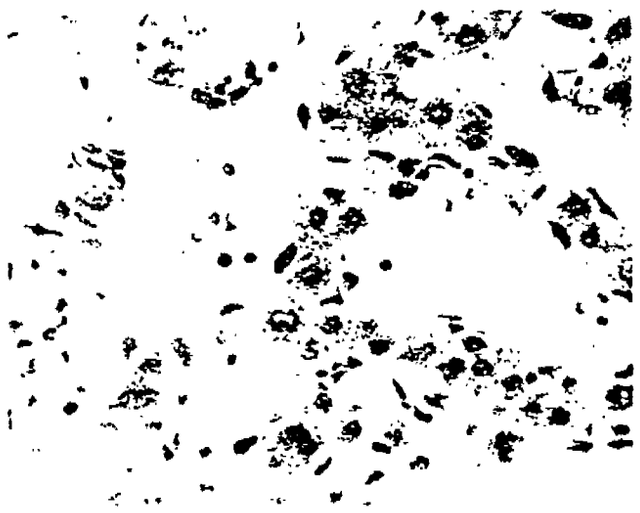

Figure 8. Irregular, focal dilatation of hepatic sinusoids. The size and number of the sinusoidal lining cells are increased. The hepatocytes also vary in size and focally appear to be proliferating. (Hematoxylin and eosin; original magnification, x 400.) NIH accession number S74-415.

In reviewing the serious hepatic lesions encountered in vinyl chloride polymerization workers, we saw that two distinctive lesions were prominent. One, angiosarcoma of the liver, has been the focus of most of the attention in this study, and its histologic features will be presented by Dr. Thomas. The second is characterized by a peculiar form of hepatic fibrosis, which, in many instances, was initially diagnosed as cirrhosis. In some of these workers, the lesion was associated with portal hypertension and variceal hemorrhage, leading to the performance of a portacaval shunt, during which time a wedge biopsy of the liver was obtained. In other cases, the lesion was diagnosed on the basis of a needle aspiration biopsy. It is of considerable interest that, in vinyl chloride workers in West Germany, severe portal fibrosis with significant portal hypertension, splenomegaly, and thombocytopenia—presumably on a splenomegalic basis—is relatively common while angiosarcoma is rare, whereas the relative incidence of these two lesions in American workers seems to be reversed.

The typical lesion of vinyl chloride-associated hepatic fibrosis consists of three features (Figure 7). The first is a typical but nondiagnostic portal fibrosis, varying in degree throughout the liver. In places, it seems aggressive, in that fibrous tissue extending into the lobular parenchyma distorts the limiting plate. It also extends into the walls of the portal vein branches, separating their muscular fibers. Sometimes accumulation of dense connective tissue is associated with proliferation of bile ductules and periductular inflammation. Inflammatory cells also aggregate around some bile ducts, and this pericholangitis might explain the focal canalicular cholestasis seen in some specimens. The second is capsular and subcapsular fibrosis, in a nodular form; it is the most characteristic lesion, particularly visible if the surface of the liver is inspected on peritoneoscopy, as reported from Bonn (7, 20). Distinction from cirrhosis by gross inspection is difficult. The third type of fibrosis is subtle and reflected in a focal intralobular accumulation of connective tissue fibers, recognized distinctly by light microscopy only in connective tissue stains. Electron microscopy (17), by contrast, detects a dense but thin connective tissue coat surrounding the hepatocytes and associated with many fibroblasts and fat-containing mesenchymal cells—Ito cells (41) or lipocytes (42)—which have been postulated to be precursors of fibroblasts (43). This fibrosis differs morphologically from that seen both in chronic active hepatitis and in alcoholic liver disease and suggests a different mechanism of development. Where the intralobular fibrosis is conspicuous, it is associated with bulging sinusoidal lining cells with a prominent diastase-resistant, periodic-acid Schiff reaction of the cytoplasm, which is sometimes granular, as in phagocytosing macrophages, but more typically diffuse. The hepatocytes show no significant changes except that, in the areas of prominent sinusoidal lining cells, they show variation in size of both cytoplasm and nuclei in that large hepatocytes, sometimes even multinuclear, alternate with small cells (Figure 8). In some instances, particularly in autopsy specimens, regenerative nodules are seen without cirrhosis.

Besides this pattern of fibrosis seen in *all* specimens, some specimens—both from patients apparently free of angiosarcoma and in two in whom angiosarcoma was later found—a focal irregular sinusoidal dilatation was noted (Figure 8). It did not seem to be related to passive congestion, since it was not predominantly in the centrolobular area. The same type of focal sinusoidal dilatation, progressing almost to peliosis, has been observed in patients after treatment with anabolic steroids (44) and contraceptive pills (45). However, in these latter instances, there have not been any reports of progression to angiosarcoma. In our vinyl chloride cases, the sinusoidal dilatation is associated with proliferation of the sinusoidal lining cells, which become enlarged and show bizarre nuclei and, as will be discussed below, progress to angiosarcoma. Thus, we can conclude that this sinusoidal dilatation is a precursor to the tumor in vinyl chloride exposure, but we have not yet clearly identified the nature of the cell undergoing malignant transformation.

In cases where we were able to study the spleen, we found it distinctly enlarged, and, particularly on laparoscopy, it showed a nodular but irregular type of capsular fibrosis (7). On the cut surface, the follicles were conspicuously enlarged, in contrast to cirrhotic fibrocongestive splenomegaly. Histologically, fresh hemorrhage was seen in most spleens, although some showed calcification and iron incrustation in the form of Gamna-Gandi bodies. The cords of the pulp contained many erythrocytes, characteristic of hemolysis. The lining cells of the sinuses were activated but, for the most part, not fibrotic.

Study of the pathogenesis of vinyl chloride-associated liver injury (46) showed that vinyl chloride or its metabolites seem to induce a hyperplasia of two types of cells: the mesenchymal sinusoidal lining cells in liver and spleen, and the hepatocytes. In the spleen, activation of the sinusoidal cells may result in splenomegaly, and, in the liver, in fibrosis contributing both to portal hypertension and the formation

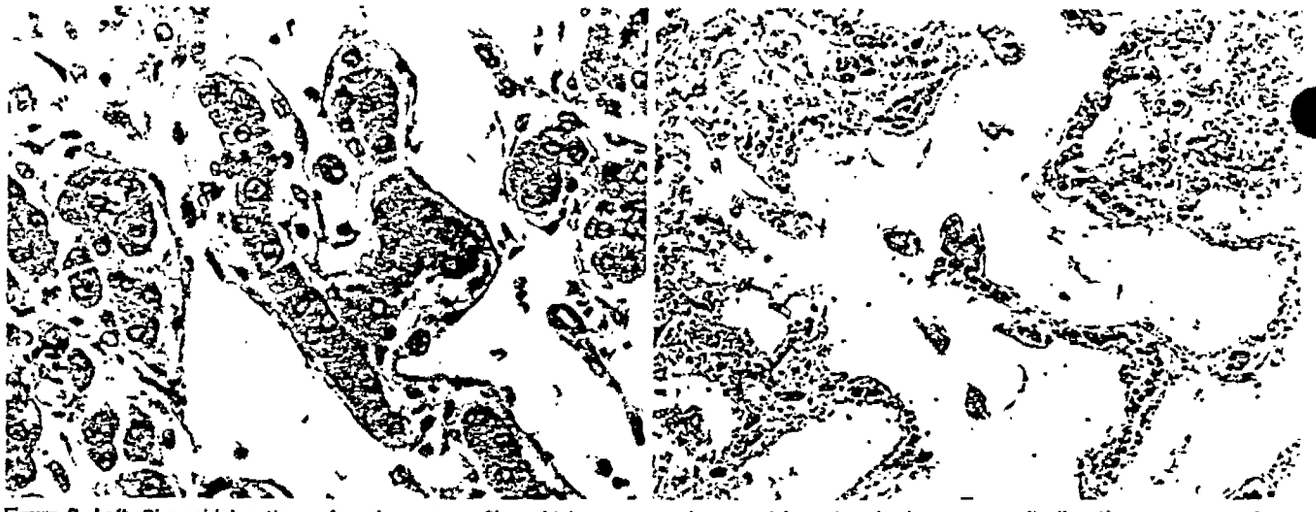

Figure 9. Left. Sinusoidal pattern of angiosarcoma. Sinusoidal spaces are large and irregular. Angiosarcoma cells line these spaces and ensheath hepatic cord cells and an increased number of non-neoplastic-appearing cells in the space of Disse. Bile plugs are present in bile canaliculi. (Hematoxylin and eosin; original magnification, x 392.) NIH accession number A74-31. (Reproduced by permission. *Ann NY Acad Sci* 246:268-277, 1975.) Right. Another area of the same angiosarcoma shown in Figure 4. Spurlike projections of hepatic tissue project into larger, more cavernous cystic spaces. The hepatic cords are disrupted by degeneration of hepatocytes. The tissue space of Disse contains increased collagen and reticulin fibers. (Hematoxylin and eosin; magnification, x 100.) NIH accession number A74-31.

of septa. These septa may progress to cirrhosis. Although we have not as yet noted this progression, it has been observed by others. Sinusoidal dilatation develops, and in this setting angiosarcoma ensues. The role of the activation of the hepatocytes, which seemingly form a scaffold, is problematic. However, it is worth noting that we have anecdotal evidence of primary hepatocellular carcinoma in persons exposed to vinyl chloride.

While studying these cases, we became aware that prolonged exposure to trivalent inorganic arsenicals, such as Fowler's solution used to treat psoriasis, results in the same clinical and histologic picture (47-49). Histologic examination of two cases made available to us by Dr. Peter Scheuer showed the same histologic changes as in vinyl chloride-induced fibrosis. Moreover, exposure to such arsenicals has also been associated with hepatic angiosarcoma (25). In vineyard workers, in addition, it has been associated with primary hepatic carcinoma and cirrhosis (24). The mechanism of toxicity of arsenic has been reported to occur through its reaction with 6,8-dithiooctanoic acid (α-lipoic acid), with the arsenic forming a stable bridge between the two sulfhydryl groups (32). Certain hypothetical metabolites of vinyl chloride formed by the mixed function oxidases would also be expected to react in a similar fashion with α-lipoic acid, possibly explaining the similar pattern of both fibrosis and neoplasia seen with these two superficially unrelated chemicals. Interestingly, thorium dioxide (thorotrast) also produces the same spectrum of lesions (22).

The pathogenesis of the noncirrhotic portal hypertension in all these instances may be related to a discrepancy between increased splenic blood flow, induced by the hyperplastic splenomegaly, and to the impairment of the distensibility of the hepatic vascular bed produced by the various forms of fibrosis, which by themselves are not conspicuous (50). The fact that this type of portal hypertension has been produced by chemicals, at least some of them environmental, suggests that idiopathic portal hypertension, found only sporadically in the Western world but more often in some areas of Asia and Africa, might result also from toxic, possibly environmental, chemicals.

Histologic Features: Angiosarcoma

Dr. Louis B. Thomas*: We have reviewed the available pathologic material for a group of vinyl chloride workers who had a peculiar hepatic fibrosis and focal sinusoidal dilatation associated with proliferation of sinusoidal lining cells. In addition, we have studied the hepatic lesions of 15 vinyl chloride workers who developed angiosarcoma of the liver. The hepatic changes described by Dr. Popper were also seen in the nonangiosarcomatous areas of the liver in all cases in which suitable sections were available for review. From these observations, we concluded that a continuous spectrum of changes occurs, starting with multifocal areas of stimulated sinusoidal cells, followed by increasing degrees of atypia and proliferation of these cells, and culminating in progressively growing, multicentric, infiltrative angiosarcomas (51).

Of those patients with angiosarcoma who died and were autopsied, postmortem examination of the livers showed massive involvement by cystic, blood-filled tumors that replaced most of the tissue. The liver weights ranged from 1860 g to 7300 g. The average weight was 4236 g. In the larger specimens some of the cystic spaces were several centimeters in diameter and were associated with large areas of hemorrhage and necrosis. Rupture of these large cavernous cysts followed by intraperitoneal hemorrhage occurred both spontaneously and at the time of surgery, and it was the immediate anatomical cause of death in several patients. The liver tissue was also irregularly replaced by small cysts that varied in size from 1 mm to 1 cm in diameter. Most of them were filled with blood; only

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