Vinyl Chloride-Associated Liver Disease

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Although polyvinyl chloride has been produced from vinyl chloride monomer for more than 40 years, recognition of toxicity among vinyl chloride polymerization workers is more recent. In the mid 1960s, workers involved in cleaning polymerization tanks were found to have acro-osteolysis. In 1974, the same population of workers was found to be at risk for an unusual type of hepatic fibrosis and angiosarcoma of the liver. We describe two cases of vinyl chloride-associated liver injury, one of hepatic fibrosis and one of angiosarcoma. Histologic features of these lesions are similar to the hepatic fibrosis and angiosarcomas resulting from chronic exposure to inorganic arsenicals. Preliminary studies suggest that the toxicity of vinyl chloride may result from formation, during high-dose exposure, of active metabolites by mixed function oxidases of the liver. Epidemiologic studies indicate an increased incidence not only of liver disease, but also of cancers of the brain, lung, and possibly other organs.

DR. PAUL D. BERK*: Polyvinyl chloride, the most widely used synthetic plastic, has been manufactured for more than 40 years by polymerization of vinyl chloride monomer gas at a number of plants in the United States and in many other countries. Worldwide production of polyvinyl chloride in 1972 totaled more than 18 billion pounds, of which about 25% was made in the United States, and items manufactured from polyvinyl chloride are virtually ubiquitous. They include building materials such as pipes and cables, home furnishings, [illegible] records and other recreational items, clothing, packaging material, and a host of other products. Although for many years vinyl chloride monomer and polyvinyl chloride were thought to be entirely inert, in the mid-1960s Wilson and associates (1) in Louisville and investigators in Europe (2–4) reported that the development of acro-osteolysis, a skin disorder characterized by Raynaud's phenomenon, scleroderma-like dermal induration, and bone lesions, was an occupational hazard to workers involved in the polymerization of vinyl chloride monomer. At greatest risk were those who manually cleaned the tanks in which the polymerization reaction occurs.

At the same time, other reports described nonspecific alterations of hepatic structure and function in vinyl chloride monomer polymerization workers (5), and a poorly characterized condition called "chronic epithelial hepatitis" was reported in roughly 15% of such workers examined in Russia (6). These reports of hepatic abnormalities in the vinyl chloride industry, published for the most part in the industrial hygiene literature of eastern Europe, attracted little attention until very recently. Then the situation was dramatically altered by the almost simultaneous reports of hepatic fibrosis, splenomegaly, and portal hypertension among vinyl chloride workers in Germany (7), and the discovery of three cases of angiosarcoma of the liver, an otherwise rare tumor, in workers involved in the production of polyvinyl chloride from vinyl chloride monomer at a plant in this country (8, 9). The subsequent introduction into this plant of a systematic surveillance system has thus far resulted in the detection of several more cases of angiosarcoma, as well as many instances in which abnormal findings in liver function tests, or scintigraphic evidence of splenomegaly, or both, were detected in otherwise asymptomatic workers. Similar screening efforts at polyvinyl chloride production plants in several countries quickly brought the number of workers with documented angiosarcoma to 19 (10)†. Not all of these cases have been published.

Results of animal experiments strongly support the presumed relation between exposure to vinyl chloride gas and hepatic angiosarcoma. Italian investigators (11) had reported several years ago that rats subjected to prolonged inhalation of high concentrations of vinyl chloride developed carcinomas of the zymbal gland and other organs. More recently, Maltoni and Lefemine (12) presented data

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† As of 10 February 1975, the Center for Disease Control [illegible] cases of hepatic angiosarcoma in vinyl chloride workers [illegible] United States, 6 in Canada, 2 in the United Kingdom, 3 in Germany, and 1 in France.

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Figure 1. Surgical liver biopsy obtained from Patient 1 in 1972. Normal lobular architecture is maintained, but there is enlargement and fibrosis of the portal tracts, with bile ductular proliferation. (Hematoxylin and eosin; original magnification, x 50.) NIH accession number S74-411. (Reproduced by permission, *Ann NY Acad Sci* 246:70-77, 1975.)

indicating the development of angiosarcomas of the liver and other organs, and the development of nephroblastomas in rats exposed to various concentrations of vinyl chloride gas, including levels within the 500 ppm limit that, until recently, had been the accepted occupational standard. Exposure to as little as 50 ppm has produced angiosarcoma of the liver in rats and mice, with a clearcut dose-response relation seen in mice (13). To date, most cases of hepatic fibrosis and angiosarcomas in man have been detected in workers exposed to vinyl chloride monomer gas during the production of polyvinyl chloride, and, as with acro-osteolysis, those who clean the polymerization tanks seem to be particularly at risk. If confirmed, preliminary anecdotal reports of hepatic fibrosis and an isolated instance of angiosarcoma of the liver in workers exposed only to the finished polymer will significantly increase the population potentially at risk. Data collected by the U. S. Public Health Service show that about 6500 workers in the United States are exposed to vinyl chloride monomer during the production of the gas (14 plants) and during its polymerization to polyvinyl chloride (37 plants). However, another 30 000 to 50 000 workers are employed in hundreds of plants that convert the polyvinyl chloride resin to finished products (14). To date, there is no evidence of risk to consumers from these finished products.

The discovery of both benign and malignant liver disease in vinyl chloride workers raises several critical questions, including the following.

1. Who is at risk of developing vinyl chloride-associated liver injury? Is the danger restricted to workers exposed to the monomer, or is there also a danger to the much larger force of polyvinyl chloride workers? Is either the incidence of liver injury or the nature of the lesion influenced by simultaneous exposure to various copolymers or other substances? Are there risks to consumers, such as those who use vinyl chloride sprays, those who eat food packaged in vinyl chloride wrapping, or those whose homes contain polyvinyl chloride plumbing?

2. What is the clinical, biochemical, and histologic spectrum of vinyl chloride associated liver injury? In particular, is there a relation between the peculiar hepatic fibrosis reported in vinyl chloride workers, and the increased incidence of angiosarcoma of the liver? What is the relative incidence of these two lesions?

3. What is the natural history of established, vinyl chloride-associated liver injury, both in patients who have had continued exposure and in those patients removed from further exposure?

4. What tests will constitute an effective screening regimen for detecting vinyl chloride-associated liver injury and for following patients with established disease?

5. What are the cellular and molecular mechanisms by which vinyl chloride produces hepatic fibrosis and angiosarcoma?

6. What is the optimum therapy for patients with both hepatic fibrosis and angiosarcoma associated with vinyl chloride exposure?

7. What preventive measures will be necessary and sufficient to protect vinyl chloride workers from future injury from this compound?

As yet, we have few definitive answers to these questions. The following case reports of two patients recently evaluated at the National Institutes of Health help to illuminate several points that will be discussed here. They also illustrate some of the difficulties in defining the scope of the problem.

Case Summaries

Dr. James F. Martin*: Patient 1 is a 30-year-old white man who worked at a plastic and rubber manufacturing plant in Louisville, Kentucky, for 8 years. For the first 5½ years, his job involved cleaning the tanks in which vinyl chloride polymer was polymerized.

Two and a half years ago, during evaluation of a nonspecific illness characterized by fever and weight loss, he was found to have hepatosplenomegaly, mildly elevated serum bilirubin and SGOT concentrations, and abnormal 45-minute plasma BSP retention (10.6%). Nonspecific symptoms persisted for 3 months, after which he was hospitalized for an acute hepatitis-like illness. Since the patient had been on sick leave for almost 3 months before he developed jaundice, the relation of this latter episode to vinyl chloride exposure is unclear.

Because his physicians were concerned about the persisting hepatosplenomegaly, they did a diagnostic laparotomy during the recovery phase of his illness. The surgeon described the liver as showing multiple areas of fine nodularity with fibrosis. An enlarged spleen was removed at the operation.

A liver biopsy done during surgery showed that the principal abnormality was intense portal fibrosis with bile ductular proliferation (Figure 1). Additional features, to be described later, included capsular and intrasinusoidal fibrosis, activation of sinusoidal lining cells, and marked anisocytosis hepatis. These features are now known to be typical of the lesions seen in workers exposed to vinyl chloride. There was no evidence of viral hepatitis, alcoholic liver disease, or any other hepatic disease that could be diagnosed morphologically. Histologic examination of the enlarged spleen showed lymphoid and reticuloendothelial proliferation without significant fibrosis.

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After surgery the patient returned to work at the plant. However he was transferred to another area in the factory with no appreciable further exposure to vinyl chloride, and he continued in this new assignment for 2½ years. When it recently became evident that vinyl chloride exposure was associated with both malignant and nonmalignant disease of the liver, Dr. John Creech referred him to the National Institutes of Health for further study. This patient thus represents a unique follow-up of a case of apparent vinyl chloride-induced liver injury 2½ years after removal from exposure.

At admission to the Clinical Center, the patient seemed to be a healthy, well-developed, and well-nourished young man. Except for several small vascular spiders on his anterior chest, physical examination was normal. Complete blood count and chest X ray were unremarkable. However, pulmonary function studies showed evidence of mild restrictive pulmonary disease. All routine tests of liver function, including serum bilirubin concentration, transaminases, alkaline phosphatase, serum protein electrophoresis, and 45-minute BSP retention (3.4%), had normal findings, as did the liver scan. Because we thought it important to confirm whether histologic improvement accompanied the apparent biochemical improvement in liver function, peritoneoscopy and liver biopsy were done with the fully informed consent of the patient.

The liver was again seen to be covered with multiple, tiny, rice-grain-size nodules, and histologic examination again showed extensive portal fibrosis with bile ductular proliferation. Cords of hepatic cells were surrounded by fibrosis. At least one area had bridging between portal areas and hepatic veins (Figure 2). Areas of intense lymphoid proliferation were seen in some of the portal tracts, and again, as in the earlier biopsy, there was considerable proliferation of collagen within the hepatic sinusoids. Although the parenchymal cells seemed relatively normal, the fibrosis in both the portal areas and within the sinusoids was at least as severe at this recent biopsy, as it had been 2½ years earlier.

Because of the tissue abnormality, hepatic vein catheterization studies were done. Total hepatic blood flow, indocyanine green extraction, and hepatic oxygen consumption were normal. However, the wedged hepatic venous pressure was slightly elevated to 12 mm Hg with a free value of 3 mm Hg.

Because we wanted to find some test of liver function that would reflect the histologic abnormalities and therefore serve as a suitable screening test, we did several special kinetic studies. Disappearance curves with radiolabeled bilirubin, BSP, and two different doses of indocyanine green (one of the most sensitive studies of liver function available) were all normal (15). Only the bile-acid disappearance test, which is an experimental procedure, had abnormal findings (16).

Figure 2. Needle biopsy of the liver from Patient 1 in early 1974. There is extensive fibrosis of the portal tracts and hepatic lobules, with bile ductular proliferation. Distortion of the normal lobular architecture is indicated by the fibrotic bridge (arrow) linking the portal tract to a central vein. (Hematoxylin and eosin; original magnification, x 50.) NIH accession number S74-870. (Reproduced by permission, Ann NY Acad Sci 246:70-77, 1975.)

In summary, our study of this vinyl chloride polymerization worker suggests that vinyl chloride associated hepatic fibrosis persists after removal from further exposure, and that the lesion may be quite extensive even though standard liver function tests have completely normal findings.

Dr. Robert C. Young*: Patient 2 is a 42 year-old white man who was first hospitalized at the Clinical Center in March 1974 for evaluation of angiosarcoma of the liver. The patient had worked in a plastics production plant in Louisville, Kentucky, for 20 years and had been involved in the polymerization of vinyl chloride monomer to polyvinyl chloride. During the course of his employment, he was involved in cleaning the polymerization vats, a phase of the operation associated with particularly high exposure to vinyl chloride monomer.

A routine general examination in November 1973 showed an elevated alkaline phosphatase level. The blood chemistry screening procedures were repeated in February 1974, and, besides the persistently elevated alkaline phosphatase, the serum bilirubin concentration was abnormal. A liver scan done in February 1974 showed mottling of the right lobe of the liver consistent with cirrhosis or with tumor.

The patient was admitted to St. Anthony's Hospital in Louisville, Kentucky, on 26 February 1974. Hepatic artery angiography was done, and the findings were consistent with tumor in the right lobe of the liver (Figure 3*A*). An exploratory laparotomy done in March 1974 showed a 4-cm bluish elevated mass in the right lobe of the liver, and small nodules in both the right and left lobes. Over the rest of the surface of the liver there was small, irregular, raised rice-like speckling. Biopsy of the large lesion in the right lobe showed angiosarcoma. Biopsy of the left lobe was reported to show both cirrhosis and hepatic fibrosis. Because of the degree of generalized cirrhosis, right hepatic lobectomy was advised against as being too hazardous.

The patient's earlier medical history is significant in that he had been a heavy alcohol consumer intermittently over the previous 5 to 6 years, during 3 of which he required hospitalization for alcohol withdrawal. The patient estimated maximum alcohol consumption during those periods at one pint of whiskey a day.

When he was first hospitalized at the Clinical Center later in March 1974, the positive physical findings were limited to the chest, where there was an increased anteroposterior diameter with a few rales at the left base. Right paramedian and left flank incisions on the abdomen were well healed. The liver was 10 cm in span by percussion but was not palpable. There was no splenomegaly, no other abdominal masses were found, and the rest of the physical examination findings were within normal limits.

Pertinent laboratory information included serum alkaline phosphatase, 87 IU/litre (normal, <78); LDH, 124 IU/litre (normal, <340); SGOT, 21 IU/litre (normal, <52); SGPT, 38 IU/litre (normal, <45); bilirubin, 0.4 mg/dl total, with 0.05 mg/dl direct-reacting. Chest X ray showed only atelectatic changes in both bases. A selective hepatic angiogram showed mottling of the liver consistent with cirrhosis, a 6-cm x 6-cm tumor mass in the right lobe, and tortuosity of the intrahepatic vessels (Figure 3*B*). The left lobe of the liver was felt to be small, as seen in the hepatic scans of 14 March 1974 (Figure 3*C*, *D*). Surgical intervention was considered once more and again rejected because of the generalized cirrhosis. The patient was started on adriamycin chemotherapy, 60 mg/m² body surface area intravenously every 3 weeks, because of the known effectiveness of this drug against other disseminated sarcomas. After the initiation of chemotherapy, the patient was followed with serial liver function tests and serial liver scans.

Subsequent scans showed no alteration in the size of the mass lesion, and the patient remained asymptomatic. He toler-

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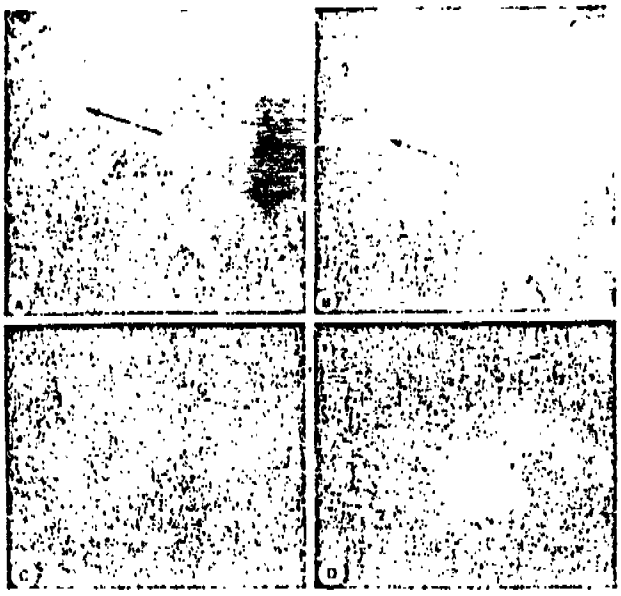

Figure 3A, B. Hepatic arteriograms in Patient 2 on 26 February 1974 (*A*) and 16 October 1974 (*B*) show a tumor in the right lobe (arrows) and tortuosity of the intrahepatic vessels. C, D. Anteroposterior and lateral hepatic scan in Patient 2 on 14 March 1974. The large tumor is apparent as a filling defect within the right lobe.

ated chemotherapy well, with nadir leukocyte counts between 2000 mm³ and 3000 mm³, and nadir platelet counts of approximately 80 000 mm³. Because of the dose-limiting cardiac toxicity of adriamycin, the patient received a maximum total dose to 480 mg/m², and the last dose was given on 27 August 1974. He had transient elevations of alkaline phosphatase during April and May 1974 associated with the resumption of heavy alcohol intake. With abstinence, liver function returned to normal, and the patient remained asymptomatic.

The patient was readmitted in October 1974 for reevaluation. Repeat angiography and scans indicated persistent lesions without definite change in size. Repeat liver function tests had normal findings except for a BSP retention of 9% at 45 minutes. Because of the total dose limitations of adriamycin, the patient was started on intermittent cyclophosphamide chemotherapy.

The patient died in March 1975 of massive intra-abdominal hemorrhage resulting from a ruptured angiosarcomatous cyst in the liver. At autopsy, there was extensive angiosarcoma in both lobes of the liver, with direct extension to the diaphragm. Metastases were found in regional lymph nodes, lung, skull, and scalp.

Industrial Screening

Dr. John Creech*: Data were obtained from examination of approximately 1200 employees of a plastic and rubber manufacturing factory in Louisville, Kentucky. The chemicals most often used in the manufacturing processes at this factory were vinyl chloride, vinylidene chloride, and vinyl acetate for plastic production and butadiene, acrylonitrile, and styrene for rubber production. When three employees were found to have angiosarcoma of the liver (8), an intensive study of the health of both present and former employees was begun. Two major routes of investigation were undertaken.

Before the initiation of a surveillance program to screen for liver disease among present employees, investigators searched for autopsy and biopsy tissue from past and present employees. Several abnormalities were repeatedly seen in these tissues, including angiosarcoma of the liver, portal fibrosis, esophageal varices, and splenomegaly. There were obvious potential interrelations among these abnormalities. Pertinent findings from the first eight positive cases detected by this search are summarized in Table 1. All patients had had a relatively high exposure to the chemicals used in plastic production, especially vinyl chloride. Furthermore, there was a very long history of exposure in these eight workers: more than 10 years in seven cases and 20 or more years in five.

Figure 4 shows the proposed screening method. Eleven hundred eighty-three employees have had many of the appropriate tests in this program. However, some of the proposed segments of the program have not yet been started. We are now doing a wide range of tests, but the results are constantly being evaluated and the program modified to use those tests that prove most sensitive and specific. Some of the more elaborate studies done in patients with known liver disease are specifically intended not only to be of diagnostic value, but also to provide information about the pathophysiology of liver injury in this population. Appropriate consent is obtained for all procedures. Besides the studies shown in Figure 4, other tests are done when indicated, for example, HB$_s$Ag and anti-HB$_s$Ag to rule out viral B hepatitis, reticulocyte counts if hemolysis is suspected, and so forth.

All employees of the plant have now been tested, some of them several times, with a standard SMA-12, augmented by an SGPT and gamma-glutamyl transpeptidase (GGTP). As noted in Figure 4, a liver/spleen scan is also routine. To date, 650 scans have been done, 193 in workers from areas with high vinyl chloride monomer (VCM) exposure (high VCM) and 457 in employees with low vinyl chloride monomer exposure (low VCM). Among the significant abnormalities reported in these scans are decreased hepatic uptake, defect within the liver, and splenomegaly (Table 2). The total percentage of abnormal liver/spleen scans in the high-VCM workers (12.4%) was comparable to that in the low-VCM group (11.8%). The only significant difference between these groups was in hepatic filling defects, which were almost three times more common in the high-

Table 1. Hepatic Disease in Workers in Louisville, Kentucky, with High Exposure to Vinyl Chloride: Features of the First Eight Cases

Patient	Duration of Exposure	Primary Disease Found		Associated Findings	
		Angiosarcoma	Portal Fibrosis	Esophageal Varices	Splenomegaly
	yrs				
1	21	+	+	+	+
2	30	−	+	−	−
3	18	+	+	+	+
4	20	+	+	−	−
5	14	+	+	+	+
6	28	+	+	−	+
7	24	−	+	+	+
8	6	−	+	−	+

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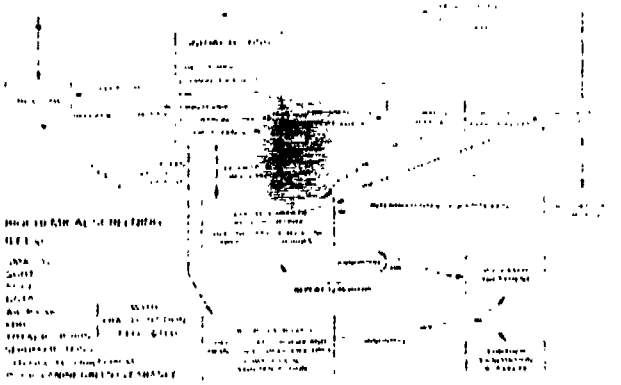

Figure 4. A program for the systematic screening of liver disease in vinyl chloride workers (see text for details). As indicated by an asterisk, the frequency of routine screening is determined by the extent of exposure to vinyl chloride monomer within the plant. CBC = complete blood count; GI = gastrointestinal; LFT = liver function test; SGOT = serum glutamic oxalacetic transaminase; SGPT = serum glutamic pyruvic transaminase; GGTP = gamma-glutamyl transpeptidase; LDH = lactic dehydrogenase; Alk. P'tase = alkaline phosphatase.

VCM group (Table 2). Filling defects included not only angiosarcomas, of which there were two, but also lesions apparently produced by a prominent porta hepatis, vascular abnormalities, or even congenital mal-location of the gall bladder. These diagnoses were ultimately confirmed by angiography or oral cholecystography, or both. In our series, the most common explanation for false-positive liver scans was benign vascular defect.

Scintigraphy showed enlarged spleens (> 14 cm in greatest dimension) in 55 workers, or 8.4% of the total group examined. The abnormal spleens ranged from 15 cm to 19 cm. Again, the incidence of splenomegaly was no greater in workers using monomers other than vinyl chloride, principally for rubber production, than in those with high-VCM exposure from plastic production (Table 2).

To date, liver biopsies have been done on 47 workers, 18 in the high-VCM group and 29 in the low-VCM group. Indication for liver biopsy has been persistent abnormal findings in the biochemical tests or an abnormal finding in the liver/spleen scan. Fatty metamorphosis of varying severity has been found in 44% of the biopsies from workers with high-VCM exposure and in 34% of those from the low-VCM areas. Interestingly, the low-VCM group has shown a higher prevalence of portal fibrosis (44%) than the high-VCM group (33%), although these differences are not statistically significant. Two more cases of angiosarcoma have been diagnosed by liver biopsy, both from workers with high exposure to vinyl chloride monomer.

An important part of the program is to determine which tests will best predict abnormal hepatic histology. Of the patients with abnormal findings from biopsies, 87% had an abnormal SGPT level, 80% an abnormal SGOT level, and 80% an abnormal GGTP level. Within our relatively small biopsy series, every patient in whom all three of these tests showed abnormal results or in whom plasma indocyanine green clearance was abnormal, had abnormal liver biopsy findings, whereas completely normal values for each of these variables was always associated with a normal liver biopsy. Splenomegaly was not a useful predictor of hepatic histology, as the prevalence of biopsy-proved portal fibrosis was essentially the same in patients with normal spleens (50%) as in those with splenic enlargement (44%).

Prospective employees at this plant now undergo our routine biochemical tests during their pre-employment physicals. The original testing of all 1183 employees showed a 26% rate of abnormal findings in one or more "liver function tests." The abnormality rate in the pre-employment group, consisting of supposedly healthy men with no exposure to vinyl chloride or other monomers, was 24% (that is, 44 of 181). Alcohol consumption probably accounts for some of this high "background" rate of abnormal liver function tests.

One other preliminary observation is worth noting. We have recorded elevated inferior vena caval pressures (> 10 mm Hg) in 21 of 40 patients with splenomegaly or abnormalities in other preliminary screening tests. We have no definitive explanation for this.

Based on our own data and those of others, we plan to continue an expanded screening program that will include indocyanine green and bile-acid clearance studies in the initial evaluation. Other tests we may do more routinely are [1] barium swallow for varices and upper gastrointestinal series in patients with splenomegaly; [2] more extensive hemodynamic evaluation, including umbilical vein catheterization for portal pressure measurement and portal venography to determine the role of portal hypertension in the splenomegaly of vinyl chloride-associated portal fibrosis; and [3] extensive pulmonary evaluation, including pulmonary artery catheterization, pressure studies, and possible biopsy, to evaluate the high prevalence of elevated vena caval pressures.

Dr. Paul Berk: Dr. Creech has been in the forefront of developing a screening program for vinyl chloride workers. Some of the problems he faces are clearly indicated in Table 3, which summarizes data obtained during admission to the Clinical Center of five workers from two vinyl chloride plants, including the two patients presented in detail here. Three, including the first patient discussed, had significant hepatic fibrosis seen on light microscopy, and, in the fourth, a characteristic pattern of fibrosis was seen with electron microscopy (17). Patient 5 had an extensive angiosarcoma and severe alcoholic liver disease with cirrhosis. Note that only this patient had biochemical abnormalities or defective BSP or indocyanine green metabolism.

Table 2. Results of 650 Liver/Spleen Scans in Workers Exposed to Vinyl Chloride Monomer (VCM) at a Plastics and Rubber Manufacturing Plant

Finding	High VCM (193 scans)	Low VCM (457 scans)
	%	
Decreased hepatic uptake	1.55	2.19
Hepatic filling defect	2.59	0.88
Enlarged spleen	8.29	8.75
Total Abnormalities	12.43	11.82

Table 1. Laboratory Data in Vinyl Chloride (VC)-Associated Liver Disease, HIH Cases.

Patient	Hepatomegaly	Splenomegaly	Routine Liver Function Tests*	45 Minute BSP Retention	Indocyanine Green Rate Constants (min⁻¹)		Histologic Diagnosis
					0.5	5.0	
				%	mg/kg		
1	+	+	Normal	3.8	. . .	0.17	VC-associated hepatic fibrosis (severe)
2	+	+	Normal	2.9	0.21	0.20	VC-associated hepatic fibrosis (severe)
3	−	−	Normal	2.1	0.25	0.20	VC-associated hepatic fibrosis (mild)
4	−	−	Normal	2.1	0.24	0.23	No pathologic diagnosis†
5	−	−	Occasional increased alkaline phosphatase	9.3	. . .	0.10	Angiosarcoma, hepatic fibrosis, cirrhosis
			Normal mean	<5	0.22	0.18	
			± SD		0.03	0.02	

* Bilirubin, SGOT/SGPT, alkaline phosphatase.
† Changes indicative of VC injury by electron microscopy.

The normal values recorded for so many tests of liver function in Patients 1-4, despite the significant histologic lesion, pose serious problems for designing an adequate screening regimen to detect liver injury among vinyl chloride workers. Furthermore, as shown by the first case presented, follow-up studies of patients with proven vinyl chloride-associated liver injury, after their removal from exposure, cannot be based on any routine tests now available. The suitability of bile-acid clearance studies for this purpose certainly merits further evaluation, as these seem to be the most sensitive indicators of hepatic disease that we have, short of liver biopsy. The absence of abnormalities in so many tests of hepatocellular function probably reflects the fact that the principal anatomic lesion in vinyl chloride-associated liver disease is fibrosis, with relative sparing of the hepatocytes. Unfortunately, we have no simple test for hepatic fibrosis without hepatocellular dysfunction.

Epidemiologic Studies: A Cohort Approach

Dr. Irving Selikoff*: With Dr. Creech's discovery last year of his clinical cases of angiosarcoma, we have come full-circle. Since Rehn first reported aniline-induced bladder cancer in 1895, there has been a discrepancy that has bothered many scientists; that is, although many chemical carcinogens have been identified in laboratory studies, very little human cancer has been reported to be associated with industrial exposure in the vast and growing petrochemical industry. The paradox is, therefore, one of many *laboratory* carcinogens but relatively few *human* neoplasms. Aside from bladder cancer due to beta-naphthylamine and benzidine, and coal-tar skin cancers, we simply had not seen much malignancy among chemical workers that was related to occupation. Recently, with the recognition of bischloromethyl ether cancers of the lung and upper respiratory tract, our suspicions were heightened, but, until last year when Dr. Creech and associates (8, 9) reported their cases and Drs. Maltoni and LeFemine (12) presented their elegant laboratory studies, we could not explain the discrepancy between our anticipated clinical findings and what actually occurred.

Recognition of this problem actually began a few years ago, in the mid-1960s, when simultaneous reports came from Europe (2, 3), the United States (1), and Great Britain (4) describing acro-osteolysis among workers exposed to vinyl chloride monomer. The syndrome is characterized by a Raynaud-like phenomenon, pseudo-clubbing of the fingers resulting from the collapse of the terminal phalanx, and local bone resorption. At the suggestion of the British plastics industry, a study of the pathology of acro-osteolysis was begun.

Rats were exposed to gaseous vinyl chloride in concentrations of up to 30 000 ppm. The investigators (11) reported an interesting set of data on the pathology of the bone changes and also described the appearance of zymbal cell tumors in the rats. Unfortunately, these findings did not receive much attention.

Such zymbal cell tumors had been seen with polycyclic aromatic hydrocarbons, benzidine, and several other carcinogenic chemicals. It was perhaps thought that this was yet another laboratory curiosity. Nevertheless, the bone changes and some fragmentary information about liver changes did attract attention, especially because of the excellent work of investigators at the University of Bonn. They found that many of their patients with acro-osteolysis also had liver disease (7, 18, 19). However, they reported no cancer (20).

That was the state of our knowledge at the end of 1973. Matters changed in 1974, with the report of three cases of angiosarcoma of the liver among workers at the plastics and rubber plant in Louisville (8, 9) and the reports of more animal studies (13) that appeared soon after. Vinyl chloride monomer was seen to be a carcinogen both in animals and humans. Because the chemical and its polymer, polyvinyl chloride, were widely used in industry and elsewhere, it was important to elucidate the true dimensions of the problem.

First, we examined and did biochemical tests on more

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than 1200 workers at three plants, representing three different sets of conditions and three different sets of exposures. These were in addition to the 1200 workers examined by Dr. Creech in Louisville. Only one test, an elevated alkaline phosphatase, proved valuable in screening these workers for vinyl chloride-associated liver injury. Several other laboratory studies that were done showed many other abnormalities, but the overlap with 400 simultaneous controls and the variability in split samples rendered them inefficient as diagnostic aids. We found no guide predictive of either liver injury or angiosarcoma (5). Whether ultrasound (21) or liver scanning will ultimately prove to be of greater help, we do not know.

When angiosarcoma of the liver was described, we all appreciated that it was, in general, rare. There has been only one established case of angiosarcoma in 52 000 consecutive autopsies at the Los Angeles County Hospital. A search of the files at the Bronx Veterans Administration Hospital showed not a single case of angiosarcoma in 30 000 autopsies. We saw a significant number of cases only at Mount Sinai Medical Center in Dr. Popper's files, where one can find everything hepatic, culled from many sources.

Besides vinyl chloride, at least two other chemicals were known to cause hepatic angiosarcoma in man: thorotrast (22, 23) and arsenicals. Arsenical-induced hepatic angiosarcoma had been seen among vineyard workers exposed to arsenical-containing insecticides used to spray the grapevines (24), and in patients chronically ingesting inorganic medicines containing arsenicals (25). Thorotrast angiosarcomas were particularly worrisome. Although the material had been used in diagnostic radiology since 1926, the first hemangiosarcomas were not seen until 20 years later. This was consistent with the long period of clinical latency we knew to be characteristic of most environmental and occupational cancers. It also correlated with what Dr. Creech saw in Louisville. The plant had been opened in 1940, and the first angiosarcomas were seen in 1968. Dr. Creech showed us one case of angiosarcoma seen 12 years after onset of exposure, but most of the others became apparent after a considerably longer time.

Although the plastics industry is relatively new—many major segments began operating during World War II—we have known of vinyl chloride for a long time. The United States began producing it on a very tentative experimental basis in 1928, and on a small commercial scale in 1938. However, production has markedly expanded only in the past decade. In 1950, the total worldwide production of plastic was only about a million and a half tons; in 1970, it was roughly 15 times more.

The question now is whether the current cases of hepatic angiosarcoma are derived from the small population of 1940-1960 workers, or from the full pool of vinyl chloride workers, including those beginning work from 1960-1974. Experience suggests that current cases are derived primarily from the early group. Lloyd (26) analyzed the first 19 cases reported in this country and abroad. The average period of clinical latency, from first exposure to diagnosis, was about 20½ years, with a range of 11 to 30 years. Most of the people in this group had begun work in the 1940s and 1950s. The cases of angiosarcoma now being seen are therefore derived from a very small population. Because most of the total work force was first exposed to vinyl chloride in the late 1950s and early 1960s, angiosarcomas are not yet seen among them.

These and other data have established that vinyl chloride-induced cancer, as well as most other environmentally induced neoplasms, has a long period of clinical latency. We do not know, however, if it will be a common problem or an infrequent one. Three groups are studying this, using a cohort approach. First, the plastics industry has mounted an extensive investigation (27). The cohort is composed of all men known to have worked in the relevant facilities for a year or more before 31 December 1972. Data have been gathered on 8384 men, but, unfortunately, about 1200 men still have not been traced. Thus it is difficult to evaluate the information gathered. Nonetheless, the data now available point to increased risk of cancers of the lung, brain, buccal cavity, and pharynx (27). Although more information is needed for conclusive evidence, the same suggestions have been found in a second study, done at the National Institute for Occupational Safety and Health (28).

Our laboratory has searched for information on a key question: How common will angiosarcoma be among populations exposed to vinyl chloride monomer? At one plant we identified all workers employed there for 5 years or more from the time the plant opened in 1948 to 1964. There were 257 such workers, all of whom have been traced. There were 25 deaths, of all causes. Three died of hemangiosarcoma of the liver, each confirmed by autopsy and reviewed by Dr. Popper and Dr. Thomas. Thus, approximately 1 of 8 deaths among vinyl chloride workers, at least in this plant, was due to angiosarcoma of the liver. Another death, caused by ruptured esophageal varices, was also related to vinyl chloride exposure. If these data are confirmed by other studies, it would seem that the risk of death from angiosarcoma of the liver will increase greatly 15 to 25 years after the initial exposure in those who began working with the chemical between 1955 and 1974.

In closing, it is worth noting that until recently, the chemical industry had been making plastics, not doing medical research. We in the medical profession had been doing research, but not paying great attention to the chemical industry. In both instances, changes are being made.

Further Epidemiologic Studies

Dr. Henry Falk*: The first four cases of vinyl chloride monomer-induced hepatic angiosarcoma identified in Louisville were all in polyvinyl chloride polymerization workers. This is as expected, since the job done by these workers, opening and cleaning the reactor vessels after polymerization, involves the highest exposure to vinyl chloride monomer. Other cases of the tumor have since been identified in workers, primarily among those involved in the polymerization process. We now know of 15 cases

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Table 4. Cases of Hepatic Angiosarcoma in Polyvinyl Chloride Polymerization (PVC) Workers (U.S.) (Center for Disease Control, Atlanta, November 1974)

Patient*	Age	Race	Sex	Date of Initial PVC Work	Date of Diagnosis	Date of Death	Duration from Initial PVC Work to Diagnosis
	yrs						yrs
1	52	W	M	6/44	4/61	4/61	19
2	43	W	M	7/52	8/67	1/68	15
3	41	W	M	5/50	3/69	3/69	19
4	36	W	M	11/55	5/70	9/71	14
5	49	W	M	12/48	3/73	3/73	24
6	58	W	M	11/45	12/73	12/73	28
7	45	W	M	1/62	2/74		12
8	43	W	M	5/45	2/74		28
9	43	W	M	6/55	11/74		19
10	40	W	M	10/46	4/61	8/61	14
11	54	W	M	6/51	5/68	5/68	16
12	60	W	M	10/46	3/70	3/70	23
13	45	W	M	8/44	3/68	3/68	23
14	52	W	M	8/45	4/74	7/74	29
15	50	W	M	9/49	5/69	5/69	19

* Patients 1 through 9 were exposed to PVC at a plant in Louisville, Kentucky. Patients 10 through 14, in Niagara Falls, New York; Patients 13 and 14, in South Charleston, West Virginia; and Patient 15, in Pottstown, Pennsylvania.

in the United States (Table 4), 9 in workers at the Louisville plant and 6 at three other plants. Of particular concern is that cases seem to be increasingly more common in recent years.

Table 4 shows several important epidemiologic features. The latency period, as reviewed in detail by Dr. Selikoff, is quite long. The duration of exposure for most of these workers is equally long. However, Patient 3 worked with polyvinyl chloride only 4 years and developed a tumor 15 years later. This suggests that relatively brief periods of exposure can be significant, and that, once started, the carcinogenic process may not always be reversible with removal from the monomer exposure. It has been widely assumed that the highest sustained levels of vinyl chloride monomer exposure occurred in the early years of the industry (1940s and early 1950s), and that most of the patients had begun work during those years. Patient 7, however, first began working in 1962, and, therefore, the carcinogenic risk factors were still present in the early 1960s. Most cases of spontaneous hepatic angiosarcoma (and other hepatic sarcomas) occur in older age groups, whereas, despite the long latency period, the vinyl chloride monomer-induced cases occur at a relatively early age for this type of tumor.

In the epidemiologic study of these initial cases, two important questions arise: [1] Why is the disease more prevalent at the plant in Louisville; and [2] Why, in particular, did it develop in these 15 workers; that is, might factors such as chemical exposure, work patterns, or past medical history serve to promote or accelerate the development of angiosarcoma in these particular persons?

The Louisville plant, which opened in 1942, is the second oldest polyvinyl chloride polymerization plant in the United States. Only seven such plants started operating before 1950, and the four plants in which cases of angiosarcoma have occurred are in this group. The age of the plant is, therefore, important, and this probably relates to both the long latency period for the disease and the less stringent precautions for working with the monomer that pertained in the early years of the industry.

Many of these cases of angiosarcoma were initially misdiagnosed. Most were called hepatoma or cirrhosis, and it is only with a full review of mortality data, including re-examination of autopsy or biopsy tissues, that some cases have been uncovered. Moreover, the last three cases at the Louisville plant were identified during intensive screening programs, as described earlier by Dr. Creech. Therefore, this initial clustering in Louisville may be only artificial, and, with continued surveillance in the industry, a more uniform distribution of cases may become apparent.

On the other hand, there are specific conditions in the Louisville plant and in the 15 reported cases that may be significant in the early appearance of angiosarcoma in these workers. There are four polymerization buildings in the plant. The two that produce the straightforward polyvinyl chloride resins show the least relation to the cases of angiosarcoma, whereas the two that produce more complex products have a stronger relation (14). One of the latter two buildings produces polyvinyl chloride latex (an aqueous suspension of polyvinyl chloride), and the work practices in this building differ slightly in that it takes longer and is more difficult to clean these reactor vessels than the ones that produce polyvinyl chloride resins. Other factors that might also lead to greater exposure while cleaning a reactor include age and size of the reactor vessels, frequency of cleaning, and the lining material used in the reactor. Differing work practices related to product and equipment may, therefore, be important in the distribution of angiosarcoma.

The building most directly associated with the angiosarcoma cases is the one where copolymers and terpolymers are produced. In this process, vinyl chloride monomer and other monomers are polymerized together to produce polymers with different qualities. Several of the monomers that are reacted with vinyl chloride monomer, such as vinylidene chloride (vinyl dichloride) and acrylonitrile (vinyl cyanide), have similar structures and chemical

characteristics. The six workers in other plants were also exposed to these and other vinyl compounds. One man in the Niagara Falls plant worked in the polymerization of polyvinyl alcohol for 5 years before switching to polyvinyl chloride. Since knowledge about the long-range effects of some of these chemicals is limited, we must consider the possibility that they may interact with or enhance the effects of vinyl chloride monomer. Thus, these initial cases may be related to exposure to multiple chemicals, and in particular, to the vinyl chloride monomer—vinylidene chloride mixture, which is found mainly in the Louisville plant.

Chemicals similar to vinyl chloride monomer are also used outside the polyvinyl chloride industry, for example, vinyl fluoride and chlorobutadiene (vinyl vinyl chloride), and it will probably be some time before we appreciate the full scope of the hazards posed by this class of compounds.

A recent study (26) has also suggested that cases of angiosarcoma may not be limited to the high-risk polymerization workers but may also occur in workers who use the plastic resins to produce a variety of plastic products and in people who reside near polyvinyl chloride plants. Of the six patients with hepatic angiosarcoma in the Connecticut Tumor Registry, two lived within the vicinity of polyvinyl chloride fabrication plants, one was an accountant at a polyvinyl chloride fabrication plant, and one (whose tumor, on pathology review at NIH, is classified as an indeterminate type of sarcoma and only possibly an angiosarcoma) worked at a plant where he applied both polyvinyl chloride and non-polyvinyl chloride insulation to electrical wires (29).

Maltoni and LeFemine's (12, 30) inhalation studies showed that vinyl chloride monomer can induce cancers of the lung, brain, and kidney as well as angiosarcoma. Several recent studies suggest that the incidence of lung cancer, brain cancer, and lymphoreticular malignancies may also be higher in vinyl chloride monomer workers. The number of deaths among the younger workers at these polyvinyl chloride plants is still small, however, and continued surveillance will be necessary to confirm these associations (27, 28, 31).

The most ominous feature of the vinyl chloride studies is the unknown in the future. It is possible that, in time, we will begin to see not only more patients with angiosarcoma, but also significant numbers of patients with other, as yet unrecognized, manifestations of vinyl chloride monomer-induced disease. And we do not know whether, in what has been called the "plastics age," related chemicals will pose similar hazards.

Metabolism of Vinyl Chloride

Dr. P. G. Watanabe*: Angiosarcomas, zymbal gland carcinomas, and nephroblastomas developed in rats exposed to from 50 ppm to 10 000 ppm of vinyl chloride monomer 4 h per day, 5 days per week, for 12 months (12). The rats were observed until death occurred. The incidence of tumors at 50 ppm was less than that predicted from a log dose-response curve generated from the tumor incidences at higher exposure levels of 250 ppm to 10 000 ppm. Because the tumor incidence at 50 ppm was lower than expected, and since vinyl chloride monomer per se is not a potent alkylating agent, we thought that the carcinogenic potential of vinyl chloride monomer might be mediated through the formation of reactive metabolites. Therefore, studies were designed to elucidate what happens to vinyl chloride monomer after it is inhaled.

A 4.7-litre Plexiglas inhalation chamber was designed and constructed so that four rats could be simultaneously exposed to various concentrations of vinyl chloride monomer. To minimize contamination of fur and skin, only the nares of the rats protruded through a rubber membrane into the chamber. The appropriate initial concentration of vinyl chloride monomer (Matheson Gas Products, East Rutherford, New Jersey, 99.9% pure) was generated in the chamber at the start of the experiment and continuously analyzed by circulation through an infrared spectrophotometer. Carbon dioxide was continuously removed from the recirculating system by absorption on an Ascarite® (Arthur H. Thomas Co., Philadelphia, Pennsylvania) column. Filtered air was metered into the system to replenish the oxygen consumed. Since the amount of air consumed was within ± 10% of baseline for all experiments, respiratory variables were not significantly changed during the approximately 1-h exposures. Rats used in these studies were Sprague-Dawley, Spartan substrain (Spartan Laboratories, Haslett, Michigan), ranging in weight from 165 g to 200 g.

Disappearance of vinyl chloride monomer from the recirculating inhalation chamber, resulting from uptake and subsequent metabolism, occurred according to apparent first order kinetics as described by the formula:

$$-\frac{dc}{dt} = kC.$$

In this equation, C is the concentration of vinyl chloride monomer (ppm) at time t (minutes), and k (minutes^{-1}) is the rate constant for the decline in vinyl chloride monomer concentration. The rate of disappearance of the monomer and the corresponding half-life ($t_{\frac{1}{2}}$) were calculated from the slopes of the log concentration-time curves. The rate of decline of vinyl chloride monomer in the empty chamber due to nonspecific leakage was also determined at each initial concentration, and the disappearance rates in the animal studies were corrected for this loss.

The rate of disappearance of vinyl chloride monomer from the chamber was slower at concentrations of 1000 ppm than 50 ppm (Figure 5). Other experiments using initial concentrations ranging from 50 ppm to 105 ppm of vinyl chloride monomer showed a linear disappearance with a rate of (8.03 ± 3.40) times 10^{-3} minutes^{-1} (mean ± SD), corresponding to an average $t_{\frac{1}{2}}$ of 86 minutes. At initial concentrations of 220 ppm to 1167 ppm of vinyl chloride monomer, the rate of disappearance was much slower (2.65 ± 1.35) × 10^{-3} minutes^{-1}, corresponding to an average $t_{\frac{1}{2}}$ of 261 minutes. Assuming that the rate of disappearance from the inhalation chamber was primarily

* Toxicology Research Laboratory, Dow Chemical Co., Midland, Michigan. Dr. Watanabe's associates in these studies were Drs. R. E. Hefner, Jr., G. R. McGowan, and P. J. Gehring.

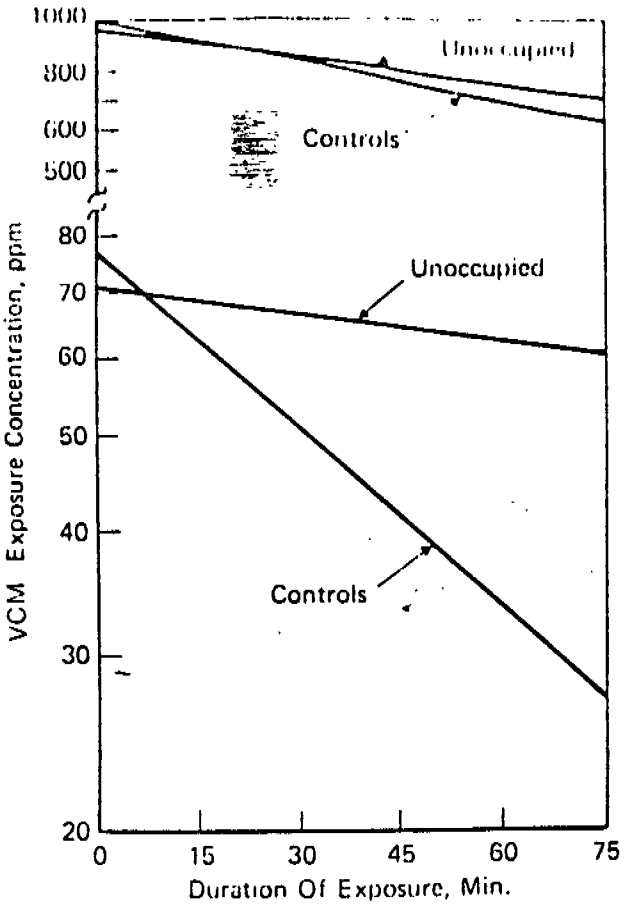

Figure 5. Decrease, with time, of vinyl chloride monomer (VCM) concentrations in the inhalation apparatus. Rats were exposed to initial VCM concentrations of approximately 50 and 1000 ppm. Also shown are the respective declines in VCM concentration from the unoccupied inhalation apparatus. The rate constants and half-lives of disappearance of VCM from the system during metabolic experiments were corrected for nonspecific leakage from the chamber (see text).

a function of metabolism, the slower rate of disappearance of the monomer at concentrations of 220 ppm or greater suggested that the metabolism of vinyl chloride monomer may be a dose-dependent process whereby low concentrations are metabolized through pathways that, with higher doses, become saturated.

Vinyl chloride monomer metabolism was also studied using metabolic inhibitors. Pretreatment of rats with ethanol (5 mg/kg body weight, by intraperitoneal injection) 1½ h before exposure to the monomer caused almost complete inhibition in rats exposed to 50 ppm, but only a mild inhibition in rats exposed to 1000 ppm (Figure 6). SKF-525A (Smith Kline & French Laboratories, Philadelphia, Pennsylvania), a commonly used inhibitor of certain reactions mediated by the mixed function oxidase enzymes, did not inhibit metabolism of the monomer in rats exposed to concentrations up to 50 ppm, but did cause a slight, approximately 20%, inhibition at 1000 ppm. Considering these data, we hypothesized that the monomer is metabolized by several different pathways, dependent on the dose. At concentrations up to 100 ppm, it is metabolized predominantly by a single pathway that, we have speculated, may involve alcohol dehydrogenase. However, at concentrations exceeding 220 ppm, this pathway becomes saturated and the significance of secondary pathways, presumably including microsomal mixed function oxidases, increases (32).

Dose-dependent processes for the elimination of a chemical are of prime importance in toxicology. Many metabolic and excretory processes have finite capacities, and, as doses of a chemical are increased, these processes become saturated or overwhelmed. If the function of the saturated process is detoxification, a disproportionate increase in toxicity may ensue *above* those doses that cause saturation. Therefore, toxicity occurring with large doses of a chemical must be interpreted cautiously, and it does not necessarily imply that comparable types of toxicity will be observed at lower doses.

Gillette (33) recently summarized the role of chemically reactive metabolites of foreign compounds in toxicity. Compounds such as bromobenzene (34) and acetaminophen (35, 36) induce hepatic necrosis only after the dose is high enough to overwhelm the detoxification pathways for the reactive metabolites of these compounds. Conjugation of the alkylating metabolites of these chemicals with glutathione is a primary mechanism for detoxification. Necrosis is induced only after the glutathione levels are reduced sufficiently to allow the reactive metabolites to

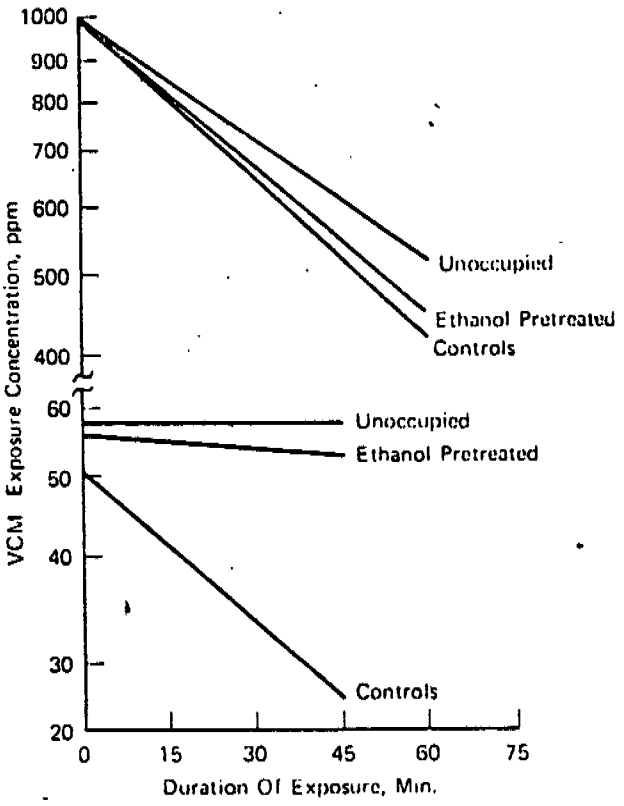

Figure 6. Declines in vinyl chloride monomer (VCM) concentrations, with time, at initial concentrations of 56 and 1034 ppm, for both pretreated and control rats. Also shown are the respective declines in VCM concentration from the unoccupied inhalation apparatus.

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 not 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 it still unpublished, has included studies in rats on the fate of ingested ^{14}C-vinyl chloride monomer, and the isolation and identification of the major urinary metabolites of this chemical. The ingestion study showed that vinyl chloride monomer is metabolized to polar products that are readily excreted in the urine. These results also support the hypothesis of dose-dependent metabolism. The hepatic nonprotein sulfhydryl content of rats exposed to vinyl chloride monomer showed a progressive depression related to the concentration and duration of exposure. Consistent with the sulfhydryl depression, two of the three urinary metabolites isolated thus far have been identified as thiodiglycolic acid and *N*-acetyl-*S* (2-hydroxyethyl) cysteine. Thus it seems that vinyl chloride monomer or its reactive metabolites covalently bind with hepatic glutathione and are subsequently hydrolyzed and excreted in the urine as conjugates of cysteine.

Other studies on vinyl chloride monomer continue to support the hypothesis that its carcinogenicity is related to the metabolic formation of reactive metabolites. Studies (37-39) have shown an enhanced positive mutagenic response in certain strains of *Salmonella typhimurium* exposed to vinyl chloride monomer if microsomal enzymes or fortified liver homogenates are present. The metabolites of the monomer identified in the urine of rats exposed to the chemical 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.

* Gustave L. Levy Distinguished Service Professor, Mount Sinai School of Medicine, New York, New York.

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

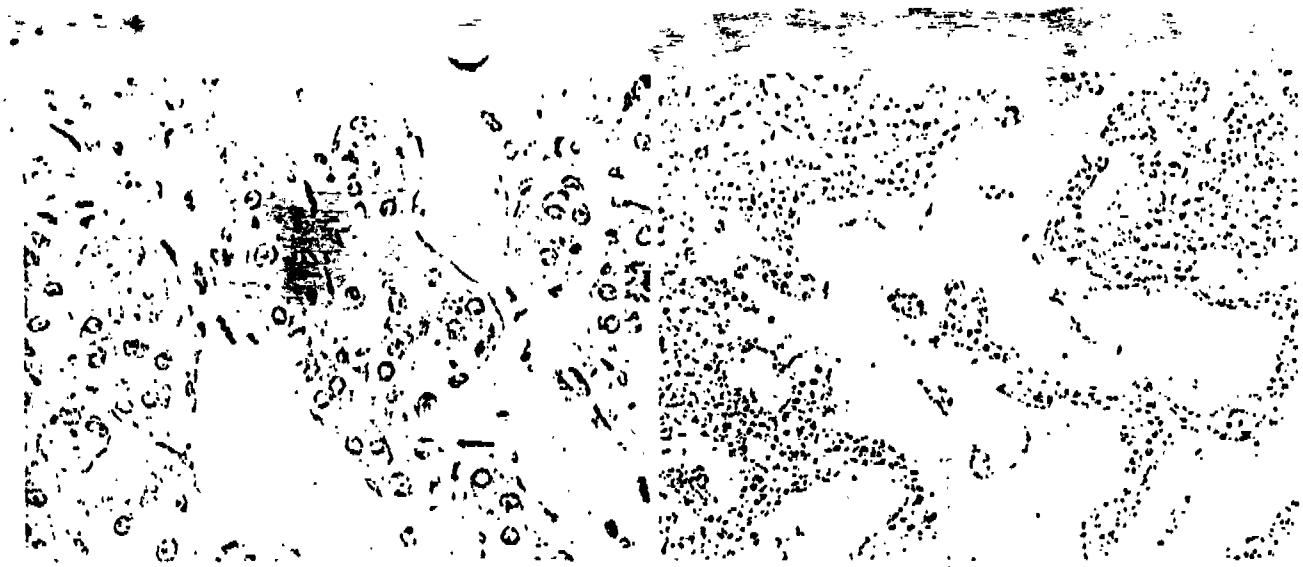

Figure 9. Left. Sinusoidal pattern of angiosarcoma. Sinusoidal spaces are large and irregular. Angiosarcoma cells line these spaces and ensheath hepatic cord cells and an increased number of non-neoplastic-appearing cells in the space of Disse. Bile plugs are present in bile canaliculi. (Hematoxylin 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

* Chief, Laboratory of Pathology, National Cancer Institute.

a few confirmed thrombi. The hepatic surface was irregular due to bulging masses of the angiosarcoma, and portions of the liver not involved grossly by angiosarcomas had irregularly shaped areas of capsular and portal tract fibrosis. The formalin-fixed livers were dark green because of bile stasis.

Microscopic examination showed the angiosarcomas to be multicentric, with several structural patterns. The sinusoidal pattern, illustrated in Figure 9*A*, consisted of neoplastic-appearing sinusoidal cells that had enveloped cords of hepatocytes and formed irregular sinusoidal spaces. In these areas, the hepatocytes were well preserved and bile-secreting, with no degeneration or necrosis. Macrophages, fibroblasts, and lipocytes proliferated in the space of Disse. With increased growth of the angiosarcoma cells, the hepatocytes atrophied and disappeared, and the space of Disse was filled with collagen fibers, so that larger, cystic spaces formed. The papillary growth pattern was an intermediate stage characterized by papillary projections of blind-ending, disrupted hepatic cords extending into the irregular, multiloculated cystic spaces of the angiosarcoma (Figure 9*B*). These spurlike projections were covered by angiosarcoma cells that ensheathed the various other cells in the space of Disse and the residual fragments of hepatic cord cells. The cavernous pattern had larger blood-filled spaces surrounded by a thick wall of dense, relatively acellular fibrous connective tissue. These larger, cystic spaces were lined by variable, sometimes quite scanty, layers of angiosarcoma cells. We also observed more nodular, anaplastic focuses of angiosarcoma in some of the livers, but in every instance these specimens also had other focuses of angiosarcoma that showed the progression from sinusoidal to papillary to cavernous growth patterns.

Thus, this series of changes seems to represent multicentric development of angiosarcoma, starting with the precursor lesions of sinusoidal cell proliferation and atypia. Some of these precursor lesions progress to the fully developed cavernous angiosarcomas, which become clinically evident. We do not have adequate morphologic criteria to separate the earliest histologic changes of sinusoidal cell atypia and hyperplasia from the progressive, fully developed angiosarcomas. As noted earlier, proliferation of hepatocytes and of non-neoplastic-appearing fibrocytes, lipoblasts, and macrophages plays some role in the development of the angiosarcomas. Proliferation of these cells was especially noted in the areas of sinusoidal and papillary growth patterns within the angiosarcomas.

Many of the histologic features observed in the evolution of the hepatic angiosarcomas in the vinyl chloride workers have also been described in the hepatic angiosarcomas caused by thorotrast and by inorganic arsenicals. Studies are now in progress to evaluate the similarities and dissimilarities between the lesions produced by these chemicals and the hepatic angiosarcomas of unknown cause that occur sporadically.

Summary

Dr. Berk: We began this conference with a list of questions that I thought might define the scope of the problem of vinyl chloride-associated liver disease. Although at least some progress has been made toward answering all of them, it is clear that there has not yet been enough progress to fully answer one of them.

It is worth recalling that we have been aware of the problem for less than a year (as of November 1974). The efforts presented at this conference represent an outstanding example of effective, if often informal, collaboration among university, industry, and government scientists, which, if pursued at its current pace, is certain to provide us in the near future with answers to at least some of the critical questions.

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References

1. Wilson RH, McCormick WE, Tatum CF, et al: Occupational acroosteolysis: report of 31 cases. *JAMA* 201:577-581, 1967
2. Suciu I, Drutman I, Valaskai M: Contribuţii la studiul imbolnăvirilor produse de clorura de vinil. *Med Interna (Bucur)* 15:967-978, 1963
3. Cordier JM, Fievez C, Le Fever M, et al: Acro-ostéolyse et lesions cutanees associees chez deux ouvriers affectes au nettoyage d'autoclaves. *Cah Med Trav* 4:14, 1966
4. Harris DK, Adams WGF: Acro-osteolysis occurring in men engaged in the polymerization of vinyl chloride. *Br Med J* 3:712-714, 1967
5. Ellis R, Anderson H, Nicholson WJ, et al: Prevalence of disease among vinyl chloride and polyvinyl chloride workers. *Ann NY Acad Sci* 246:22-41, 1975
6. Pushin GA: [Affection of the liver and bile ducts in workers engaged in the production of some types of plastics.] (Rus.) *Sov Med* 28(2):132-135, 1965
7. Marsteller HJ, Luibach WK, Muller R, et al: Chronisch-toxische Leberschaden bei Arbeitern in der PVC-produktion. *Disch Med Wochenschr* 98:2311-2314, 1973
8. Creech J, Johnson MN, Buck B: Angiosarcoma of the liver among polyvinyl chloride workers—Kentucky. *Morbid Mortal Weekly Rep* 23(6):49-50, 1974
9. Creech JL Jr, Johnson MN: Angiosarcoma of the liver in the manufacture of polyvinyl chloride. *J Occup Med* 16:150-151, 1974
10. Editorial: Vinyl chloride, P.V.C., and cancer. *Lancet* 1:1323-1324, 1974
11. Viola PL, Bigotti A, Caputo A: Oncogenic response of rat skin, lungs and bones to vinyl chloride. *Cancer Res* 31:516-522, 1971
12. Maltoni C, Li Felmine G: Carcinogenicity bioassays of vinyl chloride: current results. *Ann NY Acad Sci* 246:195-218, 1975
13. Gordon DE, Thomas LB, Kent G, et al: Hepatic angiosarcoma in man and rodents following prolonged exposure to vinyl chloride (abstract). *Gastroenterology* 67:794, 1974
14. Falk H, Creech JL, Heath CW Jr, et al: Hepatic disease among workers at a vinyl chloride polymerization plant. *JAMA* 230:59-63, 1974
15. Berk PD, Martin JF, Waggoner JG: Persistence of vinyl chloride-induced liver injury after cessation of exposure. *Ann NY Acad Sci* 246:70-77, 1975
16. LaRusso NF, Hoffman NE, Hofmann AF, et al: Validity and sensitivity of an intravenous bile acid tolerance test in patients with liver disease. *N Engl J Med* 292:1209-1214, 1975
17. Teiche T, Nanba K, Ishak K, et al: Hepatic ultrastructural changes in vinyl-chloride workers (abstract). *Clin Res* 23:259, 1975
18. Veltman G, Lange CE, Jihle S, et al: Clinical manifestations and course of vinyl chloride disease. *Ann NY Acad Sci* 246:6-17, 1975

19. [illegible] CL, [illegible] S, [illegible], et al: [illegible] in polyvinyl chloride production workers. *Ann NY Acad Sci* 246:[illegible], 1975
20. Marsteller HJ, Lelbach WK, Müller R, et al: Klinische und laparoskopische Aspekte der Leberschaden bei Chemiearbeitern in der Vinylchlorid-Polymerisation. *Leber Magen Darm* 5:196-207, 1975
21. Taylor KJ, Williams DM, Smith PM, et al: Grey-scale ultrasonography for monitoring industrial exposure to hepatotoxic agents. *Lancet* 1:1222-1224, 1975
22. Da Silva Horta J: Late lesions in man caused by colloidal thorium dioxide (thorotrast)—a new case of sarcoma of the liver 22 years after the injection. *Arch Pathol* 62:403-418, 1956
23. Visfeldt J, Poulsen H: On the histopathology of liver and liver tumours in thorium dioxide patients. *Acta Pathol Microbiol Scand* [A] 80:97-108, 1972
24. Roth F: Arsen-leber-tumoren: (hämangioendotheliom) *Z Krebsforsch* 61:468-503, 1957
25. Regelson W, Kim U, Ospina J, et al: Hemangioendothelial sarcoma of the liver from chronic arsenic intoxication by Fowler's solution. *Cancer* 21:514-522, 1968
26. Lloyd JW: Angiosarcoma of the liver in vinyl chloride/polyvinyl chloride production workers. *J Occup Med* 17:333-334, 1975
27. Tabershaw IR, Gaffey WR: Mortality study of workers in the manufacture of vinyl chloride and its polymers. *J Occup Med* 16:509-518, 1974
28. Waxweiler RJ, Stringer W, Wagoner JK, et al: Neoplastic risk among workers exposed to vinyl chloride. *Ann NY Acad Sci* 271:40-48, 1976
29. Landrigen PJ, Heath CW Jr: Hemangiosarcoma of the liver, Connecticut. *USPHS Report:* EPI-74-104-2, 1974. Atlanta, Center for Disease Control
30. Maltoni C, Lefemine G: Carcinogenicity bioassays of vinyl chloride. (1) Research plan and early results. *Environ Res* 7:387-405, 1974
31. Monson RR, Peters JM, Johnson MN: Proportional mortality among vinyl chloride workers. *Lancet* 2:397-398, 1974
32. Hefner RE Jr, Watanabe PG, Gehring PG: Preliminary studies of the fate of inhaled vinyl chloride monomer in rats. *Ann NY Acad Sci* 246:135-148, 1975
33. Gillette JR: A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity. *J Biochem Pharmacol* 23:2785-2794, 1974
34. Jollow DJ, Mitchell JR, Zampaglione N, et al: Bromobenzene induced liver necrosis, protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology* 11:151-169, 1974
Mitchell JR, Jollow DJ, [illegible] WZ, et al: Acetaminophen induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther* 187:211-217, 1973
36. Jollow DJ, Thorgeirsson SS, Potter WZ, et al: Acetaminophen induced hepatic necrosis. *Pharmacology* 12:251-271, 1974
37. Bartsch H, Malaveille C, Montesano R: Human, rat and mouse liver-mediated mutagenicity of vinyl chloride in *S. typhimurium* strains. *Int J Cancer* 15:429-437, 1975
38. Rannug U, Johansson A, Ramel C, et al: The mutagenicity of vinyl chloride after metabolic activation. *Ambio* 3:194-197, 1975
39. Malaveille C, Bartsch H, Barbin A, et al: Mutagenicity of vinyl chloride, chloroethyleneoxide, chloroacetaldehyde and chloroethanol. *Biochem Biophys Res Commun* 63:363-370, 1975
40. Popper H: The heuristic importance of environmental pathology: lessons from the vinyl chloride problem. *Arch Pathol* 99:69-71, 1975
41. Ito T: Recent advances in the study of the fine structure of the hepatic sinusoidal wall. *Gunma Rep Med Sci* 6:119-163, 1973
42. Bronfenmaier S, Schaffner F, Popper H: Fat storing cells (lipocytes) in human liver. *Arch Pathol* 82:447-453, 1966
43. Schnack H, Stockinger L, Wewalka F: Adventitious connective tissue cells in the space of Disse and their relation to fiber formation. *Rev Intern Hepat* 17:855-859, 1967
44. Bagheri SA, Boyer JL: Peliosis hepatis associated with androgenic-anabolic steroid therapy. A severe form of hepatic injury. *Ann Intern Med* 81:610-618, 1974
45. Poulsen H, Winkler K: Liver disease with periportal sinusoidal dilatation (abstract). *Digestion* 8:441, 1973
46. Popper H, Thomas LB: Alteration of liver and spleen among workers exposed to vinyl chloride. *Ann NY Acad Sci* 246:172-194, 1975
47. Viallet A, Guillaume E, Cote J, et al: Presinusoidal portal hypertension following chronic arsenic intoxication (abstract). *Gastroenterology* 62:177, 1972
48. Morris JS, Schmid M, Newman S, et al: Arsenic and noncirrhotic portal hypertension. *Gastroenterology* 66:86-94, 1974
49. Knolle J, Förster E, Roessner A, et al: Die nicht-zirrhotische portale Fibrose (Hepatoportale Sklerose) nach chronischer Arsenvergitung. *Dtsch Med Wochenschr* 99:903-908, 1974
50. Thomas LB, Popper H, Berk P, et al: Vinyl chloride-induced liver disease: from idiopathic portal hypertension (Banti's syndrome) to angiosarcoma. *N Engl J Med* 292:17-22, 1975
51. Thomas LB, Popper H: Pathology of angiosarcoma of the liver among vinyl chloride-polyvinyl chloride workers. *Ann NY Acad Sci* 246:268-277, 1975

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